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A Virtual Insight into Bioactive Compounds of Ginger as Potential Inhibitors of NEK7 for Cancer Management

Akram Ahmed Aloqbi

Abstract

Background: NIMA-related kinase 7 (NEK7) is a serine/threonine kinase that is required for cell cycle progression, particularly mitotic spindle formation and cytokinesis, and has been linked to various cancers. Furthermore, NEK7 modulates the NLRP3 inflammasome, which activates Caspase-1 and causes cell pyroptosis. A growing body of studies indicates that ginger has the ability to prevent and treat a variety of health issues.

Methods: In this study, 383 distinct bioactive compounds were extracted from the LOTUS database from *Zingiber officinale* and were screened against NEK7 protein using the PyRx 0.8 software. The top six hits' physicochemical characteristics, molecular descriptors, and natural product-likeness ratings were assessed using the LOTUS database.

Results: The compounds LTS0101379, LTS0022317, TS0094590, LTS0231343, LTS0085481, and LTS0110340 showed high affinity for NEK7, forming interactions with critical amino acid residues. Notably, these compounds had multiple interactions with amino acid residues similar to those of the control compound Dabrafenib. Furthermore, the molecular properties and descriptors of these compounds indicated favorable drug-like properties.

Conclusion: These findings highlight the potential of these compounds as NEK7 inhibitors for cancer management, though additional experimental validation is required.

Introduction

Cancer is still a growing and formidable global public health threat. According to the 2020 global cancer statistics, there were 18.1 million newly diagnosed cases and 10.0 million cancer-related deaths [1]. Human cancer is distinguished by uncontrolled cell proliferation, aggregation, and abnormal cell cycle progression. Protein kinases play an important role in the normal control mechanisms for cell division [2]. Despite significant advances in various therapeutic modalities such as surgery, radiotherapy, and chemotherapy, cancer continues to claim a significant number of lives globally [3].

Kinases play an important role in the regulation of essential cellular processes. Changes in the expression and activity of kinases have been linked to the onset of a variety of diseases, including cancer. Kinase research has received significant financial and scientific resources, resulting in significant progress. NIMA-related kinases (NEKs) from NEK1 to NEK11 are a subset of serine/threonine kinases that influence mitotic spindle formation and cytokinesis [4]. The smallest members of the NEK family, NEK7 and NEK6, have more than 85% sequence identity [5]. Previous research has found NEK7 overexpression in human cancers affecting the head and neck [6], breast [7], pancreas [8], liver [9], as well as the colorectum and lungs [10]. Furthermore, in addition to its role in cell proliferation, NEK7 has been linked to tumorigenesis and metastasis [6-10].

Zingiber officinale (Ginger) is a commonly used spice. The medicinal benefits of ginger are mostly owing to its phenolic components, including gingerols and shogaols. Studies have demonstrated that ginger possesses a variety of biological actions, including anti-inflammatory, antibacterial, and anticancer effects [11-13]. Furthermore, studies have suggested that ginger can prevent and treat a wide range of health disorders, including neurodegenerative illnesses, obesity, diabetes, chemotherapy-induced nausea and emesis, and respiratory ailments [14-18]. The purpose of this study was to identify natural NEK7 inhibitors from ginger bioactive compounds using an in-silico approach for cancer management.

Methods

Protein preparation

The 3D structure of NEK7 (PDB ID: 2WQN) was retrieved from the Protein Data Bank. Heteroatoms, comprising four ligands, and water molecules were eliminated. Further investigation showed that the protein contained incomplete portions, especially missing residues. To overcome these limitations, the structure was modeled with the SWISS-MODEL tool. This computational technique aided in the restoration of the missing sections, culminating in a full depiction of the NEK7 protein. The optimized model was then stored in the .pdb file for future analysis.

Preparation of the Ginger Compound Library

The LOTUS database resulted in 383 unique bioactive chemicals from *Z. officinale*. The compounds were collected in .sdf format. The PyRx program was used to prepare compounds for docking simulation. The preparation process included energy minimization with the mmff94 force field and converting the compounds to the .pdbqt format.

