Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2023 20(2): 549-556. **OPEN ACCESS**

Curcumin as a potent inhibitor molecule for mixed lineage kinase: a molecular docking and *in vitro* study for potential cancer treatment

Misbahuddin M Rafeeq[1], Saad Alghamdi[2], Ahmed H Qasem[2], Samah J. Almehmadi[2], Issa Saad Al-Moraya[3], Afnan Alkathiri[4], Reema Mohammed Almotairi[5,6]and Qamre Alam[7*]

[1]Department of Pharmacology, Faculty of Medicine, Rabigh, King Abdulaziz University, Jeddah, 21589, **Kingdom of Saudi Arabia**
[2]Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, **Saudi Arabia**
[3]Forensic Medicine Center, Ministry of Health, **Saudi Arabia**
[4]Medical Genetics, Laboratory Medicine Department, Faculty of Applied Medical Sciences, Albaha University, Albaha, **Kingdom of Saudi Arabia**
[5]Medical Laboratory Technology Department, Faculty of Applied medical sciences, University of Tabuk, **Saudi Arabia**
[6]Prince Fahad Bin Sultan Chair for Biomedical Research, University of Tabuk, Tabuk, **Saudi Arabia**
[7]Molecular Genomics and Precision Medicine Department, ExpressMed Diagnostics and Research, Block, 359, Zinj, **Kingdom of Bahrain**

***Correspondence:** alamqa2022@gmail.com, qamrealam@expressmedlabs.com**Received: 20-04-2023, Revised: 08-06-2023, Accepted: 11-06-2023 e-Published: 16-06-2023**

Mixed lineage kinases (MLK) have emerged as potential therapeutic targets for the treatment of cancer, as they are often overexpressed or dysregulated in cancer cells, leading to aberrant signaling pathways that promote cancer cell proliferation, survival, and metastasis. Targeting MLK using small molecule inhibitors represents a promising therapeutic strategy for various diseases, and ongoing research efforts aim to develop novel and more effective MLK inhibitors with improved target specificity and reduced off-target effects. Here, we proposed to study the binding affinity and effect of curcumin on the activity of MLK. We employed molecular docking, fluorescence-based inhibition assay, and kinase activity assay to determine the binding affinity and inhibition of MLK. Further, MTT assay was used to determine the effect of curcumin on the viability of Hek 293 cells and to determine the IC_{50} concentration of curcumin which can be used for cancer cell line experiments. Molecular docking results showed binding affinity of -7.0 kcal/mol of curcumin and reflected it as potent inhibitor of MLK. Further, fluorescence inhibition and kinase activity assays validation the curcumin as potent inhibitor of MLK. MTT assay results showed that there was no significant effect of increasing concentrations of curcumin on the viability of Hek 293 cells. The IC_{50} concentration of curcumin was found as 12.0 ± 1.99 µM. In conclusion, the findings of this study highlight the importance of using a combination of in silico and in vitro techniques to evaluate the potential of natural compounds as therapeutic agents. Here we report curcumin as potent inhibitor molecule for MLK.

Keywords: Cancer, Curcumin, Inhibition, Mixed Lineage Kinase, Molecular Docking.

INTRODUCTION

Cancer is considered one of the principal causes of morbidity and mortality across the globe. As per the statistics of World Health Organization (WHO), cancer was responsible for approximately 9.6 million fatalities in the year 2018, thereby emerging as the second primary cause of death worldwide, after cardiovascular disease (Bray et al. 2018; Singh, Gurung et al. 2023). It is anticipated that the worldwide prevalence of cancer will persistently increase, as it is estimated that by 2030, there will be approximately 21.5 million new cases and 13.0 million deaths attributable to this disease. The prevalence of cancer varies by region, with the highest rates of cancer incidence and mortality observed in low- and middle-income countries (Fitzmaurice et al. 2018; Sultan et al. 2017). This is partly due to factors such as aging populations, increasing exposure to risk factors such as tobacco and alcohol, and limited access to cancer prevention, screening, and treatment services (Allemani et al. 2018; Sultan et al. 2018). In 2018, more than half of all new cancer cases and deaths occurred in Asia, with the highest rates observed in China, India, and Indonesia (Global Burden of Disease Cancer Collaboration, 2019). In developed countries, the incidence and mortality rates of some cancers have declined in recent years, largely due to improvements in cancer prevention and early detection,

as well as advances in cancer treatment (Siegel et al. 2020; Sultan et al. 2022).

