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Short Communication

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Identification of Novel Glycogen Synthase Kinase-3β Inhibitor through Combined Shape-Based Screening and Molecular Docking Approach

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ABSTRACT

Glycogen synthase kinase-3β (GSK-3β) is an important class of therapeutic drug target currently receiving wide attention. In our computational approach, shape-based similarity search was used to screen the SPECS database, based on the shape of Tideglusib molecule; a known GSK-3β inhibitor. The resulting virtual hits were applied for docking studies on the known binding pockets of GSK-3β. A novel compound [7,10-dioxo-4,5-dihydro-7H,10H-pyrano[3,2,1-ij]quinolin-8-yl acetate] proposed from docking results in the substrate site of GSK-3β was found to have inhibitory activity (IC_{50}) above 100µM concentration in ADP-Glo™ Kinase assay. This communication aims to put forward in identifying newer hit on GSK-3β target via virtual screening approach.

Keywords: Glycogen synthase kinase-3β, Tideglusib, Virtual screening, ADP-Glo™ Kinase assay.

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INTRODUCTION

GSK-3β is a multifunctional serine/threonine kinase and the
as
Alzheimer's disease (AD) [1-2], bipolar disorder [3], various forms of cancer [4-5], and diabetes. [6] In the course of recent decades several diverse heterocyclic compounds and novel chemotypes have been reported as potent GSK-3β modulators. [7] Unlike other kinases, the ATP-binding site of GSK-3β is highly conserved. The molecules developed so far failed in the preclinical or clinical stages because of limited specificity and unfavorable off-target effects. [8] One exception is Tideglusib [9], an ATP-non competitive GSK-3β

inhibitor that reached phase II clinical trials for cognitive disorders (Alzheimer's disease and Progressive Supranuclear Palsy). [10-11] Currently, this drug is being evaluated in adolescents with Autism Spectrum Disorders (ASD) to improve their social behavior. [12] All currently available molecules that specifically target the substrate binding site (L807mts peptide and 5-Imino1,2,4-Thiadiazoles) [13-14] and allosteric site (a quinoline derivative V.P.0.7) [15] provides selective and subtle modulation of kinase. However, a journey to the clinic with a series of highly selective and potent small molecule GSK-3β targeted therapy is still a challenging task.

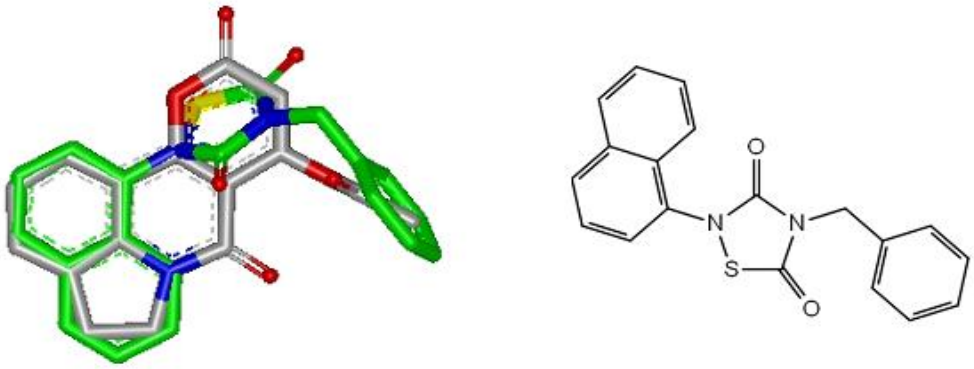

Fig. 1: Overlay of Tideglusib reference compound (green stick representation) with 2D structure representation and SPEC database compound AO-476/41610153 (gray stick representation) with ROCS similarity screening.

