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REVIEW ARTICLE

GSK-3 Inhibitors as New Leads to Treat Type-II Diabetes

Gowru Srivani[1], Ramyakrishna Sharvirala[2], Prabhakar Reddy Veerareddy[3], Dilipkumar Pal[4] and Gangarapu Kiran[5,*]

[1]Department of Bioscience and Biotechnology, Banasthali University, Vanasthali, Rajasthan- 304022, India; [2]University College of Technology, Osmania University, Hyderabad-5000 07, Telangana, India; [3]Department of Pharmaceutics, University College of Pharmaceutical Sciences, Palamuru University, Mahabubnagar-509001, Telangana, India; [4]Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, (A Central University), Koni, Bilaspur, C.G., India; [5]School of Pharmacy, Anurag Group of Institutions, Hyderabad-500 088, Telangana, India

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Abstract: In India as well as globally, diabetes is in a state of high risk and next to cardiovascular disease. As per the WHO, the risk of diabetes is expected to rise about 511 million by 2030. In quest of novel targets for type-2 diabetes, many targets were elucidated, such as Glycogen Synthase Kinase-3 (GSK-3), Dipeptidyl Peptidase (DPP-IV), PPAR-γ, α-Glucosidase, α-Amylase, GLP-1, and SGLT. Among the targets, GSK-3 was reported to be an effective target for the treatment of diabetes. In the metabolism of glycogen, GSK is a regulatory enzyme for the biosynthesis of glycogen (glycogenesis). It catalyzes the synthesis of a linear unbranched molecule with 1,4-α-glycosidic linkages. GSK-3 family has two isoenzymes, namely α and β, which differ in their N- and C- terminal sequences and are semi-conservative multifunctional serine/threonine kinase enzymes. In this chapter, we discuss an overview of general diabetic mechanisms and how GSK-3 modulation may influence these processes. Mutation in the GSK-3 complex causes diabetes. Synthetic and natural scaffolds modulate GSK-3 against diabetes and leading to its optimization for the development of GSK-3 inhibitors. This review mainly focuses on the development of GSK-3 inhibitors and highlights current and potential future therapeutic approaches that support the notion of targeting glucose metabolism with novel antidiabetic agents.

Keywords: Diabetes, Target, GSK-3, lead optimization, GSK-3β inhibitors.

1. INTRODUCTION

A pandemic disease, Type 2 Diabetes, is the world's most prevalent metabolic disorder. It is characterized by glycosuria, hyperglycemia, negative nitrogen balance, and ketonemia [1]. WHO and International Diabetes Federation (IDF) (Belgium) statistics revealed that every year the number of people affected with diabetes is increasing, and diabetes will be the primary cause of deaths all over the world. It is known that timely medication with proper procedures can easily control diabetes. Currently employed pharmacological approaches are not satisfactory in improving the consequences of insulin resistance. And also, there is no particular method to cure, and combinational therapy is usually required [2].

Type 2 diabetes and obesity are due to insulin resistance, leading to the failure of tissue response to insulin, which results in enhanced hepatic glucose output and reduced utilization of glucose by peripheral tissues. The mechanistic molecular studies have revealed that insulin resistance in cells and tissues occurs at post-receptor regions of the signaling pathway. Inhibition of the signaling pathway, especially at the downstream insulin receptor, could be a potential target for antidiabetic drugs. A serine/threonine kinase called Glycogen synthase kinase-3 (GSK-3) is one of the main downstream targets of the insulin-activated signaling pathway. The catalytic domains of two isoforms of GSK-3 have 98% homology. These isoforms have similar biochemical properties and are ubiquitously expressed in cells and tissues [3].

In resting cells, GSK-3 is actively constituted and further inhibited by the activation of extracellular signaling pathways [4-6]. This property differentiates it from other known protein kinases, making it a promising target in the drug discovery of insulin resistance and Type 2 diabetes therapy. Insulin negatively regulates GSK-3 activity, and this appears to be a general event in many cell types, including fibroblasts, adipocytes, and myoblasts [7-9]. GSK-3 phosphorylates the glycogen synthase (GS), the rate-limiting enzyme of glycogen metabolism.

The inhibition of GSK-3 by insulin is mediated through the activation of PI3 kinase (phosphatidylinositol kinase-3) and its downstream target, protein kinase B (PKB). The Direct phosphorylation of GSK-3 by PKB on a serine residue (Ser9 in GSK-3β and Ser21 in GSK-3α) inhibits the activity of the enzyme. The other known protein kinases such as protein kinase C (PKC), cAMP-dependent kinase (PKA), and the S6 ribosomal protein kinase p90RSK notably inhibit

*Address correspondence to this author at the School of Pharmacy, Anurag Group of Institutions, Hyderabad-500 088, Telangana, India;
E-mail: gangakiran1905@gmail.com

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GSK-3 by phosphorylation of the same serine residue [11, 12].

2. ACTIVITY OF GSK-3 IN SKELETAL MUSCLES

Skeletal muscle makes up 40% of human and other mammalian body mass. For the peripheral disposal of glucose, skeletal muscle is the major tissue responsible for transport in response to an insulin stimulus [13, 14]. Also, in the etiology of skeletal muscle, it is revealed that insulin resistance leads to malfunction in the expression of multiple elements in the signaling cascade of insulin that regulates the glucose transport process. These accumulating evidence indicate the role of GSK-3 in the development of insulin resistance. GSK-3 is the potential site of action for increasing insulin action in resistant insulin conditions.

GSK-3 needs to be inhibited to prevent its own inhibitory effect on its downstream substrates. Glycogen Synthase (GS) is the rate-limiting enzyme of glycogen synthesis, and its phosphorylation by GSK-3 inhibits its activity. It is also evident that abnormal GSK-3 hyperactivity causes the feedback inhibition of GS enzyme in Type 2 diabetes, and glycogen synthesis and GS activity are severely impaired. In the biopsy studies of muscle, it is indicated that when compared to the control group, the activation of glycogen synthase by insulin is lower in Type 2 diabetes patients. NMR studies also demonstrated that there was about a 60% reduction in glycogen synthesis in the Type 2 diabetes subject's muscles when compared to non-diabetic matched controls [15, 16]. Hence, it is indicating that GSK-3 might be one of the factors responsible for this defect in GS activity.

3. GSK-3 IN DIABETIC TISSUES

In a study conducted by Eldar-Finkelman, H. *et al.*, mice that were fed with a high-fat diet developed obesity and insulin resistance. And similarly, in their adipose tissue, the GSK-3 activity was 2-folds greater than the diabetic mice fed with a low-fat diet. GSK-3 activity was also significantly higher in the mice that were fed with a high-fat diet when compared with normal mice strains that were resistant to diet-induced diabetes. Thus, this suggests that GSK-3 could be an endogenous factor that would promote Type 2 diabetes during the high-fat diet [17]. In humans also, the levels of expression and GSK-3 activity are significantly higher in the Type 2 diabetes patient's skeletal muscles as compared to the non-diabetic control [18].

4. INACTIVATION OF GSK-3 BY PHOSPHORYLATION

GSK-3 is involved in several biotic activities including, cell fate determination, metabolism, and proliferation [19]. In 1980, GSK-3 was identified as one of the several protein kinases that can phosphorylate glycogen synthase. In addition to that, it is one of the last steps catalytic enzyme in glycogen synthesis. In contrast to various kinases, which trigger the substrate activation, the function of GSK-3 suppresses glycogen synthase. Later insulin-induced glycogen synthase activation leads to dephosphorylation at the same Ser (serine residues) targeted by GSK-3. This is emphasized that insulin signaling might suppress GSK-3 activity [20]. After a decade, researchers identified that Akt was responsible for GSK-3 inhibition *via* catalyzing the phosphorylation of a Ser residue at the N-terminus of GSK-3 mediated through insulin induction. This, in turn, showed that the identification of Akt activates the PDK1 (phosphoinositide-dependent protein kinase 1), which activates the PKB/AKT pathway. In the presence of PtdIns 3, 4, 5-P3 (phosphatidyl inositol-3, 4, 5,-triphosphate), PDK1 activates PKB/AKT, which is formed by the action of PI3K binds on PtdIns (4, 5) P2. PI3K inhibitors were identified to suppress all the metabolic functions of insulin, and PtdIns (3, 4, 5) P3 was already known as a significant mediator of the insulin actions. However, PtdIns (3, 4, and 5) P3 signaled mechanism in cell interior is not well-known. Further, PDK1 activates the PKB/AKT, which leads to phosphorylation and insulin suppression of GSK-3 activity.

