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Recent Advances in Drug Discovery for Diabetic Kidney Disease

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ABSTRACT

***Introduction*:** Diabetic kidney disease (DKD) is a leading cause of end-stage renal disease (ESRD), and 40% of patients with diabetes develop DKD. Although some pathophysiological mechanisms and drug targets of DKD have been described, the effectiveness or clinical usefulness of such treatment has not been well validated. Therefore, searching for new targets and potential therapeutic candidates has become an emerging research area.

***Areas covered*:** The pathophysiological mechanisms, new drug targets and potential therapeutic compounds for DKD are addressed in this review.

***Expert opinion*:** Although preclinical and clinical evidence has shown some positive results for controlling DKD progression, treatment regimens have not been well developed to reduce the mortality in patients with DKD globally. Therefore, the discovery of new therapeutic targets and effective target-based drugs to achieve better and safe treatment is urgently required. Preclinical screening and clinical trials for such drugs are needed.

Keywords: diabetic complications, diabetic nephropathy, drug discovery, endothelin receptor blockers, oxidative stress, renin-angiotensin-aldosterone system, SGLT-2 inhibitors

Article highlights.

- Diabetic kidney disease (DKD) is the single most common cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD), which is clinically characterized by persistent albuminuria of 30-300mg/day.
- Multiple pathophysiological mechanisms have been proposed; therefore, the treatment could be difficult with single-drug therapy.

- Current treatment of DKD is not able to stop its pathological progression completely and reduce mortality rate. Novel target discovery and therapeutic strategy development are urgently required.
- This article mainly focuses on new potential drug targets and related new compounds for the potential treatment of DKD.

The box summarizes key points contained in the article.

1. Introduction

Diabetic kidney disease (DKD), can be defined as diabetes with persistent microalbuminuria (ratio of urine albumin to creatinine ≥30mg/g) and impaired glomerular filtration rate (<60mL/min/1.73m^2) leading to the deterioration of renal function and ultimately morbidity and mortality of patients[1-3]. Albuminuria is the characteristic clinical biomarker of DKD observed in patients with both type 1 and type 2 diabetes mellitus (T1DM and T2DM). In 2013, the Global Burden of Disease Study reported a systematic analysis and estimated that the age-standardized mortality from DKD had increased by 106% compared to the year 1990[4]. However, a recent study has found that DKD prevalence is not so stable and the death rate is increasing at the global level[5]. As the prevalence of both T1DM and T2DM patients is increasing, the incidence of macro- and microvascular complications, such as stroke, coronary heart disease, diabetic retinopathy, ischaemic nephropathy and kidney interstitial fibrosis, is also significantly increased[6]. The pathophysiological consequences of DKD under hyperglycaemia have been described in Figure 1. The current therapeutic strategy for DKD is to control proteinuria, hyperglycaemia, and high blood pressure to reduce cardiovascular and renal failure risk. Inhibition of the renin-angiotensin-aldosterone system (RAAS) and sodium-glucose cotransporter-2 (SGLT-2) has significant clinical

outcomes such as reduction in renal progression, all-cause mortality, proteinuria, and cardiovascular events[7, 8]. A clinical trial with empagliflozin (EMPA-KIDNEY (the study of heart and kidney protection with empagliflozin) is ongoing (WWW.ClinicalTrials.gov, Identifier: NCT03594110). These treatments are promising for DKD; however, it is still proving difficult to halt the progression of DKD in patients mainly due to genetic and epigenetic variabilities[6, 9]. Therefore, development of new therapeutic strategies is required to target the underlying pathophysiological mechanisms. Some new drug targets have been recently identified, which could be helpful to develop new drugs to prevent renal and vascular damage in patients with DKD. Development of personalized medicine based on genetic and epigenetic variabilities could also be an approach to improve renal and cardiovascular outcomes in patients with DKD. In this review, we have covered recent advances in the pathophysiological mechanisms, potential new therapeutic targets and candidates for DKD.

2. Pathophysiological mechanisms

Multiple pathophysiological mechanisms are involved in the pathogenesis of DKD, including genetic and epigenetic aspects, abnormal renal haemodynamics, mitochondrial dysfunction and oxidative stress, ischaemia and inflammation, and podocyte injury and autophagy suppression. A schematic diagram for the mechanisms and possible drugs and their targets is shown in Figure 2.

2.1. Genetic and epigenetic aspects of DKD development

During the past 20 years, substantial evidence has been collected to understand the genetic basis of DKD in both T1DM and T2DM patients, such as familial clustering of DKD, the heritability and DKD -related traits, gene mapping for DKD in the genome-wide association study (GWAS)[10, 11]. It has been found that not all diabetic patients

have developed DKD due to the difference in genetic susceptibility even though glycemia is not regularly controlled[12]. Due to the variation in genetic predisposition, the prevalence of DKD is higher in certain ethnic groups such as in native Americans, African Americans, and Mexican Americans than in other populations like European Americans[10]. The genes such as APOL1, RND3/RBM43, SLITRK3, ENPP7, and GNG7 make African American populations more prevalent to DKD[10, 13]. Identification of such risk genes is a direction for future personal medicine.

The epigenetic modification including histone posttranslational modifications (PTMs), non-coding RNAs and methylation of DNA, may influence the development of DKD. Some pathologic conditions can modify the epigenetic profiles such as hyperglycaemia, hypoxia, inflammation, and release of some inflammatory cytokines[9, 14, 15]. The importance of DNA methylation on regulating DKD-related genes has been reported[15], and the different degrees of DNA methylation has been demonstrated in T2DM patients with or without DKD[16]. The gene, UNC13B, has been reported to involve in the initial pathogenesis of DKD and cause apoptosis of glomerular cells in hyperglycaemic condition[16, 17]. The gene, RASALI, was found to be involved in hypermethylation, and therefore increased Ras activation in fibroblasts and led to tissue proliferation and fibrosis[18]. A case-control association study on blood-derived DNA from T1DM patients has revealed distinct methylation patterns in genes which were related to mitochondrial function and confirmed the influence of DNA methylation in DKD progression[19]. Epigenetic modifications in the kidneys of db/db T2DM diabetic mice have been well studied. Hypomethylation was reported for SGLT2, PCK1 and G6PC genes that capable of encoding proteins which involved in glucose metabolism as well as the gene that encodes hepatocyte nuclear factor 4 alpha (HNF4A), a transcription factor, which regulates transporters for reabsorption in the proximal tubule[20]. The persistence of

abnormal DNA methylation pattern was observed in db/db mice when pioglitazone was administered to reduce blood glucose levels and albuminuria, suggesting an important clue of epigenetic metabolic memory in the progression of DKD[20]. Histone methylation is also involved in the gene expression of transforming growth factor-beta 1 (TGF-β1) mediated extracellular matrix (ECM)[21]. Applications of histone deacetylase (HDAC) inhibitors such as sodium butyrate, trichostatin, valproate and vorinostat in the streptozotocin (STZ)-induced diabetic rats have shown significant protective effects by decreasing proteinuria, glomerular injury, oxidative stress, fibrosis, and inflammation[22-24]. Furthermore, microRNAs (miRNAs) are abundantly expressed in the kidney. These small non-coding RNAs are involved in the degradation of mRNA and promote translation repression, which have been shown to affect DKD progressing[25]. In the early stage of DKD, an increased level of miRNA-192, a key gene regulator, was reported[26]. The induction of miRNA-192 in DKD was mediated by histone acetylation[27]. In mesangial cells, miRNA-192 up-regulates collagen type II (COLA2) and collagen type IV alpha 1 (COL4A1), and promotes the autoregulation of TGF-β1 by regulating other miRNAs and amplifying the response of p53 to TGF-β1[27-29]. Furthermore, miRNAs and long non-coding RNAs (lncRNAs) have been reported to play an essential role in the regulation of gene expression in DKD, such as the inhibition of lnc-MGC showed reduction of hypertrophy as well as glomerular extracellular matrix accumulation in diabetic mice[30]. Nuclear factor (erythroid-derived 2)-like 2 (NRF2) is a transcription factor which is reported to have protective effects in diabetes by neutralizing reactive oxygen species (ROS) through the activation of the intracellular antioxidation systems and thus maintaining the balance of redox homeostasis[31, 32], the inhibition of TGF-β1 and downregulation of cyclin-dependent kinase inhibitor 1A, and decrease of ECM production[33]. More studies are required to

