Article

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Identification of High-Affinity Inhibitors of TANK-Binding Kinase 1 (TBK1): A Promising Frontier for Controlling Inflammatory Signaling in Cancer

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Background: TANK-binding kinase 1 (TBK1) is an important serine/threonine kinase involved in inflammatory signaling pathways, influencing cellular processes such as proliferation, programmed cell death, autophagy, and immune response regulation. Dysregulation of TBK1 has been linked to cancer progression and neurodegenerative disorders, making it an attractive target for therapeutic development. This study aimed to identify potential TBK1 inhibitors using a structure-based virtual screening approach.

Methods: We conducted a comprehensive screening of the ZINC database to identify compounds with high binding affinity for TBK1, employing molecular docking as the primary selection criterion. The top candidates were then subjected to extensive 200 ns molecular dynamics (MD) simulations to assess the conformational dynamics of TBK1 and the stability of the protein-ligand complexes, with a focus on ZINC02095133 and ZINC02130647.

Results: The findings revealed that TBK1 forms stable complexes with ZINC02095133 and ZINC02130647, demonstrating consistent interactions throughout the MD simulations. This suggests that these compounds hold promise as potential lead molecules for future therapies targeting TBK1.

Conclusions: This study identifies ZINC02095133 and ZINC02130647 as promising TBK1 inhibitors with therapeutic potential. However, further experimental validation and optimization are required to develop novel inhibitors for diseased conditions associated with TBK1 signaling. These findings pave the way for future investigations into the clinical utility of these compounds in combating TBK1-related pathologies.

Keywords: TANK-binding kinase 1; drug discovery; natural products; virtual screening; molecular dynamics simulations; inflammatory diseases

Introduction

TANK-binding kinase 1 (TBK1), an extensively expressed serine/threonine kinase plays a vital role in numerous biological pathways at their core [1,2]. TBK1 governs several crucial cellular processes, encompassing cell cycle regulation, inflammation, apoptosis, and autophagy [3,4]. TBK1 functions as a kinase that forms a connection with the IκB kinase (IKK) complex, thereby enabling the phosphorylation and activation of interferon regulatory factors (IRFs) and nuclear factor kappa B (NF-κB) [5]. TBK1 possesses the capability to initiate the activation of NF-κB and interferon (IFN) signaling pathways, thus assuming a crucial role in the host cell's defense mechanisms [6]. TBK1 is critical in driving inflammation and innate immunity by actively participating in essential functions associated with these processes [7]. By phosphorylating and activating downstream targets such as interferon (IFN) regulatory factor 3 (IRF3) and REL proto-oncogene (c-Rel), TBK1 facilitates the activation of NF-κB signaling in cancer. The regulation of these signaling pathways plays a critical role in cell proliferation and is implicated in various forms of cancer [8].

The overexpression and mutations of TBK1 have been associated with the progression of numerous human cancers [9–11]. TBK1's involvement in cancer-related pathways and its activation has been linked to the development and advancement of various types of cancer [12–14]. TBK1 has been extensively documented as an oncogene in many cancers. Its aberrant activation or overexpression is frequently observed in cancer cells and promotes tumor growth, survival, invasion, and metastasis. The oncogenic role of TBK1 underscores its significance as a potential tar-

get for cancer therapy and research [13,15,16]. TBK1 has been linked with RAS-mediated non-small cell lung cancer (NSCLC) [17]. Activated TBK1 triggers the phosphorylation of AKT serine/threonine kinase 1 (Akt) and leads to the activation of NF-κB, impacting cell survival and anti-apoptotic pathways [18]. Studies have shown that TBK1 exerts a regulatory influence on the activity of mechanistic target of rapamycin complex 1 (mTORC1) by phosphorylating regulatory-associated protein of mTOR (Raptor) at Ser877 [19]. The *TBK1* gene, responsible for encoding, is positioned at 12q14.1 on the human chromosome.

The *TBK1* gene consists of 21 exons and encodes an 84 kDa protein. The TBK1 protein comprises 729 amino acids and contains four distinct domains. These domains include a kinase domain (KD) located at the N-terminal region, a ubiquitin-like domain (ULD), a coiled-coil domain (CCD1), and a second coiled-coil domain (CCD2) [20]. These domains contribute to the functional properties and interactions of TBK1 in various cellular processes. The crystal structure of TBK1 in a complex with an inhibitor reveals the binding mode of the inhibitor to the adenosine triphosphate (ATP)-binding site of TBK1 in a type I binding manner. In this binding mode, the pyridine nitrogen of the inhibitor forms a hydrogen bond with Glu87 of TBK1, while the amine group forms a hydrogen bond with Cys89 [21]. These interactions between the inhibitor and TBK1 residues contribute to stabilizing the inhibitor within the ATP-binding site of TBK1. They may serve as a platform for the development of novel TBK1 inhibitors.

Indeed, TBK1 has emerged as a promising target for the treatment of various cancer types and neurodegenerative complexities. Numerous inhibitors specifically designed to target TBK1 have been identified, highlighting the potential therapeutic roles of TBK1 inhibition in diseases associated with aberrant TBK1 signaling. The exploration of TBK1 as a therapeutic candidate provides opportunities for drug discovery and the development of targeted therapies aimed at modulating its activity to combat cancer and other TBK1-related disorders.

