Research Article

Hacettepe University Journal of the Faculty of Pharmacy
Special Issue / International Multidisciplinary Symposium on
Drug Research and Development, DRD-2023/July 2024 / pp.11-16

In Silico Drug Repurposing As Inhibitors Against GSK-3β

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Received date : 16.09.2023
Accepted date : 01.11.2023

DOI: 10.52794/hujpharm.1361472

ABSTRACT

Tau, a protein associated with microtubules, is widely distributed throughout the central nervous system and promotes the polymerization, assembly, and stability of microtubules. Hyperphosphorylation of tau proteins leads to intracellular neurofibrillary tangles, which are the pathological hallmark of numerous neurodegenerative diseases and are collectively referred to as "tauopathies". The most notable kinase identified in tau phosphorylation is glycogen synthase kinase 3 (GSK-3). Among the GSK-3 isoforms, GSK-3β has been linked to the pathophysiology of neurodegenerative diseases. Pharmacological inhibition of GSK-3β has been suggested as a potential therapeutic target for these diseases. In this study, the literature and databases were searched for potential inhibitory drugs against GSK-3β and 58 drugs were found. The drugs were filtered according to physicochemical-pharmacological properties and toxicity profiles via SwissADME, pkCSM, and ProTox-II, free web tools. After pre-filtration, molecular docking was performed against GSK-3β with the remaining seven drugs (Nabumeton, Loxoprofen, Ketoprofen, Oxytetracycline, Benzoyl Peroxide, Naproxen, and Epinephrine Hydrochloride). According to the results, nabumetone had the best binding energy (-7.39 kcal/mol) and inhibition ability at the lowest concentration (3.8 μM) against GSK-3β among the seven drugs [compared to PF-04802367, a highly selective brain-penetrant kinase inhibitor]. Our results suggest that nabumetone may be a potential inhibitor of GSK-3β.

Keywords: Drug Repurposing, GSK-3β, *In Silico* Analysis, Nabumetone, Tauopathies

1. Introduction

Tau protein is a microtubule-associated protein mostly present in neurons. It plays a key role in stabilizing microtubules, which are structures that maintain the cell's shape and provide a framework for cellular transport [1].

In healthy cells, tau is typically phosphorylated at particular sites and binds to stabilize microtubules. However, extra phosphate groups are added to the tau protein when hyperphosphorylation occurs. Hyperphosphorylated tau leads to the instability of the neuronal cytoskeleton. In these conditions, tau assembles inside neurons to form neurofibrillary tangles (NFTs), which are insoluble aggregates. These NFTs are one of the pathological hallmarks of Alzheimer's disease and other tauopathies [1].

The exact mechanisms underlying tau hyperphosphorylation have yet to be fully understood. However, the aberrant hyperphosphorylation of tau is thought to be caused by imbalances in the protein kinases and phosphatases that control tau phosphorylation [1, 2].

The field of neurodegenerative diseases is actively researching to better understand the mechanisms driving tau hyperphosphorylation and to create treatment approaches to prevent or minimize it. Potential strategies being investigated to create therapies for tauopathies include targeting the enzymes responsible for tau phosphorylation, promoting tau dephosphorylation, and preventing the aggregation of hyperphosphorylated tau [1-3].

One of the enzymes that plays an important role in tau phosphorylation is glycogen synthase kinase-3β (GSK-3β). Multiple locations on tau are known to be phosphorylated by GSK-3β. GSK-3β-mediated tau hyperphosphorylation disrupts its normal function and leads to the accumulation of NFTs. Recent studies have extensively investigated GSK-3β and tau hyperphosphorylation as potential therapeutic targets for tauopathies. Preclinical research has indicated that inhibiting GSK-3 activity or reducing tau hyperphosphorylation may be effective ways to slow the progression of neurodegenerative disorders [4, 5]. However, traditional drug development is time-consuming and costly [6].

In this *in silico* study, we first searched the literature and databases for potential inhibitory drugs against human GSK-3β. Subsequently, we filtered the drugs according to their absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles. Finally, we docked the prefiltered drugs against GSK-3β.