understand the role of non-coding RNAs that could serve as molecular modulators in DKD. Collectively, these studies provide evidence that genetic difference and epigenetic modifications could explain the variability of drug responses and the molecular mechanisms involved in DKD progressing, which could pave the way for the development of personalized medicine.

2.2. Abnormal renal haemodynamics

Hyperfiltration by the glomeruli leads to the occurrence of DKD. Hyperglycaemia causes chronic metabolic and hemodynamic changes by modulating signalling pathways, transcription factors, cytokines, chemokines, and growth factors[9, 13, 34, 35]. It also leads to afferent arteriolar dilatation by increasing the release of vasoactive mediators, such as insulin-like growth factor 1 (IGF-1), glucagon, nitric oxide (NO), vascular endothelial growth factor (VEGF) and prostaglandins[9]. Due to the haemodynamic changes in diabetic patients, they may predispose DKD through both episodes of acute kidney injury and chronic progression[36]. Furthermore, upregulation of SGLT-2 in poorly glycaemic controlled diabetic patients was reported, which causes hyperfiltration of glucose as well as increased reabsorption of both glucose and sodium in proximal tubule[37]. Hence, a small amount of sodium reaches the macula densa, causing constriction of the afferent arteriole via tubuloglomerular feedback. Simultaneously, the efferent arteriole also constricts due to the high levels of angiotensin II (Ang II), causing glomerular hyperfiltration and hypertension[38]. Recently, it has been reported that Ang-II mediates MYH9 (a gene expressed in podocytes and involved in glomerular pathophysiology) downregulation that leads to podocyte structural and functional damage in DKD[2]. Ang-II also mediates transient receptor potential cation channel, subfamily C, member 6 (TRPC6) activation and reduces MYH9 expression[2].Therefore, the Ang-II-mediated MYH9 depletion in DKD

increases the filtration barrier permeability by inducing apoptosis of podocyte via TRPC6 calcium channels and Ca^{2+} influx[2]. Hence, MYH9 could be regarded as a potential target for DKD. High oral intake of sodium also activates the RAAS system and causes blunting of the antihypertensive effects of angiotensin-converting enzyme inhibitors (ACEI) and angiotensin-II receptor blockers (ARB)[2, 39-42]. Aldosterone is also reported to be involved in the pathophysiology of DKD through plasminogen activator inhibitor 1 (PAI-1) and TGF-β promoting macrophage infiltration and renal tissue fibrosis[43].

Moreover, vascular homeostasis is regulated by endothelin-1 (ET-1), a potent vasoactive peptide produced by endothelial cells, via the activation of endothelin receptor. Compensatory high insulinaemia seen in T2DM increases ET-1 secretion resulting in vasoconstriction and vascular dysfunction[44, 45].

2.3. Mitochondrial dysfunction and oxidative stress

Hyperglycaemia is thought to play an essential role in mitochondrial dysfunction through overloading the electron transport chain system that causes reduction of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity, estimulating excess ROS production, and causes oxidative injury to podocytes and consequently their apoptosis[46, 47]. Excess ROS leads to deleterious effects on DNA, lipids and proteins and hence results in cell death, fibrosis, and eventual kidney dysfunction. Also, there is impairment of glycolytic metabolism that leads to accumulation of upstream glycolytic intermediates which shift the metabolic flux to other metabolic pathways (those are involved in kidney injury) such as polyol pathway, hexosamine pathway, activation of PKC and production of advanced glycation end products (AGEs) precursors relating to cellular dysfunction, DNA damage, vascular leakage, thrombosis, fibrosis, angiogenesis and inflammation[46-48]. It has been well investigated that hyperglycaemia

leads to upregulation of the polyol pathway. The glucose converted to sorbitol via the enzyme, aldose reductase and then the sorbitol is oxidised to fructose. The increase level of sorbitol leads to reduction of intracellular antioxidant, i.e. glutathione (GSH) which is thought to accelerate the intracellular oxidative stress and apoptosis. The fructose is the end product of the polyol pathway and increase of fructose concentration leads to inhibition of glycolysis. Fructose is also considered as a potential nephrotoxin. The endogenous production of fructose via the polyol pathway has led to the increased level of proteinuria, reduction of GFR, and increased of glomerular and proximal tubular injuries which have been investigated in a diabetic murine mice model. Furthermore, these mice also reported to express high levels of superoxide and inflammatory cytokine like NF-κB[6]. On the other hand, it has been found that the DKD kidney contains fewer mitochondria, reduced mitochondrial superoxide formation, and therefore has reduced activity of electron chain complexes[49]. Stimulation of inflammatory processes via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and vascular dysfunction was also identified due to the decreased mitochondrial superoxides that reduced the endothelial nitric oxide synthase (eNOS) phosphorylation process and AMP-activated protein kinase (AMPK) activity[49]. Hyperglycaemia and AGEs activate the RAAS system and induce renin and angiotensin expression in renal cells through kidney-specific G protein-coupled metabolic receptor GPR91 and ROS, causing deterioration of DKD. Ang-II stimulates the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, chronic hypoxia, ROS production, inflammation, renal tissue fibrosis and renal tubular dysfunction and subsequent atrophy, and decrease of the number of small vessels[2, 39, 41]. Also, endothelial dysfunction due to the high glucose, insulinaemia and insulin resistance through intracellular mechanisms, relating to activation of PKC, increased release of

ROS, and AGEs-induced proinflammatory signalling[44]. Activation of endothelin receptor A is also involved with podocyte injury, inflammation, oxidative stress, and fibrosis in the kidney[44, 45].

2.4. Ischaemia and inflammation

Renal ischaemia and inflammation contribute to the pathophysiological progress of DKD. In developed DKD, the susceptibility of renal ischaemia is increased and this aggravates the renal inflammation by infiltration of neutrophils and macrophages in the kidney[50]. Renal medulla hypoxia occurs as the supply of oxygen is reduced due to glomerular and vascular lesions in DKD. Therefore, more ROS are generated and hypoxia-inducible factors (HIFs) are activated[51]. Along with this, intrarenal NO production is reduced[52]. Thus, the overproduction of ROS and hyperglycaemia lead to renal injury[51, 52]. Smoking also contributes to the progression of DKD in both T1DM and T2DM patients through upregulation of growth factors, escalating oxidative stress, and endothelial dysfunction[53].