TBK1 inhibitors are considered as promising therapies for cancer and neurodegenerative diseases [22]. Inhibitors targeting TBK1 have demonstrated effective efficiency in *in vivo* animal models and *in vitro* studies [21]. The recent advancements in understanding TBK1 and the availability of previously identified inhibitors have facilitated novel investigations focused on targeting TBK1. Given its potential as a drug target, extensive research is necessary to identify new TBK1 inhibitors that exhibit enhanced efficacy and specificity. These studies aim to discover novel small molecules that can effectively modulate TBK1 activity, improving treatment options for various diseases.

The broad spectrum of therapeutic functions exhibited by natural compounds makes them highly desirable molecules in drug discovery [23,24]. The wide-ranging therapeutic applications of these compounds stem from their diverse structural and chemical properties [25–29]. A thorough investigation into the interactions between key therapeutic classes, potential drugs, and plasma or target tissue proteins has long been recognized as crucial in pharmacological identification. Structure-based drug design has emerged as an integral and indispensable component of the drug discovery process. It has proven instrumental in the discovery of highly specific and efficient bioactive molecules with enhanced pharmacological properties [30–35]. Identifying small molecule inhibitors targeting TBK1 has been limited, with only a few being discovered in recent years. However, the ongoing challenge lies in the development of novel TBK1 inhibitors. The ATP-binding region of kinases has been the primary focus of research aiming to discover highly selective kinase inhibitors thus far.

Here, we employed a multitier virtual screening to discover small bioactive molecules with potential as inhibitors of TBK1 for therapeutic purposes. We focused on natural products from the ZINC database and subjected them to a structure-based virtual screening process. A comprehensive set of 32,902 natural compounds was subjected to molecular docking, and top hits exhibiting binding solid affinity and specific interaction were selected. These selected hits underwent further evaluation using the Pan-assay interference compounds (PAINS) filter, absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties assessment, prediction of activity spectra for substances (PASS) analysis, and analysis of 2D interactions with the receptor-ligand complexes. As a result, two natural compounds were identified as promising inhibitors for TBK1-mediated cancer, displaying superior affinity and specific interaction towards the binding pocket of TBK1. Additionally, both compounds showed drug-likeliness based on the predicted ADMET attributes. These findings suggest that these natural compounds are potential therapeutic candidates for targeting TBK1 in treating TBK1-mediated cancers and neurodegenerative diseases.

Material and Methods

Molecular Docking Screening

Employing a molecular docking screening method makes it highly feasible to identify compounds that exhibit a high affinity for TBK1. Several bioinformatics tools were used to analyze docking and interactions, including InstaDock (New Delhi, Delhi, India) and Discovery Studio Visualizer Biovia, D.S. (2019), San Diego., CA, USA) [36]. The Protein Data Bank (PDB) was used to extract the TBK1 crystal structure (ID: 4IWP) [20], and it was then amended to fetch the kinase domain, add hydrogens to polar groups, and the relevant atom types to get it ready for virtual screening in InstaDock v1.1. The natural compounds library was constructed from the ZINC database (http://zinc.docking.org/) and then processed in InstaDock software [36]. The search box was set to blind search space

by defining a grid of center X = 5.57, Y = –39.31, Z = –11.06 with size parameter values X = 48, Y = 54, and Z = 52. The most promising docking hits were selected using the docking score and ligand efficiency criteria, and potential docked conformers were generated. The close interactions between the compounds and TBK1 were visualized in PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC. New York City, NY, USA), highlighting the polar contacts within a distance of 3.5 Å [37]. The Discovery Studio Visualizer explored the interactions between the selected hits and TBK1. Compounds demonstrating interactions with essential residues within the TBK1 binding pocket were chosen for subsequent investigation. These residues are crucial in TBK1 function and activity [20,38,39].

ADMET Prediction

When selecting promising leads for therapeutic development, the efficacy and safety of chemical compounds play vital roles as determining criteria. Their ADMET features were thoroughly investigated to evaluate the effectiveness of the chosen compounds. The pkCSM web tool (https://biosig.lab.uq.edu.au/pkcsm/) was employed to calculate and assess the ADMET characteristics of the selected compounds. This analysis provides valuable insights into the compounds' pharmacokinetic and toxicity profiles, aiding in identifying compounds with favorable ADMET properties [40]. Both online servers in this study accept input as Simplified Molecular Input Line Entry System (SMILES) strings for the compounds. The screened hits were subjected to the PAINS filter, which helps identify compounds prone to causing false-positive results across various assays. This filter ensures that compounds with undesirable promiscuity are excluded from further analysis, maintaining the focus on more specific and reliable hit compounds [41].

PASS Analysis

The PASS webserver (https://www.way2drug.com/passonline/) was utilized to estimate the pharmacological and biological properties of the compounds. This online tool predicts various activities and effects the compounds may exhibit based on their chemical structure [42]. The PASS involves the calculation of molecular descriptors and database comparison for the target compound. The descriptors are numerical representations of a compound's chemical structure, including information about atom types, bond types, functional groups, and other structural features. The calculated molecular descriptors for the target compound are then compared to a reference database of compounds with known biological activities. The output of PASS is an "activity spectrum" that provides a profile of potential biological activities associated with the target compound. Each predicted activity in the spectrum is associated with a probability score, Pa and Pi, which indicates the confidence level of the prediction. Higher Pa scores suggest a higher likelihood that the compound will exhibit the predicted activity. Here, leveraging the PASS website gained valuable insights into the potential pharmacological and biological properties of the compounds under investigation, aiding in assessing their therapeutic potential.

Table 1. The top 30 hits and their binding affinity with TANK-binding kinase 1 (TBK1).