Insulin inhibits the GSK-3 activity, leading to dephosphorylation and activation of proteins, such as glycogen synthase and eIF2B (eukaryotic initiation factor 2 B), thus contributing to the insulin-induced activation of both protein and glycogen synthesis (Fig. **1**) [21, 22].

5. Phosphate-Binding Site

GSK-3 has a distinct specificity. Protein kinase requires a number of its substrates to be phosphorylated at Ser/Thr residues through another protein kinases named "priming phosphate-binding" at the GSK-3 phosphorylation site. For this phosphorylation, four residues were placed on the –COOH terminal of the GSK-3. Recently, it has been noticed that Arg96, Arg180, and Lys205 residues play a significant role in primate phosphate-binding site of GSK-3. Excitingly, the GSK-3 structure looks like MAPK family members, and the stimulation requires phosphorylation of a tyrosine and threonine residue fall on the TXY sequences [21]. Interestingly, the MAPK phosphothreonine residues interact with the same basic residues, which bind the priming phosphate substrates of GSK-3. Additionally, in mammalian cells, GSK-3 itself phosphorylates at tyrosine residue, which is positioned at the same phosphotyrosine residue site of MAPK. Hence, the GSK-3 activation seems to be MAPK analogue, except for the active conformation that is stimulated when the priming phosphate is associated with GSK-3 [23]. Thus, phosphorylation inhibits the activity by turning the NH-terminus of GSK-3 into a 'pseudosubstrate.' This will not only stop substrates from binding but also hinder their entry into the catalytic center [22].

6. ROLE OF SIGNALING PATHWAYS IN GSK-3 INHIBITION

Amino acids stimulate the protein kinase activity of the mTOR pathway that triggers the S6 kinase. It is an indirect mechanism yet to be illustrated, but it might be associated with the protein phosphatase inhibition that inactivates and dephosphorylates the S6K55 [24]. Immediately S6K phosphorylates the same Ser residue on GSK-3, which is targeted

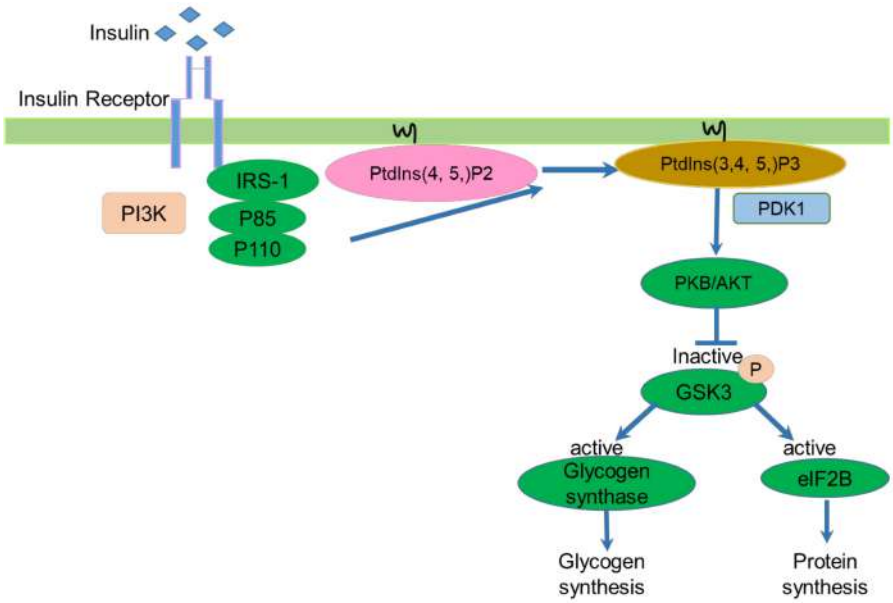


Fig. (1). The insulin signaling pathway through insulin suppression of GSK-3 and contributes to the activation of glycogen and protein synthesis. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

by PKB/Akt, thereby suppressing its function [25, 26]. Various growth factors induce the conversion of RAS into its active GTP–bound state, which stimulates the MAPK signaling cascade activation. MAPK-APK1 (MAPK activated protein kinase 1), also called RSK, is the most down-regulated kinase of this pathway that suppresses the GSK-3 *via* phosphorylating the same residue, which is targeted by PKD/Akt. Depending on the growth factors, the cell milieu suppresses the GSK-3 by PtdIns (3, 4, 5)P3- dependent PKB/Akt pathway (Fig. **2**) [27].

A.A: Amino acids; G.F: Growth Factors;

Two autonomously regulated pools of GSK-3 present in the cell are 1) Wnt signaling cascade and 2) PI3k/Akt signaling cascade (Fig. **3**). In the presence of Wnt negative regulators (DKK1- Dickkopf-1) or absence of extracellular Wnt ligands, the β-catenin, which is a transcriptional coactivator, phosphorylated through GSK-3 complex that contains scaffolding protein axin and APC cells (tumor inhibitor adenomatous polyposis coli) [28]. Afterward, phosphorylated β-catenin undergoes proteasome-dependent degradation. Conversely, extracellular Wnt proteins, the low-density LRP5/6 (Lipoprotein Related Protein-5 and 6), and Frizzled receptors are stimulated. This event results in the enrollment of Dv11 (disheveled mammalian homology), which leads to disrupting the GSK-3β–APC–Axin protein complex and its sequestration into MVB (multivesicular bodies). Inactivation of GSK-3 permits stabilization of β-catenin and facilitates gene expression through transcription factors such as TCF/LEF. In the PI3K/Akt signaling cascade, growth signals stimulate the catalytic subunits of PI3K that phosphorylates PIP2 (phosphatidylinositol-4, 5 bisphosphate) to produce PIP3 and triggers PDK-1. Hence, PDK1 activates the recruited serine-threonine kinase pathway. AKT/PKB phosphorylates GSK-3 to suppress its activity. GSK-3 regulates various neuronal activities by phosphorylating protein substrates associated with the control of metabolism, gene transcription, cytoskeletal dynamics, and apoptosis. To confirm the proper implementation of these functions, GSK-3 functions regulate by the interplay of localization, phosphorylation, and requisitioning through GSK-3 interacting proteins.

7. EXPERIMENTAL PROOF OF GSK-3 IN TYPE-II DIABETES

Several experimental studies revealed that GSK-3 is increased in tissues of insulin resistance animal models, such as obese rats, ZDF (Zucker diabetic fatty) rats, and high-fat diet mice. In addition, enhanced GSK-3 was observed in skeletal muscles of type-II diabetic patients and obese patients [17, 29]. The increased level of GSK-3 in skeletal muscle of diabetic patients showed that it is negatively correlated with both whole-body insulin-mediated glucose disposal and glycogen synthase activity [18]. Collectively, these inventions demonstrated that GSK-3 could indirectly regulate glucose transport as well as metabolism in skeletal muscles [30]. For insulin resistance, it was observed that serine phosphorylation of IRS-1 occurred at the binding site of phosphotyrosine, which is associated with the impairment of tyrosine phosphorylation and IRS-1 through the insulin receptor, reducing the activity of PI3 kinase. It has been observed that GSK-3 phosphorylates IRS-1 on serine molecule, and it exhibites GSK-3 associated IRS-1, which reduces insulin signaling in skeletal muscles through the phosphorylation on serine 307, another serine molecule on IRS -1. Interestingly, current *in vitro* studies exhibited that GSK-3 can phosphorylate serine 332 on IRS-1, and this effect is decreased by the Lithium, which acts as a GSK-3 inhibitor [30].