In DKD, NF-kB is activated by phosphorylation and participates in the regulation of inflammatory factors. The activation of NF-kB is due to the increased levels of ROS and tumour necrosis factor (TNF). NF-kB is also involved in proteinuria and infiltration of renal interstitial inflammatory cells[54]. It has been reported that the immune system is also involved in the development of DKD[55, 56]. Diabetic kidney cells are prone to produce pro-inflammatory factors such as danger-associated molecular patterns (DAMPs), chemokines and cell adhesion molecules (CAMs) to facilitate innate immune responses. Hyperglycaemia increases the oxidized lipoproteins content, cellular stress, and activates macrophages, which disturb the functions of mitochondria and endoplasmic reticulum. Also, hyperglycaemia leads to abnormal activation of intracellular signalling pathways that are involved in the formation of immune

complexes. Deposition of these immune complexes in the kidney cells amplifies the innate immune response through the activation of complement and immunoglobulin Fc_{γ} receptors[9, 57]. It has further been reported that Janus-kinase signal transducer and activator of transcription (JAK-STAT) pathway is involved in the progression of DKD through angiotensin receptors, cytokines and chemokines with assist innate immune responses[58]. Recently, the involvement of gut microbiome in chronic inflammation with T2DM and CKD has been identified because of the release of toxin products such as indoxyl sulfate, cresyl sulfate and beta-2 microglobulin, by them into the blood which can trigger the activation of innate immune responses and cause systemic inflammation, which is thought to be associated with the pathophysiology of DKD[59].

2.5. Podocyte injury and autophagy suppression

Podocyte injury plays an important role in the pathogenesis of DKD because it maintains the glomerular filtration barrier. The level of albuminuria is an early biomarker of the severity of DKD. There is evidence that a balance between the activation and repression of the WNT/β-catenin (CTNNB1) pathway is essential for podocyte health. The increased expression of CTNNB1 pathway components such as transcripts and proteins in podocytes has been reported in both humans and mice with DKD[60, 61]. In addition, some potential mechanisms such as RAAS (including ACE1 and ACE2), peroxisome proliferator-activated receptors (PPAR), prorenin and its receptor, AGEs, and their receptors (RAGE), ROS, prostanoids, adiponectin, and miRNAs have been reported for the irreversible podocyte injury and loss due to the contribution of diabetic milieu. Furthermore, some biomarkers that include podocyte-released microparticles and expression of podocyte-specific markers noticed in the urine of early podocyte injury have been identified[62, 63], such as nephrin, a key structural and signalling molecule in the podocyte slit diaphragm[64, 65]. Recently, the involvement of

autophagy in diabetic podocyte injuries has been reported, such as the two proteins, β-arrestins and ubiquitin carboxy-terminal hydrolase 1 (UCH-L1), through the inhibition and upregulation of autophagy respectively[66-69]. Activated podocyte autophagy has protective effects on DKD through mammalian target of rapamycin (mTOR) regulation and autophagy-related (Atg) protein conjugation system; however, activation of mTOR suppresses autophagy[69]. Podocyte injury and autophagy are important targets to limit DKD progression because it has been identified that modulation of autophagic pathways in podocytes has shown promising results in the treatment of DKD patients. Autophagy activators such as metformin, resveratrol, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AMPK activators), rapamycin and sirtuin-1(mTORC1 inhibitors) have been reported as showing significant protective effects on renal structural integrity and function, and decrease albuminuria in experimental DKD models[70]. Similarly, selective apoptosis signal-regulating kinase (ASK1) inhibitor such as selonsertib has shown to reduce the rate of eGFR decline by 71% in phase 2 clinical trial in adult T2DM patients with moderate-to-advanced DKD. Selonsertib at oral daily doses of 2, 6, or 18 mg was found to be safe with no dose-dependent adverse effects and considered to be able to slow DKD progression[71]. Recently, resveratrol (a non-flavonoid polyphenol) has shown protective effects against podocyte apoptosis by stimulating autophagy, and astragaloside IV (the active component in a traditional Chinese herbal remedy) has also been shown to promote autophagy in podocyte and to attenuate endoplasmic reticulum (ER) stress in diabetic mice models with DKD[72, 73]. These data will allow researchers to expand the discovery of targets susceptible to pharmacological manipulation and compound screening via targeting podocyte injury and activation of autophagy.

3. Therapeutic candidates and drug development for DKD

Current clinically used therapeutic drugs have shown some therapeutic efficacy for DKD, but they are unable to halt progression of the disease and stop death of patients[13]. Hence, many potential therapeutic targets have been explored in order to search alternate safe and effective therapeutic candidates. Several drug discovery programmes, such as preclinical small molecule screening (both natural and synthetic compounds) using cellular and animal models, and clinical trials on existing drugs, have been developed. The therapeutic target-based drugs used clinically (**Table 1**), and list of compounds in preclinical development (**Table 2**) for the treatment of DKD are summarized.

3.1. RAAS inhibitors

Over the last two decades, RAAS inhibitors have been the first-line treatment choice due to their successful clinical results[13]. Clinical trials on ACE inhibitors, such as captopril, and ARBs, including losartan and irbesartan, have demonstrated positive outcomes in decreasing albuminuria in DKD patients[74-77]. However, it has been reported that in clinical trials paricalcitol fails to show a renoprotective effect[78]. RAAS inhibitors have been shown to merely slow the progression of DKD, and some diabetic patients have still progressed to CKD and ESRD[13]. Inclusion of a mineralocorticoid receptor (MR) antagonist with ACE or ARB inhibitor regimen is beneficial in decreasing albuminuria, inflammation, and kidney fibrosis in patients with DKD. The risk of hyperkalaemia due to MR antagonists can be lowered using novel non-steroidal MR antagonist such as finerenone, which is an MR antagonist. Recent trials with finerenone showed reductions in proteinuria, kidney failure and DKD progression[79]. In another clinical trial, finerenone also showed to reduce cardiovascular mortality and morbidity in DKD patients[80]. Some other MR antagonist such as spironolactone has been tested in combination with antidiabetic/

renoprotective/antihypertensive drugs which showed positive results in reducing DKD progression and lowering proteinuria[81]. The finerenone is still under phase 3 clinical trial with T2DM patients to evaluate its efficacy in long-term for DKD[82]. Therefore, further investigation is still required to find better therapeutic candidates for this class of drug.

3.2. SGLT-2 inhibitors

SGLT-2 inhibitors are currently considered as the best therapeutic option for the treatment of DKD as this class of drugs have been shown to slow the progression of DKD by preventing glucose reabsorption at the proximal tubule of the kidney, and therefore lower the blood glucose levels in diabetic patients[38]. SGLT-2 inhibitors, such as canagliflozin, dapagliflozin and empagliflozin, have shown renoprotective effects in clinical trials[83].The mechanisms for renoprotection in T1DM and T2DM patients for SGLT-2 inhibitors could be due to the reduction of hyperfiltration and post-glomerular vasodilation[83, 84]. In addition, SGLT-2 inhibitors have been shown to exhibit antioxidant and anti-inflammatory effects, and downregulate RAGE expression in DKD patients[84]. Canagliflozin has been shown to reduce the levels of matrix metalloproteinase-7, fibronectin-1, tumour necrosis factor receptor 1 (TNFR1) and interleukin-6 (IL-6) to protect the kidney[85]. Dapagliflozin also had protection against proteinuria, podocyte loss and glomerular injury in DKD[86]. The renoprotective effects of lusogliflozin in an ischemia-reperfusion injury model was thought to be due to lowering hypoxia by increasing the VEGF-A level and decreasing capillary rarefaction[87]. The renoprotective effects of SGLT-2 inhibitors is also considered to be due to the increase of erythropoietin production and reduction of diuretic requirement, as a result by lowering hypertension, increasing renal perfusion and oxygenation[87]. Hence, the role of

SGLT-2 inhibitors as renoprotectors can provide critical mechanistic insights into the use of these class of drugs for treating DKD. Recent clinical trials on SGLT-2 inhibitors in the treatment of DKD have shown exciting results: 1) Empagliflozin has been shown to induce a 44% decrease in the risk of incidence or to worsen of nephropathy along with beneficial cardiovascular effects in DKD patients; 2) Canagliflozin (100 mg daily) treatment in T2DM patients has been shown to decrease 30% risk of ESRD; 3) Dapagliflozin has been shown to reduce 46% in sustained eGFR decline and to reduce ESRD risk or renal death, and shows cardiovascular protection[88-90]. Therefore, the clinical use of SGLT-2 inhibitors is currently a good option for the treatment of DKD.

