INNOVATIVE THERAPEUTICS FOR NEURODEGENERATIVE DISEASES: COMPUTATIONAL IDENTIFICATION OF ASK1 INHIBITORS FROM DIVERSE COMPOUND LIBRARIES

Thesis submitted
in Partial Fulfilment of the Requirements for the Degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

by

PALLAVI

(2K22/MSCBIO/35)

Under the Supervision of

Prof. PRAVEEN KUMAR

Professor and Dean IA

Delhi Technological University

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, Delhi - 110042

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CANDIDATE'S DECLARATION

I, **Pallavi**, bearing Roll No. 2K22/MSCBIO/35 hereby certify that the work which is being presented in the thesis entitled "**Innovative Therapeutics For Neurodegenerative Diseases: Computational Identification Of Ask1 Inhibitors From Diverse Compound Libraries**" in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Prof. Pravir Kumar.

The matter presented in the thesis has not been submitted by me for the award of any degree of this or any other Institute.

Pallavi

Candidate's Signature

This is to certify that the student has incorporated all the corrections suggested by the examiner in the thesis and the statement made by the candidate is correct to the best of our knowledge.

05/06/2024

Signature of Supervisor

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Shahbad Daulatpur, Main Bawana Road, Delhi-110042

CERTIFICATE BY THE SUPERVISOR

Certified that **PALLAVI (2k22/MSCBIO/35)** has carried out her research work presented in this thesis entitled **"Innovative Therapeutics for Neurodegenerative Diseases: Computational Identification of ASK1 Inhibitors from Diverse Compound Libraries"** for the award of the degree of Master of Science and submitted to the Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. This thesis embodies the results of original work, and studies carried out by the student himself, and the contents of the thesis do not form the basis for the award of any other degree to the candidate or anybody else from this or any other Institution.

Date:

05.06.24

Prof. Yasha Hasija
Head of Department
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05/06/2024

Prof. Pravir Kumar
Supervisor
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Lastly, I would use this opportunity to show my appreciation toward my parents and for their unwavering support and for making this journey enjoyable. Their encouragement and camaraderie were essential in helping us support each other through this process.

Place: Delhi

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Innovative Therapeutics for Neurodegenerative Diseases: Computational Identification of ASK1 Inhibitors from Diverse Compound Libraries

PALLAVI

ABSTRACT

ND characterized by the loss of essential neurons, affects around 50 million people globally, with numbers expected to triple by 2050. AD is the most common ND, predominantly affecting women, followed by Parkinson's disease. In ND, Aging, and genetics are major contributors. Misfolded protein aggregation and oxidative stress, leading to ROS production, are central to ND pathology. ROS-induced damage is exacerbated by neurons' limited regenerative capacity and low antioxidant levels. This activates the MAPK signaling cascade, with ASK1, a MAP3K, promoting apoptosis via the JNK and p38 pathways under stress conditions.

In our study, we targeted ASK1 to disrupt its signaling pathway by identifying a suitable inhibitor. We screened a library of 3,647 FDA-approved compounds and natural compounds derived from plants known for their anticancer and anti-inflammatory properties. These plant-derived compounds were sourced from literature and databases such as the IMPPAT library. Drug discovery was performed using the AutoDock tool in PyRx, and inhibitors with the highest binding affinities and favorable biological properties were selected. ADMET analysis was conducted to ensure the selected inhibitors had acceptable pharmacokinetic and toxicological profiles. The software utilized in this study is publicly accessible, facilitating further laboratory validation of these inhibitors in model organisms.

In drug discovery analysis we found the top three compounds in bot both libraries representing excellent binding affinity with the therapeutic protein. These ligands are further analyzed for their physiochemical, pharmacokinetic, and carcinogenic properties which also show remarkable results.

All the analyses show good results of leading compounds for FDA drugs and phytochemicals. Phytochemicals (IMPHY003277, IMPHY001869, IMPHY010687)

and FDA-approved drugs (amitriptylinoxide, florantyrone, ponatinib) demonstrated strong ASK1 binding, favorable pharmacokinetics, minimal toxicity, and non-carcinogenic properties, making them promising candidates for neurodegenerative disease treatment. This study highlights the importance of comprehensive evaluation in drug development targeting ASK1.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ND	Neurodegenerative disease
PD	Parkinson disease
AD	Alzheimer disease
ALS	Amyotrophic lateral sclerosis
ROS	Reactive oxygen species
MAPK	Mitogen-activated protein kinase
JNK	c-Jun N-terminal kinase
CCC	C-terminal coiled coil
ER	Endoplasmic reticulum
ERAD	ER-associated protein degradation
LB	Lewy body
GOF	Gain of function
LRRK2	Leucine-rich repeat kinase 2
LPS	Lipopolysaccharide
ASK1	Apoptotic signal kinase 1
PARP	poly (ADP-ribose) polymerase

CHAPTER 1

INTRODUCTION

ND is primarily characterized by the loss of essential neuronal cells, with dementia being the most prevalent symptom across these conditions, affecting approximately 50 million individuals worldwide [1]. Projections estimate this number could rise to 150 million by 2050 [2]. Among neurodegenerative diseases, AD is the most prevalent, with a higher incidence in women (20%) compared to men (10%). PD ranks as the second most common ND. Both aging and genetic predispositions significantly contribute to the onset of these diseases [3]. A shared hallmark of all NDs is the aggregation of misfolded proteins, which triggers various pathological pathways, including numerous oxidative stress such as endoplasmic reticulum-mediated stress, Ca2+ overload, and LPS-induced stress, with oxidative stress being the predominant factor [4]. This stress forms ROS like hydrogen peroxide (H2O2), nitric oxide (NO), hydroxyl radicals (OH-), and superoxide anions [5]. The limited regenerative capacity of neurons and reduced levels of antioxidants exacerbate the detrimental effects of ROS, ultimately leading to neuronal cell death [6].

These oxidative stress pathways activate the MAPK signaling. The MAPK pathway is a critical response mechanism to cellular stress. Within this cascade, MAPK kinase kinase kinases (MAP3Ks) activate MAPK kinase kinases (MAP2Ks), which subsequently activate the MAPKs [7]. The MAP3K family includes ASK1, a protein activated under stress conditions that drives apoptotic pathways through the JNK and p38 signaling pathways [8].

CHAPTER 2

LITERATURE REVIEW

2.1. ASK and its structural components

There are three types of Apoptotic Signal-Regulating Kinase (ASK) proteins: ASK1, ASK2, and ASK3, which are structurally homologous to each other [9]. Among these, ASK1 is the most extensively studied protein, playing a crucial role in inflammation and apoptosis signaling via cytokine and stress-induced pathways [10]. Discovered in 1997 through the identification of a putative serine-threonine kinase by PCR, ASK1, in conjunction with ASK2, forms heterocomplexes that exhibit tumor suppressor properties and regulate caspase-3 and PARP [11].

Under normal, non-stress conditions, ASK1 homodimers or ASK1/ASK2 heterodimers interact with thioredoxin (Trx) and glutaredoxin (Grx) at the Trx binding domain (TBD), while the 14-3-3 gets attached to the C-ter region between the kinase domain and the CCC domain [12]. The Trx/Grx and 14-3-3 complexes are involved in regulating ASK1 activity, forming a high-mol-weight molecule called a signalosome under a reducing environment [13]. However, during oxidative stress, this complex oxidizes and dissociates from ASK1, triggering a switch from oxidative stress to phosphorylation signaling that leads to apoptosis [14].

2.1.1. Trx Binding Domain (TBD)

The TBD is located at residues 46-277 on the N-terminal of ASK1 and is known for regulating and inhibiting its apoptotic signaling [15]. The binding dynamics depend on the cellular redox state [16]. In reducing conditions, Cys32 and Cys35 form a disulfide bond that facilitates Trx binding to the TBD. Under oxidative conditions, this disulfide bond dissociates, leading to Trx release [17]. Subsequently, intermolecular disulfide bonds form, resulting in the phosphorylation of the activating loop, which further propagates apoptotic signaling by activating downstream MAP2K family members. Additionally, the TBD can also mediate ASK1 ubiquitin degradation, which is inhibited under certain conditions by TRAF (TNF receptor-associated factor).

Structurally, the TBD comprises six beta sheets followed by 6 alpha helices and an extra loop [18].

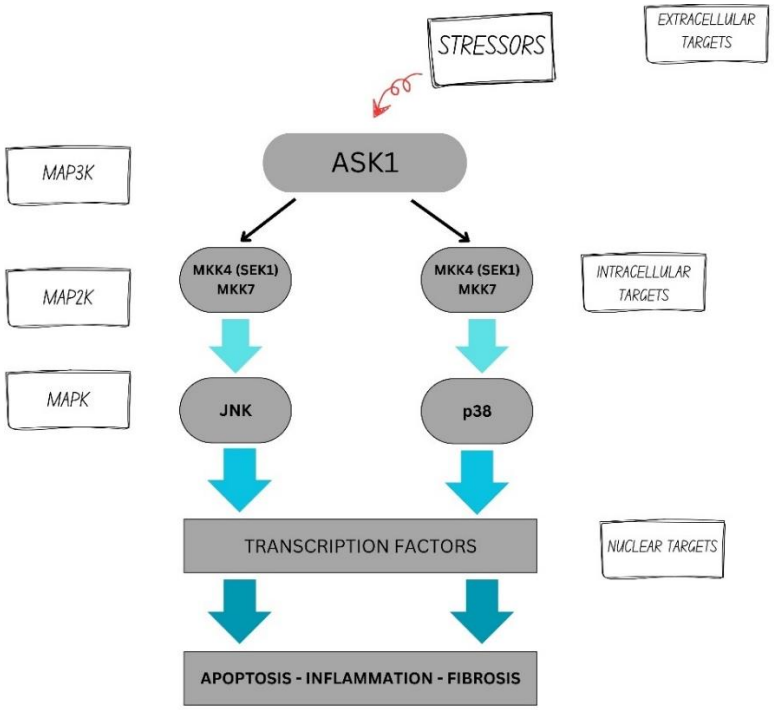


Fig. 2.1: ASK1 apoptotic signaling through MAPK pathway

The TBD contains seven consensus cysteine residues that undergo structural changes under stress conditions. Cys250 is a crucial residue that, when mutated to serine, alters the binding structure [19]. The domain also includes several methylation sites at Arg78, Arg80, and Arg89. Methylation at Arg78 and Arg80 promotes Trx binding and TRAF dissociation, thereby preventing ASK1 activation, whereas Arg89 methylation promotes the binding of Akt kinase, which phosphorylates Ser83, resulting in the downregulation of ASK1 [20].