S. No.	ZINC ID	Affinity (kcal/mol)
1.	ZINC02093555	−11.6
2.	ZINC02138708	−11.6
3.	ZINC02130599	−11.5
4.	ZINC02131906	−11.5
5.	ZINC02133362	−11.5
6.	ZINC02128472	−11.5
7.	ZINC02130647	−11.4
8.	ZINC02135959	−11.4
9.	ZINC02130521	−11.3
10.	ZINC02132169	−11.3
11.	ZINC02113878	−11.3
12.	ZINC02135236	−11.3
13.	ZINC02135418	−11.3
14.	ZINC01767075	−11.3
15.	ZINC02112816	−11.3
16.	ZINC02095133	−11.2
17.	ZINC02127651	−11.2
18.	ZINC02117173	−11.2
19.	ZINC01295691	−11.2
20.	ZINC02133022	−11.2
21.	ZINC02128142	−11.1
22.	ZINC02135470	−11.1
23.	ZINC02136713	−11.1
24.	ZINC02115467	−11.1
25.	ZINC02116784	−11.1
26.	ZINC02122421	−11.1
27.	ZINC02130066	−11.1
28.	ZINC02131030	−11.0
29.	ZINC02117169	−11.0
30.	ZINC02113447	−11.0

MD Simulations

MD simulations were conducted at 300 Kelvin using the Schrödinger simulation package on an HP Z840 computer (Palo Alto, CA, USA). The simulation protocol involved the apo and ligand-bound states of TBK1 with compounds ZINC02095133 and ZINC02130647. All-atom simulations employed the GROMOS 54A7 forcefield [43]. The topologies and force field parameters for ZINC02095133 and ZINC02130647 were created using the PRODRG website (https://www.ccp4.ac.uk/html/cprodrg.html). Both complexes and free TBK1 were placed in a cubic box with a radius of 10Å from the molecule to the box edges. Solvation in aqueous conditions was achieved by adding water molecules using the Simple Point Charge (SPC216) model. Counterions were introduced into the sys-

Table 2. ADMET parameters of the elucidated hit molecules.

S. No.	Compound ID	Absorption	Distribution	Metabolism	Excretion	Toxicity
		GI Absorption	*BBB permeation*	*CYP2D6 Inhibitor*	*OCT2 substrate*	*AMES/Hepatotoxicity*
1.	ZINC02133362	High	No	No	No	No/Yes
2.	ZINC02113878	High	No	No	No	Yes/Yes
3.	ZINC02117173	High	No	No	No	Yes/Yes
4.	ZINC02130647	High	No	Yes	No	No/No
5.	ZINC02095133	High	No	No	No	No/No
6.	ZINC02132169	High	No	Yes	No	No/Yes
7.	ZINC01295691	High	No	No	No	No/Yes
8.	ZINC01767075	High	No	No	No	No/Yes
9.	ZINC02136713	High	No	Yes	No	No/Yes
10.	ZINC02117169	High	No	No	No	No/Yes

GI, gastrointestinal; *BBB*, blood-brain barrier; *CYP2D6*, Cytochrome P450 2D6; *OCT2*, organic cation transporter 2; *AEMS*, anion exchange membranes.

tems to ensure uniform charge distribution. Before advancing to MD's production phase, we undertook preliminary measures to guarantee system relaxation and establish a stable initial state. These steps encompass energy minimization and equilibration in the constant number of particles, volume, and temperature (NVT) and constant number of particles, pressure, and temperature (NPT) ensembles. Following a 1000-ps equilibration period with constant volume conditions and the imposition of periodic boundary constraints at a fixed pressure of 1 bar, the temperature of all systems gradually ascended from 0 to 300 Kelvin. We recorded trajectories at consistent 2-femtosecond intervals to support subsequent analysis. The simulations were carried out for 200 ns, and the output was analyzed using the built-in tools provided by Schrödinger.

Results

Molecular Docking

TBK1 is a transmembrane tyrosine kinase known for oncogenic properties when overexpressed. TBK1 was regarded as a potential target, and its structural coordinates were taken from the RCSB PDB (https://www.rcsb.org/). A set of 32,902 natural products was retrieved from the ZINC database of natural products based on Lipinski's rule of five [44]. Molecular docking was a critical filter to improve the effectiveness of identifying powerful TBK1 inhibitors. To find potential inhibitors of TBK1, virtual screening was done on InstaDock based on the molecular docking approach. The top 30 compounds were selected for further study based on their significant binding affinity for the TBK1. The study reveals that the 30 compounds chosen have a significant affinity with TBK1, estimated from –11.0 to –11.6 kcal/mol (Table 1).

Table 3. The elucidated molecules and their biological activities.

Compound ID	Pa	Pi	Biological Activity
ZINC02130647	0.685	0.014	Neurotransmitter uptake inhibitor
	0.402	0.140	TP53 expression enhancer
	0.392	0.070	Kinase inhibitor
	0.365	0.064	MMP9 expression inhibitor
	0.351	0.029	Chemopreventive
ZINC02095133	0.960	0.001	Monoamine oxidase inhibitor
	0.851	0.003	Histidine kinase inhibitor
	0.580	0.036	Anti-inflammatory
	0.482	0.016	Chemo-preventive
	0.483	0.077	Antineoplastic

TP53, tumor protein p53; MMP9, Matrix metalloproteinase (MMP)-9.