There are several treatment strategies against cancer, which are often used in combination to achieve the best possible outcomes. The discovery of small molecule inhibitors has made significant contributions to the treatment of cancer (Diasio, 2018). Small molecule inhibitors are drugs that can target specific molecules or pathways involved in cancer cell growth and survival, and they work by inhibiting the activity of these targets (Wu and Nielsen, 2009). These inhibitors have several advantages over traditional chemotherapy drugs, including fewer side effects, better tolerability, and more specific targeting of cancer cells. Small molecule inhibitors have also been developed for targeted therapy in precision medicine, which involves using genetic and molecular information to tailor treatment to the specific characteristics of an individual's cancer (Kapoor et al. 2019). The application of this approach has yielded positive results in the treatment of specific cancer subtypes, including melanoma and lung cancer, leading to improved clinical outcomes for affected individuals. Overall, the discovery of small molecule inhibitors has revolutionized cancer treatment by providing targeted and effective therapies that have improved outcomes and reduced side effects. Continued research and development in this area are likely to lead to further improvements in cancer treatment and outcomes for patients.

Mixed-lineage kinases (MLKs) play a crucial role in various signaling pathways, including those that regulate cell growth, differentiation, and apoptosis (Gallo and Johnson, 2002; McDonald and Dedhar, 2022). MLKs have gained attention as promising therapeutic targets. This is due to their tendency to be overexpressed or dysregulated in cancer cells, resulting in the activation of aberrant signaling pathways that promote cancer cell proliferation, survival, and metastasis. (McDonald and Dedhar, 2022). MLKs have been implicated in various cellular processes that are relevant to cancer development and progression. For instance, MLKs have been shown to be involved in the regulation of the MAPK cascades, which play a crucial role in cell proliferation, differentiation, and survival (Cuadrado and Nebreda, 2010; Gallo and Johnson, 2002; Xie and Guo, 2019). Dysregulation of these pathways can result in aberrant cell growth and contribute to cancer development and progression (Cuadrado and Nebreda, 2010).

MLKs have also been linked to the regulation of the JNK pathway implicated in cell proliferation, differentiation, and survival (Gallo and Johnson, 2002; Johnson and Nakamura, 2007; Kyriakis and Avruch, 2012). Correspondingly, JNK pathway is associated with many cancer types, including glioblastoma, breast, and prostate cancers (Lu et al. 2019; Sabapathy and Jochum, 2018; Wagner and Nebreda, 2009). Moreover, MLKs are also dictating cellular responses to stress and inflammation, which are also implicated in cancer development and progression (Kyriakis and Avruch, 2012). For instance, MLKs have been shown to regulate the activation of NF-κB, which is important to regulate the inflammatory responses (Wang et al. 2003; Zhang et al. 2019). It has been demonstrated that the aberrant regulation of NF-κB signaling is associated with the development of several types of cancers, such as colorectal, lung, and pancreatic cancer (Hoesel and Schmid, 2013; Viala et al. 2018; Zou et al. 2019). In light of these observations, it can be inferred that MLKs are pivotal in the advancement and progression of cancer, and that directing therapeutic strategies towards the modulation of MLKs may be a hopeful avenue for cancer treatment.

These Emerging studies reflect that targeting MLKs with small molecule inhibitors represents a promising approach for the treatment of cancer. Over the past decade, several MLK inhibitors have been developed and have shown promising results in preclinical studies, including in breast cancer, prostate cancer, and glioblastoma (Cafferkey and Chauhan, 2020; Shrestha et al. 2020; Wada and Penninger, 2019; Zhang et al. 2018; Zhou et al. 2019).