2.1.2. Central Regulatory Region (CRR)

The Central Regulatory Region (CRR) is a multidomain structure containing seven tetratricopeptide repeat (TPR) motifs and a pleckstrin homology (PH) domain, spanning residues 269 to 658 [21] [22]. The CRR interacts with various TRAF proteins, predominantly TRAF2, TRAF5, and TRAF6, which are present in oxidative stress-induced ASK1 activation [23]. The seven TPR motifs consist of 14 alpha helices, while the PH domain features a C-terminal alpha helix and two antiparallel

beta sheets [21]. The TPR 6, TPR 7, and PH domains are connected through hydrophobic interactions. The PH domain, located between the kinase/catalytic domain and the TPR motifs, is responsible for MAP2K activation through phosphorylation [21]. Structurally, the PH domain is positioned close to the TBD, blocking MAP2K activation. Under oxidative conditions, Trx dissociation induces a conformational change, exposing the PH domain and enabling further activation and signaling through MAP2K [24].

2.1.3. Kinase/Catalytic Domain (KD)

The KD is situated centrally within the protein, between the N-ter PH and the C-ter 14-3-3 binding site. Crystallographic studies reveal that the KD possesses a typical kinase structure with dual lobes joined by a hinge, which serves as the site for ATP docking [25]. The KD is essential for phosphorylating MAP2K, thereby perpetuating the signaling cascade. The KD contains three autophosphorylation sites: Thr838, Thr813, and Thr842, which are crucial for oxidative stress-mediated activation of ASK1 [26]. The KD of both ASK1 and ASK2 is conserved, suggesting that in a heterocomplex, both are arranged similarly, with the KD playing a pivotal role in oligomeric complex formation [27] [28] [29].

2.2. Regulation by 14-3-3

The 14-3-3 protein family has conserved gene sequences with regulatory functions present in all cells. These proteins typically bind to phosphoserine or phosphothreonine sites, modulating the activity of partner proteins [30] [31]. In ASK1, 14-3-3 binds to Ser966, located adjacent to the KD. Mutation of the Ser966 site results in ASK1-mediated cell death. ASK1-interacting protein 1 (AIP1) interacts near the 14-3-3 binding sites [32]. Upon TNF-alpha treatment, AIP1 is phosphorylated by receptor-interacting protein 1 (RIP1) [33]. Activated AIP1 dephosphorylates Ser966, followed by the dissociation of 14-3-3, thereby regulating ASK1 activity [34].

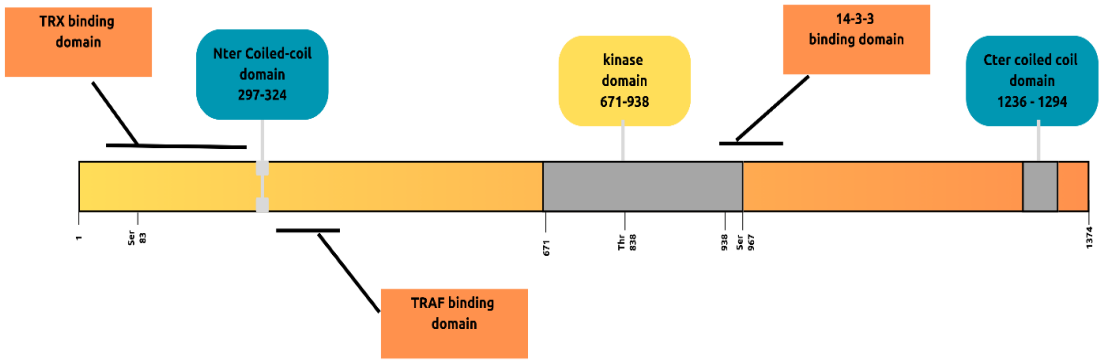


Fig. 2.2: ASK1 apoptotic signaling through MAPK pathway

2.3. Pathways

2.3.1 TNF-alpha Pathway

The TNF-alpha pathway involves the activation of ASK1, which subsequently activates JNK and p38 signaling pathways, leading to apoptosis. Experimental evidence has demonstrated that TNF-alpha induces cell death via ASK1 activation through these signaling pathways. This process requires the generation of reactive oxygen species (ROS) for ASK1 activation and signaling. The activation of ASK1 by TNF is mediated through TRAF [35] [36].

2.3.2. FAS Pathway

The FAS pathway also triggers JNK and p38 signaling via ASK1 activation. When FAS is activated, it engages the Daxx protein, which subsequently activates ASK1. The activated ASK1 then induces apoptosis through the JNK and p38 signaling pathways [37] [38].

2.3.3. TLR4 Pathway

TLR4 is a receptor on cells that recognizes and binds lipopolysaccharides (LPS) from Gram-negative bacteria, identifying them as pathogen-associated molecular patterns. LPS binding to TLR4 induces septic shock, marked by the production of NOS and TNF-alpha, resulting in ASK1-mediated cell death. TLR4-mediated ASK1 activation and subsequent JNK and p38 pathway activation lead to apoptosis, potentially involving ROS intermediates [39].

2.3.4. Calcium Pathway

Intracellular calcium ion concentration and calcium/calmodulin-dependent kinase (CaMK) play roles in neuronal behavior, plasticity, and development. Calcium influx can activate ASK1 and p38 via CaMK, leading to cell death. This activation may involve the phosphorylation of Thr845 on ASK1, facilitating its activation [40] [41].

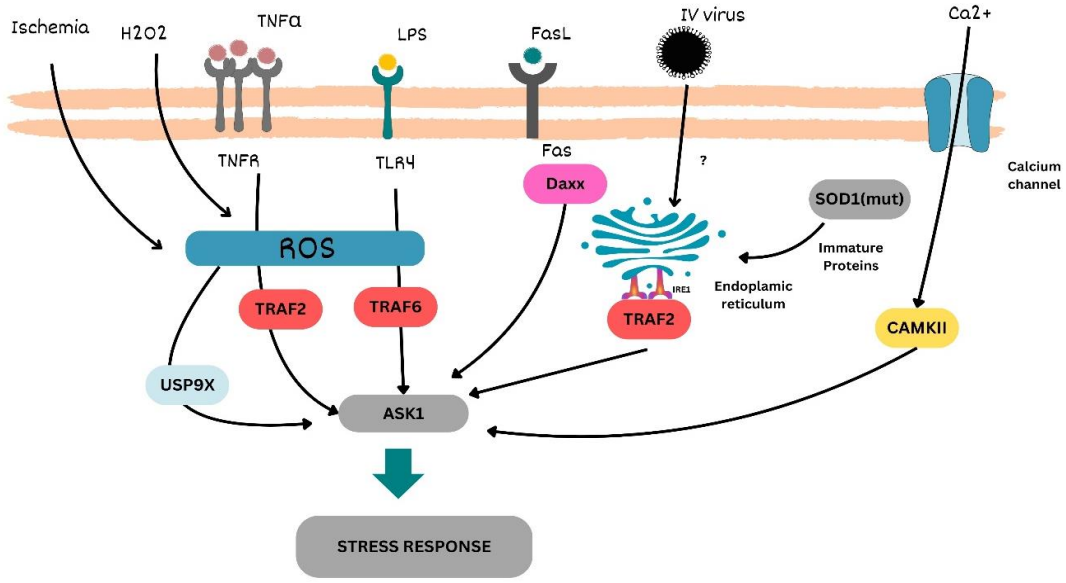


Fig. 2.3: ASK1 activation by various pathways leading to cell apoptosis

2.4. Neurodegenerative Diseases and ASK1

Neurodegenerative diseases (NDs) exhibit overlapping clinical symptoms and encompass a spectrum of disorders characterized by cognitive and motor function impairments. Common pathological features of NDs include the accumulation of misfolded proteins, faulty proteins, peptide fragments, and neurotoxicity, all of which contribute to neuronal cell malfunction and death. The ER plays a crucial role in protein quality control, ensuring that only properly folded proteins are secreted [42]. Misfolded proteins undergo multiple folding cycles, and if they remain incorrectly folded, they are targeted for degradation via the ERAD pathway [43]. The accumulation of misfolded proteins disrupts ER functionality, leading to the formation of protein aggregates and the activation of the ER stress signaling pathway through IRE1 and PERK [44] [45]. These ER transmembrane Ser/Thr protein kinases initiate ER stress signaling in an oligomerization-dependent autophosphorylation manner [46] [47].

2.4.1. Alzheimer's Disease (AD)

AD patients have dementia and is marked by the accumulation of amyloid-beta and tau proteins, which form amyloid plaques and neurofibrillary tangles, respectively [48]. These proteinaceous molecule aggregates induce stress in neurons. Interactions between amyloid precursor protein and ASK1 have been demonstrated in mouse models, indicating stress-induced ASK1 activation, which subsequently activates the p38 or JNK pathways, contributing to AD pathogenesis. Notably, ASK1-deficient mice exhibit improved cognitive function, suggesting that targeting ASK1 could be a promising therapeutic approach [49].

2.4.2. Parkinson’s Disease (PD)

PD is characterized by cognitive decline and motor function impairment, affecting approximately 3% of individuals over the age of 60 [50]. PD pathology includes the loss of dopamine in the basal ganglia due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta [51]. Alpha-synuclein, a protein prominently associated with PD, is a major component of LB and neurites. Alpha-synuclein is implicated in neuroinflammation and the activation of ASK [52]. Another key protein in PD is LRRK2, a multi-domain protein that activates ASK1, leading to neuronal cell death via direct phosphorylation. The most common LRRK2 mutation, Gly2019Ser, results in a GOF [53]. ASK1-deficient mice challenged with LRRK2 show suppressed apoptosis [54]. Familial PD involves mutations in the DJ-1 gene, which is crucial for mitochondrial function under oxidative stress [55]. These mutations lead to autosomal recessive inheritance and apoptosis via ASK1 activation and Daxx protein nuclear sequestration [56].