3.3. Endothelin receptor blockers

Selective endothelin receptor A (ETA) blockers have shown some promising results by decreasing albuminuria, hypertension, fibrosis, structural injury, and the maintenance of podocyte integrity in experimental models of DKD, and therefore, blockage of ETA has become an attractive line of approach for the treatment of DKD. Currently, atrasentan, a selective ETA antagonist, is in phase III clinical trials. Atrasentan has an antialbuminuric effect which may be due to the possible mechanism of restoring glomerular endothelial glycocalyx barrier and improving endothelial function, which were confirmed by the increase of NO concentrations, the decrease of glomerular heparinase expression and inflammation[91]. However, despite a 40% decrease in albuminuria, clinical studies on avosentan in combination with RAAS blockers for DKD has been terminated early due to high rates of cardiovascular adverse effects. These included edema, heart failure and cardiovascular deaths, which was thought to be due to high dose of avosentan that blocks both ETA and the endothelin receptor B (ETB)[92]. Therefore, it may be

assumed that selective inhibitors of ETA could be a potential approach for DKD treatment.

3.4. New targets and drug development

3.4.1. PKC inhibitors

Protein kinase C (PKC) inhibitors have shown some promising results in DKD treatment[93, 94]. Ruboxistaurin, a selective PKC-β inhibitor, decreases albuminuria and improves renal function, and this was thought to be due to the prevention of TGF-β signalling upregulation in the STZ-induced DKD model[93]. In clinical trial results, ruboxistaurin decreased albuminuria and increased eGFR in patients with DKD[94]. Moreover, the use of recombinant human GLP-1 has been reported showing protective effects in diabetic rats, which were thought to be mediated by partial PKC inhibition and PKA activation[95]. Therefore, development of novel PKC inhibitors could be a potential way to treat DKD.

3.4.2. WNT signalling pathway inhibitors

The wingless-type MMTV integration site (WNT) pathway involves in multiple physiological and pathological processes, such as angiogenesis, inflammation, and fibrosis. This signalling pathway also plays an important role in the pathogenesis of DN[96]. It has been shown that WNT pathway is activated in the kidneys of both T1DM and T2DM mice models. WNT signalling pathway inhibitors have been shown to decrease renal inflammation, fibrosis, and proteinuria in an Akita diabetic mice model. The blockade on the WNT pathway with the monoclonal antibody 2F1, which binds the ligand-binding domain of low density lipoprotein-receptor-related protein 6, has been shown to decrease proteinuria and renal fibrosis in both in db/db mice and STZ-induced T1DM rats[96]. Therefore, the WNT signalling pathway could

be a potential therapeutic target for DKD and inhibitors of the WNT pathway could provide useful new drug candidates.

3.4.3. GLP-1 receptor agonists and DPP-4 inhibitors

Two incretin hormones, glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP), are secreted from the intestine in response to food intake. This process can stimulate the secretion of insulin and maintains the homeostasis of glucose. Both peptides act on the GLP-1 receptor and decrease the concentration of glucose. However, dipeptidyl peptidase-4 (DPP-4), a proteolytic enzyme, rapidly degrades these peptides, so they exhibit a very short half-life (<2 mins). In T2DM patients, low GLP-1 levels have been reported, but patients with DKD have upregulated expression of DPP-4 in the glomeruli[97, 98]. Therefore, both GLP-1 receptor agonists and DPP-4 inhibitors have been developed to control glycemia. These classes of compounds are also reported to show nephroprotective effects and to maintain renal functions. Exedin-4, a GLP-1 analogue, is reported to decrease albuminuria, glomerular hypertrophy, mesangial expansion, collagen accumulation, inflammatory infiltration, and the number of apoptotic cells in the glomeruli in type 2 diabetic mouse model with DKD[99]. Liraglutide has also been reported to alter the proteome of diabetic kidney and increases the levels of the Na^+-H^+ antiporter 3 regulatory cofactor-2 protein and normalizes the peroxiredoxin-2 levels[100]. Linagliptin, a DPP-4 inhibitor has been reported to decrease the kidney fibrosis in STZ-induced diabetic mouse model[101]. Similarly, sitagliptin has been shown to attenuate DKD and inhibited the TGF-β1 expression[102]. More studies are required on the two classes of compounds for DKD treatment.

3.4.4. RAGE blockers

Some RAGE blockers, such as OPB-9195, LR-90, ALT-946 and pyridoxamine, have been tested in DKD models and have shown some beneficial effects both in animal models and clinical trials. The results include: 1) Pyridoxamine has been reported to improve the creatinine levels in DKD patients[13]; 2) RAGE-aptamer has beneficial effects in animal models of DKD[103]; 3) Pimagedine did not show any significant benefit in DKD with T1DM patients as it decreased the glomerular filtration rate very slowly, and at a higher dose (>300mg) caused glomerulonephritis [103, 104]. Therefore, more study is needed to evaluate the safety and potential therapeutic value for RAGE blockers.

3.4.5. MCP-1, JAK and phosphodiesterase inhibitors targeting inflammation

Inhibitors of the monocyte chemotactic protein-1 (MCP-1, also named CCL2) that can either neutralize the antibodies or antagonize the receptor (CCR2) have been shown to reduce albuminuria, kidney injury and inflammation in rodent models of DKD[105]. In phase II clinical trial, emapticap pegol, a CCL2 inhibitor, caused a 29% reduction in albuminuria[106]. Similarly, the Janus kinase 1 and 2 (JAK1 and JAK2) inhibitor baricitinib, in phase II randomized control trial on T2DM patients, has been shown to reduce albuminuria by 40% relative to placebo[107].

Patients with DKD have a high serum level of TNF-α, an inflammatory mediator, which causes progression of the disease through inflammatory pathways. As a result, pentoxifylline, a phosphodiesterase inhibitor, was selected for a clinical trial on the basis that it has anti-inflammatory properties including the ability to lower TNF-α levels. Pentoxifylline was also reported to have ability to downregulate the effect of other inflammatory mediators, such as interferon-gamma, interleukin-1, IL-6, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1[108-111]. Additionally, it has been reported that pentoxifylline in combination with

angiotensin II receptor blockers had an additive antiproteinuric effect in T2D patients[112]. Clinical evidence on anti-inflammatory agents belonging to all three being MCP-1, JAK and phosphodiesterase inhibitors class of drugs have shown to be safe and effective in the reduction of albuminuria in the treatment of DKD[106, 107, 112]. Therefore, new potential selective anti-inflammatory agents could be developed for improved outcomes for DKD patients.