MATERIALS AND METHODS

Target protein and ligand preparation

We retrieved 3D crystal structure of MLK from the Protein Data Bank (PDB ID 3DTC). The resolution of the structure was 2.60 Å and was in complex with inhibitor molecule. The 2D structure of ligand molecule namely curcumin was downloaded from DrugBank (Accession Number = DB11672). The structure of the target protein was critically analyzed by a standard receptor preparation protocol (Sultan et al. 2021, 2022). In order to remove the inhibitor molecule from the protein-inhibitor complex, Swiss-PDB Viewer was employed to carry out energy minimization by adjusting the positions of atoms to release local constraints. Various issues, such as missing side chains, atoms, or bonds, molecule-chain breaks, added water, alternate locations, and others, were identified and rectified. The target protein was supplemented with adequate polar hydrogens, and Kollman United Atom Charges were assigned. Both the target and ligand molecules were transformed to pdbqt format, which is the only file format that AutoDock Vina accepts for molecular docking. These steps were taken to ensure a promising approach for drug discovery and development.

Molecular docking and calculation of Inhibition constant (Ki; nM)

The molecular docking studies were performed using AutoDock Vina and MGL tools, following the protocol described in Jha et al. 2022 and Sultan et al. (2021, 2022). The active sites of MLK were targeted for docking with scaffolds of curcumin. The grid size for X, Y, and Z coordinates was set to 61, 74, and 70 Å, respectively, and

was centered at coordinates of −29.90, 15.84, and 63.83, respectively. The grid spacing was set to 1.00 Å and the exhaustiveness parameter was set to 8. To analyze the binding interactions of MLK with curcumin, the following steps were taken (Bhat et al., 2022; Singh, Mohsin et al. 2023): (1) bound conformations of the complexes were visualized and determined using PyMOL and Discovery Studio Visualizer, (2) bonding interactions between the protein and the small molecules were identified, (3) amino acid residues involved in the binding were determined, and (4) polar interactions between the complexes were mapped and labeled.

The inhibition constant (Ki) in nanomolar (nM) units was used as an indicator of inhibiting potency. The Ki value was calculated from the affinity (ΔG), which describes the strength of the MLK-curcumin interaction, using the following formula:

Ki = EXP ((ΔG *1000)/(R*T))

Where ΔG = docking energy; R = 1.98719 cal K-1 mol-1; T 298.15°k

Ki = EXP ((A*1000)/(198719*29815)).

A lower Ki value reflected a higher potency of the inhibitor.

MTT assay

The MTT assay is a commonly utilized technique for evaluating cell viability in cancer cell lines, as reported by Liu, Wu et al. in 2019. In this experiment, Hek 293 cells were cultured in a 96-well microplate at a density of 5,000 cells/well in 100 µL of DMEM medium and incubated in a CO2 incubator at 37°C with humidified conditions for 24 hours. After 24 hours of incubation, the MTT reagent was prepared by dissolving 5 mg of MTT in 1 mL of sterile PBS, followed by sterile filtration through a 0.22 µm filter. Subsequently, the culture medium was removed from the wells and replaced with 100 µL of fresh medium containing MTT reagent at a final concentration of 0.5 mg/mL. The cells were incubated for 4 hours at 37°C. After 4 hours, the medium containing MTT reagent was removed, and 100 µL of DMSO was added to each well to dissolve the purple formazan crystals formed by the MTT reagent. The microplate was gently shaken for 10 minutes to ensure complete dissolution of the formazan crystals, and the absorbance of each well was measured at 570 nm using a plate reader (Synergy HTX Multimode Reader, BioTek). The absorbance of the blank wells (containing only culture medium and MTT reagent) was subtracted from the absorbance of the test wells to obtain the net absorbance. The percentage of cell viability was calculated by dividing the net absorbance of the test wells by the net absorbance of the control wells (cells without any treatment) and multiplying by 100.