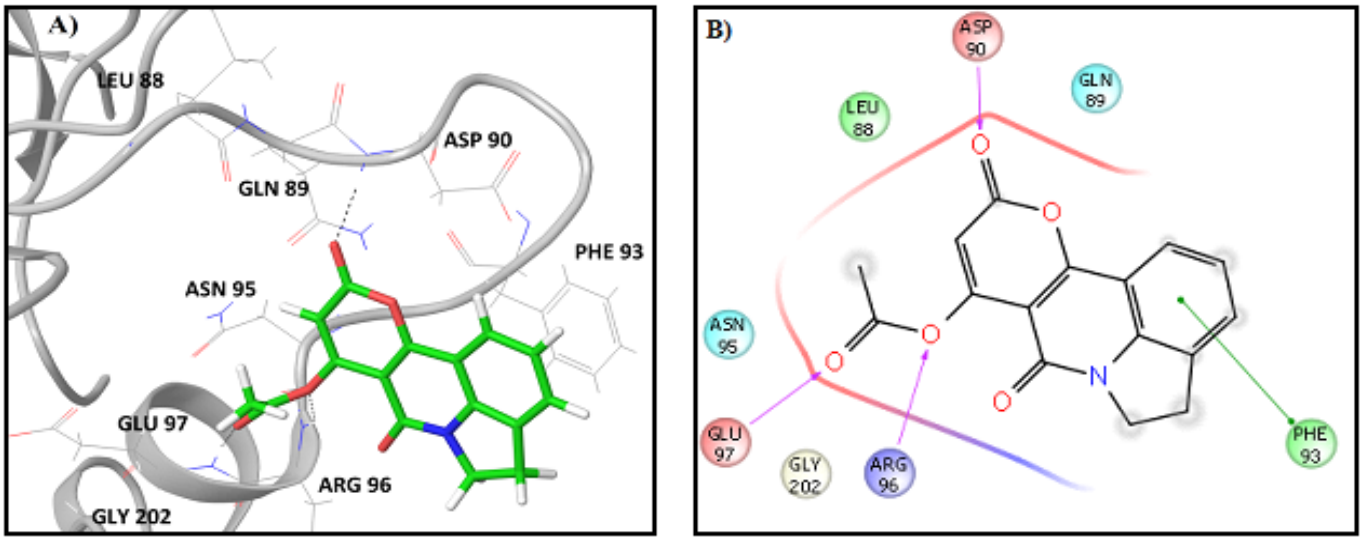

Fig. 2: A) Docked complex of SPECS database compound [7,10-dioxo-4,5-dihydro-7H,10H-pyrano[3,2,1-ij]quinolin-8-yl acetate] in the substrate binding site of GSK-3β. B) Ligplot interaction diagram of the SPECS database compound in the substrate binding site.