ADMET Evaluation

The selection and development of drug molecules are significantly influenced by the crucial role played by ADMET characteristics [45]. Having a favorable set of ADMET properties significantly increases the likelihood of success for compounds in clinical trials [46]. The physicochemical and ADMET parameters of each molecule were calculated using the pkCSM web server [40]. Several ADMET characteristics were determined for the top 30 compounds chosen from the molecular docking filter. 10 out of 30 compounds that displayed a collection of favorable physicochemical characteristics without a PAINS pattern were selected in this filter [41]. The predicted ADMET parameters of the identified compounds are presented in Table 2. The results indicate that the elucidated hit molecules exhibit similar ADMET profiles and show no signs of toxicity.

PASS Analysis

The PASS server incorporates an extensive training set of diverse bioactive compounds and their structure-activity

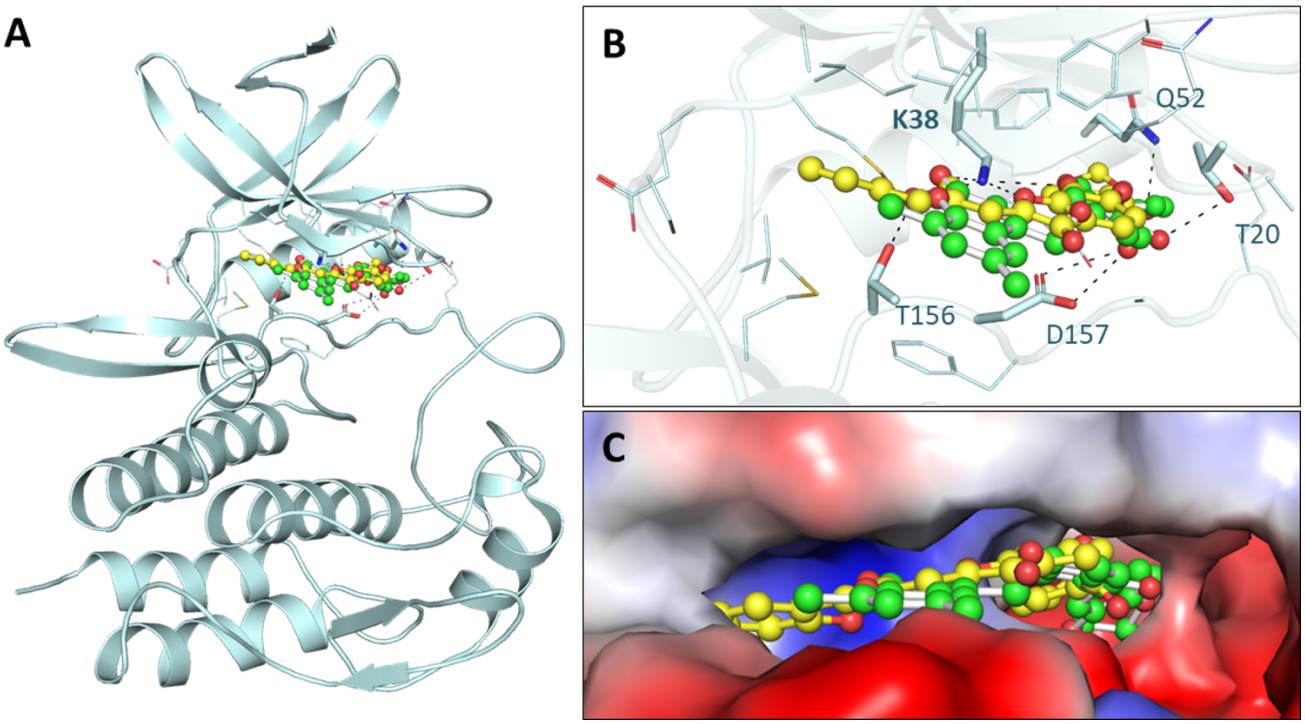

Fig. 1. TBK1 interaction with ZINC02130647 and ZINC02095133. (A) Ribbon representation of TBK1 with ZINC02130647 and ZINC02095133. (B) The close interaction of TBK1 with ZINC02130647 (green) and ZINC02095133 (yellow). (C) ZINC02130647 and ZINC02095133 occupied the binding pocket of TBK1, as observed in the surface potential view.

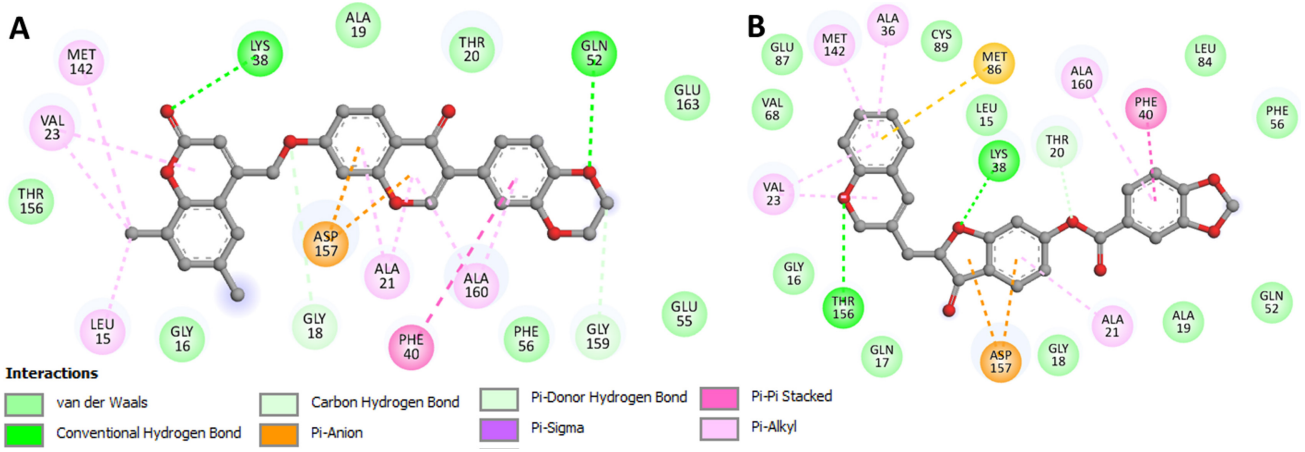

Fig. 2. 2D depiction of TBK1 residues interacting with (A) ZINC02130647 and (B) ZINC02095133.