***In vitro* (fluorescence emission spectra) based inhibition assay**

Purified MLK recombinant human protein was purchased from Thermo Fisher Scientific, USA (Cat #: A30979) and the curcumin was purchased from Sigma, USA (CAS No.: 458-37-7). Fluorescence binding studies of MLK and curcumin was studied in a JASCO 6300 spectrofluorometer in using a 1cm quartz cell. To obtain the fluorescence spectrum of the MLK and MLK-curcumin complex, we performed the following steps: (1) the MLK was excited at a wavelength of 280 nm, (2) emission from the protein was recorded in the range of 300-500 nm, and (3) the excitation and emission slit widths were set to 10 mm, and the response was set to medium. The fluorescence data obtained from the MLK-curcumin complex were analyzed to obtain different binding parameters of the MLK-curcumin complex, reflecting the inhibition of MLK by curcumin.

Kinase Assay

To check the effect of curcumin on the activity of MLK, an ATPase assay was performed (Rule et al. 2016). This assay determined the activity of MLK based on the release of inorganic phosphate (Pi) during ATP hydrolysis. Different concentrations of curcumin (0-20 µM) were added to MLK (2 µM) and incubated for 1 hour at 25°C. Then, freshly prepared ATP (200 µM) was added to the reaction mixture along with MgCl2 (10 mM), and the mixture was incubated for 30 minutes at 25°C. The reaction was stopped by adding BIOMOL® reagent, and the green-colored complex formed was read at 620 nm on a microplate reader. Malachite green reagent (Biomol, Enzo Life Sciences, New York, NY, USA) was used in this assay.

RESULTS

Molecular docking analysis and calculation of Inhibition constant (Ki; nM)

Table 1 and Figure 1 show the molecular docking results. We used molecular docking to investigate how curcumin and MLK might bind together, to explore their bonding, as well as to determine their binding affinity (Irfan Dar et al. 2023). Docking analysis generated affinity scores and docked poses, which indicated that curcumin has an appreciable binding affinity score of −7.0 kcal/mol with MLK. The docking result revealed that curcumin has a preferable interaction with the binding pocket of MLK, as illustrated in Figure 1A-D. The binding mode and interaction pattern of curcumin with MLK were further studied using PyMOL, which revealed the formation of hydrogen bonds with SER137, ARG95, and LYS135 residues of the kinase domain of MLK (Figure 1A and 1B). Curcumin also formed several hydrophobic interactions with MLK. The docking pose showed that curcumin was docked into the deep binding cavity of MLK (Figure 1C and 1D). Based on the docking results, it can be concluded that curcumin could be a promising binding and inhibitor molecule of MLK.

Table 1: Binding affinity and inhibition constant (Ki) of curcumin with MLK.

Complex	Binding Free Energy (kcal/mol)	pKi	Ligand Efficiency (kcal/mol/ non-H atom)	Torsional Energy
MLK1-Curcumin	-7.0	5.13	0.2593	3.113