3.4.6. Antioxidants targeting mitochondrial dysfunction

Based on the pathophysiological mechanisms involved in the generation of excess free radicals, and subsequent oxidative stress due to the mitochondrial dysfunction, antioxidants may play an essential role in halting the progression of DKD. This provided a potential rationale to evaluate both natural and synthetic antioxidants in DKD models or clinical trials[113]. Clinical evidence revealed that SGLT-2 inhibitors exhibiting antioxidant and anti-inflammatory effects have renoprotective activity in DKD patients[114]. Similarly, DPP-4 inhibitors have also shown to protect the kidney by stimulating antioxidant defence systems and inhibiting fibrosis[102]. Mitoquinone (MitoQ), a well-known antioxidant targeting the mitochondria, has been tested and shown to prevent kidney injury, and to reduce albuminuria in db/db mice with DKD, by improving mitochondrial structural appearance and function as well as facilitating the mitophagy of defective mitochondria[115]. Genetic, modification or pharmacological inhibition of NADPH subunit Nox4 has shown significant renoprotection in T1DM mouse models[116]. In diabetic mice, the glutathione peroxidase (GPX) mimic, ebselen, reduced the oxidative stress and attenuated diabetes-associated renal injury[117]. Several other antioxidants, such as ascorbic acid, resveratrol, and ubiquinone have been tested in the context of DKD, which have shown potential therapeutic benefits in animal models and DKD patients[72, 118].

Furthermore, the so-called "third gasotransmitter", hydrogen sulfide (H_2S), possessing antioxidant properties, has been tested in several DKD models. Evidence shows that H_2S is produced in large amount in the kidney and the enzymes responsible for its production are expressed in the glomeruli, proximal and distal tubules, and interlobular arteries. On the contrary, it has also been identified that H_2S production is low in DKD patients[119]. The H_2S supplementation has been shown to reduce oxidative stress and inflammation in the STZ-induced diabetic rats [120]. It has also been found that H_2S can activate antioxidant defence mechanisms by increasing the SOD activities, the expression of NRF2 transcription factor, and by inhibiting pro-inflammatory and profibrotic pathways[121]. Therefore, H_2S donors, such as NaSH (sodium hydrogen sulfide), have been tested in experimental DKD models and reported to show promising results including reduction of albuminuria and oxidative stress, inhibition of RAAS products, and renal fibrosis, and improvement of kidney function and glomerular filtration barrier[122, 123]. Therefore, H_2S and its analogues can be deployed in new potential drug development. Taken together, the development of new potential antioxidants could be a good approach for treating DKD.

3.4.7. Targeting ORAI and TRPC channels

The underlying molecular mechanism of impaired albumin reabsorption in DKD has been studied[124-127]. It has been reported that ORAI1-3 are well expressed in proximal tubular epithelial cells (PTECs) and downregulated in the late stage of patients with DKD. Hyperglycaemia or blockade of insulin signalling reduced ORAI1-3 expression. In addition, albumin uptake is impaired due to the inhibition of ORAI channels by both BTP2 ([N-{4-[3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl]phenyl}-4-methyl-1,2,3-thiadiazole-5-carboxamide]) and diethylstilbestrol, or

due to the silencing of ORAI expression[124]. In a transgenic mouse model, an increase in albuminuria was noticed due to the expression of a dominant-negative ORAI1 mutant (E108Q). The process of albumin endocytosis is Ca^{2+} dependent and accompanied by ORAI1 internalization. Furthermore, it has been shown that amnionless (AMN) was associated with ORAIs and forms STIM/ORAI/AMN complexes after Ca^{2+} store depletion. These mechanisms for protein reabsorption could be potential targets for the treatment of diabetic proteinuria[124]. It has also been found that mibefradil, a T-type Ca^{2+} channel antagonist, blocks ORAI channels in a dose-dependent manner by acting at the extracellular surface (IC_{50} = 52.6 μM, 14.1 μM and 3.8 μM for ORAI1, ORAI2, and ORAI3, respectively). Application of mibefradil at more than 20 μM concentration inhibited Ca^{2+} release but did not affect cytosolic STIM1 translocation. Mibefradil inhibited cell proliferation, arrested cell cycle progression and induced apoptosis, and these pharmacological actions were due linked to its interaction with ORAI channels[125]. ORAI store-operated channels were also blocked by 4-chloro-3-ethylphenol (4-CEP), an agonist of the ryanodine receptor (RyR), via direct ORAI1-3 channel inhibition. 4-CEP also caused indirect interference with STIM1 subplasmalemmal translocation and clustering after store depletion[126]. To understand the roles of ORAI and STIM in vascular endothelial cells in diabetic conditions, it has been found, using tissue from patients, *in vivo* diabetic mice and *in vitro* cell models, that hyperglycaemia (25 mM D-glucose) enhanced the store-operated Ca^{2+} entry through upregulation of ORAI/STIM via calcineurin-NFAT signalling. It was concluded that the pathway of upregulation of STIM/ORAI through Ca^{2+}-calcineurin-NFAT is a novel mechanism to cause abnormal Ca^{2+} homeostasis and endothelial dysfunction in hyperglycaemia[127]. TRPC channels are

Ca^{2+} permeable cationic channels and TRPC6 is related to kidney disease. In podocytes, TRPC6 can bind and activate calpains, which are calcium-dependent cysteine proteases that control the podocyte cytoskeleton, cell adhesion, and motility via cleavage of paxillin, focal adhesion kinase, and talin. Therefore, TRPC6 plays a scaffolding role in podocytes and thus affects the glomerular filter[128]. In addition, the TRPC5 channel was found to be activated by extracellular thioredoxin and interacted with TRPC1 to form heteromultimeric channels, and both isoforms were present in the kidney and vasculatures[129]. Hence, both ORAIs and TRPC channels are worthy of further exploration to understand better their roles in DKD, and their potential as new therapeutic targets.

4. Conclusion

It can be concluded that RAAS inhibitors and SGLT-2 inhibitors are beneficial for DKD patients, but are not able to stop DKD progression completely. SGLT-2 inhibitors possess both antioxidative and anti-inflammatory properties, which could be beneficial in terms of pathogenesis of DKD, and further SGLT-2 inhibitors need to be developed. Additionally, SGLT-2 inhibitors such as canagliflozin, dapagliflozin and empagliflozin have shown potential effects at multiple molecular targets or contributors of DKD. Therefore, it is a good indication for the discovery and development of better future therapeutics for the treatment of DKD considering these SGLT-2 inhibitors as standard in experimental models. Furthermore, potential SGLT-2 inhibitors can be designed and synthesized for biological testing. Some new therapeutic targets such as DPP-4, PKC, JAK, endothelin receptor, ORAIs/TRPCs, etc are involved in the pathological progression of DKD, and the development of inhibitors of these targets needs more investigation to validate their efficacy and safety before clinical applications. Furthermore, targeting renal circulation could be an alternative approach for DKD. For example, a combination of sacubitril and valsartan is under clinical trial phase III for DKD[1], Cinaciguat, a soluble guanylate cyclase (sGC) activator has shown promising preclinical results, and rosiglitazone, a PPARγ activator has been shown to reduce both microalbuminuria and blood pressure in T2DM patients

independently of glycaemia[130]. Long-term use of epalrestat, an aldose reductase inhibitor, on T2DM patients has shown some potential usefulness on renal functions[131]. Overall, there remains an urgent need for identification and validation of new drug targets and candidates to achieve better treatment of DKD.