2.4.3. Huntington’s Disease (HD)

HD typically manifests in individuals aged 40 or older, with patients generally succumbing 15-20 years after onset [57]. HD is exemplified by progressive neuron malfunction and demise, resulting in cognitive, motor, and psychiatric impairments [58]. The disease is caused by the expansion of polyglutamine (poly Q) repeats, which aggregate within cells due to misfolding, causing ER stress and dysfunction [59]. This aggregation, combined with oxidative stress, activates the ASK1-mediated JNK and p38 pathways, ending in neuronal cell death. HD mouse models exhibit overproduction of ROS, ASK1 activation, active caspases, and striatal cell death.

2.4.4. Amyotrophic Lateral Sclerosis (ALS)

ALS is marked by the devolution of spinal and cortical neurons, leading to motor dysfunction and the impairment of voluntary muscle control [60]. ALS is predominantly seen in European populations, with a relatively low mortality rate within 3-5 years post-onset [61] [62] [63] [64]. Individuals aged 50-75 are at higher risk. The disease involves multiple molecular pathways, with SOD1 being a major protein implicated in its progression [65]. Misfolded SOD1, along with other proteins, forms aggregates that induce ER and cellular stress. As SOD1, which normally regulates oxidative stress, becomes mutated and misfolded, it leads to significant oxidative stress and the activation of ASK1-associated JNK and p38 pathways, resulting in neuronal cell death.

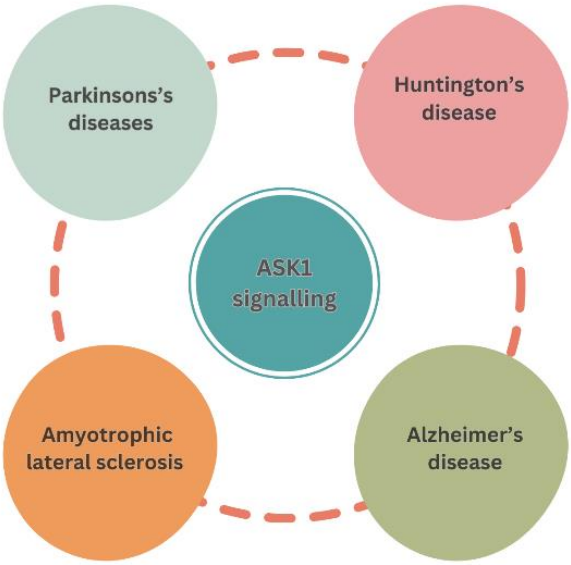


Fig. 2.4: ASK1 involvement in different NDs

CHAPTER 3

METHODOLOGY

3.1. Resources: Various databases have been utilized in this study. All of them are mentioned below:

PubMed: This database is managed by NCBI and NIH and is a free source for literature study in the field of life sciences.

PubChem: This database is managed by NIH and provides millions of data globally for free. It contains information about the chemical structure, physical properties, and other information about all sorts of compounds like carbohydrates, proteins, lipids, and nucleotides.

IMPAAT 2.0: Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 is a database for Indian medicinal plants their phytochemical along with physiochemical, pharmacokinetics, drug likeliness, and various other properties.

Protein Data Bank (PDB): This database comprises structural (3-D) information about compounds like protein and nucleotides and has been used globally for *in-silico* analysis or study.

3.2. Software utilized :

Open Babel: This open-source software is used to convert the formats of chemical files. It is said that open babel speaks many languages.

PyRx: It is a free software tool used for docking. This software has Autodock, Autodock Vina. It is used for screening drugs for target molecules.

Biovia Discovery Studio: This tool is used to visualize the various forms of compounds such as ligands, proteins, ligand-protein 2-D structure, protein-protein 2-D structure

PyMOL: This powerful tool is used for visualization of the 3-D structure of biomolecules. This tool also can be used to edit the biomolecule if it has some missing residues. It can also be used to draw 3-D interaction of ligand protein and visualize H-bond.

3.3. Workflow

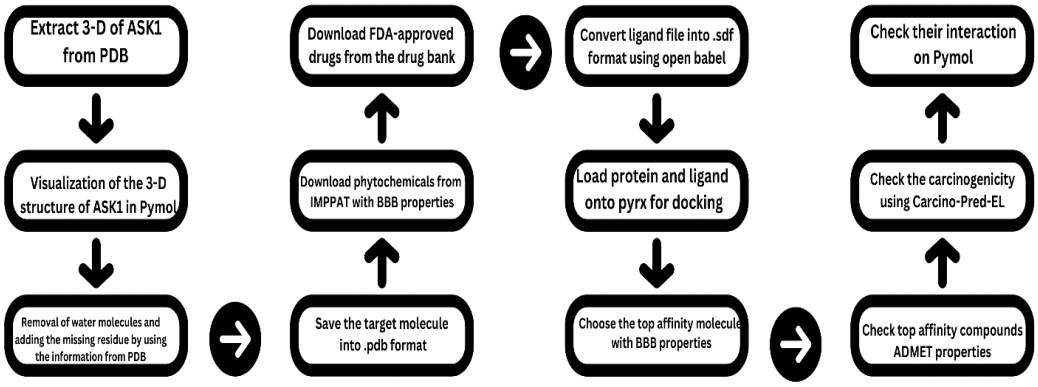


Fig. 3.1: Overview of the methodology.

3.4. Data Extraction

Data of phytochemicals has been extracted from IMPPAT 2.0 using plant properties. Specific properties that have been chosen for this study are anticancer, anti-nociceptive, anti-inflammatory, antimalarial, antiallergic, neuroprotective, anti-diabetic, anti-fungal, antimicrobial, antiulcer, antioxidant, and antidiarrhoeal, immunomodulatory, antitumor, analgesic, antipyretic, anti-plasmodic, antihistaminic, anti-proliferative, anthelmintic, astringent, anti-hyperglycaemic, anti-HIV, aphrodisiac, anticonvulsant, anti-osteoporotic,anti-cociceptive. Plant having these properties are selected and individual compounds has been checked for BBB properties and only those having BBB are downloaded having IMPPAT ID as IMPHYXXXX where X are digits uniue to each compound. Also FDA approved ligands are downloaded from DRUG bank. Total 3,674 ligands are downloaded.

Table 3.1: Phytochemicals derived from medicinal plants were selected for docking studies based on their therapeutic properties.

Name of medicinal plant	Family	Number of phytochemical entries	Source of phytochemicals	Activity	Reference
Albizia lebbeck	Fabaceae	108	Bark, flower, fruit, leaf, root, seed, wood	Anticancer,anti-nociceptive, anti-inflammatory, antimalarial, antiallergic, neuroprotective	[66] [67] [68] [69] [70]
Asparagus officinalis	Asparagaceae	51	flower, leaf, root, seed, shoot	Anti-diabetic, anti-cancer, anti-fungal, antimicrobial	[71]
Asparagus racemosus	Liliaceae	40	Bark, flower, fruit, leaf, root, wood,	Antiulcer, antioxidant, and antidiarrhoeal, antidiabetic and immunomodulatory, antitumor	[72] [73]
Bauhinia racemosa	Fabaceae	20	Bark, root, seed, stem, wood	Analgesic, antipyretic, anti–inflammatory, anti-plasmodic, antimicrobial, antihistaminic	[74][75][76][77]
Bidens pilosa	Asteraceae	190	Flower, leaf, root, stem	Anti-proliferative, anti-inflammatory, anti-diabetic, antioxidant, antimalarial	[78][79][80][81] [82][83]
Butea monosperma	Fabaceae	77	Bark, flower, plant exudate, root, seed, whole plant	Anti-tumor, anti-microbial, anthelmintic, anti-inflammatory, astringent	[84][85]
Cedrus deodara	Pinaceae	189	Bark, flower, leaf, plant exudate, root, seed, wood, whole plant	Anti-inflammatory, anti-hyperglycaemic, antimicrobial, anti-apoptotic, immunomodulatory, anti-malarial, anti-ulcer, anti-cancer, analgesic	[86]
Croton tiglium	Euphorbiaceae	33	Seed	Anti-bacterial, anti-fungal, analgesic, anti-inflammatory, anti-HIV, anti-tumor	[87] [88] [89] [90]

Datura innoxia	Solanaceae	53	Aerial part, flower, fruit, leaf, root, seed, stem	analgesic, anthelmintic, anti-inflammatory	[91]
Datura metel	Solanaceae	104	Aerial, leaf, part, stem, bark, root, flower, fruit, seed, whole plant	Anti-proliferative, anti-inflammatory, antioxidant, antipyretic, and analgesic	[92][93][94] [95]
Euphorbia hirta	Euphorbiaceae	129	Aerial, part, bark, flower, leaf, plant exudate, root, stem, whole plant	Anthelmintic, antimicrobial, antimalarial, antispasmodic	[96]
Gymnema sylvestre	Asclepiadaceae	119	Fruit, leaf	Antioxidant, anti-diabetic, antimicrobial, anti-inflammatory, anticancer	[97][98]
Inula racemosa	Compositae	48	Root	Anti-inflammatory, analgesic, anti-cancer	[99]
Moringa oliefera	Moringaceae	200	Bark, stem, flower, root, fruit, leaf, seed, whole plant	Antioxidant, anti-cancer, anti-inflammatory	[100]
Plantago major	Plantaginaceae	46	Aerial part, flower, leaf, root, seed, whole plant	Hepatoprotective, Anti-hypercholesteremia, Anti-atherosclerosis, anti-inflammatory, analgesic, anti-microbial, anti-cancer	[101][102] [103]
Pterocarpus marsupium	Fabaceae	71	Bark, root, seed, whole plant, wood	anthelminthic, antipyretic, anti-inflammatory, aphrodisiac, antiulcer	[104]
Taxus wallichiana	Taxaceae	181	Bark, fruit, leaf, Root, stem, wood	Analgesic, anti-inflammatory, immunomodulatory, antispasmodic anti-allergic, anticonvulsant, anti-osteoporotic,anti-cociceptive	[105] [106]

Urtica dioica	Urticaceae	69	Flower, leaf, plant cells/culture, rhizome, root, trichome	Antioxidant, Anti-Inflammatory, Hypoglycemic, Antiulcer, , Cardiovascular protective, Repression of prostate-cell metabolism and proliferation	[107] [108]
Vitex negundo	Verbenaceae	228	Bark, flower, fruit, leaf, root, seed, stem	Antihelmintic, anti-inflammatory, anti-proliferative, antioxidant	[109] [110]

3.5. Protein preparation:

ASK1 is downloaded from PDB in 3D .pdb format. It is visualized using pymol software where water molecule and hetatom is removed and missing residue also added using information present on PDB.