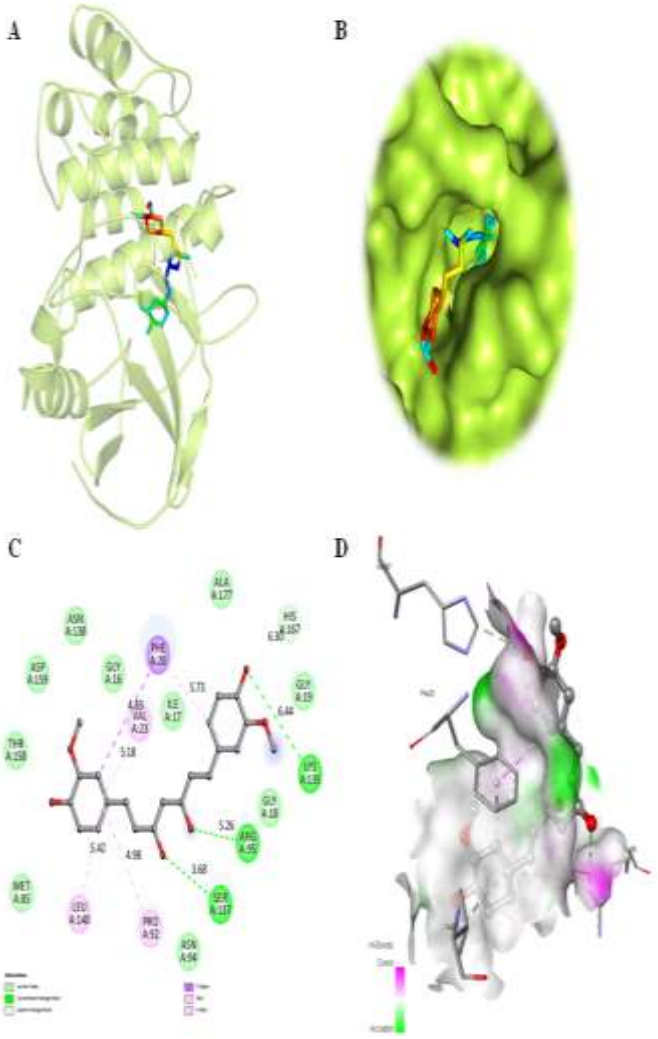

Figure 1: Molecular docking of curcumin with MLK and their interactions. (A) Cartoon plot of MLK-curcumin complex, (B) 2D plot of the MLK-curcumin complex interactions, and (C and D) curcumin showing different interactions in the binding pocket of MLK.

MTT assay

We studied the effect of curcumin on the viability of Hek 293 cells. Cells were treated with curcumin at concentrations of 0 to 100 µM. Results are presented in Figure 2. Results showed that treatment with increasing concentrations of curcumin did not significantly affect the viability of cells. Less reduction in cell viability was observed. We further proceeded with the computation of IC_{50} dose of curcumin for Hek 293 cells. The analysis of cell viability data reflected 12.0 ± 1.99 µM as IC_{50} dose of curcumin. This dose can be used to determine its effect on cancer cells.

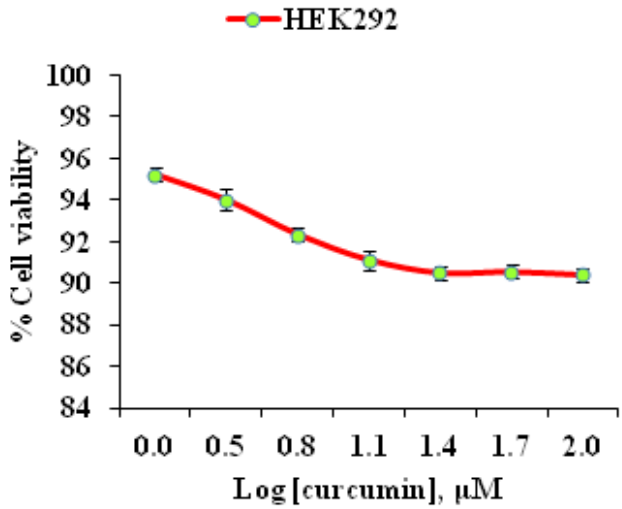

Figure 2: The cell viability showing the effect of curcumin treatment on the viability of Hek 293 cells. The cells were treated with different concentrations of curcumin for 24 h and thereafter viability of the cells was determined by MTT assay. Experiment was conducted in triplicate and the data is presented as mean ± SE.

***In vitro* (fluorescence emission spectra) based inhibition assay**

After confirming the binding of Curcumin to MLK using computer simulations, we used a fluorescence-based binding assay to measure the strength of the interaction between the two. This method measures how much the fluorescence of the protein changes when the ligand (Curcumin) binds to it. Figure 3 shows the fluorescence emission spectra of MLK with different amounts of Curcumin (0-12 µM). We found that as the concentration of Curcumin increased, the fluorescence of MLK decreased, indicating that Curcumin inhibits the activity of MLK. These results suggest that Curcumin could potentially be a powerful inhibitor of MLK, and we will test it further using the ATPase assay.

