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IDENTIFICATION OF GSK-3B INHIBITION ASSAY BY MEANS OF IN-VITRO METHODS

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ABSTRACT

Dysregulation of glycogen synthase kinase (GSK-3β) is implicated in the pathophysiology of many diseases, including type-2 diabetes, stroke, Alzheimer's, and others. A multistage virtual screening strategy designed so as to overcome known caveats arising from the considerable flexibility of GSK-3β yielded, from among compounds in our in-house database and two commercial databases, new GSK-3β inhibitors with novel scaffold structures. The two most potent and selective validated hits, a 2-anilino-5-phenyl-1,3,4-oxadiazole (24) and a phenyl methylenehydration (28), both exhibited Nano molar affinity and selectivity over CDK2 and were potent enough for direct in vivo validation. Both were able to cause significant increases in liver glycogen accumulation in dose-dependent fashion. One also exhibited excellent blood-brain barrier permeability, the other adequate for a lead compound. Analogues of the ox diazole 24 were synthesized to experimentally corroborate or rule out ligand-bound structures arising from docking studies. SAR results supported one docking study among a number of alternatives.

Keywords: -GSK, Glycogen, Pathways, Diabetes, Acid.

I. INTRODUCTION

GSK-3 IN SIGNALING PATHWAYS

As a downstream regulatory switch that controls the outcome of signaling pathways started by various stimuli, GSK-3 plays a significant function. GSK-3 is mainly found in the cytosol, cell membrane, nucleus, and cytoskeleton of cells, among other organelles. Alzheimer's disease, bipolar disorder, diabetes, and various cancers have all been linked to uncontrolled signaling pathways in which GSK-3 functions as a critical regulator.

Diabetes results from an impaired insulin signaling system. Due to GSK-3's detrimental control of the insulin signaling pathway, type 2 diabetes was the first illness to be linked to it (Figure 1). Insulin receptors in most peripheral tissues as well as in the brain stimulate a signaling cascade via insulin receptor substrate-1 (IRS-1), phosphatidyl inositol-3-kinase (PI3-kinase) and phosphatidylinositol trisphosphate (PIP3) and subsequent activation of PKB/Akt which

phosphorylates and inhibits GSK-3 leading to dephosphorylation of glycogen synthase and stimulation of glycogen synthesis.

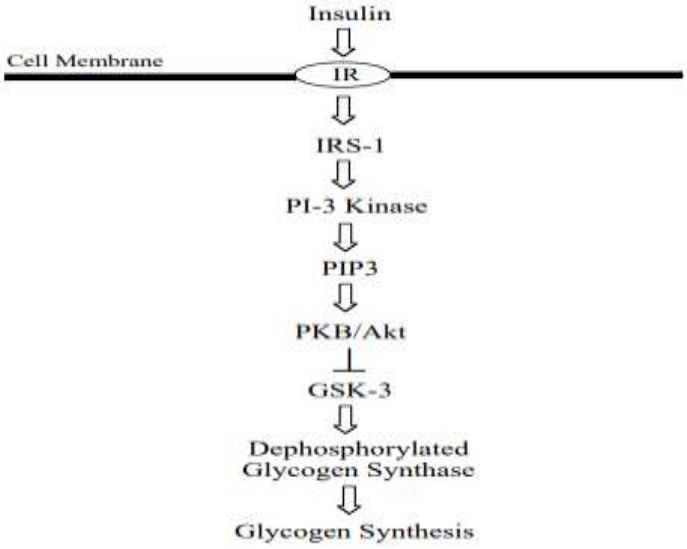

Fig 1.Schematic representation of insulin signaling pathway involved in GSK-3 activity

GSK-3 is thus seen as a negative regulator of the effects of insulin on glycogen synthase. According to Henriksen and Dokken, diabetes may cause peripheral tissues to overexpress GSK-3, and GSK-3 inhibition may help diabetic people keep their blood sugar levels under control. Potential GSK-3 activity inhibitors may imitate insulin's capacity to stimulate the conversion of glucose to glycogen in order to overcome insulin resistance.