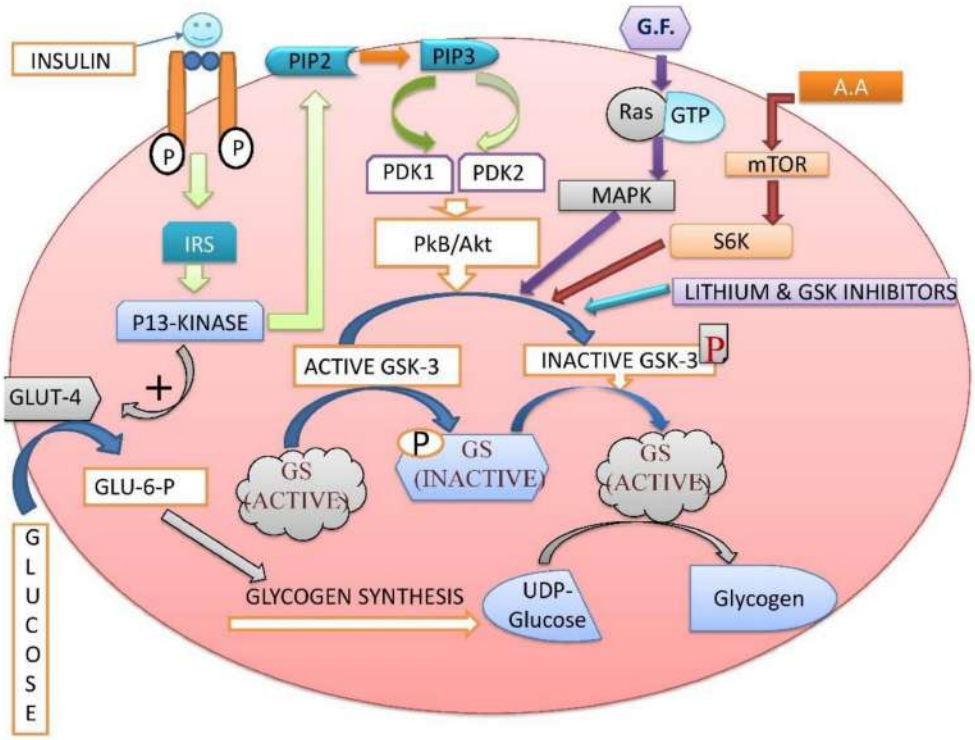


Fig. (2). Various signaling pathways induce the stimulation of GSK-3 through phosphorylating the same residue near the NH_2 terminus. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

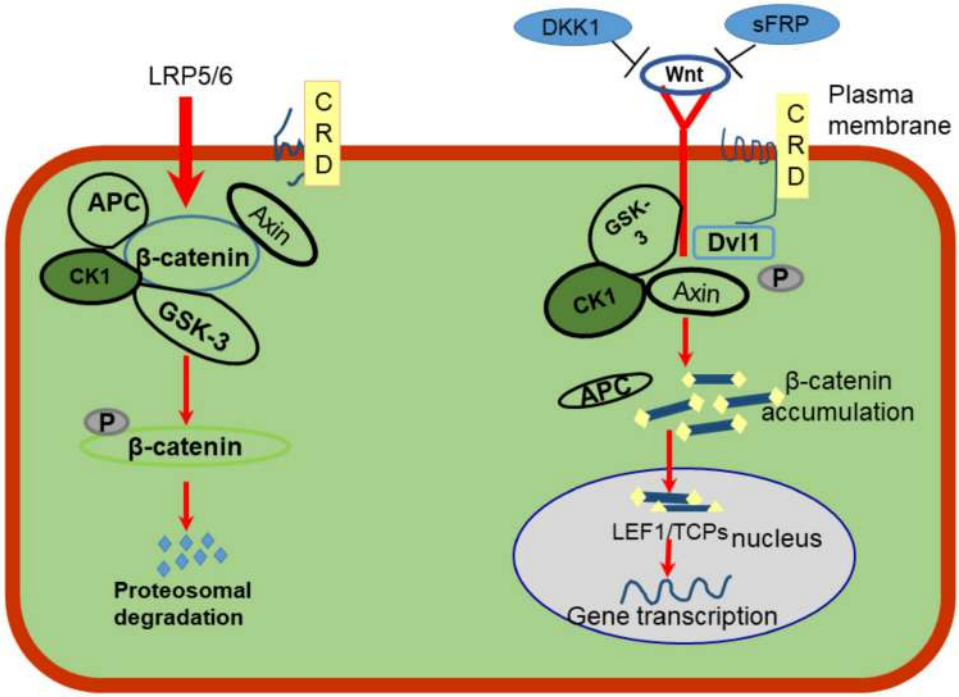


Fig. (3). GSK-3 –Wnt signaling pathway. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

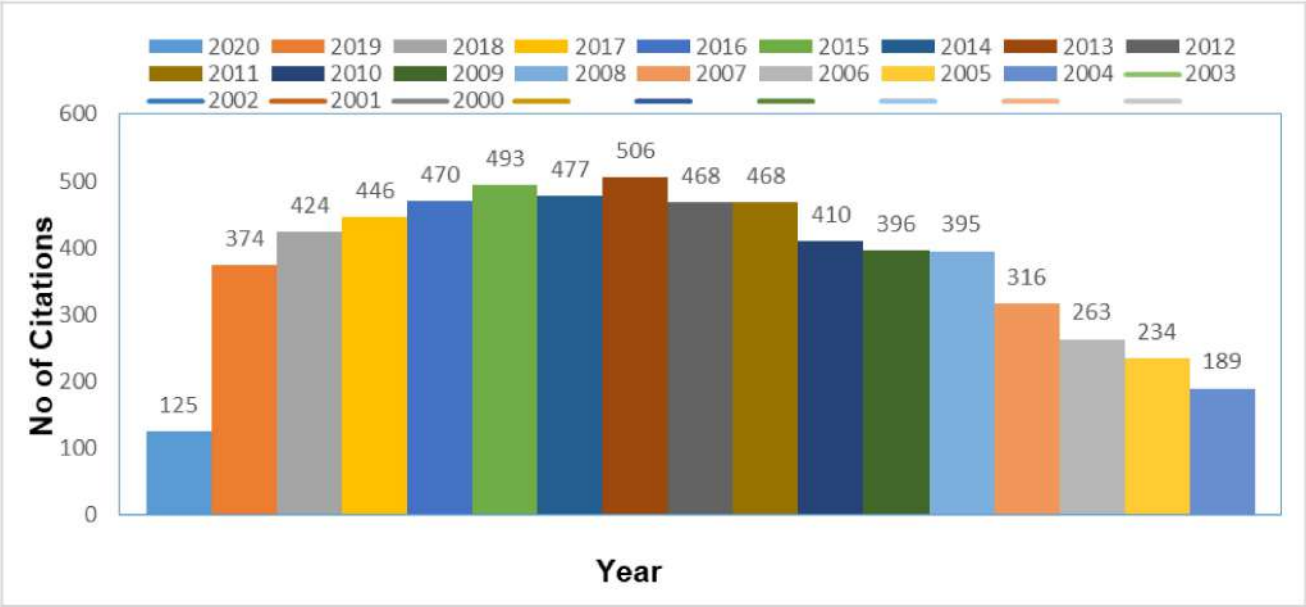


Fig. (4). PubMed citations of GSK-3 inhibitor (2000-2020). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Furthermore, alterations in serine molecule on IRS-1 in ovary cells of Chinese hamster upregulated the insulin receptors and enhanced the tyrosine phosphorylation, and increased the insulin AKT activation [31]. Researchers have recently investigated the effect of increased GSK-3 expression on total body glucose level in the animal model. Researchers developed an animal model that selectively upregulated GSK-3β in skeletal muscle by 5 to 7-fold. Compare to the control animal (non-transgenic mice), GSK-3β male transgenic mice showed the low expression muscle protein expression of IRS-1, high-fat mass, and lessened glycogen levels and glycogen synthase activity in muscles [32]. Additionally, in the oral glucose tolerance test, GSK-3β transgenic male mice exhibited a high glucose response and increased insulin ratio, showing less insulin sensitivity [32]. This experiment provided evidence that GSK-3β transgenic male mice showed dyslipidemia and increased levels of triglycerides, free fatty acids in fasting plasma[32]. Recently, researchers investigated the overexpression of GSK-3 on metabolic activities in mice by blocking the GSK-3 active forms (GSK-3α and GSK-3β). Relatively, GSK-3β overexpression in mice with GSK-3 active forms (GSK-3α/ GSK-3β) showed no changes in glycogen levels in muscle, insulin levels in plasma, and insulin-stimulated muscle glucose intake and glucose intolerance [33]. GSK-3 phosphorylates glycogen on various serine molecules and inhibits the enzyme, thus lessening glycogenesis [32, 34]. This will indirectly enhance glycogenolysis to raise hepatic glucose productivity. Furthermore, there is indirect proof of *in vitro* studies that GSK-3 in hepatic gluconeogenesis can phosphorylate on Ser129 residue and stimulate CREB protein (cAMP-responsive element-binding protein). This stimulated CREB protein enhances the PEPCK expression that leads to the upregulation of gluconeogenesis. However, careful *in vitro* suppression of GSK-3 can inhibit the G6pase gene and PEPCK activity, which results in reduced gluconeogenesis [35-37].