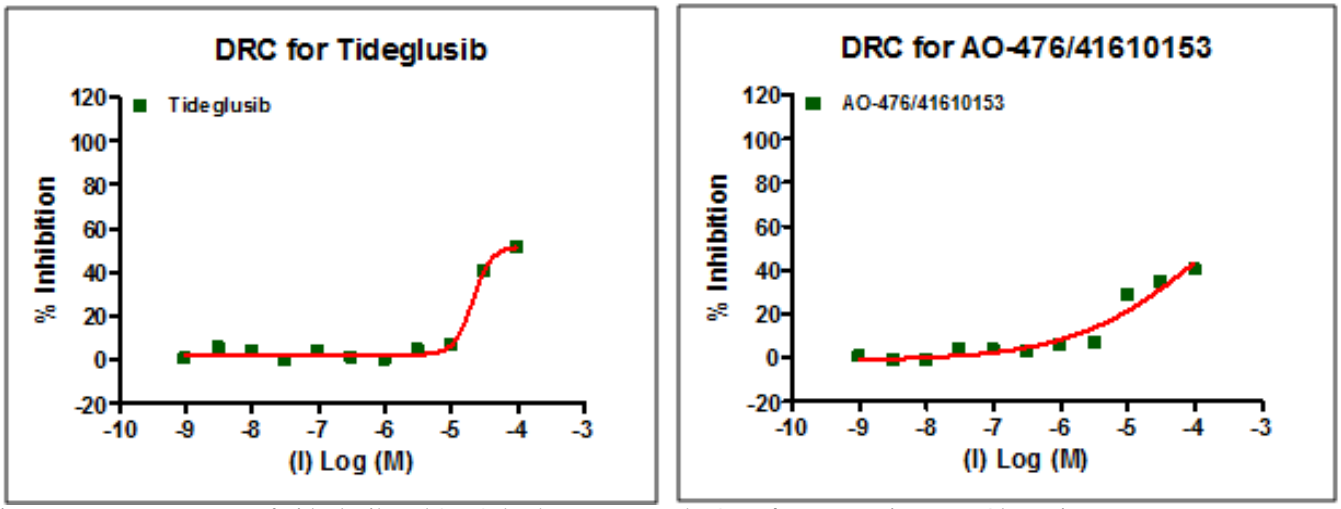

Fig. 3: Dose response curve of Tideglusib and SPEC database compound AO-476/41610153 using ADP-Glo™ Kinase assay.

MATERIAL AND METHODS

All computational and modeling studies were carried out using Schrödinger molecular modeling software and Open Eye Scientific Software on a windows Dell workstation.

Shape-based screening

In this research, with an aim to identify novel GSK-3β inhibitors we selected Tideglusib, a small-molecule drug of thiadiazolidinone (TDZD) class as a reference compound to screen the SPECS database (http://www.specs.net/). The shape-based virtual screening was performed with ROCS (Rapid Overlay of Chemical Structures; version 3.1.2) in Open Eye Scientific Software. [16] This program was designed to screen large scale chemistry databases based on the 3D shape and chemical ("color") similarity. The database was first prepared by removing undesirable compounds of toxic functionalities with FILTER (version 2.0.2) in OpenEye software. In our computations, single low-energy conformations of Tideglusib and filtered database were generated with OMEGA (Optimized Ensemble Generation Application; version 2.4.6) in OpenEye, and served as an input database (oeb.gz file format) for performing ROCS calculations. The 3D similarity scores were ranked by Tanimoto Combo score (default score) that includes both shape fit and color similarity. This score varies between range 0 and 2. Our initial shape-screening protocol thus yielded 500 top-scoring virtual hits. However, the SPCES compound Tanimoto Combo score gave a similarity score of 1.028 with Tideglusib (Fig. 1).

Molecular Docking

Molecular docking was performed with GLIDE XP (extra precision mode) in the Maestro program (version 9.3.5) of Schrödinger suite. [17] GLIDE program is designed to search for favorable interactions between one or more ligand molecules and a receptor, usually a protein. In Glide extra-precision mode is a refinement tool designed for use only on good ligand poses. Molecular docking jobs in GLIDE can be performed by the following steps: (i) Ligand preparation (ii) Protein preparation (iii) Receptor grid generation (iv) GLIDE docking.

Preparation of ligands of 500 top scoring virtual hits was performed using LigPrep module of Schrödinger suite. LigPrep produces a single, low energy, 3D conformation structures with correct chiralities for each input structures. The ionization states were generated using Epik at a default pH of 7.0 ± 2.0 (considered general pH for biological system). OPLS 2005 force field was selected for energy minimization. The resulting structures were saved in the Maestro file format.

The X-ray crystal structure of GSK-3β (PDB ID: 1PYX, 2.4 Å resolution) was extracted from the Protein Data Bank (www.rcsb.org). The dimer structure was separated by keeping chain A monomer. Subsequently, the original ligand (Phosphoaminophosphonic acid-adenylate ester) was omitted and the complex was refined with the Protein Preperation Wizard in the Maestro program using the default options settings, where hydrogen atoms were added, water molecules were removed, optimal protonation states of each ionizable residues were assigned and hydrogen-bonding network was optimized. Finally, a restrained minimization with OPLS 2005 (Optimized Potential for Liquid Simulations) force field was performed to attain relaxed state of the complex.