3.6. Ligand preparation:

Once the ligands are downloaded they are converted into .smile files using the open babel tool. These .smile files are used by SWISS-ADME webtool which reports the BBB properties of each compound and those were chosen for further drug discovery analysis. This process is followed for phytochemicals.

For FDA-approved drugs file is converted into .sdf format than after drug discovery BBB were selected.

3.7. Docking using Pyrx:

Pyrx was used for drug discovery through docking by Autodock. For docking target protein is loaded and converted into macromolecule. Subsequently, ligand is also loaded and their energy is minimized and converted into .pdbqt in open babel of pyrx software. Then for blind docking area of the grid represented on the software in the image is maximized. Then proceeded for docking.

3.8. Carcinogenicity tesusing AI model Carcino-Pred-EL

Ensemble XGBoost, Ensemble SVM, and Ensemble RF these ensemble models are used for the prediction of carcinogenicity. These three models used several molecular fingerprints for training and development. .smile file of top affinity compounds are used to make predictions. The probability of the three models ranges from 0 to 1.

3.9. ADMET analysis

This web tool is used to predict various properties like physiochemical, pharmacokinetics, drug likeliness, lipophilicity, water solubility, and others which are useful in determining the effectiveness of a drug. Boiled egg made for every molecule which represents the p-gp substrate, BBB of the compound.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Docking results:

Docking results of the plant-derived compounds and FDA-approved drugs produced very good results showing binding affinity ranging from -10.8 to -9.5 for phytochemicals and -10.8 to -10 for FDA-approved ligands. For phytochemical IMPHY001869, IMPHY010687, and IMPHY003277 show the highest binding affinity of -9.5, -10.4, and -10.8 respectively. For FDA-approved drugs highest affinity was shown by Amitriptylinoxide, Florantyrone, and Ponatinib with binding affinity -10.8, -10.7, and -10.5 respectively.

4.2 Protein-ligand interaction:

The output file from docking is used to draw 2D and 3D diagrams using Discovery Studio and Pymol respectively. The number of interactions and H-bonds with their distance is shown in the table.3. 2D diagram from Discovery Studio shows the different interactions formed between protein and ligands can be seen. Further H-bond can be drawn in Pymol and its distance can be measured. The presence of H-bond and Van-dar-waal interaction are most important of good interaction.

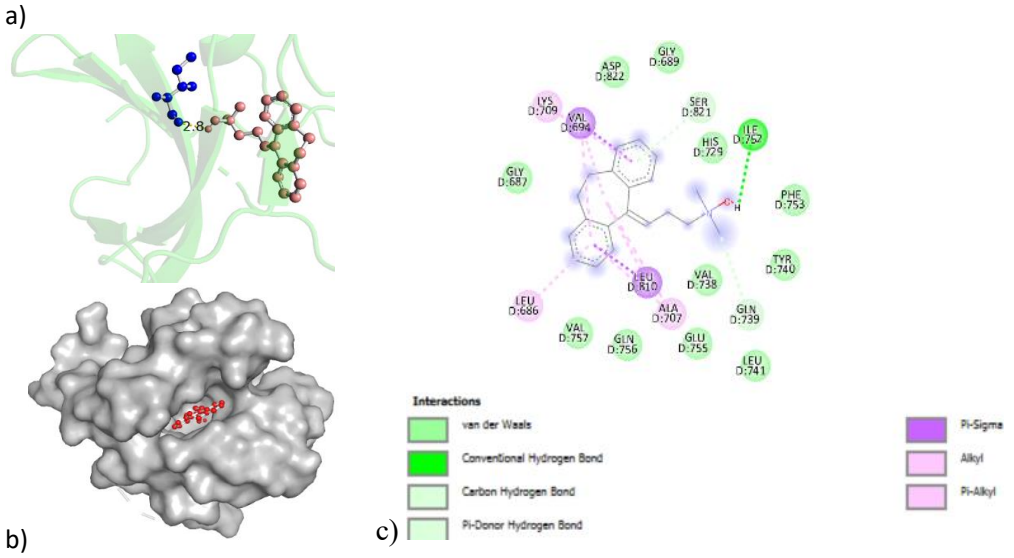


Fig. 4.1: a) represents H-bond formed between Amitriptylinoxide and ASK1 in the 3D view taken from pymol b) is the surface view of protein and ligand made using pymol c) shows a 2D depiction of various interactions formed between ASK1 and Amitriptylinoxide

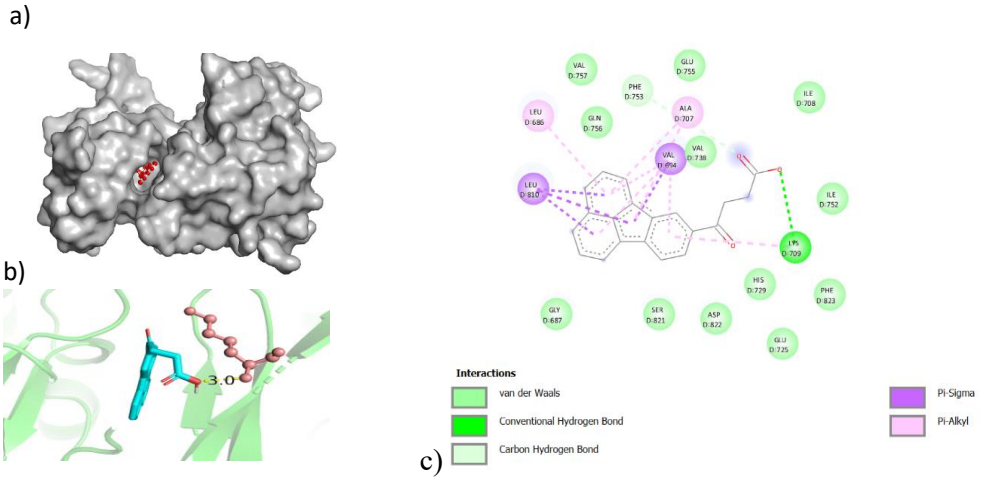


Figure 4.2: a) represents the H-bond formed between Florantyrone and ASK1 in the 3D view taken from pymol b) is the surface view of protein and ligand made using pymol c) shows a 2D depiction of various interactions formed between ASK1 and Florantyrone.

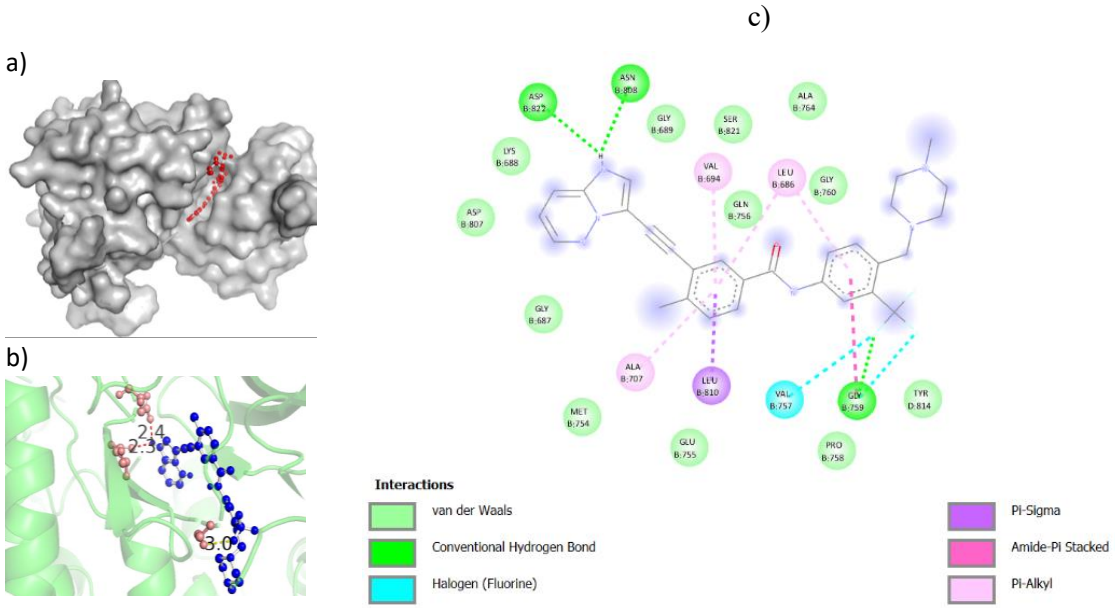


Fig. 4.3: a) represents H-bond formed between Ponatinib and ASK1 in the 3D view taken from pymol b) is the surface view of protein and ligand made using pymol c) shows a 2D depiction of various interactions formed between ASK1 and Ponatinib