5. Expert opinion

RAAS and SGLT-2 inhibitors have been used clinically to treat DKD. These current standard treatments control hyperglycaemia, high blood pressure, lipid profile etc. to manage the disease, but are not sufficient to stop the disease progression. Many new therapies for DKD (DPP-4 inhibitor-ARBs, GLP-1R agonists, DPP-4 inhibitors, paricalcitol, pentoxifylline, pyridoxamine, ruboxistaurin, soludexide, thiazolidinediones, non-steroidal mineralocorticoid receptor antagonists and JAK inhibitors) have or are being preclinically and clinically tested in order to find new effective regimens. Recently, from clinical trials, meta-analysis on efficacy and safety risks of dual blockade of the RAAS in DKD has shown that dual inhibition therapy was superior to monotherapy in controlling blood pressure and reducing proteinuria. However, this analysis did not translate into long-term improvements including reduction of ESRD progression, cardiovascular mortality, and all-cause mortality. This study concluded that a combination of ACEI and ARB or direct renin inhibitor and ACEI/ARB may be a safe and effective therapy for patients with DKD, and a combination therapy may be more suitable for patients with DKD and in controlling hypertension and microalbuminuria. Therefore, this study reveals that use of dual therapy is tolerable in terms of safety risks of drugs and can be better employed in DKD treatment and more investigation is needed for new drug discovery. However, recent reports have also brought into focus the need for identification of more specific biomarkers to better understand the detailed pathophysiological mechanisms of DKD, and hence, to discover potential therapeutic agents for better future treatment and management of DKD. The specific biomarker discovery may identify the pathway (s) involved in the DKD progression and therefore, target based either mono or multi therapy could be prescribed to treat DKD patient subpopulations and can decrease the mortality rate. Due to the multiple pathophysiological mechanisms of DKD, it may be difficult to treat DKD by single-drug therapy, and so different target-based drug discovery approaches are being sought. It may also be thought that DKD might

progress to ESRD through nonalbuminuric pathways (can be defined as 'nonalbuminuric renal impairment'), which can be possibly treated by potential antioxidants or anti-inflammatory agents. Considering the importance and potentiality, use of SGLT-2 inhibitors could be a good option. Some recent research investigations have helped to understand the role and importance of Ca^{2+} channels, such as ORAIs and TRPC, and their association with podocyte injury or renal artery circulation in DKD. This exploration should be continued in order to find new effective drug candidates via high throughput screening. The successful preclinical results of resveratrol and astragaloside IV on podocyte and animal models of DKD may motivate researchers to search for different classes of natural products. In addition, natural and synthetic antioxidants with potential free radical scavenging abilities, including H_2S donor compounds, could also become candidates for DKD therapeutics.

In summary, the genomic and epigenetic diversity and interplay of heterogeneous multiple pathophysiological mechanisms during the disease development makes the disease much more complex. Therefore, for effective treatment of DKD, novel targets need to be validated urgently so that discovery of new therapeutic drug candidates can be identified.

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Declaration of interest

The authors declare no conflict of interest.

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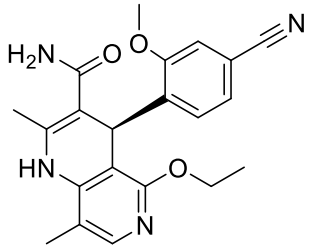
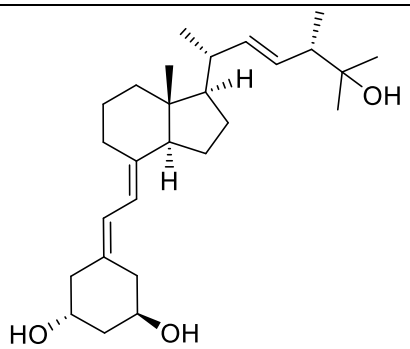
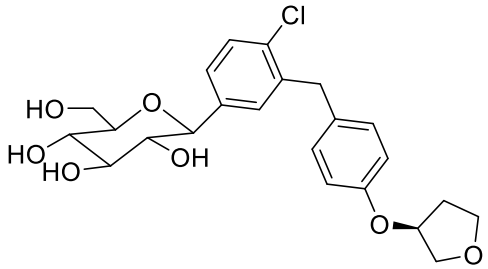
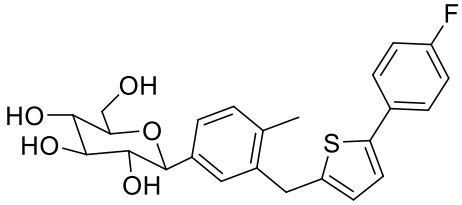
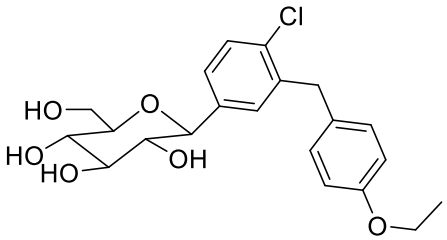
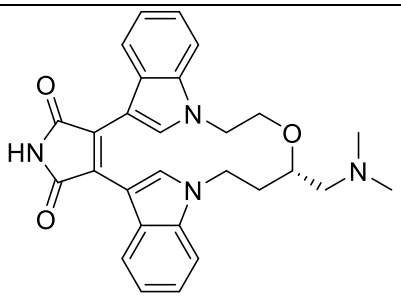
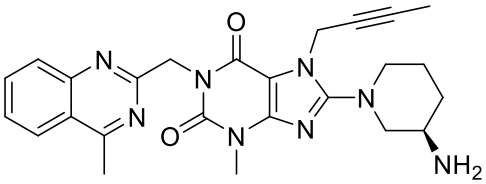
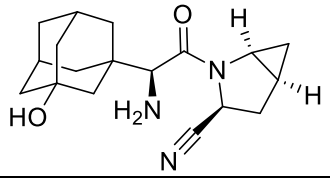
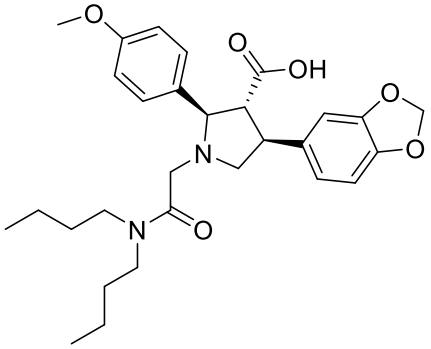
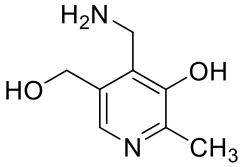
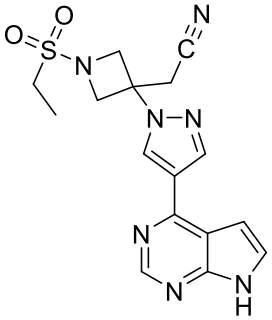
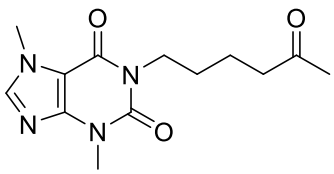
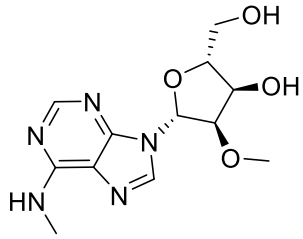
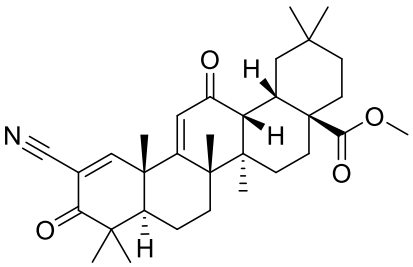
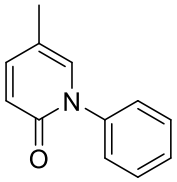
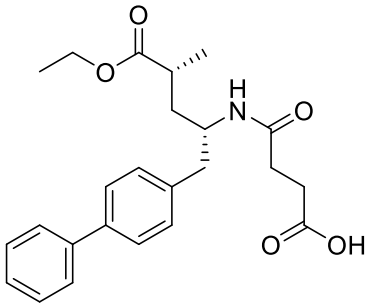
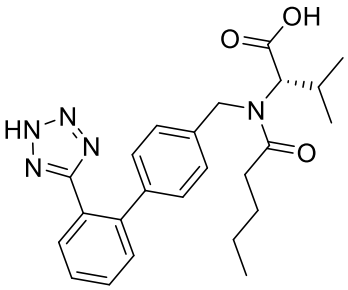
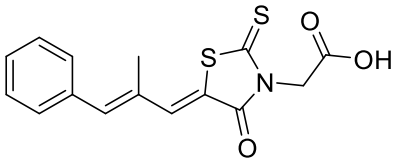
Table 1. List of therapeutic drugs with clinical trials for DKD