8. GSK-3β INHIBITORS

Many glycogen synthase kinase 3 (GSK-3) inhibitors have received considerable interest over the last two decades. Here we report the recently developed GSK3 inhibitors from synthetic and natural sources. Among FDA-approved drugs, Tideglusib 1 is a selective and irreversible GSK-3β inhibitor and belongs to the class of thiadiazolidinone and has been developed for Alzheimer's disease [38]. The mechanism involved in neuroprotection is the inhibition of the hyperpolarization of tau protein by preventing neuronal loss in hippocampal and cortical regions. Fig. (**4**) gives the number of citations for GSK-3 inhibitors; the data have taken from Pubmed.

The structure of GSK-3 protein belongs to the protein kinase family and is divided into C- and N- lobes, and the interaction of inhibitors occurs in a cleft between the two lobes (Fig. **5**).

GSK-3β is a homodimer, and each monomer consists of the N-terminal domain (β-strand) and C-terminal domain (α-helical). Its activation segment is responsible for the catalytic activity of the kinase. All protein kinase has different recognition motifs, which are stretch of amino acids that likes to phosphorylate. GSK-3 has a phosphate-binding site, which plays a key role in the activation of the loop. The substrate-binding pockets containing residues of amino acids are Arg96, Arg180, Lys205, and Val214. ATP binding site contains residues Val163, Pro136 Lys85, Glu97, and Asp200 amino acids.

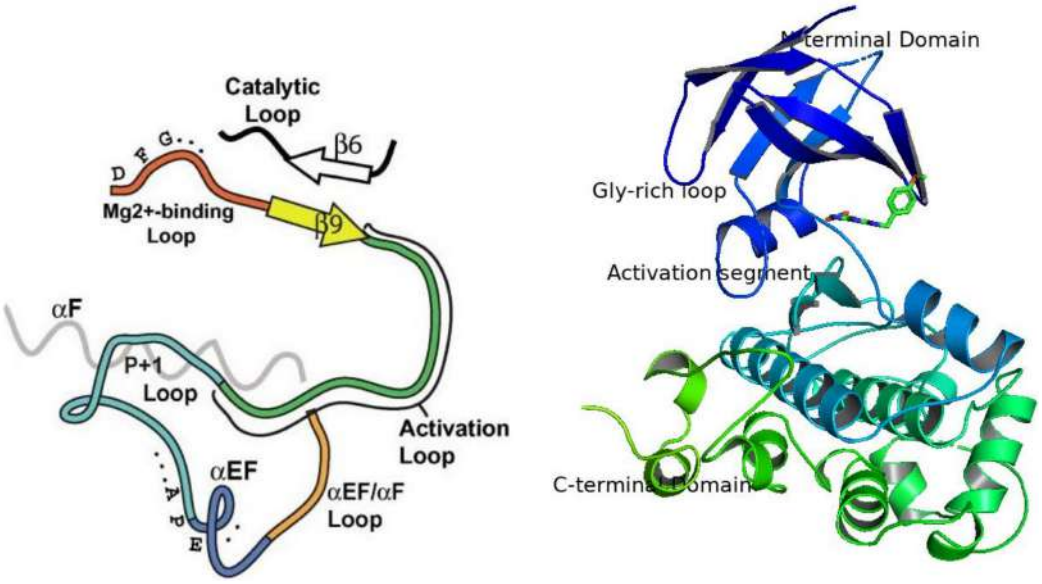


Fig. (5). Monomer of GSK-3 (PDB ID: 1Q5K). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Target-based drug design plays a crucial role in drug development and could serve a prominent role in designing the drugs against the specific target. In this instance, exploring a target and studying the mechanistic approach have become the essential parameter. One of the identified targets for diabetes is GSK-3, which has isoforms GSK-3α and GSK-3β. Modulation of GSK-3 target could be therapeutically useful in cancer, neurological and diabetic disorders.

GSK-3 inhibitors classified as i) Substrate Competitive Inhibitors ii) Non-ATP Competitive Inhibitors iii) ATP Competitive Inhibitors.

9. SUBSTRATE COMPETITIVE INHIBITORS

Previously, many ATP competitive inhibitors have developed, but the typical problem of ATP inhibitors is lack of specificity and drug-induced resistance. ATP binding site also undergoes point mutation. Whereas substrate competitive inhibitors have more specificity because the substrate-binding region is much more unique or specific to the target site, and they also have additional benefits such as safety and less drug resistance. Some of the substrate competitive inhibitors reported by different researcher groups are mentioned below.

Wagman *et al.* have synthesized libraries of pyrimidine and dihydropyrimidine derivatives as selective GSK-3β inhibitors. Among the screened, the compound with imidazole substitution **2** has shown high selectivity and potent GSK-3β inhibitory activity with an IC_{50} value of 0.0049μM [39]. To further improve the efficacy and pharmacokinetic properties of the pyrimidine derivatives, Savithri Ramurthy *et al*. have modified the scaffold by replacing the central ring of pyrimidine **2** with the pyridine ring, which enhanced the cell permeability. Furthermore, the imidazole core, which is metabolically labile in the pyrimidine core, is replaced with a monoketopiperazine group in the pyridines, which improved the pharmacokinetic and physicochemical properties (Fig. **6**).

Savithri Ramurthy group has synthesized series of 5-nitro-N2-(2-(pyridine-2ylamino)ethyl)pyridine-2,6-diamine derivatives as novel GSK-3β inhibitor. The novel pyridine compounds were synthesized from the 2,6-dichloro-3-nitropyridine by Suzuki coupling reaction with boronic acid derivatives. All the synthesized derivatives activate the insulin receptor-expressing CHO-IR cells in the hepatocytes of a rat. The compound substituted with monoketopiperazine **3** has shown effectiveness against GSK-3β with an IC_{50} value of 0.8 nM. The molecular docking studies showed that the hydrophobic and pi-pi interactions are essential for accurately fit the ligand into the binding site. In this contest, 2,4-dichlorophenyl group binds with P-loop by hydrophobic and nitroaminopyridine group binds with pi-pi interactions of GSK-3β protein. The active residues involved in the binding pocket are Ile62, Gly63, Val70, Lys85, Phe67, and Asp200 amino acid residues [40].

Qingxiu He *et al.* have studied the *in silico* computational study on (5-Imidazol-2-yl-4-phenylpyrimidin-2-yl) [2-(2-pyridylamino)ethyl]amine derivatives by 3D-QSAR, docking, and molecular dynamics simulation. They established 3D-QSAR models using 79 pyrimidine derivatives of GSK-3 inhibitors by CoMFA and CoMSIA methods and also showed good molecular interactions with protein target of GSK-3 (PDB ID: 1J1B), and further molecular dynamic simulations are found to the compounds bind by hydrogen and hydrophobic interactions at the active site. Based on these theoretical calculations, novel selective GSK-3 inhibitors could be designed as anti-diabetic agents [41].