2. Materials and Methods

2.1. Screening and selection of drugs targeting GSK-3β

The screening of drugs was identified through literature and databases (e.g., HIT 2.0, PubChem, and ChEMBL) [7, 8]. Drugs found after screening were filtered according to physicochemical-pharmacological properties (molecular weight, LogP, and other Lipinski rules), pharmacokinetics (Human intestinal absorption, Blood-brain barrier penetration, CYP inhibition), and toxicity profiles (Maximum tolerated dose, hepatotoxicity, cardiotoxicity) via SwissADME, pkCSM, and ProTox-II, free web tools [9-11]. After preliminary elimination based on the aforementioned physicochemical properties and medicinal chemistry conditions, 7 out of the initial 58 drugs remained, and these remaining drugs were then docked to GSK-3β.

2.2. Molecular docking studies of the selected drugs

The interactions of the selected drugs with their macromolecule targets were carried out under the docking conditions which have been described in detail [12, 13]. The docking studies were conducted using AutoDock 4.2 software to identify the interactions of the drugs with the binding site of the crystal structure of GSK-3β. The crystal structure of human GSK-3β (PDB ID: 5K5N) and the control ligand PF-04802367 (PDB ID: 6QH), a highly selective brain-penetrant kinase inhibitor [14], were obtained from the RCSB Protein Data Bank (https://www.rcsb.org). The molecular structures of the drugs were drawn with Gaussview 5.0 and then optimized with the DFT method using the Gaussian 03 package at the theoretical level of the B3LYP method and the 6-31G basis set. The Lamarckian genetic algorithm approach was applied in the simulations. The interactions of the crystal structures of GSK-3β with the drugs were analyzed using the Discovery Studio Client 4.1 program.

3. Results and Discussion

3.1. The drugs targeting GSK-3β

The screening resulted in 58 drugs that likely target GSK-3β. After pre-filtration, only seven drugs were found: Nabumetone, Loxoprofen, Ketoprofen, Oxytetracycline, Benzoyl Peroxide, Naproxen, and Epinephrine Hydrochloride. *In silico* molecular docking was then performed against GSK-3β using these seven drugs.

3.2. The molecular docking simulation and ADMET profile of the potential drug

As a result of molecular docking, the drug nabumetone exhibited high binding affinity (more negative value of binding energy) and low-dose inhibition against GSK-3β compared to the control among the selected drugs (Table 1).

Nabumetone forms H-bonds with the amino acids ASP200 and TYR134 in the active site of the GSK-3β enzyme. These interactions are important in ensuring the higher binding affinity of nabumetone for GSK-3β. In addition, nabumetone is associated with a pi-sigma interaction with LEU188, and it also forms pi-alkyl interactions with ALA83, LYS85, VAL110, LEU132, and CYS199 in the active site of GSK-3β. As shown in Figure 2, nabumetone has a conformation suitable for the active site with these interactions.

Nabumetone (Figure 1) is a nonsteroidal anti-inflammatory drug that reduces pain, inflammation, and stiffness caused by osteoarthritis and rheumatoid arthritis. The physicochemical properties, pharmacokinetics, and toxicities of nabumetone were also investigated to determine whether this drug may have adverse effects.

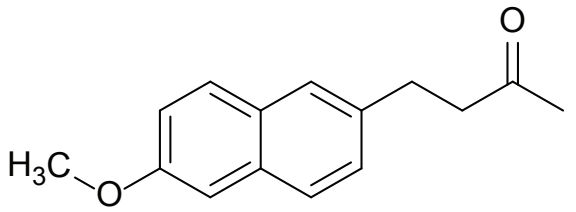


Figure 1. Molecular structure of nabumetone

According to ADME profiles by SwissADME, nabumetone had high gastrointestinal absorption and could cross the blood-brain barrier (BBB). It was not a P-glycoprotein substrate. In addition, since CYP isozymes metabolize approximately two-thirds of known drugs in humans, and 80% of this property belongs to five isozymes (1A2, 3A4, 2C9, 2C19, and 2D6), it was also determined whether Nabumetone would inhibit these CYPs. Nabumetone was a CYP1A2, CYP2C19, and CYP2D6 inhibitor but not CYP2C9 and CYP3A4 (Figure 3).