Name and References	**Drug Chemical Structure**	**Therapeutic target / mechanism**	**Clinical Status**
Finerenone [79,80,82]		RAAS inhibition	Phase 3 ongoing
Paricalcitol [78]		RAAS inhibition	Clinically failed to show renoprotective effect
Empagliflozin [83,88]		SGLT-2 inhibition	Phase 3 completed in 2015
Canagliflozin [7,83]		SGLT-2 inhibition	Phase 3 ongoing
Dapagliflozin [8,83,90]		SGLT-2 inhibition	Phase 3 ongoing

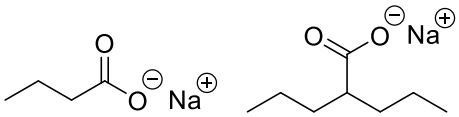
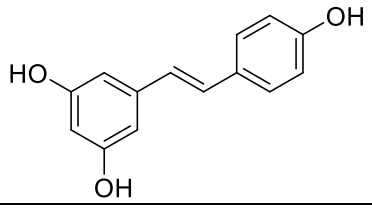
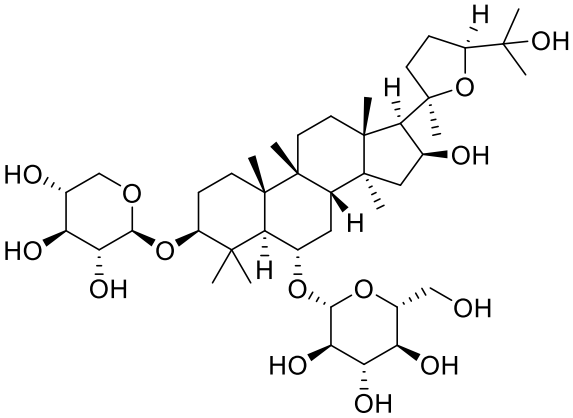
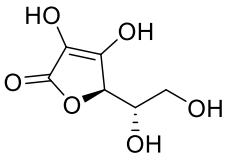
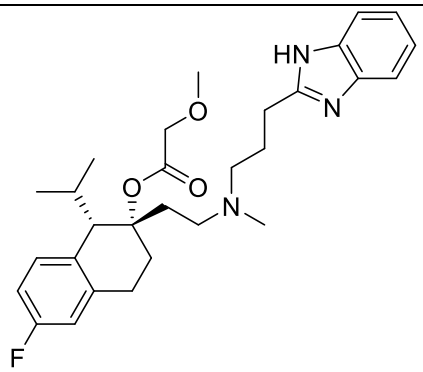
Ruboxistaurin [94]		PKC-β inhibition and oxidative stress reduction	Phase 2 completed
Linagliptin [78]		DPP-4 inhibition	Under clinical trial
Saxagliptin [9]		DPP-4 inhibition	Under clinical trial
Atrasentan [9,44,78]		Selective ETA antagonism	Phase 3 ongoing
Pyridoxamine [9, 13,78]		Blocking AGEs synthesis, and scavenging free radicals and carbonyl products	Phase 3 completed in 2017
Baricitinib [9,78,107]		Selective JAK-1 and JAK-2 inhibition	Phase 2 completed in 2017
Pentoxifylline [108-112]		Phosphodiesterase inhibition	Under clinical trial

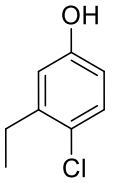
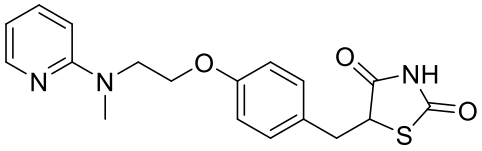
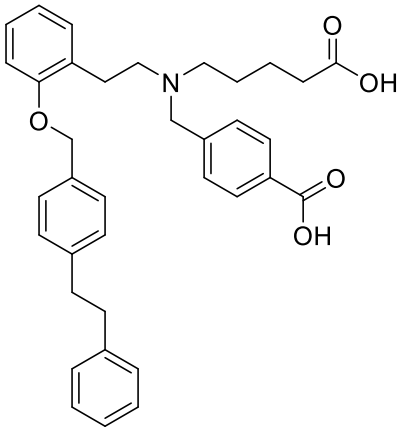
Sulodexide [9,78]		Restoration of anionic heparan sulphate charges at the glomerular basement membrane	Phase 4 terminated in 2008
Bardoxolone methyl [9,78]		NRF2 activation and NF-kB pathway inhibition	Phase 2 completed in 2017
Pirfenidone [9,78]		Blocking TGF-β	Phase 1 & 2 completed in 2009
Sacubitril* [1]		NEP inhibition	Phase 3 ongoing
Valsartan***** [1]		NEP inhibition	Phase 3 ongoing
Epalrestat [131]		Aldose reductase inhibitor	Under clinical trial

*****Both drugs have tested clinically in combination form as LCZ696.

RAAS: renin angiotensin aldosterone system; SGLT2: sodium glucose cotransporter 2; DPP-4: dipeptidyl peptidase-4; AGE: advanced glycation end products; ETA: endothelin receptor A; PKC: Protein kinase C; JAK: Janus kinase; NEP: neprilysin; NRF2: Nuclear factor (erythroid-derived 2)-like 2; NF-kB: nuclear factor kB; TGF: transforming growth factor.

Table 2. List of compounds in preclinical development for the treatment of DKD.