Virtual screening

The prepared *Z. officinale* compound library was screened against the NEK7 protein with PyRx 0.8 software. The X, Y, and Z coordinates were defined as -11.348222, -32.512407, and -46.605296, respectively. The coordinates were determined based on the ligand's natural position within the protein crystal structure. The screening yielded a large number of results, which were carefully analyzed to determine the binding affinities of the compounds. The docking score of each compound was compared to that of Dabrafenib, a well-known control compound, to identify potential drug candidates that interact positively with the NEK7 protein.

Physiochemical, molecular descriptor, and NP-likeness properties

Using the LOTUS database (https://lotus.naturalproducts.net/), the physicochemical characteristics, molecular descriptors, and natural product-likeness ratings of the top six hits were evaluated. The LOTUS database, renowned for its vast natural product data collection, simplified the process of analyzing the molecular makeup of each compound [19].

Results

This study used a thorough computational screening process to identify the natural substances that inhibit the NEK7 protein. The NEK7 protein is regarded as a key target for drug development in cancer treatments. The bioactive compounds found in *Z. officinale* were chosen to determine their ability to bind to the active site of the NEK7 protein. The original 3D conformation of NEK7, obtained from the PDB was found to be missing certain residues, resulting in an incomplete structure. To address this issue and ensure a thorough examination, the structure was rebuilt using the SWISS-MODEL online tool. SWISS-MODEL used the original 2WQN structure as a foundational template to accurately model the NEK7 protein's missing segments, preparing it for further computational screening.

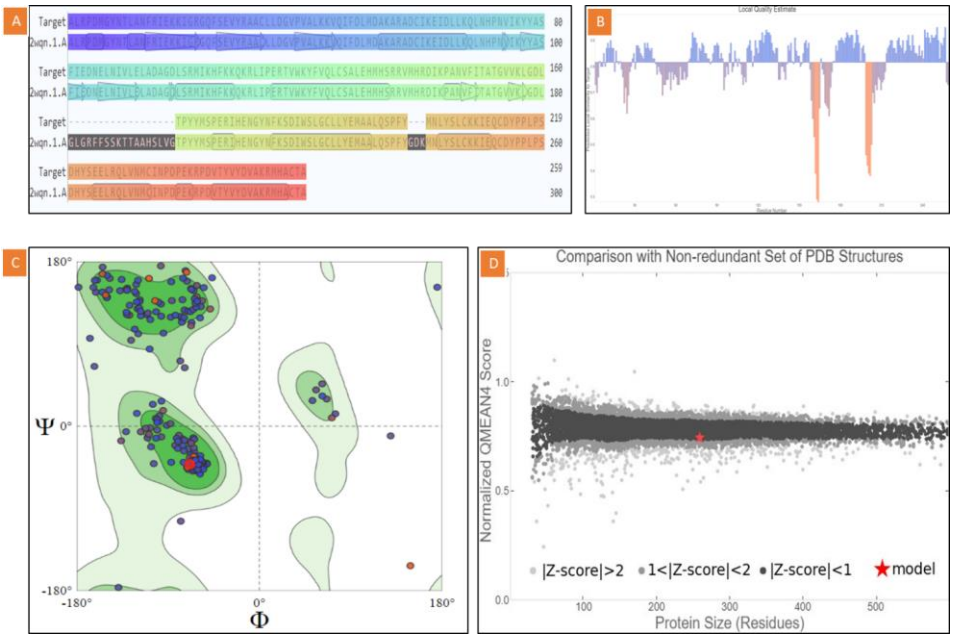

Figure 1: Structure assessment of the modeled structure of NEK7. Sequence alignment (target is the downloaded structure's sequence, 2wqn is the original sequence) (A), QMEANDisCo Local Quality Estimate (B), Ramachandran plot (C), and Z Score (D).

The resulting model's integrity and accuracy were thoroughly evaluated with SWISS-MODEL's integrated quality estimation tools. These validation measures revealed a high-quality model, demonstrating its structural dependability and applicability for further research (Figure 1).