In silico toxicity assessment using the pkCSM and ProTox-II web tools showed that nabumetone did not exhibit serious toxic effects. It showed no hepatotoxic, cardiotoxic, carcinogenic, immunogenic, mutagenic, or cytotoxic effects. The acute oral LD_{50} of nabumetone predicted by the Protox-II web tool was

Table 1. The docking scores of the hit drugs against human GSK-3β (PDB ID: 5K5N).

Compounds	Binding Energy (kcal/mol)	Ligand Efficiency	Inhibitory Conc. (μM)
Control ligand [PF-04802367 (PDB ID: 6QH)]	-7.66	-0.31	2.41
Nabumetone	**-7.39**	**-0.43**	**3.80**
Loxoprofen	-7.20	-0.40	5.31
Ketoprofen	-7.01	-0.37	7.28
Oxytetracycline	-6.97	-0.21	7.83
Benzoyl Peroxide	-6.93	-0.39	8.27
Naproxen	-6.76	-0.40	7.96
Epinephrine	-3.85	-0.30	1500

ISSN: 2458 - 8806

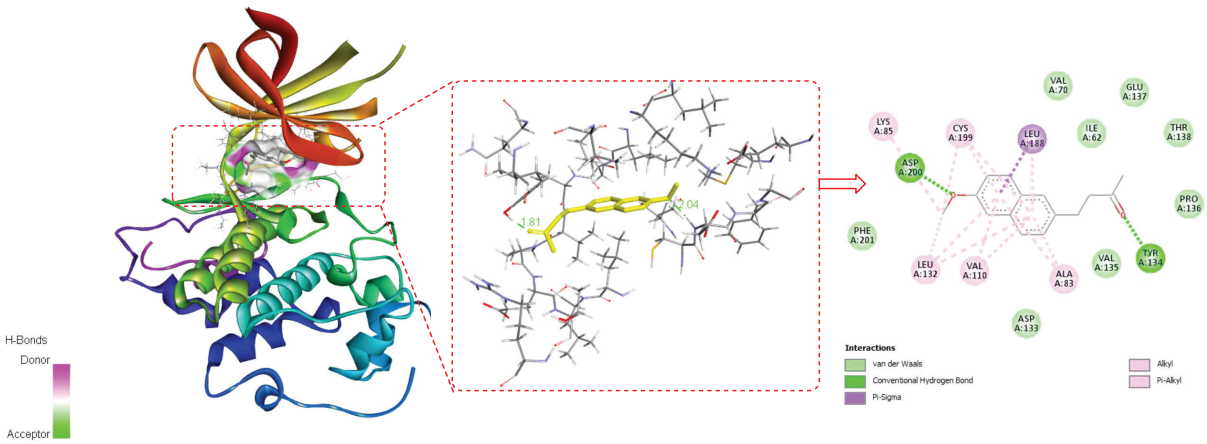
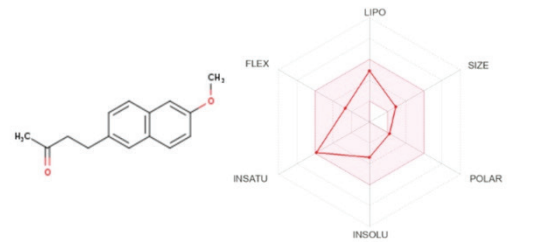


Figure 2. The 3D and 2D interactions of Nabumetone with GSK-3β.