relationships, extensively utilized in clinical and preclinical research [42]. The PASS server bases its bioactivity prediction for a chemical on the involved training set. From the ADMET screening, we have projected the biological activities of the selected molecules. Only two molecules, in this case, ZINC02130647 and ZINC02095133, passed the PASS screening of predicted desirable biological activity. The findings indicate that both substances have antineoplastic and kinase-inhibitory potential. They also showed high predictions for anti-inflammatory, monoamine oxidase inhibitory potential, and tumor protein p53 (TP53) expression enhancer potential, with the Pa values ranging from 0.351 to 0.960 when Pa > Pi (Table 3). Higher Pa scores indicate a more substantial likelihood that the compound will demonstrate the predicted biological activity.

Interaction Analysis

The shortlisted molecules' binding conformations were examined using interaction analysis for their specific interactions with TBK1. During the investigation, ZINC02130647 and ZINC02095133 interacted with mutual residues, including the ATP binding sites Lys38. The binding prototype of ZINC02130647 and ZINC02095133 is illustrated in Fig. 1. It was discovered that ZINC02130647

and ZINC02095133 interact with the key residues of the TBK1 binding pocket similarly (Fig. 1B). They overlap one another and interact closely with the TBK1 active site in a variety of ways. Both molecules fit into the deep cavity with favorable complementarity and obstruct TBK1's binding site (Fig. 1C). ZINC02130647 and ZINC02095133 bind to TBK1, which indicates that their stability may prevent TBK1 from being accessible to ATP and so inhibit its function.

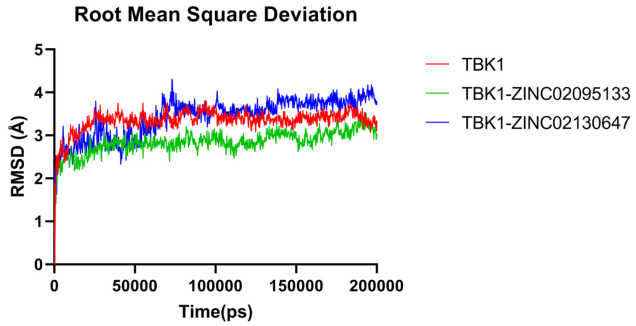

Fig. 3. Root mean square deviation (RMSD) of three compounds simulated for 200 ns.

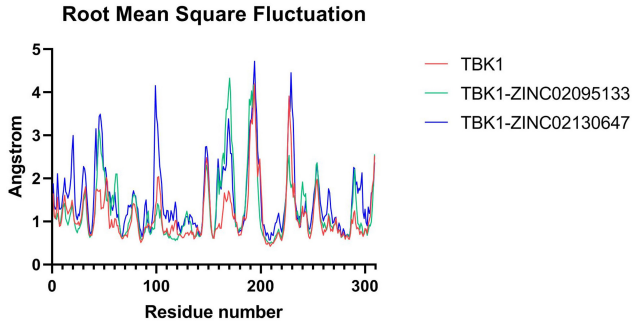

Fig. 4. Root mean square fluctuation (RMSF) of TBK1 after simulation for 200 ns.

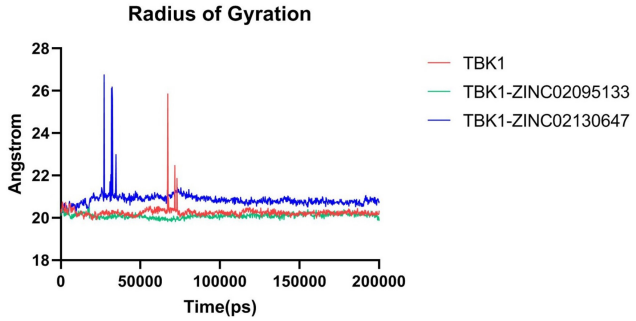

Fig. 5. Radius of gyration of TBK1 after simulation for 200 ns.

By employing Discovery Studio Visualizer, we examined the binding patterns of the identified hits. Analyzing the 'out-files' of the two selected natural compounds resulted in 18 possible docked conformers. Discovery Studio Visualizer was utilized to analyze all the interactions between the elucidated hit molecules and TBK1. Notably, TBK1's kinase domain residues exhibited significant interactions with the identified compounds. Both compounds were observed to interact with the ATP binding sites or closely associate with them. Furthermore, a hydrogen bond was detected between both compounds and Lys38, an amino acid within the ATP binding site of TBK1.