Virtual screening was performed on the 3D structure of NEK7 employing 383 optimized *Z. officinale* compounds, with Dabrafenib acting as a positive control. Of the 383 compounds that were looked at, Table 1 indicates that 17 of them had binding energies that were either higher than or comparable to the control.

Compound ID	Binding affinity (kcal/mol)
LTS0101379	-8.9
LTS0022317	-8.7
LTS0094590	-8.7
LTS0231343	-8.6
LTS0085481	-8.6
LTS0110340	-8.4
LTS0168132	-8.3
LTS0210386	-8.3
LTS0071838	-8.3
LTS0203280	-8.3
LTS0266647	-8.3
LTS0031222	-8.2
LTS0032006	-8.2
LTS0035327	-8.2
LTS0051594	-8.2
LTS0055885	-8.2
LTS0068205	-8.2
Dabrafenib (control compound)	-8.2

Table 1: List of best screened compounds including the control compound and their binding affinity values.

Upon executing a visual assessment of the binding poses of the top 17 compounds using both 3D and 2D interaction evaluation, it was observed that 6 compounds exhibited better binding and interaction with the majority of NEK7's active site residues. Figure 2 illustrates the regions in which the control and top six compounds bind. These compounds lie in the same binding pocket and have most of the active site residues in common.

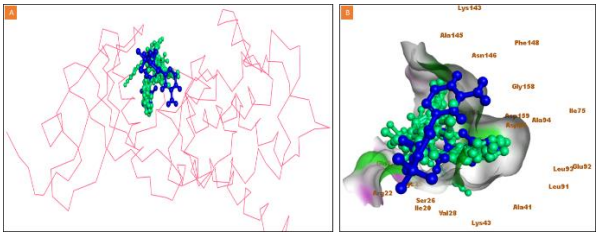

Figure 2: Binding interaction of *Z. officinale* compounds (top 6) and control (blue) in the NEK7 binding pocket. Protein in CA wire display style and binding poses of top hits and control (A), and NEK7 interacting residues and surface view of ligand interaction (B).

The 6 top hits namely LTS0101379, LTS0022317, TS0094590, LTS0231343, LTS0085481, and LTS0110340 were further extensively analyzed by their interaction with active site residues of NEK7. LTS0101379 was found to interact with Thr161, Lys43, Asp159, Glu92, Ile75, Ala94, Asp95, Leu93, Leu91, Phe148, Ala41, Ile20, Val28, Ala145, Lys143, and Asn146 residues of NEK7 (Figure 3A). LTS0094590 interacted with Val28, Gly21, Lys143, Gln24, Pro162, Gly23, Phe25, Ser26,

Indicators	LTS0101379	LTS0022317	LTS0094590	LTS0231343	LTS0085481	LTS0110340
Molecular Properties						
Total atom number	45	57	65	59	39	57
Heavy atom number	21	29	33	31	15	25
Bond count	21	31	34	33	16	25
Number of carbons	17	22	25	23	15	21
Minimal number of rings	1	3	2	3	2	1
Maximal number of rings	1	3	2	3	3	1
Molecular Descriptors						
NP-likeness score	1.02	1	1.01	1	1	1.04
Alogp	3.45	2.9	4.13	3.03	4.89	5.26
Alogp2	11.9	8.42	17.1	9.18	23.95	27.65
Apol	49.131	63.0042	71.7534	65.5662	42.403	61.5054
Bpol	29.111	38.2738	44.5626	39.2318	26.237	38.8146
Eccentric Connectivity Index Descriptor	451	677	838	717	149	689
Fmf Descriptor	0.2857	0.6897	0.5758	0.6452	0.7333	0.24
Fsp3	0.4706	0.4545	0.44	0.4348	0.7333	0.619
Fragment Complexity Descriptor	1605.04	2669.07	3300.08	2791.08	1390	2649.04
Petitjean Number	0.5	0.5	0.5	0.5	0.5	0.5
Lipinski's Rule (Failures)	0	0	1	0	1	2
Wiener Path Number	1166	2436	3592	2852	316	2052
Xlogp	2.711	2.595	3.906	3.443	5.558	5.406
Zagreb Index	94	148	158	158	80	110
TopoPSA	66.76	97.61	111.52	114.68	0	63.6

Table 2: Molecular Properties and Molecular Descriptors of top hits (LTS0101379, LTS0022317, LTS0094590, LTS0231343, LTS0085481, and LTS0110340).