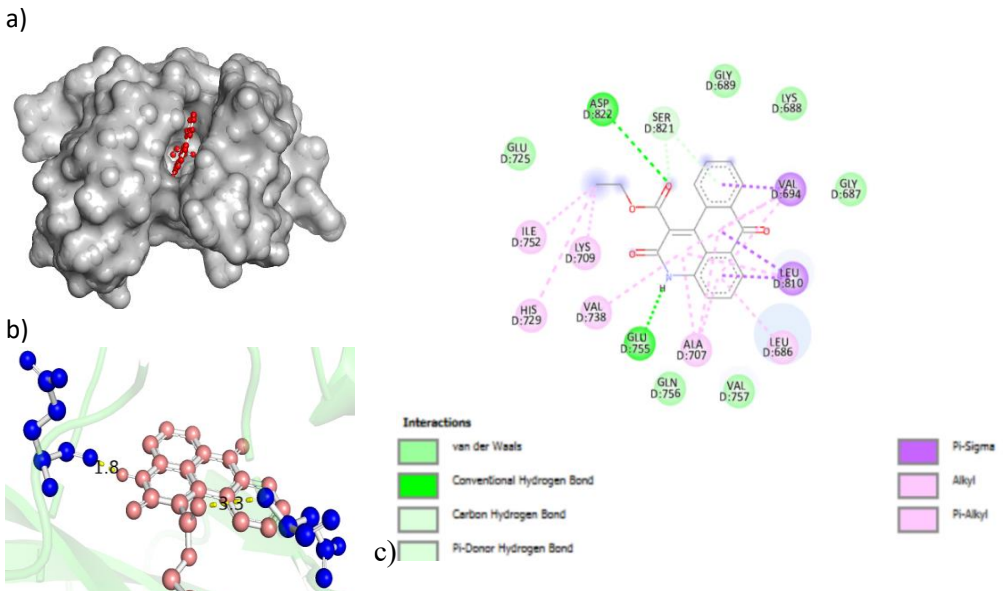


Fig. 4.4: a) represents the H-bond formed between NQDI (reference drug)and ASK1 in the 3D view taken from pymol b) is the surface view of protein and ligand made using pymol c) shows a 2D depiction of various interactions formed between ASK1 and NQDI

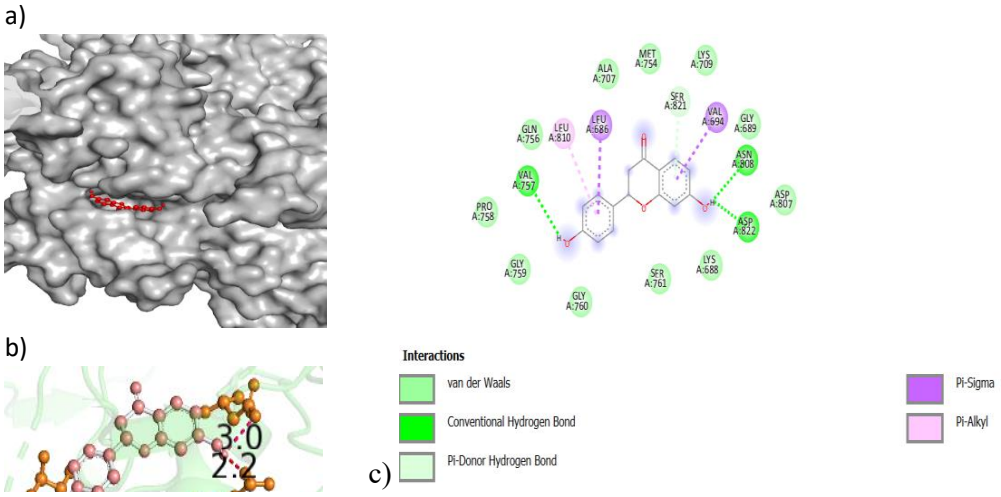


Fig. 4.5: a) represents the H-bond formed between IMPHY001869 and ASK1 in the 3D view taken from pymol b) is the surface view of protein and ligand made using pymol c) shows a 2D depiction of various interactions formed between ASK1 and IMPHY001869

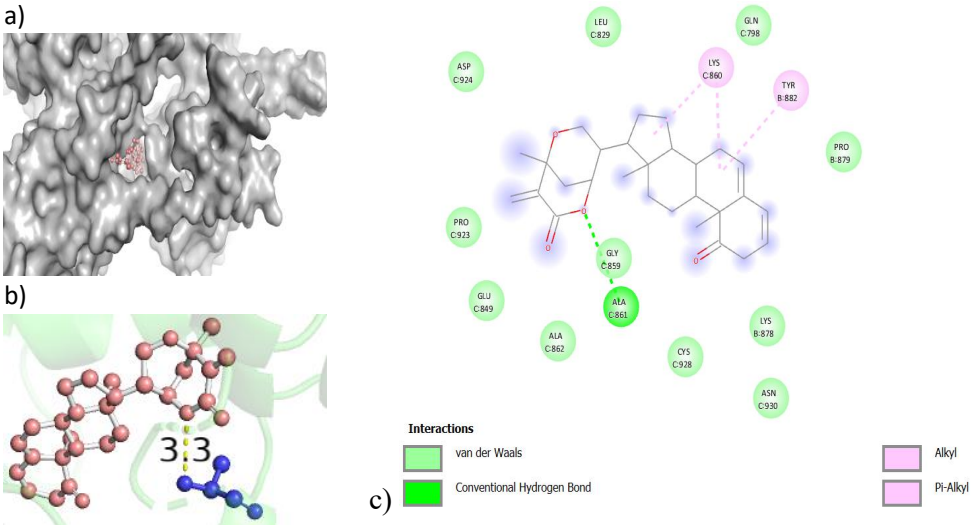


Fig. 4.6: a) represents H-bond formed between IMPHY010687 and ASK1 in 3D view taken from pymol b) is the surface view of protein and ligand made using pymol c) shows 2D depiction of various interactions formed between ASK1 and IMPHY010687

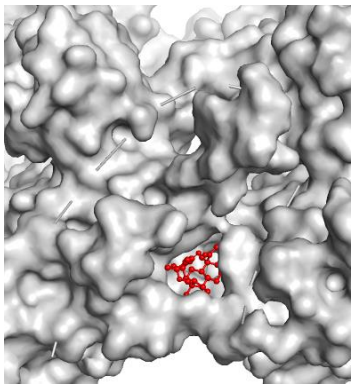
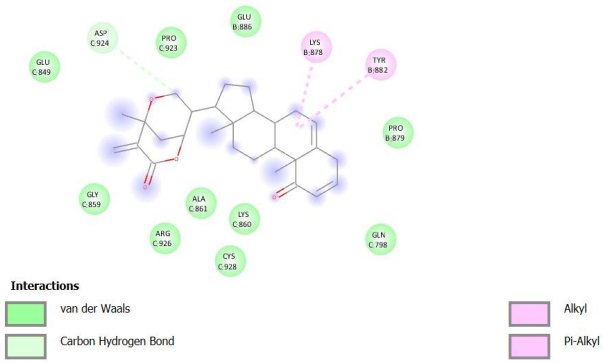
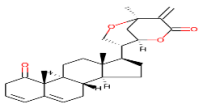
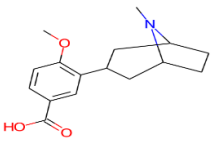
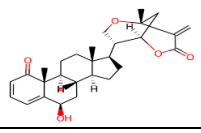
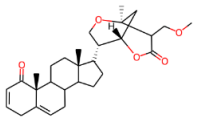
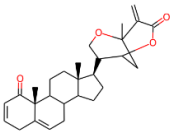
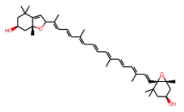
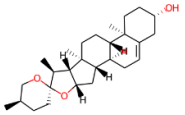
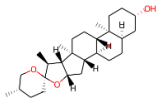
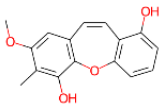
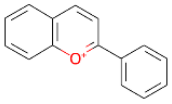
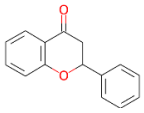
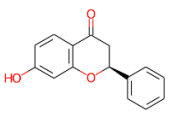
a)  b) 

Fig. 4.7: a) is the surface view of protein and ligand made using pymol b) shows a 2D depiction of various interactions formed between ASK1 and IMPHY003277

Table 4.1: Top affinity phytochemicals with their binding score and structural diagram

S.no	Ligand	Binding energy	Name of medicinal plant	Phytochemical name	Structural Diagram
1.	IMPHY010687	-10.4	Datura metel	Isowithametelin	
2.	IMPHY009440	-8.7	Datura metel	Datumetine	
3.	IMPHY009120	-9.2	Datura metel	Withametelin B	

4.	IMPHY004278	-9.2	Datura metel	Datumetelin	
5.	IMPHY003277	-10.8	Datura innoxia	withametelin, withametelin (daturilin)	
6.	IMPHY002029	-9.1	Urtica diocia	Luteoxanthin	
7.	IMPHY003681	-9.3	Asparagus racemosus	Diosgenin	
8.	IMPHY012274	-9.3	Asparagus officinalis	Sarsasapogenin	
9.	IMPHY001963	-9.2	Bauhinia racemosus	Pacharin	
10.	IMPHY002588	-8.9	Euphorbia hirta	Flavylium	
11.	IMPHY001315	-9.2	Vitex negundo	Flavanone	
12.	IMPHY011492	-9.3	Pterocarpus marsupium	(2S)-7-hydroxyflavanone	

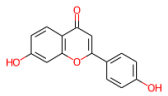
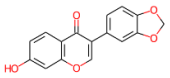
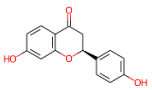
13.	IMPHY010592	-9.4	Pterocarpus marsupium	7,4'-Dihydroxyflavone	
14.	IMPHY004580	-9.5	Pterocarpus marsupium	Pseudobaptigenin	
15.	IMPHY001869	-9.5	Pterocarpus marsupium	Liquiritigenin	

Table 4.2: FDA-approved ligands and phytochemicals exhibiting the highest binding affinity along with various interactions