We can better understand the precise types of non-covalent interactions and their types using thorough interaction analysis. Further research was conducted on the selected interaction mechanisms of ZINC02130647 and ZINC02095133 for their detailed interactions with TBK1. For the putative interactions between TBK1 and ZINC02130647, and ZINC02095133, 2D graphs were created (Fig. 2). The 2D plots clearly illustrate the interactions between ZINC02130647 and ZINC02095133 with the ATP binding site, particularly with the critical residue "Lys38" (Fig. 2A,B). Both compounds exhibit multiple interactions with important residues within the ATP-binding pocket of TBK1, including the crucial site "Lys38". Based on the comprehensive analysis of their interactions, ZINC02130647 and ZINC02095133 demonstrate similar binding patterns to TBK1 (Fig. 2).

MD Simulation Analysis

The MD simulation results for 200 ns of TBK1 and its docked complexes were analyzed for various parameters, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), the radius of gyration (*R*g), and solvent-accessible surface area (SASA). The protein structure RMSD was observed to be averaged at 3.5 Å, and it was quite stable with minor fluctuations (Fig. 3). The protein-ligand complex of TBK1-ZINC02095133 was noted that till 200 ns simulation time, the docked complex was stable, but later, after around 110 ns, the complex was observed to have minor fluctuations with an RMSD of nearly 3.5 Å (**Supplementary Fig. 1**). The TBK1-ZINC02130647 complex was detected that till 75 ns simulation time, the docked complex was stable, but later, at around 80 ns, it was observed to have minor fluctuations with an RMSD of nearly 4 Å (**Supplementary Fig. 2**). Protein-Ligand RMSD shows that both their trajectories made contacts at 0–75 ns on and off, 125 ns and later continuously from 160–200 ns (**Supplementary Figs. 1,2**). RMSD analysis suggests that TBK1 was stable with docked ZINC02095133 and ZINC02130647 with minimal fluctuations.

To analyze the residual fluctuations in TBK1 in its free state and complex with the identified compounds, the mean fluctuation of each residue was computed using RMSF. TBK1 exhibits random residual oscillations from the N- to

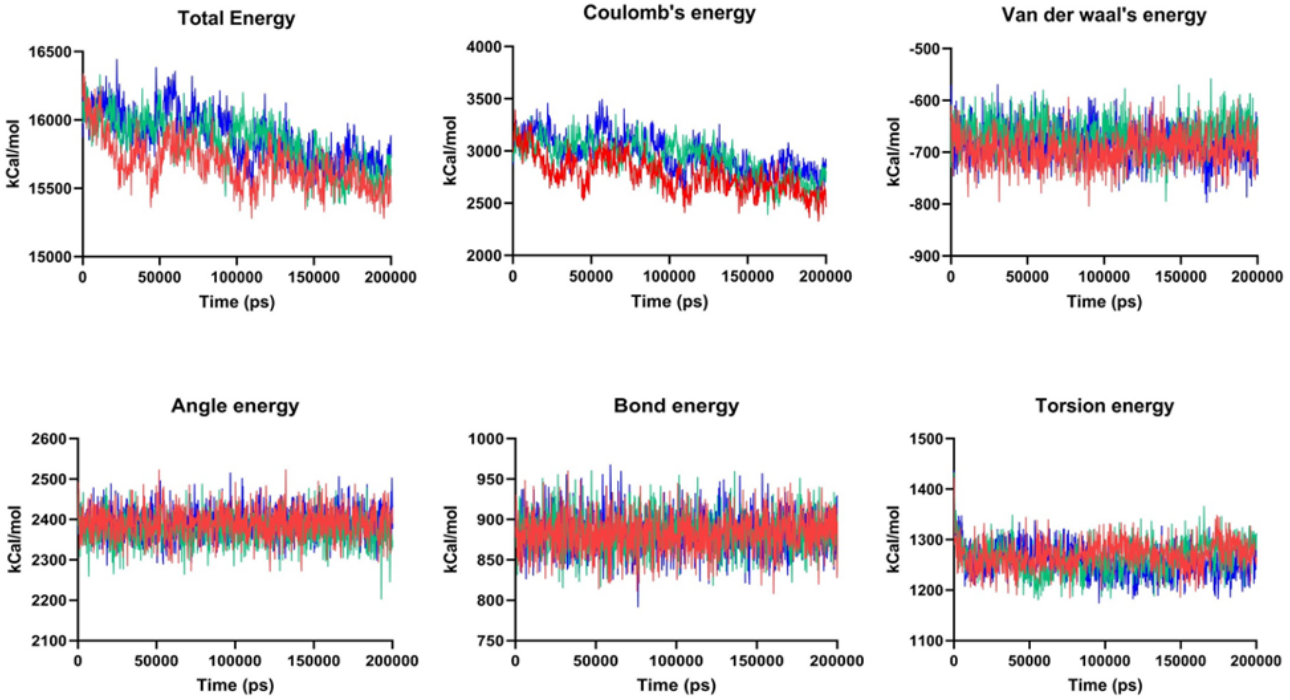

Fig. 6. Energy plots of three compounds after simulation for 200 ns. Red, green, and blue represent TBK1, TBK1-ZINC02095133, and TBK1-ZINC02130647, respectively.

the C-terminal in various locations. RMSF plot revealed that amino acids 150, 180–200, and 200–250 showed significant fluctuations (Fig. 4). RMSF plot revealed that amino acid residues show higher fluctuations (up to 4.5 Å) when bound to ZINC02095133 and ZINC02130647. According to the study of the RMSF plot, there aren't many changes in the residual fluctuations in the area where the compounds are binding. However, fewer enhanced fluctuations are also observed in TBK1 when compounds bind, perhaps due to the protein's binding pocket adjusting its conformation. The SASA of TBK1 and its docked complexes is also associated with the RMSF analysis, as depicted in **Supplementary Figs. 3,4**.