Met51, Ile47, Ile63, Leu87, Ile89, Cys59, Val45, Thr161, Cys43, Asp159, Ala41, Glu92, Ile75, Phe148, Leu91, Ala94, Leu93, and Leu93 residues of NEK7 (Figure 3B). LTS0022317 was found to interact with Pro162, Gln24, Lys143, Ala145, Asn146, Phe148, Asp159, Ile75, Glu92, Leu91, Asp95, Leu93, Ala94, Ile20, Ala41, Val28, Phe25, Thr161, Gly23, and Ser26 residues of NEK7 (Figure 3C). LTS0231343 interacted with Asp98, Ala145, Asn146, Lys143, Pro162, Gln24, Thr161, Phe25, Gly23, Ser26, Lys43, Val28, Asp159, Leu91, Ile75, Ala41, Ala94, Glu92, Leu93, Phe148, and Gly97 residues of NEK7 (Figure 3D). LTS0085481 interacted with Glu92, Leu93, Ile20, Asp95, Gly21, Val28, Lys43, Asp159, Phe148, Leu91, Ala41, and Ala94 residues of NEK7 (Figure 3E). Further, LTS0110340 was found to interact with Arg22, Gly21, Gly23, Val28, Ala41, Lys43, Asp159, Leu91, Ile75, Glu92, Asp95, Ala94, Leu93, Phe148, Thr161, Ile20, Ser26, Asn146, and Ala145 residues of NEK7 (Figure 3F).

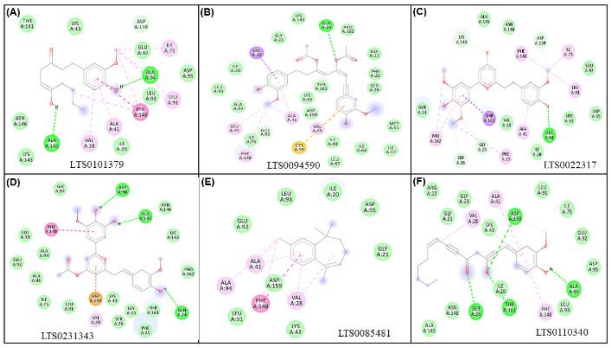

Figure 3: Interacting residues of NEK7 protein with LTS0101379 (A), TS0094590 (B), LTS0022317 (C), LTS0231343 (D), LTS0085481 (E), and LTS0110340 (F).

Furthermore, the control Dabrafenib was found to interact with Asp98, Ala94, Gly21, Val28, Ala41, Leu93, Asp159, Glu92, Leu91, Lys43, Phe148, Ile20, Lys18, Arg30, Asp95, Gly97, Ile149, and Ala96 residues of NEK7 (Figure 4).

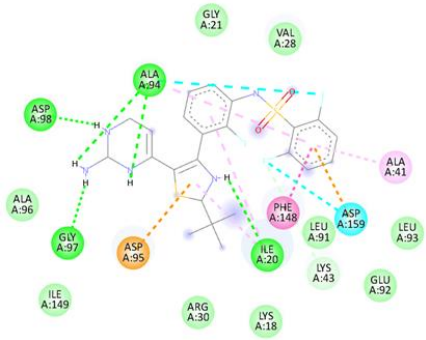

Figure 4: 2D representation of the interaction of Dabrafenib with NEK7 protein.