Name and References	**Drug Chemical Structure**	**Therapeutic target / mechanism**	**Clinical Status**
Sodium butyrate and Valproate [21-23]		HDAC inhibition	Preclinical stage
Resveratrol [72]		Autophagy stimulation to protect podocyte apoptosis	Preclinical stage
Astragaloside IV [73]		Attenuation of ER stress and promotion of autophagy in podocyte	Preclinical stage
Ascorbic acid [118]		Antioxidant	Preclinical and clinical stage
Sodium hydrogen sufide (H_2S donor) [119-121]	NaSH	Antioxidant, reduction of albuminuria, oxidative stress, RAAS products and renal fibrosis	Preclinical stage
Mibefradil [125]		T-type Ca^{2+} channel blocker, ORAI channels inhibitor	Preclinical stage

4-Chloro-3-ethylphenol [126]		Ryanodine receptor agonist, ORAI channels inhibitor	Preclinical stage
Rosiglitazone [9,78, 130]		PPARγ activation	Preclinical stage
Cinaciguat [1,13, 130]		sGC activation	Preclinical/early clinical stage

HDAC: histone deacetylase; ER: endoplasmic reticulum; RAAS: renin angiotensin aldosterone system; PPAR: peroxisome proliferator-activated receptor; sGC: soluble guanylate cyclase.

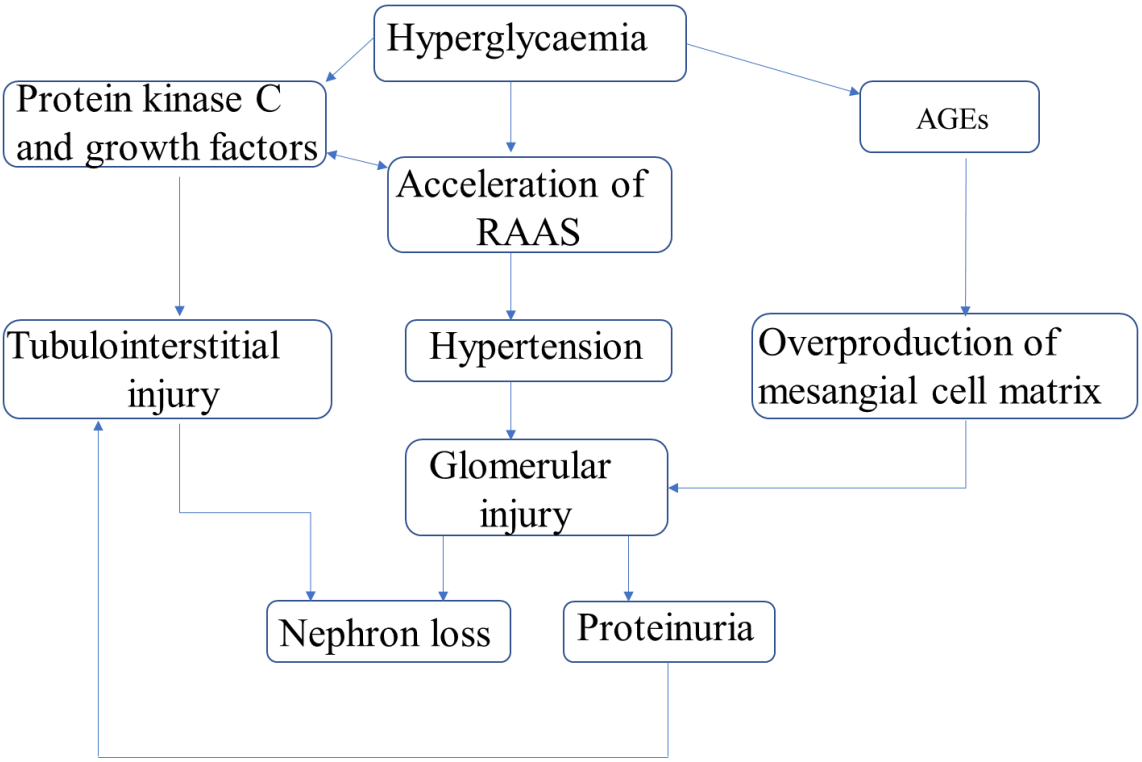


Figure 1. The pathological consequences of hyperglycaemia in DKD. RAAS: renin angiotensin aldosterone system; AGEs: advanced glycation end products.

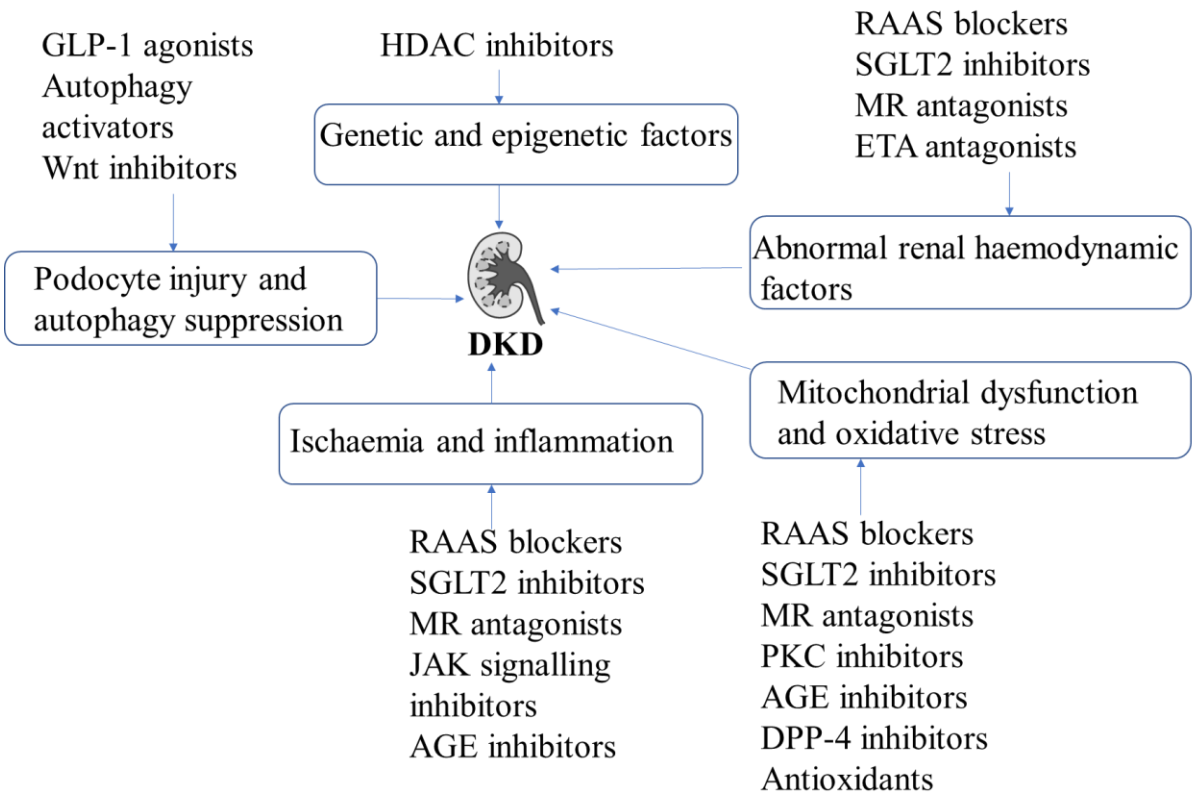


Figure 2. A schematic diagram describing the pathophysiological mechanisms of DKD and the novel therapeutic approach for its treatment. HDAC: histone deacetylases; RAAS: renin angiotensin aldosterone system; SGLT2: sodium glucose cotransporter 2; MR: mineralocorticoid receptor; ETA: endothelin receptor A; PKC: Protein kinase C; AGE: advanced glycation end products; DPP-4: dipeptidyl peptidase-4; JAK: Janus kinase; GLP-1: glucagon-like peptide-1.