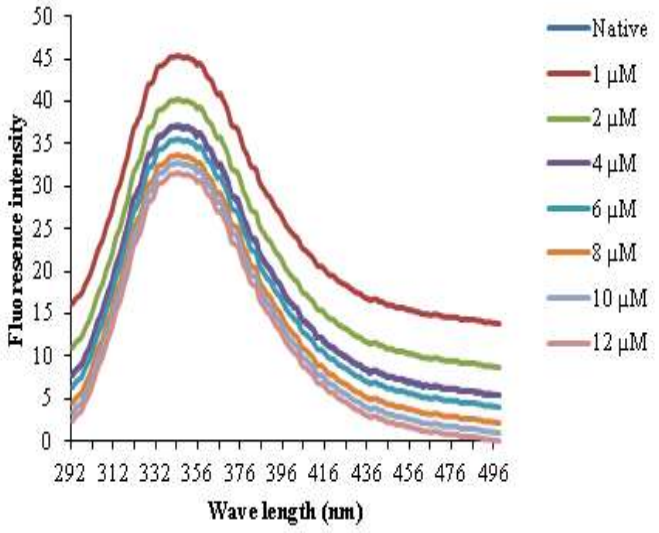

Figure 3: Fluorescence emission spectra of MLK in the absence and presence of different concentration (0-12 µM) of curcumin. The MLK protein was excited at 280 nm with emission recorded in the range of 300-500 nm.

Kinase Assay reflects inhibition of MLK by curcumin

Our next aim was to see how curcumin affects the activity of MLK. We conducted a kinase assay with different concentrations of curcumin, as shown in Figure 4. We considered the activity of MLK without curcumin as 100%. The results show that as the concentration of curcumin increases, the activity of MLK decreases, indicating that curcumin inhibits the kinase activity of MLK.

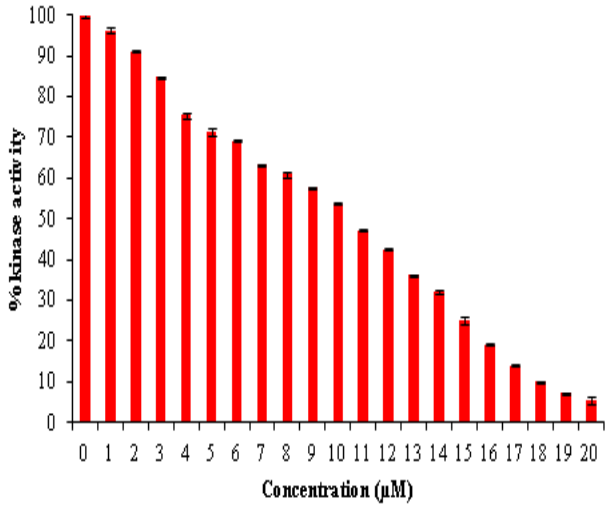

Figure 4: ATPase assay of MLK with curcumin. The concentration of MLK was fixed while curcumin was titrated from 0-20 µM.

DISCUSSION

Cancer is a major health problem worldwide and is a leading cause of death (Sultan et al. 2017, 2018). Conventional treatment options such as chemotherapy, radiation therapy, and surgery have been used to treat cancer, but they are often associated with significant side effects and limitations (Sultan et al. 2018). Therefore, there is a need for advanced treatment options that are more effective and less toxic. One approach to treating cancer is the inhibition of proteins that are highly expressed in cancers. These proteins play a critical role in the growth and spread of cancer cells and are potential targets for drug development. Small molecule inhibitors are promising agents for targeting these proteins due to their potential to inhibit the downstream signaling cascades activated by these proteins. Several small molecule inhibitors have been developed and are being studied for their effectiveness in preclinical and clinical trials (Cafferkey and Chauhan, 2020; Shrestha et al. 2020; Wada and Penninger, 2019; Zhang et al. 2018; Zhou et al. 2019).

MLKs are an emerging class of therapeutic targets for various diseases, including cancer and neurodegenerative disorders. Small molecule inhibitors are considered as promising agents for targeting MLKs due to their potential to inhibit the downstream signaling cascades activated by MLKs (Shrestha et al. 2020; Wada and Penninger, 2019; Zhang et al. 2018;). Several small molecule inhibitors have been developed to target MLKs, such as URMC-099, CEP-1347, and URMC-182 (Cafferkey and Chauhan, 2020; Shrestha et al. 2020; Wada and Penninger, 2019; Zhang et al. 2018; Zhou et al. 2019). These inhibitors have shown efficacy in preclinical studies, demonstrating their ability to inhibit MLKs and improve outcomes in animal models of various diseases (Gallo and Johnson, 2002; Johnson and Nakamura, 2007; Kyriakis and Avruch, 2012). Advances in drug discovery technologies have facilitated the development of more specific and potent MLK inhibitors, which hold promise for the treatment of diseases associated with MLK dysregulation. Overall, targeting MLKs using small molecule inhibitors represents a promising therapeutic strategy for various diseases, and ongoing research efforts aim to develop novel and more effective MLK inhibitors with improved target specificity and reduced off-target effects. All these statements together suggest that MLK can be considered as therapeutic target for several diseases including cancer. Bioinformatics techniques have been the most effective methods for identifying biologically active hits against molecular targets (Pinzi and Rastelli, 2019). Use of these techniques has widened the research area towards novel drug discovery in disease horizons.