The radius of gyration (*R*g) has been used to comprehend a protein's compactness and folding behavior. It is closely related to the tertiary structure and global conformation [47]. We evaluated the stability of TBK1 and its docked complexes by computing their *R*g. The *R*g plot showed that the protein structure had high fluctuations from 70 ns to 75 ns; after that, it regained stability (Fig. 5). *R*g plot showed that the docked system had lower fluctuations from 50 ns to 75 ns; after that, it regained its stability. *R*g plot showed that the docked structure had higher fluctuations from 25 ns–40 ns; after that, it regained stability (Fig. 5).

Various kinetic parameters were calculated and analyzed to determine the equilibration and stability of TBK1 and its docked complexes. The total energy of the protein TBK1 was 16,400 kcal/mol, with a gradual decrease in the fluctuations at 50 ns. At the same time, coulomb's energy showed a similar pattern of fluctuations as that of total energy, with a gradual decrease in the fluctuations at 50 ns. However, the energy was 3400 kcal/mol. The van der Waal's energy was –600 kcal/mol and showed minor fluctuations over 200 ns within the range of –600 to –800 kcal/mol. At the same time, the torsion energy was 1400 kcal/mol with fluctuations observed from 0–20 ns, after that, the torsion energy was relatively stable. Angle energy was observed to be 2500 kcal/mol, showing fluctuations in the range of 2300–2500 kcal/mol throughout the simulations. The bond energy was 975 kcal/mol, with a fluctuation observed at 75 ns; after that, the fluctuations were minor throughout the trajectories (Fig. 6).

The protein-ligand interaction plot suggested that several residues of TBK1 were found to be interactive with the elucidated compounds, including Leu15, Gln17, Thr20, Ala21, Val23, Ala36, Lys38, Asp135, Ile141 and Val171. Protein-Ligand RMSD shows that both trajectories continuously made contact at 30–70 ns (Fig. 7).

Discussion

Identifying high-affinity inhibitors for TBK1 is a promising endeavour with significant implications for controlling inflammatory signaling in cancer and other related conditions [48,49]. Here, we employed a structure-based virtual screening approach to identify potential TBK1 inhibitors from a vast database of natural products. The study begins with screening over 32,000 natural products for their binding affinity to TBK1. The molecular docking analysis identified the top 30 compounds with the most significant