Ligands	**Binding energy (kcal/mol)**	**P-gp substrate**	**Type of Interactions**			**Chain**
			H-bond with bond length in Å	**Wan-der-Vaal**	**Other non-polar interactions**	
Ponatinib	-10.5	Yes	D-822(2.3) N-808(2.4) G-759(3.0)	K-688 D-807 G-687 M-754 E-755 P-758 Y-814 G-760 Q-756 A-764 S-821 G-689	V-694 L-686 A-707 L-810 V-757	B
Florantyrone	-10.7	No	K-709(3.0)	V-757 Q-756 E-755 V-738 I-708 I-752 F-838 H-729 E-725 D-822 S-821 G-687	F-755 A-707 V-694 L-810 L-686 L-709	D
Amitriptylinoxide	-10.8	No	I-752 (2.8)	D-822 G-689 H-729 F-753 Y-740 V-738 L-741 E-755 Q-756 V-757 G-687	K-709 V-694 L-686 L-810 A-707 Q-739 S-821	D

NQDI (reference)	-9.6	No	D-822(3.3) E-755 (1.8)	E-725 G-689 K-688 G-687 V-757 Q-756	I-752 V-738 K-709 H-729 A-707 L-686 L-810 V-694 S821	D
IMPHY001869	-9.5		V-757 (2.3) N-808(3.3) D-822(2.2)	A-707 M-754 K-709 G-689 D-807 S-761 K-688 G-760 G-759 P-758 G-756	S-821 L-810 L-686 V-694	A
IMPHY010687	-10.4		C chain: A-861 (3.3)	C chain: L-829 G-798 N-930 C-928 A-862 E-849 P-923 D-924 G-859 B chain : K-878 P-879	C Chain: K-860 B chain: Y-882	B&C
IMPHY003277	-10.8		C chain: D-924	C chain: E-849 P-923 Q-798 K-860 C-928 R-926 A-861 G-859 B Chain: P-879 E-886	B-Chain: K-878 Y-882	B&C

4.3. ADMET properties analysis:

ADME analysis of leading drugs of FDA-approved drugs and phytochemicals shows significant results shown in the table below. All properties of ADME such as pharmacokinetics, drug likeliness, water solubility, and other properties mentioned.

Table 4.3: ADMET properties of pharmacokinetics and Drug likeliness of top affinity FDA-approved drugs and phytochemicals

Ligands	**Solubility class**	**BBB permeant**	**Lipinski's rule of 5**	**Bioavailability score**
IMPHY001869	Soluble	Yes	Passed (0 violation)	0.55
IMPHY010687	Moderately soluble		Passed	

		Yes	(1 violation)	0.55
IMPHY003277	Moderately soluble	Yes	Passed (1 violation)	0.55
Amitriptylinoxide	Moderately soluble	Yes	Yes (0 violation)	0.55
Florantyrone	Moderately soluble	Yes	Yes (0 violation)	0.85
Ponatinib	Moderately soluble	Yes	Yes (1 violation)	0.55

Table 4.4: Pharmacokinetic properties of top affinity FDA-approved drugs and Phytochemicals

Ligands	**GI absorption**	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**CYP3A4**
IMPHY001869	High	Inhibition	No Inhibition	Non Inhibition	No Inhibition	No Inhibition
IMPHY010687	High	No Inhibition	No Inhibition	Inhibition	No Inhibition	No Inhibition
IMPHY003277	High	No Inhibition	No Inhibition	Inhibition	No Inhibition	No Inhibition
Amitriptylinoxide	High	No Inhibition	No Inhibition	Inhibition	Inhibition	Inhibition
Florantyrone	High	Inhibition	No Inhibition	Inhibition	No Inhibition	No Inhibition
Ponatinib	High	No Inhibition	Inhibition	Inhibition	Inhibition	No Inhibition

Table 4.5: Physiochemical properties of top affinity FDA-approved drugs and Phytochemicals

Ligand	Mol. weight (g/mol)	H-bond acceptors count	H-bond donors count	Rotatable bonds count	TPSA
IMPHY001869	256.26	4	2	1	66.76 Å²
IMPHY010687	436.59	4	0	1	52.6 Å²
IMPHY003277	436.58	4	0	1	52.60 Å²
Amitriptylinoxide	293.40	1	0	3	29.43 Å²
Florantyrone	302.32	3	1	4	54.37 Å²
Ponatinib	532.56	8	1	6	65.77 Å²

4.4 Carcinogenicity prediction:

Carcinogenicity prediction made by using the CarcinoPred-EL AI model shows significant results of all three models shown in the table.

Table 4.6: Carcinogenicity probability prediction using CarcinoPred-EL for IMPHY001869

Method	Average probability	Carcinogen
RF	0.39	No
SVM	0.33	No
XGBoost	0.44	No

Table 4.7: Carcinogenicity probability prediction using CarcinoPred-EL for IMPHY010687

Method	Average probability	Carcinogen
RF	0.41	No
SVM	0.41	No
XGBoost	0.45	No

Table 4.8: Carcinogenicity probability prediction using CarcinoPred-EL for IMPHY003277

Method	Average probability	Carcinogen
RF	0.31	No
SVM	0.33	No
XGBoost	0.43	No

Table 4.9: Carcinogenicity probability prediction using CarcinoPred-EL for Amitriptylinoxide

Method	Average probability	Carcinogen
RF	0.44	No
SVM	0.46	No
XGBoost	0.45	No

Table 4.10: Carcinogenicity probability prediction using CarcinoPred-EL for Florantyrone

Method	**Average probability**	**Carcinogen**
RF	0.40	No
SVM	0.42	No
XGBoost	0.48	No

Table 4.11: Carcinogenicity probability prediction using CarcinoPred-EL for Ponatinib

Method	**Average probability**	Carcinogen
RF	0.39	No
SVM	0.41	No
XGBoost	0.43	No

4.4. Discussion :

Apoptotic signal-regulating kinase 1 (ASK1), a part of the MAP3K family, has a crucial role in stress-induced apoptotic signaling via the MAPK pathway. Targeting ASK1 offers a promising therapeutic approach for neurodegenerative diseases (ND). In our study, screening of FDA-approved drug libraries and phytochemicals revealed several potent candidates. Among the phytochemicals, IMPHY003277, also known as daturilin, exhibited a max binding affinity of -10.8 kcal/mol. Other notable phytochemicals, IMPHY001869 and IMPHY010687, demonstrated binding affinities of -9.5 and -10.4 kcal/mol, respectively. These compounds also showed favorable ADME (Absorption, Distribution, Metabolism, and Excretion) profiles, with a bioavailability score of 0.55, indicative of good oral bioavailability. This prediction is supported by their physicochemical properties, such as the number of H-bond acceptors and donors, molecular weight, and solubility.

All three phytochemicals passed Lipinski's rule of 5, indicating drug-likeness, and exhibited minimal to no inhibitory effects on cytochrome P450 isoforms, suggesting low toxicity and reduced potential for drug-drug interactions. Their molecular sizes are within the optimal range for absorption, contributing to their good gastrointestinal (GI) absorption profiles. They possess a favorable number of H-bond acceptors, fewer H-bond donors, and topological polar surface areas (TPSA) below 140 $Å^2$, underscoring their excellent physicochemical properties.

Carcinogenicity predictions using the AI model CarcinoPred-EL, which utilizes seven molecular fingerprints and three models, indicated that all three compounds have an average carcinogenicity probability of less than 0.5. This suggests that they are non-carcinogenic and safe for use.

Among the FDA-approved drugs, amitriptylinoxide, florantyrone, and ponatinib emerged as leading compounds with binding affinities of -10.8, -10.7, and -10.5 kcal/mol, respectively. Structural and interaction analyses revealed a significant number of hydrogen bonds, which are critical for robust drug-target interactions. These compounds also displayed favorable bioavailability scores, suggesting good oral absorption and efficacy. Like the phytochemicals, they passed Lipinski's rule with minimal violations and showed negligible inhibition of cytochrome P450 isoforms, except for CYP2C9, which is inhibited by all three. This minimal inhibition indicates low toxicity and efficient metabolism, preventing accumulation in the biological system.

The FDA-approved drugs also exhibit optimal molecular weights, facilitating easy GI absorption. They possess a balanced number of H-bond acceptors, fewer H-bond donors, and a suitable number of rotatable bonds, enhancing molecular flexibility. Their TPSA values are well below the threshold of 140 $Å^2$, highlighting their excellent physicochemical properties.

Carcinogenicity predictions using CarcinoPred-EL for the FDA-approved drugs yielded favorable results, with average probabilities below 0.5, confirming their non-carcinogenic nature and safety for therapeutic use.

In summary, both the plant-derived compounds and FDA-approved drugs investigated in this study demonstrated strong binding affinities to ASK1 and exhibited desirable

pharmacokinetic and pharmacodynamic properties. The phytochemicals IMPHY003277 (daturilin), IMPHY001869, and IMPHY010687, along with the FDA-approved drugs amitriptylinoxide, florantyrone, and ponatinib, show great potential as therapeutic agents for neurodegenerative diseases. Their good oral bioavailability, minimal toxicity, non-carcinogenic nature, and excellent physicochemical properties make them viable candidates for further development. This comprehensive evaluation underscores the importance of integrating binding affinity, ADME profiles, physicochemical characteristics, and carcinogenicity predictions in drug discovery and development, particularly for targeting critical proteins like ASK1 in neurodegenerative disease therapies.

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Dear Author(s),

Greetings from **SJC Institute of Technology, Chikkaballapur!**

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in 2nd **International Conference on Knowledge Engineering and Communication Systems** held at **SJC Institute of Technology** in association with **IEEE Bangalore Section**, April 18 & 19 2024.