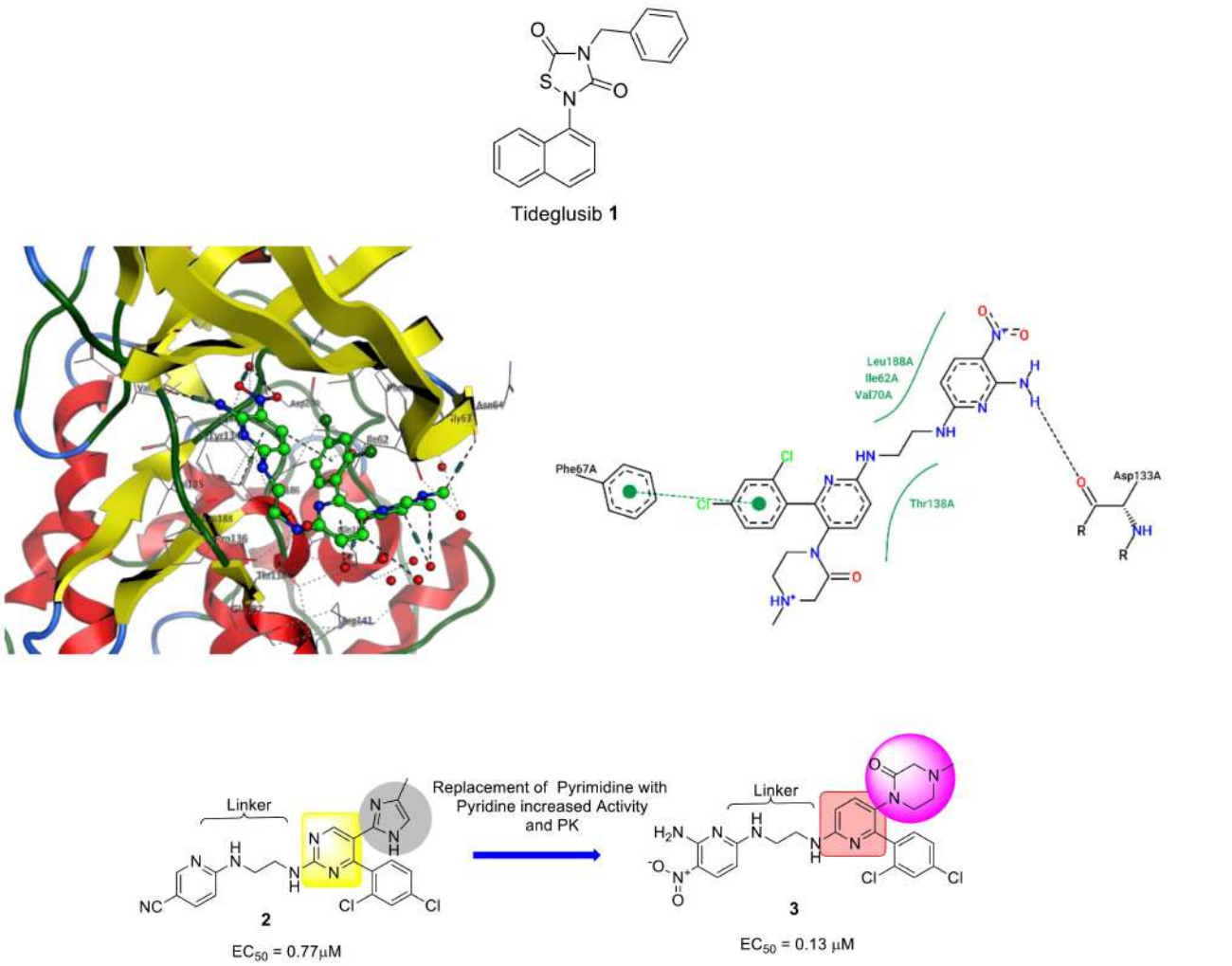


Fig. (6). Design of Potent GSK-3β Inhibitors. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Natalia A. Lozinskaya and co-workers developed a novel class of 3-arylidene2-oxindole derivatives as selective GSK-3β inhibitors and anti-diabetic agents. The synthesis of these scaffolds was carried out by condensation of isatin or 2-oxindoles with aromatic aldehydes using piperidine, which forms 3-arylidene indolinones. Amongst them, compound 3-(2-pyridinylmethylene)-2-oxindole **4** showed remarkable potent GSK-3β inhibitory activity with an IC_{50} value of 4.19 nM and in cell-based assays found to be moderately cytotoxic with a prominent anti-hyperglycemic activity in diabetic insulin-resistant rats. These results could indicate that these scaffolds could be efficient leads for the therapeutic use of Type 2 diabetes [42].

Chengfu Su and co-workers have investigated the anti-diabetic activity of amentoflavone **5** in diabetic mice. The biochemical analysis results revealed that Amentoflavone **5** increases the glucose metabolism by inhibiting the activity of GSK-3, which could have shown the effect by activating the PI3K/Akt pathway. Similarly, Kyle from Montclair State University has studied the effect of astragaloside IV **6** on GSK-3β by phosphorylation, which could regulate glycogen synthesis and activate the carbohydrate metabolism [43].

Shigeki Seto *et al.* group have designed 6-6-7 tricyclic quinolones with moiety containing strained spirocycle and evaluated against the target GSK-3β. Among the synthesized compounds, **7** with cyclobutane ring on a spirocycle showed prominent GSK-3β inhibitory activity with IC_{50} value 36 nM and EC_{50} value 3.2 μM in both cell-free and cell-based assays, respectively. The lead molecule **7** has reduced the plasma glucose concentration in dose-dependent manner in OGTT in mice [44].

GSK-3β inhibitors have been reported to possess the neuroprotective effect, particularly in Alzheimer's disease, which has been studied extensively by Stefan Berg *et al.* group from AstraZeneca R&D, Sweden, and the X-ray crystal structure has reposited in Protein Data Bank with PDB ID's 6HK3, 6HK4 and 6HK7 [45].

10. NON-ATP COMPETITIVE INHIBITORS

Initially, many non-ATP competitive inhibitors have been identified, and they belong to structure classes of thiadiazolidinone, quinolone, benzothiazepines, benzothiazinone, and thienyl and phenyl α-Halo ketones.

The thiadiazolidindione is reported as one of the novel GSK-3 inhibitors and is the well-known ATP-noncompetitive selective inhibitor. Evidently, *in vivo* studies showed that thiadiazolidindione acts as an effective neuroprotective agent. TDZD-8 and tideglusib **1**, derivatives of thiadiazolidindione, have also clinically proven as reversible covalent inhibitors of the enzyme. They mainly bind at the active site of Cys-199 directed by the electrostatic repulsions followed by covalent binding. Both highly selective non- ATP competitive inhibitors significantly inhibit GSK-3 activity with an IC_{50} value of 17.1μM. In addition to that, tideglusib exhibits improved cognitive activity in Alzheimer's patients [46-48]. Halomethylketone is an effective irreversible ATP competitive inhibitor of GSK-3. In addition to that, it exhibitspotential cell-permeability and is able to decrease tau phosphorylation in an ATP-non-competitive way [49].

Conde *et al*. discovered a novel class of molecules as non–ATP competitive inhibitors of GSK-3, such as halomethyl phenyl ketones and chloromethyl thienyl ketones [50]. Among them, manazamines, a β-carboline alkaloid, has also been found to suppress the GSK-3 activity in a non-ATP competitive way, reducing the hyperphosphorylation of tau in neuroblastoma cells. Additionally, they reported other natural compounds, tricantin, and sesquiterpene palinurin, as effective GSK-3 inhibitors [51].

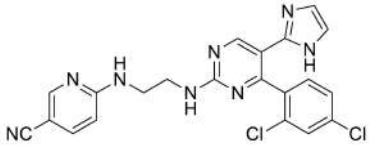
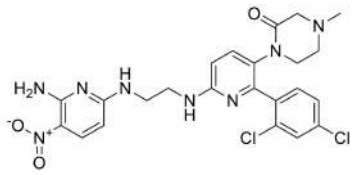
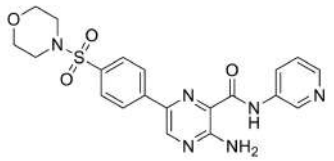
Yngve *et al*. identified a novel analogue of imidazopyridine as a potential GSK-3 inhibitor. They used hydrogen donor and acceptor motifs to bind the kinase residues and form effective interactions with the kinases. These inhibitors exhibit greater inhibition of GSK-3 with the IC_{50} values of 1.4 and 3.0 nM, respectively [52].

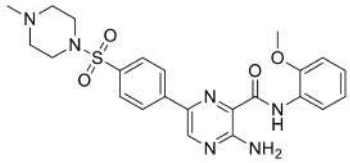
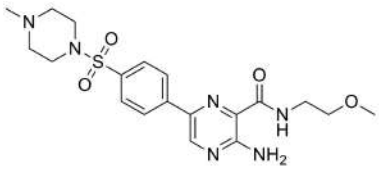
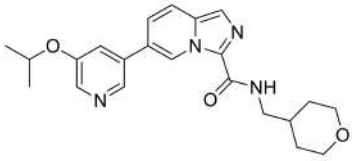
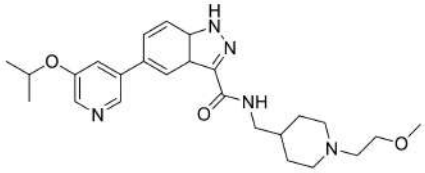
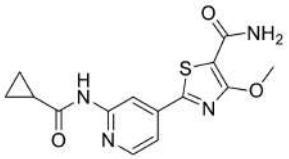
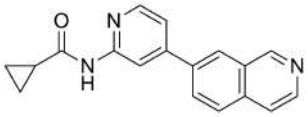
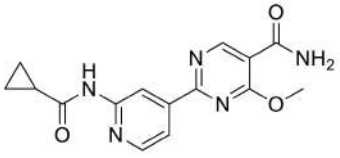
11. ATP COMPETITIVE INHIBITORS

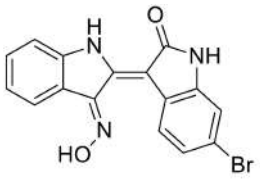
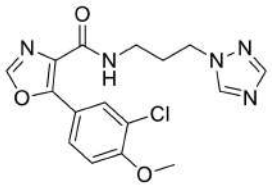
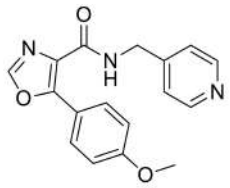
GSK-3β inhibitors, which bind at the ATP site, are widely developed and reposited as a crystal structure in Protein Data Bank. ATP competitive inhibitors belong to structural classes of inorganic compounds (Lithium) viz., Pyrazolopyridines [53], Thiazoles [54], Maleimides [55], Oxadiazole [56], pyridines [57] and natural marine sources *viz*., Indirubins, Hymenialdisine [57, 58].