Alzheimer's disease (AD) is a neurological ailment that is gradual and irreversible and is characterised by memory loss and diminished cognitive abilities. Neurofibrillary tangles (NFTs) and amyloid beta plaques (A) buildup between neurons in the human brain is one of the symptoms of AD. While amyloid beta plaques are produced by the proteolytic cleavage of the amyloid precursor protein (APP), NFTs are hyper phosphorylated forms of the microtubule-associated protein tau. These protein fragments combine to create tough, soluble plaques between neurons in the brains of AD patients (Hooper et al.).

Studies in genetics and epidemiology suggest that GSK-3 plays a significant role in the etiology of Alzheimer's disease (AD). Through modifications in the intermediates of the Wingless (Wnt) and insulin signaling pathways upstream, GSK-3 activity is dysregulated in AD patients.

In AD, tau hyper phosphorylation, increased amyloid beta formation, and local plaque-associated microglial-mediated inflammatory responses are all attributed to over-expression of GSK-3,

which results in progressive memory loss and thinking capacity (Jope). In animal experiments, GSK-3 pharmacological inhibitors such Tideglusib have shown a reduction in the quantity of altered tau protein (Eldar-Finkelman and Ana Martinez). For the treatment of Alzheimer's disease, this medication has completed phase 2 of its clinical study.

GSK-3 has been linked to many roles in cancer, including "tumour promoter" and "tumour suppressor," however these roles are still debatable. The 'tumour promoter' GSK-3 may be important for cell growth. Colon, pancreatic, liver, and ovarian cancers are only a few of the tumour types that have been related to overexpression of GSK-3. According to McCubrey et al., inhibition of GSK-3 may be beneficial in treating various cancer types.

On the contrary, GSK-3 also plays significant role as 'tumour suppressor'. GSK-3 can restrain the Wnt/β-catenin pathway by phosphorylating β-catenin which results in the ubiquitin/proteasome-dependent degradation of β-catenin (Luo et al.). β- Catenin is a cotranscriptional factor whose increased expression promotes the growth of cancer. Three cell-cycle regulators, cyclin D1, cyclin E, and c-Myc, whose overexpression is associated with cancer (Shang et al.), are stabilized as a result of the suppression of GSK-3 expression.

II. STRUCTURE OF GSK-3

Mammalian GSK-3α and GSK-3β are two distinct isoforms that are 98% identical to one another in the kinase catalytic domain. The brain also contains GSK-3β 2, a splice variation (Meijer et al.). Due to its greater bulk (GSK-3α has a molecular weight of 47 kDa compared to 51 kDa for GSK-3β), GSK-3α includes an extra glycine-rich N-terminal extension. The two isoforms are structurally quite similar, yet they have different functional purposes. GSK-3β , for instance, controls glycogen storage predominantly in the liver, while GSK-3α, in the skeletal muscle, (Doble).

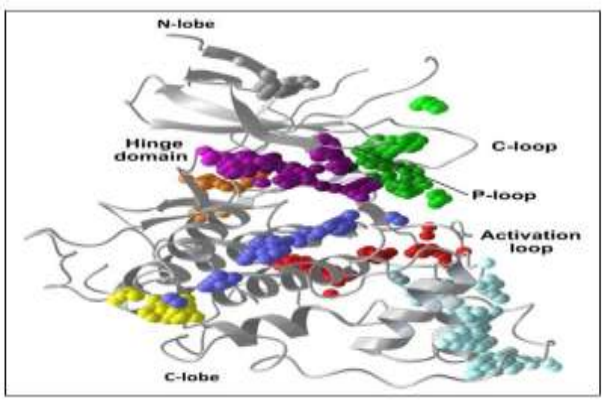

Figure 2. The different structural regions of GSK-3β

Reprinted from Acta Pharmaceutica Sciencia [Chauhan and Gajjar, "Classifying Druggability on Potential Binding Sites of Glycogen Synthase Kinase-3β: An in-Silico Assessment." Acta Pharmaceutica Sciencia, vol. 55, no. 3, 2017, pp. 43–60] with permission from publisher.