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Employing Multifaceted Bioinformatics Strategies for the Discovery of Novel ASK1 Inhibitors Targeting Neurodegenerative Disorders

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***Abstract*—Neurodegenerative diseases, characterized by reactive oxygen species-induced oxidative perturbation, ER stress, intracellular stress, and inflammation, often lead to neuronal cellular toxicity and death. The MAP3K ASK1 plays a pivotal role in this process, mediating the downstream activation of MAP2K and MAP1K family proteins. Given its involvement in various neurodegenerative diseases and cancer, ASK1 emerges as a promising therapeutic target. To identify potential inhibitors, a comprehensive approach was undertaken. Docking simulations involving 3,674 FDA-approved drugs were conducted, aiming to discover compounds with superior affinity compared to existing drugs. This extensive screening process narrowed down the candidates, and subsequent SWISS-ADME analysis was performed to evaluate the physicochemical properties, bioavailability, solubility, and lipophilicity of the selected ligands. Among the examined drugs, one emerged as a particularly promising candidate for targeting ASK1. The selection was based not only on its binding affinity but also on favorable physicochemical characteristics crucial for drug development. This robust methodology combines molecular docking, leveraging the vast library of FDA-approved drugs, with advanced ADME analysis, enhancing the likelihood of identifying a drug with therapeutic potential. This integrated approach holds promise in uncovering novel ASK1 inhibitors, providing a foundation for further preclinical and clinical investigations. The identified candidate exhibits strong potential for therapeutic intervention in neurodegenerative disorders, offering new avenues for drug development and treatment strategies.**

Keywords— ASK1, MAP3K, JNK, p38, SWISS ADME, binding affinity, BBB, PGP substrate.

I. Introduction

Apoptotic signal kinase 1 (ASK1) belongs to the mitogen-activated kinase kinase kinase (MAP3K) protein family. Apoptotic signal kinase protein has 3 proteins ASK1, ASK2, and ASK3. ASK1 is a studied protein involved in crucial cell growth regulation pathways. ASK1 functions as a homodimer or heterodimer with ASK2 in ER stress, cellular stress, inflammatory signal, and oxidative stress.

It induces cellular apoptosis through activation of the extracellular signal-regulated protein kinases (ERKs), c-Jun N-terminal kinases (JNKs), and p38 MAPKs pathways [1]. The signaling pathway includes the activation of ASK1 which activates the MAP2K family which will activate the MAP1K family that functions to regulate cell fate by activating different signaling pathways. ASK1 homo/heterodimer forms are regulated by glutaredoxin(Grx), thioredoxin(Trx), and 14-3-3 forming the signalosome of ASK1. Thioredoxin ubiquitinates ASK1 when bound to it but under stress conditions, oxidoreductase (Trx, Grx) dissociated and ASK1 becomes activated and proceeds its signaling pathway.

A. Structural information

ASK1 has different domains present in it. Ask2 and Ask3 shows >80% sequence similarity to ASK1. It consists of the Trx binding domain (TBD) present on the N-terminus. The binding of Trx to TBD will result in ubiquitination or inactivation of ASK1. The joining of both proteins occurs through the Cys residue of Trx. In the oxidizing condition, 7 conserved sequences of Cys in TBD form an intramolecular disulfide bond and prevent Trx binding [2]. The next domain is a central regulatory domain (CRR), which consists of PH (pleckstrin homology) and TPR (tetratricopeptide repeat) regions. It provides a site for binding TRAF2, TRAF5, and TRAF6 which act as ASK1 activators [3]. PH has 2 antiparallel beta-strands and 1 alpha-helix. This domain is responsible for TRAF binding and substrate binding which results in the phosphorylation of the kinase domain. The Kinase domain (KD) has multiple phosphorylation sites the most important one being Thr-838. The kinase domain comprises a diminutive N-terminal lobe and an extensive C-terminal lobe that engages in mutual interaction via the hinge region. N-ter have 5 beta-strands and 1 alpha-strand [4]. Adjacent to this present 14-3-3 binding motif which is responsible for the regulation of ASK1 activity. At the C- terminus sterile alpha motif (SAM) is present which has multiple phosphorylation sites that are involved in the regulation of ASK1[5]. This domain differs from others in terms of oligomerization. It has a high propensity for oligomerization. SAM of ASK1 and ASK2 forms a heterocomplex which is formed by mid-loop end helix, mutation in which can render formation of heterocomplex.

B. ASK1 in different neurodegenerative diseases

Parkinson's disease (PD) leads to death and toxicity of dopaminergic neuronal cells. The crucial genes involved in PD is alpha-synuclein, protein deglycase, and leucine-rich repeat kinase 2 which are engaged in the initiation or triggering of ASK1 and thus responsible for neuronal cell death. Alzheimer's disease (AD) is associated with the accumulation of improper folded amyloid protein or mutation in amyloid precursor protein. AD can also be caused by trans-

fatty acids. All of them are responsible for ROS-mediated or Ca^{2+} activation of the ASK1/p38 signaling pathway. Huntington's disease is marked by multiple repeats of CAG on the htt gene which form polyQ sequences and induce ER stress. This ER stress is responsible for ASK1 activation. Amyotrophic lateral sclerosis (ALS) patients are seen to have a loss of motor neurons. Mainly Zn-Cu superoxide dismutase mutation is responsible for the oxidative stress in the cell which will activate ASK1 and hence, the apoptotic signaling pathway [6].

II. Methodology

A. Protein preparation

ASK1 is downloaded from the protein data bank in the .sdf (structural data file) format. Water molecules and removal of side chains done in protein preparation on Biovia Drug Discovery Studio Visualizer 2020... Finally, save the protein in .pdb format. ASK1(5VIO) downloaded from PDB is of 2.4 Å resolution which is present with a potent inhibitor that is removed using symbol. Missing residues are also added using Pymol by taking reference from the sequence present on PDB.

B. Ligand preparation

To find the ASK1 protein library inhibitor, 3,674 FDA-approved drugs were used. Drugs were in SDF format and converted into .pdbqt format using Open Babel software present in Pyrx. Here the energy of the ligand is also minimized.

C. Docking using Pyrx

The main application of the Python library Pyrx is molecular docking research. It facilitates the simulation of the interaction between target proteins and small molecules by scientists and researchers. Drug discovery relies heavily on molecular docking, which predicts how prospective drug compounds might bind to a target protein and helps identify promising candidates for further investigation. The first step is to put the main protein into the program and transform it into a macromolecule. Ligands are selected for docking after being converted into the .pdbqt format and going through an energy minimization process. Blind docking then occurs, in which Vina Wizard's "maximize" function is used to maximize the docking site; the final dimensions are x-118.1468 Å, y-99.1581 Å, and z-76.8338 Å. Following docking, data with each ligand's binding affinity for the macromolecule is stored. Each docked ligand's output file is saved in a designated area for later 2D analysis that clarifies the interactions and hydrogen bonds that occur between the ligand and macromolecule.

D. BBB permeability and ADME analysis

A web tool called SwissADME is used to predict different aspects of pharmacokinetics and how drugs-like a compound will be. Regarding the blood-brain barrier (BBB), SwissADME offers approximations for a compound's capacity to cross it based on a number of factors, including P- glycoprotein substrate, Lipinski's rule of five, and the expectation of central nervous system activity. It makes predictions in order to verify a compound's true ability to cross the BBB, experiments are frequently needed. In the realm of medicinal chemistry, Lipinski's Rule comprises a set of criteria employed for assessing the drug-likeness of a molecule. As per these criteria, a molecule should possess a molecular weight below 500 Daltons, have fewer than five hydrogen bond donors, exhibit fewer than ten hydrogen bond acceptors, and demonstrate a computed LogP (partition coefficient) value lower than five to qualify as orally active. These criteria are used to predict whether a compound is likely to have favorable pharmacokinetic properties for oral administration [7].

III. Result and Discussion

A. BBB permeability and drug target analysis

Out of the 3,647 FDA-approved ligands, 37 ligands were selected with the variation of binding affinity from -10 to -12.4 kcal/mol. Out of them 19 of the compounds can pass the BBB and their binding affinity varies from -10 to -10.8 kcal/mol. Amitriptylinoxide, Florantyrone, and Ponatinib have the highest binding affinity (-10.8, 10.7, 10.5 kcal/mol respectively) forming 1, 1, and 3 H-bonds respectively and a significant number of van-der-Waal interactions and other bonds and interactions are shown in Table 1. Reference drug has a lower binding affinity (-9.6 kcal/mol) than the top affinity compounds.

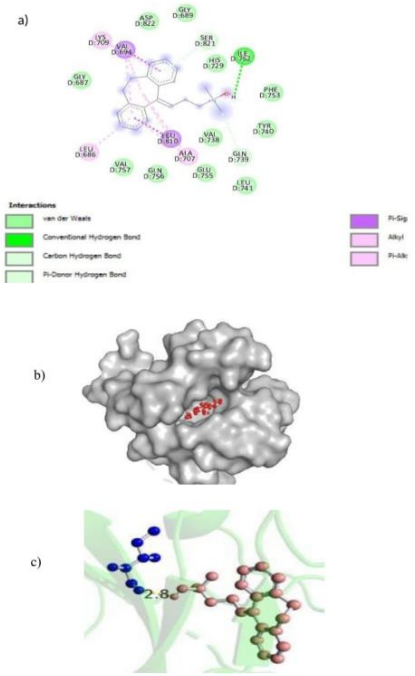

Fig.1. a) is a 2D structure c) is a 3D structure showing an H-bond with distance, and b) is a 3D structure representing a surface structure with a binding pocket of Amitriptylinoxin.