GSK-3 inhibitors potentiate the insulin action in skeletal muscle and improve glucose utilization. These inhibitors might offer a therapeutic advantage. From the above-detailed review, GSK-3 could have therapeutic benefits in the treatment of Type 2 diabetes, particularly in insulin resistance. Indeed, numerous selective GSK-3 inhibitors were recently developed and mechanistically exhibit insulin-like effects and proven to be insulin sensitisers in both *in vivo* and *in vitro* studies (Table **1**).

Table 1. PDB ID's and Co-crystal Ligands of some GSK-3β Inhibitors.

S. No	PDB ID	Co-Crystal Ligand	IC50 μM	Pharmacological Activity	References
1	6B8J	 6-((2-((4-(2,4-dichlorophenyl)-5-(1*H*-imidazol-2-yl)pyrimidin-2-yl)amino)ethyl)amino)nicotinonitrile **(2)**	0.0049μM	Anti-diabetic	[39]
2	6V6L	 1-(6-((2-((6-amino-5-nitropyridin-2-yl)amino)ethyl)amino)-2-(2,4-dichlorophenyl)pyridin-3-yl)-4-methylpiperazin-2-one **(3)**	08nM	Anti-diabetic	[40]
3	6HK4	 3-azanyl-6-(4-morpholin-4-ylsulfonylphenyl)-~{N}-pyridin-3-yl-pyrazine-2-carboxamide **(4)**	Ki = 0.67 nM	Neuroprotective activity: Alzheimer's Disease	[59]

S. No	PDB ID	Co-Crystal Ligand	IC50 µM	Pharmacological Activity	References
4	6HK3	 3-azanyl-~{N}-(2-methoxyphenyl)-6-[4-(4-methylpiperazin-1-yl) sulfonylphenyl]pyrazine-2-carboxamide **(5)**	Ki = 74 nM	Neuroprotective activity: Alzheimer's Disease	[59]
5	6HK7	 3-azanyl-~{N}-(2-methoxyethyl)-6-[4-(4-methylpiperazin-1-yl) sulfonylphenyl]pyrazine-2-carboxamide **(6)**	Ki = 90 nM	Neuroprotective activity: Alzheimer's Disease	[59]
6	6Y9S	 {N}-(oxan-4-ylmethyl)-6-(5-propan-2-yloxypyridin-3-yl) imidazo[1,5-a]pyridine-3-carboxamide **(7)**	0.072 µ M	CNS activity	[60]
7	6Y9R	 5-(5-isopropoxypyridin-3-yl)-*N*-((1-(2-methoxyethyl)piperidin-4-yl)methyl)-3a,7a-dihydro-1*H*-indazole-3-carboxamide **(8)**	0.009µM	CNS activity	[60]
8	4PTC	 2-[2-(cyclopropylcarbonylamino)pyridin-4-yl]-4-methoxy-1,3-thiazole-5-carboxamide **(9)**	1.1 nM	Neuroprotective activity: Alzheimer's Disease	[61]
9	4PTE	 N-[4-(isoquinolin-7-yl)pyridin-2-yl] cyclopropanecarboxamide**(10)**	74 nM	Neuroprotective activity: Alzheimer's Disease	[61]
10	4PTG	 2-{2-[(cyclopropylcarbonyl)amino]pyridin-4-yl} -4-methoxypyrimidine-5-carboxamide **(11)**	0.0059 µM	Neuroprotective activity: Alzheimer's Disease	[61]

S. No	PDB ID	Co-Crystal Ligand	IC50 µM	Pharmacological Activity	References
11	1UV5	 (2*Z*,3*E*)-6'-bromo-3-(hydroxyimino)-[2,3'-biindolinylidene]-2'-one	0.005 µM	neurodegenerative diseases OR diabetic disorders	[62]
12	5K5N	 5-(3-chloro-4-methoxy-phenyl)-~{N}-[3-(1,2,4-triazol-1-yl) propyl]-1,3-oxazole-4-carboxamide	2.1 nM	Neuroprotective	[63]
13	4AFJ	 5-(4-methoxyphenyl)-*N*-(pyridin-4-ylmethyl) oxazole-4-carboxamide	0.07943 µM	Neuroprotective	[64]

12. AUTHORS' INSIGHT ON THE TOPIC

GSK-3 was known in 1970 and is a constitutively active, ubiquitous expressed Ser/Thr kinase that is involved in many signaling pathways such as insulin, Wnt, Reelin, Notch, and Hedgehog signaling, *etc*. GSK-3 is a novel drug discovery target for diabetes, neurodegenerative and affective disorders. Apparently, GSK-3 is a protein kinase, and its activity is elevated in these pathological disorders and leads to hyperactivation of kinase and causes or will actually change several processes in the cells and will contribute to the development and severity of these pathological disorders. In diabetes, GSK-3 refers to important targets of insulin signaling, the IRS protein and glycogen synthase, and by phosphorylation, it inhibits their function. So, GSK-3 is a negative regulator in insulin signaling, and inhibition of GSK-3 will have an advantage, and therefore there is a need for a strategy to design specific GSK inhibitors as future drugs for treating diabetes. The approaches for developing novel GSK-3 inhibitors could be peptide inhibitors, small molecules, and virtual screening against GSK-3 targets and repurposing the FDA-approved drugs. In this contest, several researchers in groups or independently are trying and testing the GSK-3 inhibitors for the development of anti-diabetic drugs.

CONCLUSION

New approaches are needed for the treatment of insulin resistance. In this review, GSK-3 is a significant drug target for Type 2 diabetes as well as insulin resistance. The IRS-1 and glycogen synthase of the insulin pathway are the two key targets of GSK-3. Insulin phosphorylates and inhibits them, thus eventually acting as a negative regulator. Among the genome analysis, the GSK-3 gene defects were not found in Type 2 diabetes, showing that intracellular biochemical functions deregulated the enzymes in diabetic conditions. Hence, selected GSK-3 inhibitors could represent the potential therapeutics against Type 2 diabetes. Moreover, the insulin-like function of GSK-3 inhibitors plugs the potential benefits of these inhibitors in the treatment of Type 1 diabetes and decreases the basic need for exogenous insulin doses. GSK-3 activity is a crucial factor for cell survival, and its severe inhibition may lead to unwanted results.

Therefore, controlled administration of suitable GSK-3 inhibitor dose, possibly in combination with tissue-targeting strategy, might be a harmless method in the usage of these inhibitors in Type 2 diabetes. The data achieved from the acute and chronic suppression of GSK-3β based on *in vitro* and *in vivo* studies will be crucial for further investigations to enhance the sensitivity of insulin and utilize GSK-3β inhibitors as selective anti-diabetic agents.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Bahare R, Gupta G, Malik S, Sharma N. New emerging targets for type-2 diabetes. Int J Pharm Tech Res 2011; 3(2): 809-18.

[2] Vats RK, Kumar V, Kothari A, Mital A, Ramachandran U. Emerging targets for diabetes. Curr Sci 2005; •••: 241-9.