The receptor grid generation panel is used to specify a receptor structure in a protein. In Glide, grids were generated keeping the default parameters of van der Walls scaling factor 1.00 and partial charge cut off with 0.25 values. The bounding box was set to 10Å (default value) along each axis (x, y and z) from the centroid of Arg96 residue of GSK-3β (PDB ID: 1PYX) and the receptor grid for the substrate site was prepared for docking calculations. All prepared 500 ligand hits were subjected to GLIDE docking with the extra precision mode. Glide sort its results by Glide Score. However, without any assumption of the exact binding mode these ligands were docked into the three well-known binding sites of GSK-3β: (i) the ATP site (ii) the substrate site (iii) allosteric site (referred to as the binding site of V.P0.7 molecule). Here we report the SPECS database compound [7,10-dioxo-4,5-dihydro-7H,10H-pyrano[3,2,1-ij]quinolin-8-yl acetate] which showed the highest XP Glide Score -4.2 kcal/mol. in the substrate site. Key hydrogen-bonds are indicated by dashed lines between the ligand with Arg96, Glu97 and Gln89 residues and pi-stacking with Phe93 residue in the substrate site of GSK-3β (Fig. 2A). LigPlot view of the docked structure is shown in Fig. 2B. This 'cherry picked' compound was purchased from SPECS (Bleiswijkseweg, The Netherlands) for further biological evaluation.

RESULTS AND DISCUSSION

Biological evaluation and hit validation

ADP-Glo™ Kinase assay of Promega corporation (www.promega.com) was used to measure the potency (IC_{50}) of selected hit compound AO-476/41610153. This is a luminescent type of assay that quantifies kinase activity by measuring the amount of ADP (Adenosine diphosphate) formed during a kinase reaction. The ADP-Glo™ Kinase assay kit (Catalogue number V9101) consists of: (i) ADP-Glo™ reagent (ii) kinase detection reagent (prepared by mixing kinase detection buffer with a lyophilized kinase detection substrate) and (iii) ultrapure Adenosine triphosphate (ATP) and ADP. Other components of ATP such as ADP could result in high background. Upon completion of kinase reaction the assay procedure consists of two steps: (i) addition of ADP-Glo™ reagent for termination of kinase reaction and depletion of remaining ATP (ii) the kinase detection reagent is added to convert ADP to ATP and the newly generated ATP by reaction with luciferase/luciferin produces luminescence. The luminescent signal generated is proportional to the ADP concentration produced and is correlated with the kinase activity. The kinase assays were performed at GVKBio, Hyderabad, India.

Active GSK-3β enzyme (catalogue number G09-10G) and GSK-3 substrate (glycogen synthase-1; catalogue

number G50-58) were procured from SignalChem. Tideglusib (Sigma-Aldrich; product catalogue number SML0339) was used as a reference compound. The ADP-Glo™ Kinase assay was performed according to the supplier protocol (Promega; catalogue number V9101) and the method described by Jolanta Vidugiriene *et.al.* [18]

To determine the half maximal inhibitory concentration (IC_{50}) values of test compound and tideglusib, these compounds with different concentrations ranging from 0.003µM to 100µM of 3-fold dilutions (dissolved in DMSO; Dimethyl sulphoxide), were diluted with 0.048 ml of assay buffer, and 7.5ng of enzyme solution were added to each well (384 well plate format) and incubated at 30°C for 60 minutes. 0.005 ml of assay buffer containing 2µM substrate and 10µM ATP was added to the reaction mixture. After 15 minutes of incubation at room temperature, the enzymatic reaction was stopped with 0.005 ml of kinase Glo™ reagent by further incubation of the reaction mixture for 40 minutes followed by the addition of Kinase detection reagent and then incubated further for 30 minutes at room temperature. The luminescence data obtained through Envision (Perkin Elmer) was analyzed by GraphPad Prism 6 software. In the GraphPad Prism the compound concentrations used for GSK-3β inhibition was taken on to the x-axis and the luminescence data obtained for the compound was taken on the y-axis for obtaining Dose Response Curve (DRC). All experiments were performed at least in duplicates. The half maximal inhibitory concentration (IC_{50}) analyzed for Tideglusib was found to be 46µM and the selected hit compound AO-476/41610153 showed an IC_{50} value above 100µM as shown in Fig. 3. Results from this biological evaluation conclude the hit compound as a weak inhibitor of GSK-3β.