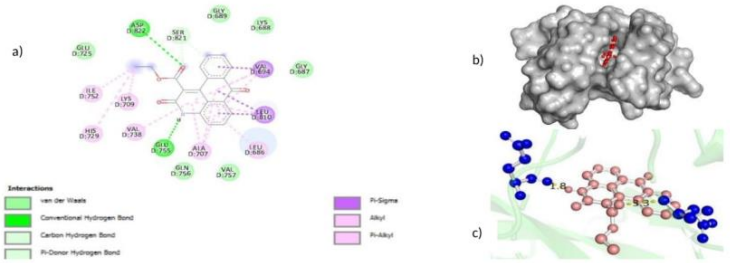

Fig.2. a) is a 2D structure c) is a 3D structure showing an H-bond with distance, and b) is a 3D structure representing a surface structure with a binding pocket of NQDI1

TABLE 1. BBB PERMEABLE LIGANDS OF TOP BINDING AFFINITY WITH ASK

Ligands	Binding energy (kcal/mol)	P-gp substrate	Type of Interactions			Chain
			H-bond with bond length in Å	*Wan-der-Vaal*	*Other non-polar interactions*	
Atovaquone	-10.4	negative	-	S-821 D-822 H-729 E-755 V-757 Q-756 G-759 G-760 S-761	V-694 K-709 I-752 V-738 A-707 L-810 L-686	D
Ponatinib	-10.5	Yes	D-822(2.3) N-808(2.4) G-759(3.0)	K-688 D-807 G-687 M-754 E-755 P-758 Y-814 G-760 Q-756 A-764 S-821 G-689	V-694 L-686 A-707 L-810 V-757	B
Elliptinium	-10.4	Yes	V-757(1.8) L-686(2.7)	G-759 P-758 G-760 S-761 G-687 K-688 D-807 G-689 K-709 D-822 M-754 S-821 V-738 A-707 Q-756 Y-814	L-686 L-810 V-694	A
Dosulepin	10.2	No	-	S-821 D-822 G-689 K-688 S-761 V-757 Q-756 E-755 V-738	K-709 M-754 D-807 G-687 L-686 L-810 A-707 V-694	C
Prasterone	-10.1	No	-	E-886 D-924 P-923 C-928 A-861 K-860 Q-798 R-767	Y-882 K-878 P-879	B
Mequitazine	-10.1	Yes	-	H-729 I-752 F-753 E-755 Q-756 V-757 G-687 K-688 G-689 D-822	A-707 L-686 L-810 S-821 V-738 V-694 K-709	D
Periciazine	-10	Yes	S-763(2.5) S-761(2.5) D-807(3.6)	R-767 A-764 G-760 K-688 G-689 D-822 S-821 V-738 E-755 Q-756 V-757	G-687 L-686 K-709 V-694 M-754 L-810 A--707	A
Florantyrone	-10.7	No	K-709(3.0)	V-757 Q-756 E-755 V-738 I-708 I-752 F-838 H-729 E-725 D-822 S-821 G-687	F-755 A-707 V-694 L-810 L-686 L-709	D
Fendosal	-10	Yes	C chain :R-926 (2.1)(2.3)	B chain : R-767 Y-882 E-886 C chain : G-859 Q-798 L-829 D-924 K-925 P-923 A-927 I-864	B chain: P-879 K-878 C chain: K-860 C-928 A-861	B,C
Promestriene	-10.1	No	-	B chain: Y-882 R-767 C chain: A-862 G-859 E-849 N-930	B chain : K-878 P-879 G-877 C chain : P-923 A-861 K-860 C-928 P-923 A-861 K-860 C-928	B,C
Nevirapine	-10	Yes	L-686 (2.8)	K-688 G-689 D-822 K-709 S-821 755 Q-756 V-757 G-760	G-687 V-694 L-810 V-738 A-707 M-754	A
Cyclobenzaprine	-10.3	No	-	G-687 K-688 G-689 D-822 K-709 H-729 F-753 V-738 E-755 Q-756 V-757	L-810 V-694 S-821 L-686 A-707 I-752	D
Benztropine	-10	No	-	D-822 G-689 K-688 N-808 V-757 Q-756 I-708 E-755 F-753 Y-740 L-741 H-729 V-738	S-821 Q-739 V-694 I-752 K-709 A-707 L-686 L-810	D
Amitriptylinoxide	-10.8	No	I-752 (2.8)	D-822 G-689 H-729 F-753 Y-740 V-738 L-741 E-755 Q-756 V-757 G-687	K-709 V-694 L-686 L-810 A-707 Q-739 S-821	D
Apomorphine	-10.2	Yes	L-686(2.0)	G-760 G-687 K-688 G-689 D-822 S-821 L-709 V-738 E-755 Q-756 V-757	L-686 A-707 L-810 V-694	D
Asenapine	-10.1	Yes	-	A chain: F—937 G-781 K-785 Q-778 C chain: S-701 N-702	A chain: L-938 F-782	A, C
Astemizole	-10	Yes	-	C chain: R-705 Y-814 A chain: M-754 K-688 G-687 D-807 S-761 G-760 V-757 K-769 T-813 Y-814 G-759 P-758 Q-756 S-821 V-738	A chain: L-810 V-694 A-707 E-755 L-686	A, C
Bagrosin	-10.4	Yes	-	I-752 K-709 E-755 L-686 G-687 D-807 K-688 N-808 G-689 H-729 D-822 F-823 E-725	L-810 V-738 A-707 V-694 S-821	D
NQDI (reference)	-9.6	No	D-822 (3.3) E-755 (1.8)	E-725 G-689 K-688 G-687 V-757 Q-756	I-752 V-738 K-709 H-729 A-707 L-686 L-810 V-694 S821	D

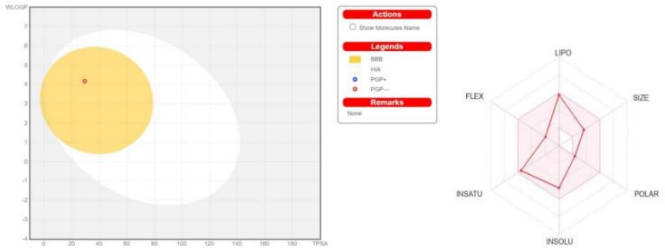

Fig. 3. Boiled egg and pharmacokinetics analysis using ADME of Amitriptylinoxide

B. ADME analysis

ADME evaluation of high-affinity substances. Both of them display noteworthy outcomes. An image is displayed that illustrates several physicochemical parameters based on which bioavailability of ligand is expected. High bioavailability is exhibited by compounds whose characteristics are entirely contained in the colored zones in Figure 3. The average lipophilicity of five different types of lipophilicity calculation methodologies consensus log po/w is 3.72, 2.98, and 4.30 for Florantyrone, Amitriptylinoxide, and Ponatinib respectively. The solubility in water is modest for all three compounds. All three compounds absorb well in the GI tract.

Ponatinib is PGP sue other two are PGP substrates-. Its skin permeation, or Log Kp of Florantyrone, Amitriptylinoxide, and Ponatinib is noteworthy at -5.57 cm/s, -4.85 cm/s, -6.63 cm/s respectively. The bioavailability score outcome is 0.85 for Florantyrone and 0.55 for both Amitriptylinoxide and Ponatinib. Lipinski's analysis reveals no violations for Florantyrone, Amitriptylinoxide, and 1 violation of >500g/mol molecular weight for ponatinib (532 g/mol) and shows favorable outcomes. Synthetic accessibility is 2.44, 3.47, and 3.97 for Florantyrone, Amitriptylinoxide, and Ponatinib respectively.

IV. Conclusion

With illnesses like Alzheimer's disease, Parkinson's disease, and Huntington's disease placing a heavy strain on patients, caregivers, and healthcare systems globally, neurodegenerative disorders represent a huge and expanding health concern. Effective treatments for many illnesses are still elusive despite decades of research. To combat neurodegenerative disorders, new therapeutic techniques must be discovered and developed. Drug development is drawn to ASK1 inhibition since it has been demonstrated to have neuroprotective benefits in preclinical models of neurodegenerative disorders. Researchers can expedite the drug development process by identifying promising drug candidates with high affinity and specificity for ASK1 by utilizing several bioinformatics methodologies.

The top 19 compounds with the highest binding affinities all exhibit binding at one or both of the significant inhibitory sites, gln-756 and val-757. Three compounds that exhibit maximal inhibition and better binding affinity than the reference (NQDI) medication with BBB permeability were produced through docking. Although Amitriptylinoxide is the most efficient and produces the best results, according to the ADME analysis and binding affinity with target protein ASK1, it is PGP substrate- that indicates that this medication will be absorbed by brain cells without being transported outside via the PGP transporter. The other parameter values show good solubility, physicochemical characteristics, and bioavailability for both aminoquinuride and amitriptylinoxide, and they are appropriate with substantial values.

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UNIVERSITY EDUCATION

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Master of Science, Biotechnology	DELHI TECHNOLOGICAL UNIVERSITY	8.4/10 (up to 3rd semester)	2022-2024
Bachelors Of Science, Zoology	UNIVERSITY OF DELHI	8.419/10	2018- 2021

SCHOOL EDUCATION

Class	Affiliating Board	Percentage/GPA	Year
Intermediate	Board of School Education Haryana	85.6	2018
High School	Board of School Education Haryana	80.08	2016

Experience

1.) Structural Biology Laboratory, JAMIA MILLIA ISLAMIA
New Delhi
Research Intern (June 2023 – August 2023)
Under the guidance of Prof. MD Imtaiyaz Hassan

- **Investigated the role of PIM-1 kinase in prostate and breast cancer.**
- Conducted DNA isolation procedures utilizing various extraction methods.
- Executed protein isolation techniques from biological samples.
- Conducted quantitative protein analysis using spectrophotometry or other appropriate methods.
- Conducted qualitative protein analysis using techniques such as SDS-PAGE or mass spectrometry.

2.) CIIDRET, UNIVERSITY OF DELHI
New Delhi
Research Trainee (February 2023)

- Conducted immunobiological experiments under senior researcher supervision.
- Executed ELISA assays for protein quantification.
- Prepared and maintained experimental cell cultures.
- Conducted Western blotting to analyze protein expression.
- Contributed to data analysis and interpretation.

3.) **Decode Life Bioinformatics Training Institute**

May to June 2023

-The training was organized in an online mode, during which several basic techniques of computational biology used in drug discovery were demonstrated, and we were enlightened by talks from eminent scientists.

Skills

Technical: Bioinformatics tools such as Protein structure analysis, homology searches, Molecular docking

Laboratory

- Sample culturing techniques
- Staining methods for various samples
- Quantitative and qualitative analysis of proteins and carbohydrates
- Gel electrophoresis proficiency
- DNA isolation techniques
- Proficient in ELISA assays using both 96 wells and 384 wells plates
- Operation of automated ELISA washer
- SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) expertise
- Western blotting proficiency
- Fluorescence microscopy skills, specifically using the EVOS M7000 system

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Author name: Pallavi and Prof. Pravir Kumar

Name of conference: 2nd IEEE International Conference on Knowledge Engineering and Communication Systems

Status of Paper: Accepted for Presentation and publication