[3] Woodgett JR. Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J 1990; 9(8): 2431-8. http://dx.doi.org/10.1002/j.1460-2075.1990.tb07419.x PMID: 2164470

[4] Frame S, Cohen P. GSK3 takes centre stage more than 20 years after its discovery. Biochem J 2001; 359(Pt 1): 1-16. http://dx.doi.org/10.1042/bj3590001 PMID: 11563964

[5] Grimes CA, Jope RS. The multifaceted roles of glycogen synthase kinase 3β in cellular signaling. Prog Neurobiol 2001; 65(4): 391-426. http://dx.doi.org/10.1016/S0301-0082(01)00011-9 PMID: 11527574

[6] Eldar-Finkelman H. Glycogen synthase kinase 3: an emerging therapeutic target. Trends Mol Med 2002; 8(3): 126-32. http://dx.doi.org/10.1016/S1471-4914(01)02266-3 PMID: 11879773

[7] Cross DA, Alessi DR, Vandenheede JR, McDowell HE, Hundal HS, Cohen P. The inhibition of glycogen synthase kinase-3 by insulin or insulin-like growth factor 1 in the rat skeletal muscle cell line L6 is blocked by wortmannin, but not by rapamycin: evidence that wortmannin blocks activation of the mitogen-activated protein kinase pathway in L6 cells between Ras and Raf. Biochem J 1994; 303(Pt 1): 21-6. http://dx.doi.org/10.1042/bj3030021 PMID: 7945242

[8] Welsh GI, Proud CG. Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. Biochem J 1993; 294(Pt 3): 625-9. http://dx.doi.org/10.1042/bj2940625 PMID: 8397507

[9] Moule SK, Welsh GI, Edgell NJ, Foulstone EJ, Proud CG, Denton RM. Regulation of protein kinase B and glycogen synthase kinase-3 by insulin and β-adrenergic agonists in rat epididymal fat cells. Activation of protein kinase B by wortmannin-sensitive and -insensitive mechanisms. J Biol Chem 1997; 272(12): 7713-9. http://dx.doi.org/10.1074/jbc.272.12.7713 PMID: 9065430

[10] Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 1995; 378(6559): 785-9. http://dx.doi.org/10.1038/378785a0 PMID: 8524413

[11] Goode N, Hughes K, Woodgett JR, Parker PJ. Differential regulation of glycogen synthase kinase-3 beta by protein kinase C isotypes. J Biol Chem 1992; 267(24): 16878-82. PMID: 1324914

[12] Tsujio I, Tanaka T, Kudo T, *et al.* Inactivation of glycogen synthase kinase-3 by protein kinase C δ: implications for regulation of τ phosphorylation. FEBS Lett 2000; 469(1): 111-7. http://dx.doi.org/10.1016/S0014-5793(00)01234-5 PMID: 10708767

[13] DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J. Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. Diabetes 1983; 32(1): 35-45. http://dx.doi.org/10.2337/diab.32.1.35 PMID: 6336701

[14] Baron AD, Brechtel G, Wallace P, Edelman SV. Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. Am J Physiol 1988; 255(6 Pt 1): E769-74. PMID: 3059816

[15] Roden M, Shulman GI. Applications of NMR spectroscopy to study muscle glycogen metabolism in man. Annu Rev Med 1999; 50(1): 277-90. http://dx.doi.org/10.1146/annurev.med.50.1.277 PMID: 10073278

[16] Shulman GI. Cellular mechanisms of insulin resistance in humans. Am J Cardiol 1999; 84(1A): 3J-10J. http://dx.doi.org/10.1016/S0002-9149(99)00350-1 PMID: 10418851

[17] Eldar-Finkelman H, Schreyer SA, Shinohara MM, LeBoeuf RC, Krebs EG. Increased glycogen synthase kinase-3 activity in diabetes- and obesity-prone C57BL/6J mice. Diabetes 1999; 48(8): 1662-6. http://dx.doi.org/10.2337/diabetes.48.8.1662 PMID: 10426388

[18] Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. Diabetes 2000; 49(2): 263-71. http://dx.doi.org/10.2337/diabetes.49.2.263 PMID: 10868943

[19] Cohen P, Frame S. The renaissance of GSK3. Nat Rev Mol Cell Biol 2001; 2(10): 769-76. http://dx.doi.org/10.1038/35096075 PMID: 11584304

[20] Parker PJ, Caudwell FB, Cohen P. Glycogen synthase from rabbit skeletal muscle; effect of insulin on the state of phosphorylation of the seven phosphoserine residues in vivo. Eur J Biochem 1983; 130(1): 227-34. http://dx.doi.org/10.1111/j.1432-1033.1983.tb07140.x PMID: 6402364

[21] Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol Ther 2015; 148: 114-31. http://dx.doi.org/10.1016/j.pharmthera.2014.11.016 PMID: 25435019

[22] Frame S, Cohen P, Biondi RM. A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. Mol Cell 2001; 7(6): 1321-7. http://dx.doi.org/10.1016/S1097-2765(01)00253-2 PMID: 11430833

[23] Maqbool M, Hoda N. GSK3 inhibitors in the therapeutic development of diabetes, cancer and neurodegeneration: past, present and future. Curr Pharm Des 2017; 23(29): 4332-50. http://dx.doi.org/10.2174/1381612823666170714141450 PMID: 28714403

[24] Showkat M, Beigh MA, Andrabi KI. mTOR Signaling in Protein Translation Regulation: Implications in Cancer Genesis and Therapeutic Interventions. Mol Biol Int 2014; 2014: 686984. http://dx.doi.org/10.1155/2014/686984 PMID: 25505994

[25] Zhang HH, Lipovsky AI, Dibble CC, Sahin M, Manning BD. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. Mol Cell 2006; 24(2): 185-97. http://dx.doi.org/10.1016/j.molcel.2006.09.019 PMID: 17052453

[26] Shin S, Wolgamott L, Yu Y, Blenis J, Yoon S-O. Glycogen synthase kinase (GSK)-3 promotes p70 ribosomal protein S6 kinase (p70S6K) activity and cell proliferation. Proc Natl Acad Sci USA 2011; 108(47): E1204-13. http://dx.doi.org/10.1073/pnas.1110195108 PMID: 22065737

[27] Qin Y, Li L, Pan W, Wu D. Regulation of phosphatidylinositol kinases and metabolism by Wnt3a and Dvl. J Biol Chem 2009; 284(34): 22544-8. http://dx.doi.org/10.1074/jbc.M109.014399 PMID: 19561074

[28] MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009; 17(1): 9-26. http://dx.doi.org/10.1016/j.devcel.2009.06.016 PMID: 19619488

[29] Dokken BB, Sloniger JA, Henriksen EJ. Acute selective glycogen synthase kinase-3 inhibition enhances insulin signaling in prediabetic insulin-resistant rat skeletal muscle. Am J Physiol Endocrinol Metab 2005; 288(6): E1188-94. http://dx.doi.org/10.1152/ajpendo.00547.2004 PMID: 15671078

[30] Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem 2002; 277(2): 1531-7. http://dx.doi.org/10.1074/jbc.M101521200 PMID: 11606564

[31] Liberman Z, Eldar-Finkelman H. Serine 332 phosphorylation of insulin receptor substrate-1 by glycogen synthase kinase-3 attenuates insulin signaling. J Biol Chem 2005; 280(6): 4422-8. http://dx.doi.org/10.1074/jbc.M410610200 PMID: 15574412

[32] Pearce NJ, Arch JR, Clapham JC, *et al.* Development of glucose intolerance in male transgenic mice overexpressing human glycogen synthase kinase-3β on a muscle-specific promoter. Metabolism 2004; 53(10): 1322-30. http://dx.doi.org/10.1016/j.metabol.2004.05.008 PMID: 15375789

[33] McManus EJ, Sakamoto K, Armit LJ, *et al.* Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. EMBO J 2005; 24(8): 1571-83. http://dx.doi.org/10.1038/sj.emboj.7600633 PMID: 15791206

[34] Cross DA, Watt PW, Shaw M, *et al.* Insulin activates protein kinase B, inhibits glycogen synthase kinase-3 and activates glycogen synthase by rapamycin-insensitive pathways in skeletal muscle and adipose tissue. FEBS Lett 1997; 406(1-2): 211-5. http://dx.doi.org/10.1016/S0014-5793(97)00240-8 PMID: 9109420

[35] Lochhead PA, Coghlan M, Rice SQ, Sutherland C. Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolypyruvate carboxykinase gene expression. Diabetes 2001; 50(5): 937-46. http://dx.doi.org/10.2337/diabetes.50.5.937 PMID: 11334436

[36] Fiol CJ, Williams JS, Chou C-H, Wang QM, Roach PJ, Andrisani OM. A secondary phosphorylation of CREB341 at Ser129 is required for the cAMP-mediated control of gene expression. A role for glycogen synthase kinase-3 in the control of gene expression. J Biol Chem 1994; 269(51): 32187-93. PMID: 7798217

[37] Yeagley D, Agati JM, Quinn PG. A tripartite array of transcription factor binding sites mediates cAMP induction of phosphoenolpyruvate carboxykinase gene transcription and its inhibition by insulin. J Biol Chem 1998; 273(30): 18743-50. http://dx.doi.org/10.1074/jbc.273.30.18743 PMID: 9668047