The molecular properties and descriptors of the top hits LTS0101379, LTS0022317, LTS0094590, LTS0231343, LTS0085481, and LTS0110340 were found to be favorable as shown in Table 2. The natural product-likeness assessment for each compound provided scores between -5 and +5, where -5 denoted the least probable resemblance and +5 signified the most probable resemblance to natural products. Every compound demonstrated score exceeding +1, which signifies a significant predilection for attributes resembling those of natural products. Positive scoring suggests that the substances in consideration might exhibit resemblances to established natural products, which bode well for subsequent research endeavors in the field of drug discovery. In addition, a comprehensive analysis utilizing Lipinski's Rule of Five

revealed an intricate profile of these hits, assessing the chemical structure of the drug to forecast its oral bioavailability. The oral bioavailability of LTS0094590, LTS0085481, and LTS0110340 may be compromised due to occasional violations of the rule. On the other hand, LTS0101379, LTS0022317, and LTS0231343 demonstrated a high level of suitability for further drug development considerations. They all met the criteria and did not show any violations.

Discussion

A wide range of regulatory proteins contribute to cell division fidelity, with kinases emerging as particularly important players. Protein kinases serve a variety of activities throughout the cell cycle, including checkpoint control and involvement in cancer etiology [20-22]. Protein kinase inhibition has proven to be an effective therapeutic method in cancer treatment [23,24]. NEK7, an essential component in spindle assembly and mitotic division, holds pivotal significance. Dysregulated expression of NEK7 is linked to the advancement of many cancers and related malignancies [25], making it a promising target for the development of highly selective and powerful anti-cancer therapies. In this study, *Z. officinale* bioactive compounds were tested against the NEK7 protein. Among them, six compounds (LTS0101379, LTS0022317, TS0094590, LTS0231343, LTS0085481, and LTS0110340) displayed better binding affinity and interaction with NEK7's active site residues.

To gain a better understanding of the interaction between the hit compounds and NEK7 residues, an analysis of the interaction between the control compound (Dabrafenib) and the NEK7 protein was performed. This analysis showed that several NEK7 residues, including Asp98, Ala94, Gly21, Val28, Ala41, Leu93, Asp159, Glu92, Leu91, Kys43, Phe148, Ile20, Lys18, Arg30, Asp95, Gly97, Ile149, and Ala96, played critical roles in binding with Dabrafenib. Interestingly, the top 5 compounds (LTS0101379, LTS0022317, TS0094590, LTS0231343, LTS0085481, and LTS0110340) were observed to interact with most of these NEK7 residues.

H-bonding has an important role in stabilizing the ligand-protein complex [26-28]. Accordingly, the hit compounds exhibited various H-bonding with NEK7 residues. The Ala94 and Ala145 residues of NEK7 formed H-bonds with LTS0101379, while the Gln24 residue formed H-bonds with LTS0094590. LTS0231343 also formed hydrogen bonds with the Asp98, Ala145, and Gln24 residues of NEK7, while LTS0022317 formed hydrogen bonds with the Ala94 residue of NEK7. Furthermore, LTS0110340 showed hydrogen bonding with Asp159, Ala94, Thr161, and Ser 26 residues of NEK7.

A pronounced negative binding energy indicates a strong interaction within the ligand-protein complex, which corresponds to a lower ligand dissociation rate [27,29,30]. Notably, the hit compounds (LTS0101379, LTS0022317, TS0094590, LTS0231343, LTS0085481, and LTS0110340) had higher negative binding energies than Dabrafenib. This observation indicates that these compounds have strong interactions with the NEK7 protein.

Field research conducted in several Asian and African regions has revealed the historic use of ginger in cancer treatment. The scientific community has extensively researched the biological properties of ginger extracts and extracted compounds such as gingerols, shogaols, zingiberene, and zingerone, using both in vitro and vivo approaches. Ginger and its active components have shown inhibitory actions against key enzymes related to cancer progression [31]. The identified ginger compounds in this study had a strong binding affinity for the NEK7 protein, indicating their potential utility in cancer management.

Any alteration in the normal expression or genetic sequence of NEK7 can result in the development of cancerous malignancies. This study screened the *Z. officinale* compounds against the NEK7. LTS0101379, LTS0022317, TS0094590, LTS0231343, LTS0085481, and LTS0110340 were found to interact strongly with NEK7. In addition, these compounds have several amino acid residues interactions in common with the Dabrafenib. These compounds can be used as NEK7 inhibitors in cancer management. However, further experimental validation is required.

Conflict of Interest

The author declare that there is no conflict of interest regarding the publication of this paper.

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