In summary, this communication aims to present the preliminary part of the research work as identification of a validated newer hit on GSK-3β target by the use of combined shape-based screening and molecular docking approach. However, the exact binding mode and molecular inhibition mechanism of this compound would be confirmed by kinetic experiments. More work would be focused on the synthesis and structural variations on several parts of the molecule to identify analogues with better potency.

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REFERENCES

1. Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. J Neurochem. 2008; 104:1433-1439.
2. Llorens-Marítin M, Jurado J, Hernández F, Ávila J. GSK-3beta, a pivotal kinase in Alzheimer disease. Front Mol Neurosci. 2014; 7:46.
3. Li X, Liu M, Cai Z, Wang G, Li X. Regulation of glycogen synthase kinase-3 during bipolar mania treatment. Bipolar Disord. 2010; 12:741-752.
4. Manoukian AS, Woodgett JR. Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. Adv Cancer Res. 2002; 84:203-229.
5. Piazza F, Manni S, Semenzato G. Novel players in multiple myeloma pathogenesis: role of protein kinases CK2 and GSK3. Leukemia Res. 2013; 37:221-227.
6. 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; 49:263-271.
7. Khan I, Tantray MA, Alam MS, Hamid H. Natural and synthetic bioactive inhibitors of glycogen synthase kinase. Eur J Med Chem. 2017; 125:464-477.
8. Eldar-Finkelman H, Licht-Murava A, Pietrokovski S, Eisenstein M. Substrate competitive GSK-3 inhibitors – strategy and implications. Biochim Biophys Acta. 2010; 1804:598-603.
9. 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; 45:1292-1299.
10. Lovestone S, Boada M, Dubois B, Hüll M, Rinne JO, Huppertz HJ, Calero M, Andrés MV, Gómez-Carrillo B, León T, Del Ser T. ARGO investigators. A phase II trial of tideglusib in alzheimer's disease. J Alzheimers Dis. 2015; 45:75-88.
11. Tolosa E, Litvan I, Höglinger GU, Burn D, Lees A, Andrés MV, Gómez-Carrillo B, León T, Del Ser T. TAUROS Investigators. A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. Mov Disord. 2014; 29:470-478.
12. Tideglusib vs. Placebo in the Treatment of Adolescents with Autism Spectrum Disorders (TIDE). Clinical trials.gov. https://clinicaltrials.gov/ct2/show/NCT02586935 [Last accessed 5 March 2017].
13. Licht-Murava A, Paz R, Vaks L, Avrahami L, Plotkin B, Eisenstein M, Eldar-Finkelman H. A unique type of GSK-3 inhibitor brings new opportunities to the clinic. Sci Signal. 2016; 9:ra110.
14. Palomo V, Perez DI, Perez C, Morales-Garcia JA, Soteras I, Alonso-Gil S, Encinas A, Castro A, Campillo NE, Perez-Castillo A, Gil C, Martinez A. 5-imino-1,2,4-thiadiazoles: first small molecules as substrate competitive inhibitors of glycogen synthase kinase 3. J Med Chem. 2012; 55:1645-1661.
15. Palomo V, Soteras I, Perez DI, Perez C, Gil C, Campillo NE, Martinez A. Exploring the binding sites of glycogen synthase kinase 3. Identification and characterization of allosteric modulation cavities. J Med Chem. 2011; 54:8461-8470.
16. OpenEye Scientific Software. Inc., Santa Fe, NM, USA. 2012.
17. Schrödinger LLC. Schrödinger Suite 2012. New York, NY. 2012.
18. ADP-Glo™ Kinase assay, Protocol KA. ADP-Glo™ Kinase Assay.

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