Nabumetone – SwissADME results



SMILES COc1ccc2c(c1)ccc(c2)CCC(=O)C

Physicochemical Properties	
Formula	C15H16O2
Molecular weight	228.29 g/mol
Num. heavy atoms	17
Num. arom. heavy atoms	10
Fraction Csp3	0.27
Num. rotatable bonds	4
Num. H-bond acceptors	2
Num. H-bond donors	0
Molar Refractivity	70.03
TPSA	26.30 Å²

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-5.51 cm/s

Figure 3. Physicochemical properties and pharmacokinetics of nabumetone.

3880 mg/kg. The maximum tolerated dose of nabumetone in humans was 0.494 log (mg/kg/day) [Low < 0.477 log (mg/kg/day) < High)] (Figure 4).

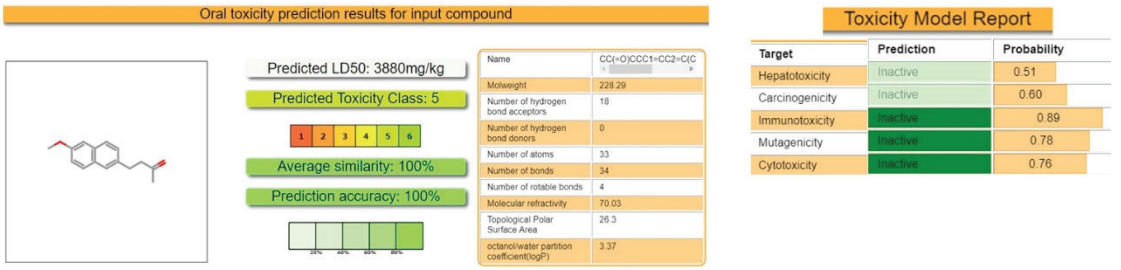
Inhibition of GSK-3β activity is one of the most important targets in tauopathies. Finding potential inhibitor drugs by drug repurposing approach is more efficient and safe. The molecular docking simulations with the seven drugs showed that nabumetone had high binding energy (-7.39 kcal/mol) and inhibition potential at low concentrations (3.8 µM) (Table 1), suggesting that this drug may have strong GSK-3β inhibitory potential.

Nabumetone is an NSAID, and common side effects include stomach upset, nausea, vomiting, diarrhea, headache, dizziness, and drowsiness. According to results of *in silico* toxicity research tools there is not any important predicted toxicity (Figure 4). This *in silico* research has shown that targeting GSK-3β

A) Nabumetone - pkCSM results

Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.494	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.246	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.049	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	1.404	Numeric (log ug/L)
Toxicity	Minnow toxicity	-0.166	Numeric (log mM)

B) Nabumetone - Protox-II results



Toxicity Model Report

Target	Prediction	Probability
Hepatotoxicity	Inactive	0.51
Carcinogenicity	Inactive	0.60
Immunotoxicity	Inactive	0.89
Mutagenicity	Inactive	0.78
Cytotoxicity	Inactive	0.76

Figure 4. Toxicity profiles of nabumetone according to pkCSM and Protox-II web tools.

with marketed drugs may be beneficial for tauopathies. These results can be a basis for *in vitro* and *in vivo* studies of nabumetone to GSK-3β.

4. Conclusion

The present study highlights the importance of GSK-3β in the hyperphosphorylation of tau proteins. Inhibition of GSK-3β activity may prevent the formation of abnormal tau protein aggregates (NFTs) and thereby prevent neuronal damage. In this study, nabumetone was found with the drug repurposing approach to inhibit GSK-3β without any toxic effect. This study could lead the way for the development of drugs that will inhibit GSK-3β.

Acknowledgments

The authors also thank Van Yüzüncü Yıl University, Faculty of Pharmacy, for the opportunity required for this study.

Conflict of Interest

The authors report there are no competing interests to declare.

Statement of Contribution of Researchers

Concept – F.K., B.K.; Design – F.K., B.K.; Supervision – B.K.; Resources E.D., F.K.; Materials –E.D., F.K., B.K.; Data Collection and Processing – E.D., F.K., B.K.; Analysis an Interpretation – F.K., B.K.; Literature Search – F.K., B.K.; Writing – E.D., F.K., B.K.; Critical Reviews – F.K., B.K.

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