GSK-3 has a two-domain kinase structure that consists of a helix domain and a strand domain structurally. The N-lobe has a β-strand domain between amino acids 25 and 138, while the C-lobe contains a -helical domain between amino acids 139 and 343. The ATP-binding site (depicted by a pink sphere) is located deep inside the interface between the α-helix and β-strand domains, and it is sandwiched between a hinge region and a loop that is rich in glycine and known as the "P-loop." According to figure 2. (Beurel et al.), the C-loop and the activation loop encircle the substrate binding area, denoted by green spheres.

Ser9 and Tyr216 residues, two distinct phosphorylation sites, control the catalytic activity of GSK-3 β. GSK-3 β is inactivated by Ser9 phosphorylation, but the activation loop's Tyr216 phosphorylation boosts GSK-3 β's catalytic activity. According to Panda and DeGrado, phosphorylation at Ser21 makes GSK-3 inactive. The -strand and -helical domains must be adjusted in a catalytically active conformation for efficient substrate binding prior to phosphorylation. Axin, tau, -catenin, c-Jun, and c-Myc are just a few of the substrates that GSK-3 prephosphorylates. In some cases, however, the substrate must first be primed by another kinase before the target may be phosphorylated (Sutherland).

III. CURRENT STRATEGIES FOR DESIGNING SELECTIVE GSK-3B INHIBITORS

A vast majority of GSK-3β inhibitors currently available are ATP-competitive. These inhibitors' binding mechanism has been determined by crystallographic investigations. They establish vital H-bond connections with Asp133 and Val135's backbone amino acid residues. In certain complexes, Pro136 is also shown to improve the interaction between the ligand and the hingebackbone. According to Chun et al., the hinge region is substantially conserved in almost all kinases.

By concentrating on GSK-3'βs substrate and allosteric pockets, high selectivity may be achieved. This may be explained by the fact that substrate binding to the correct region is inhibited by inhibitors that target Arg96, Arg180, Lys205, and adjacent residues in the substrate site (Martinez et al.; Patel et al.). The GSK-3β allosteric modulators bind to certain areas of the protein, causing conformational changes that prevent the substrate from attaching to the correct location. An allosteric GSK-3 inhibitor named V.P. 0.7 was discovered by Institute of QuimicaMedica researchers (Palomo et al.). This method produced GSK-3's specific inhibition.

Tideglusib has been found to selectively inhibit GSK-3β by Martinez et al. According to this hypothesis, Tideglusib interacts to the active site by a covalent contact with the Cys199 residue, rendering the enzyme inactive. The most promising candidates to address the problems of cross-kinase effects are anticipated to be non-ATP competitive, substrate competitive, and allosteric modulators of GSK-3β.

IV. CONCLUSION

Serine/threonine kinase Glycogen Synthase Kinase-3 (GSK-3) was first discovered thirty years ago. The over-activity of GSK-3 leads to the development of several diseases, including Alzheimer's disease, type 2 diabetes, mental disorders, and different cancers, including colon, liver, ovarian, and pancreatic tumours. Although a large number of GSK-3β inhibitors from diverse chemical families have been created so far, none have been commercially successful. The selectivity and safety versus other kinases are the main issues. GSK-3β , which allosteric modulators may prove to be effective and secure medications for long-term use. Such inhibitors must traverse the blood-brain barrier in order to control the aberrant GSK-3β levels in the brain. The preferred oral administration route for treating CNS disorders, such as chronic AD, exacerbates this issue because it is very challenging to balance the molecular hydrophilicity needed for oral administration with the molecular lipophilicity needed to enter the brain. This requirement has eliminated several promising GSK-3β inhibitors in both pre-clinical and clinical stages.

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