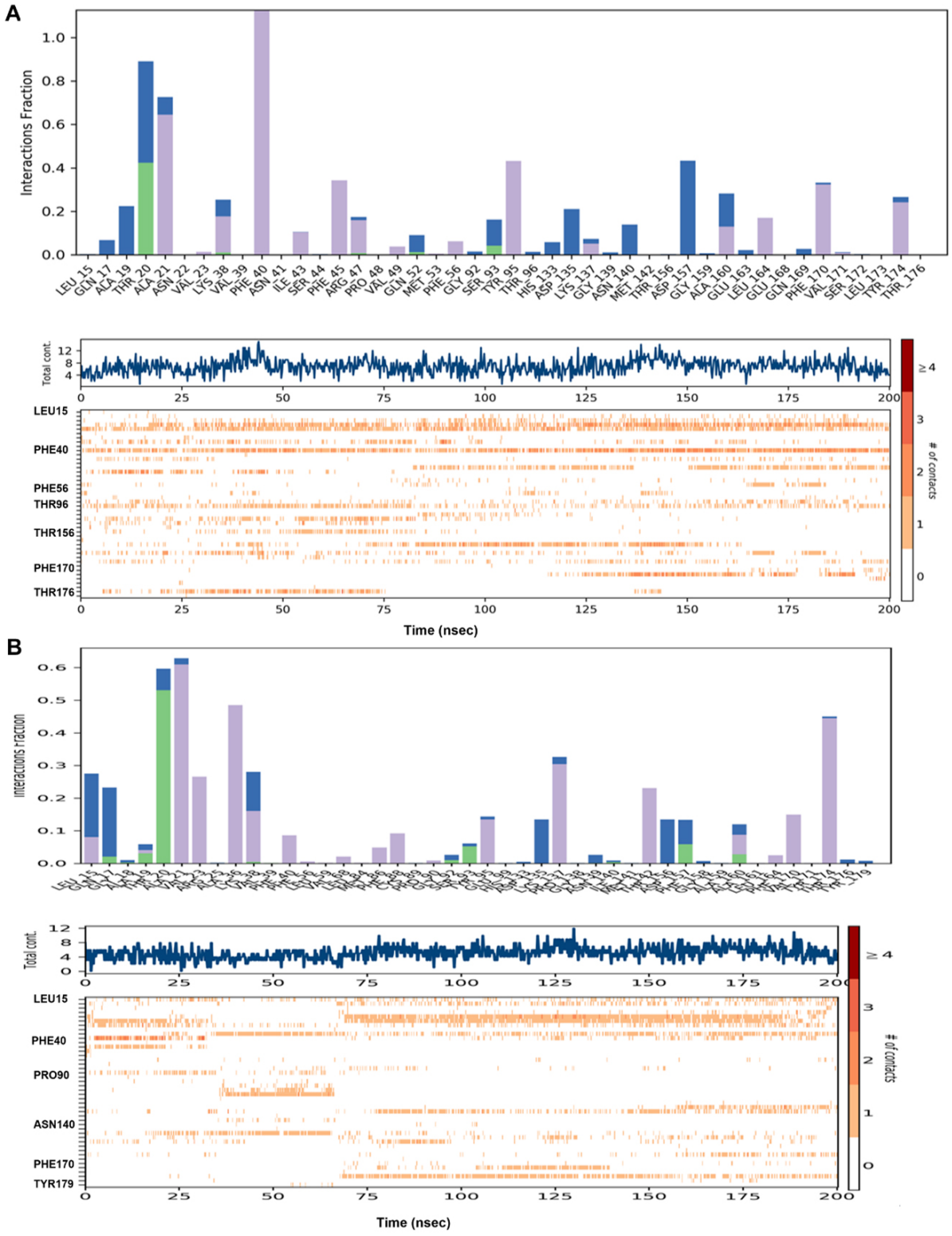

Fig. 7. Protein-ligand interaction plot of (A) TBK1-ZINC02095133 and (B) TBK1-ZINC02130647. The upper panels show the interaction fraction of each interacting residue. At the same time, the middle and lower panels show the time evolution of interacting residues.

binding affinity, ranging from –11.0 to –11.6 kcal/mol. This step narrows down the candidate compounds that have the potential to bind TBK1 effectively. The selection of drug candidates goes beyond binding affinity and involves assessing their ADMET profiles. The selected compounds exhibited favorable ADMET characteristics without any

adverse signs of toxicity. This is a promising indication of their potential as drug candidates, as compounds with favorable ADMET properties are more likely to succeed in clinical settings.

The therapeutic targeting of TBK1 in cancer and neurodegenerative diseases represents a significant breakthrough, particularly in the context of drug discovery [50]. Identifying potent and selective TBK1 inhibitors adds a new dimension to the arsenal of therapeutic molecules [21]. Furthermore, TBK1 is well-established for coordinating innate immune responses against viruses and other pathogens. In a recent study, a surprising revelation that TBK1 is implicated as a candidate immune-evasion gene in the context of cancer adds complexity to our understanding of its role in immunology. The multifaceted role of TBK1 in cancer, neurodegenerative diseases, innate immune responses and its significant involvement in immune evasion significantly influences drug discovery and therapeutic targeting [51]. Our findings advocate further evaluating and developing potent and selective TBK1 inhibitors to establish directed therapeutic strategies and provide a comprehensive framework for assessing therapeutic potential [52]. As research in this field progresses, the therapeutic landscape may witness the integration of TBK1-targeted strategies to address cancer therapy [53].

We used the PASS server to evaluate the selected compounds' biological activity further. This analysis revealed that two compounds, ZINC02130647 and ZINC02095133, passed the screening with predicted desirable biological activities. These compounds showed potential as antineoplastic agents, kinase inhibitors, anti-inflammatory agents, and more. An in-depth interaction analysis of the shortlisted compounds with TBK1 was conducted to understand the nature of their binding and potential inhibitory mechanisms. Both ZINC02130647 and ZINC02095133 were found to interact with critical residues, including the ATP binding site Lys38, which is essential for TBK1's function. This interaction pattern suggests that these compounds may prevent TBK1 from accessing ATP, modulating its kinase activity. MD simulations were employed to assess the stability of TBK1 in complexes with the identified compounds. The RMSD, RMSF, *R*g, and SASA analyses provided valuable insights into the structural dynamics and stability of the protein-ligand complexes. The kinetic energy parameters also revealed that the protein-ligand complexes have maintained stability during the simulations.

In summary, the study has identified two potential TBK1 inhibitors, ZINC02130647 and ZINC02095133, which have demonstrated high binding affinity, favorable ADMET properties, and significant interactions with key residues of TBK1. The results of this study lay the foundation for developing novel therapeutics targeting TBK1 in the context of cancer, neurodegenerative diseases, and other TBK1-associated inflammatory conditions [3,54,55]. While these findings are promising, further experimental validation and optimization are necessary before these compounds can be considered in clinical settings. Additionally, further studies should focus on *in vitro* and *in vivo* experiments to confirm the efficacy and safety of these potential inhibitors.

Conclusions

TBK1 is a crucial drug target because it regulates cancer progression, proliferation, and migration positively. Cancer cells' metabolic switching complexity will be better understood when new efficient and targeted therapy methods are developed. State-of-the-art methods from bioinformatics are instrumental in designing therapeutic molecules using natural leads as potent TBK1 inhibitors. Following systematic virtual screening, we discovered two bioactive molecules, ZINC02095133 and ZINC02130647, evaluated as potent TBK1-binding partners and potential inhibitors. The interaction between the identified hits and TBK1 was determined using molecular docking, which showed that the binding mechanism of both molecules is essential to the critical residues in TBK1. According to the results of the docking analysis, these substances bind to TBK1 using a similar set of amino acid residues, which was supported by all-atom MD simulations. Our findings strongly suggest that the elucidated molecules can function as promising TBK1 inhibitors and can be used as a starting point to develop potent and specific inhibitors to prevent TBK1-mediated cancer growth.

Availability of Data and Materials

All data generated or analyzed during this study are included in this manuscript.

Author Contributions

DSJ and MIH conceptualized the study; DKY, TM, MFA and AH performed the research; DSJ, DKY and TM collected and analyzed the data; DKY, TM, MIH and AH performed writing and draft preparation; MIH, AH, MFA did project supervision. DSJ, AH, MFA and MIH have been involved in drafting the manuscript or revising it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202436180.12.

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