[38] Palomo V, Martinez A. Glycogen synthase kinase 3 (GSK-3) inhibitors: a patent update (2014-2015). Expert Opin Ther Pat 2017; 27(6): 657-66. http://dx.doi.org/10.1080/13543776.2017.1259412 PMID: 27828716

[39] Wagman AS, Boyce RS, Brown SP, *et al.* Synthesis, binding mode, and antihyperglycemic activity of potent and selective (5-imidazol-2-yl-4-phenylpyrimidin-2-yl)[2-(2-pyridylamino) ethyl] amine inhibitors of glycogen synthase kinase 3. J Med Chem 2017; 60(20): 8482-514. http://dx.doi.org/10.1021/acs.jmedchem.7b00922 PMID: 29016121

[40] Ramurthy S, Pfister KB, Boyce RS, *et al.* Discovery and optimization of novel pyridines as highly potent and selective glycogen synthase kinase 3 inhibitors. Bioorg Med Chem Lett 2020; 30(4): 126930. http://dx.doi.org/10.1016/j.bmcl.2019.126930 PMID: 31926786

[41] He Q, Han C, Li G, *et al.* In silico design novel (5-imidazol-2-yl-4-phenylpyrimidin-2-yl)[2-(2-pyridylamino)ethyl]amine derivatives as inhibitors for glycogen synthase kinase 3 based on 3D-QSAR, molecular docking and molecular dynamics simulation. Comput Biol Chem 2020; 88: 107328. http://dx.doi.org/10.1016/j.compbiolchem.2020.107328 PMID: 32688011

[42] Lozinskaya NA, Babkov DA, Zaryanova EV, *et al.* Synthesis and biological evaluation of 3-substituted 2-oxindole derivatives as new glycogen synthase kinase 3β inhibitors. Bioorg Med Chem 2019; 27(9): 1804-17. http://dx.doi.org/10.1016/j.bmc.2019.03.028 PMID: 30902399

[43] Tuohy K J. Effects of Astragaloside IV on GSK-3β and S6K1 Phosphorylation in C2C12 Muscle Cells. 2019.

[44] Seto S, Yumoto K, Okada K, *et al.* Quinolone derivatives containing strained spirocycle as orally active glycogen synthase kinase 3β (GSK-3β) inhibitors for type 2 diabetics. Bioorg Med Chem 2012; 20(3): 1188-200. http://dx.doi.org/10.1016/j.bmc.2011.12.046 PMID: 22261023

[45] Berg S, Bergh M, Hellberg S, *et al.* Discovery of novel potent and highly selective glycogen synthase kinase-3β (GSK3β) inhibitors for Alzheimer's disease: design, synthesis, and characterization of pyrazines. J Med Chem 2012; 55(21): 9107-19. http://dx.doi.org/10.1021/jm201724m PMID: 22489897

[46] Martínez A, Dorronsoro I, Alonso M, *et al.* GSK-3 inhibitors. US08686042, 2014.

[47] Martinez A, Alonso M, Castro A, Pérez C, Moreno FJ. First non-ATP competitive glycogen synthase kinase 3 β (GSK-3β) inhibitors: thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. J Med Chem 2002; 45(6): 1292-9. http://dx.doi.org/10.1021/jm011020u PMID: 11881998

[48] Aguilar-Morante D, Morales-Garcia JA, Sanz-SanCristobal M, Garcia-Cabezas MA, Santos A, Perez-Castillo A. Inhibition of glioblastoma growth by the thiadiazolidinone compound TDZD-8. PLoS One 2010; 5(11): e13879. http://dx.doi.org/10.1371/journal.pone.0013879 PMID: 21079728

[49] Hamann M, Alonso D, Martín-Aparicio E, *et al.* Glycogen synthase kinase-3 (GSK-3) inhibitory activity and structure-activity relationship (SAR) studies of the manzamine alkaloids. Potential for Alzheimer's disease. J Nat Prod 2007; 70(9): 1397-405. http://dx.doi.org/10.1021/np060092r PMID: 17708655

[50] Conde S, Pérez DI, Martínez A, Perez C, Moreno FJ. Thienyl and phenyl α-halomethyl ketones: new inhibitors of glycogen synthase kinase (GSK-3β) from a library of compound searching. J Med Chem 2003; 46(22): 4631-3. http://dx.doi.org/10.1021/jm034108b PMID: 14561081

[51] Martinez A, Gil C, Perez D I. Glycogen synthase kinase 3 inhibitors in the next horizon for Alzheimer's disease treatment. International journal of Alzheimer's disease 2011; 2011 http://dx.doi.org/10.4061/2011/280502

[52] Yngve U, Söderman P, Svensson M, Rosqvist S, Arvidsson PI. Imidazopyridine-based inhibitors of glycogen synthase kinase 3: synthesis and evaluation of amide isostere replacements of the carboxamide scaffold. Chem Biodivers 2012; 9(11): 2442-52. http://dx.doi.org/10.1002/cbdv.201200308 PMID: 23161627

[53] Witherington J, Bordas V, Garland SL, *et al.* 5-aryl-pyrazolo[3,4-b]pyridines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). Bioorg Med Chem Lett 2003; 13(9): 1577-80. http://dx.doi.org/10.1016/S0960-894X(03)00134-3 PMID: 12699759

[54] Bhat RV, Berg S, Burrows J, Lindquist J. GSK-3 inhibitors for the treatment of Alzheimer's disease.Alzheimer's Disease. Springer 2007; pp. 137-74.

[55] Zhang H-C, Boñaga LV, Ye H, Derian CK, Damiano BP, Maryanoff BE. Novel bis(indolyl)maleimide pyridinophanes that are potent, selective inhibitors of glycogen synthase kinase-3. Bioorg Med Chem Lett 2007; 17(10): 2863-8. http://dx.doi.org/10.1016/j.bmcl.2007.02.059 PMID: 17350261

[56] Lo Monte F, Kramer T, Gu J, *et al.* Structure-based optimization of oxadiazole-based GSK-3 inhibitors. Eur J Med Chem 2013; 61: 26-40. http://dx.doi.org/10.1016/j.ejmech.2012.06.006 PMID: 22749643

[57] Rawlings D A, Witherington J. Pyrazolo [3, 4-c] pyridines as gsk-3 inhibitors. Google Patents 2006.

[58] Alonso D, Martínez A. Marine compounds as a new source for glycogen synthase kinase 3 inhibitors. Glycogen synthase kinase 3 (GSK-3) and its inhibitors 2006; 307-31.

[59] Gobbo D, Piretti V, Di Martino RMC, *et al.* Investigating Drug--Target Residence Time in Kinases through Enhanced Sampling Simulations. J Chem Theory Comput 2019; 15(8): 4646-59. http://dx.doi.org/10.1021/acs.jctc.9b00104 PMID: 31246463

[60] Buonfiglio R, Prati F, Bischetti M, Cavarischia C, Furlotti G, Ombrato R. Discovery of Novel Imidazopyridine GSK-3β Inhibitors Supported by Computational Approaches. Molecules 2020; 25(9): 2163. http://dx.doi.org/10.3390/molecules25092163 PMID: 32380735

[61] Sivaprakasam P, Han X, Civiello RL, *et al.* Discovery of new acylaminopyridines as GSK-3 inhibitors by a structure guided in--depth exploration of chemical space around a pyrrolopyridinone core. Bioorg Med Chem Lett 2015; 25(9): 1856-63. http://dx.doi.org/10.1016/j.bmcl.2015.03.046 PMID: 25845281

[62] Meijer L, Skaltsounis A-L, Magiatis P, *et al.* GSK-3-selective inhibitors derived from Tyrian purple indirubins. Chem Biol 2003; 10(12): 1255-66. http://dx.doi.org/10.1016/j.chembiol.2003.11.010 PMID: 14700633

[63] Liang SH, Chen JM, Normandin MD, *et al.* Discovery of a Highly Selective Glycogen Synthase Kinase-3 Inhibitor (PF-04802367)

That Modulates Tau Phosphorylation in the Brain: Translation for PET Neuroimaging. Angew Chem Int Ed Engl 2016; 55(33): 9601-5.
http://dx.doi.org/10.1002/anie.201603797 PMID: 27355874

[64] Gentile G, Merlo G, Pozzan A, *et al.* 5-Aryl-4-carboxamide-1,3-oxazoles: potent and selective GSK-3 inhibitors. Bioorg Med Chem Lett 2012; 22(5): 1989-94.
http://dx.doi.org/10.1016/j.bmcl.2012.01.034 PMID: 22310227