The current study investigated the potential of curcumin as an inhibitor of MLK using a combination of in silico and in vitro techniques (Sultan et al. 2021, 2022). We first utilized molecular docking to predict the binding affinity of curcumin with MLK. We demonstrated that

curcumin had a high binding affinity (-7.0 kcal/mol with MLK, forming hydrogen bonds and hydrophobic interactions with key residues in the kinase domain. Subsequently, we conducted a cell viability assay to evaluate the effect of curcumin on Hek 293 cells. We found that increasing concentrations of curcumin did not significantly affect the viability of cells, and the IC_{50} dose of curcumin was determined to be 12.0 ± 1.99 µM. Furthermore, we performed a fluorescence-based binding assay to determine the actual affinity of curcumin with MLK, which showed a decrease in fluorescence intensity of MLK with increasing concentrations of curcumin, indicating the formation of a complex between the protein and the ligand. The inhibition of MLK was saturated at 12 µM concentration of curcumin reflection that this concentration is sufficient to inhibit the activity of MLK in several diseases including cancer. Finally, a kinase assay was conducted to examine the impact of curcumin on the functional aspect of MLK. The results demonstrated a noticeable decrease in the kinase activity of MLK with increasing concentrations of curcumin, indicating its inhibitory effect.

Overall, the study provides evidence that curcumin has the potential to act as a potent MLK inhibitor, suggesting its potential therapeutic use in diseases associated with MLK dysregulation, such as cancer and neurodegenerative disorders. However, further studies are needed to evaluate the efficacy and safety of curcumin as an MLK inhibitor, and to optimize its dosage and delivery for clinical use.

CONCLUSION

Targeting MLKs using small molecule inhibitors represents a promising therapeutic strategy for various diseases, and ongoing research efforts aim to develop novel and more effective MLK inhibitors with improved target specificity and reduced off-target effects. This suggests that MLK can be considered as therapeutic target several diseases including cancer. The findings of this study highlight the importance of using a combination of in silico and in vitro techniques to evaluate the potential of natural compounds as therapeutic agents. Here we report curcumin as potent inhibitor molecules for MLK.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

ACKNOWLEDGEMENT

The authors express heartfelt gratitude towards the Express Med laboratories, Zinj, Kingdom of Bahrain, for providing all the obligatory facilities in the course of this work.

AUTHOR CONTRIBUTIONS

MMR, SA and AHQ; designed and first draft the manuscript. SJA and ISA, data analysis and revised the manuscript. AA and RMA, data collection and edit the manuscript. QA performed the experiment and reviewed the manuscript. All authors read and approved the final version.

MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Allemani, C., Matsuda, T., Di Carlo, V., et al. (2018). Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): analysis of individual records for 37 513 025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. The Lancet, 391(10125), 1023-1075.

Bhat, S. A., Siddiqui, Z. I., Parray, Z. A., Sultan, A., Afroz, M., Azam, S. A., Farooqui, S. R., & Alqahtani, S. (2022). Naturally occurring HMGB1 inhibitor delineating the anti-hepatitis B virus mechanism of glycyrrhizin via in vitro and in silico studies. Journal of Molecular Liquids, 356, 119029.

Bray, F., Ferlay, J., Soerjomataram, I., et al. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 68(6), 394-424.

Cafferkey, C., & Chauhan, A. (2020). Mixed lineage kinase inhibitors: a new class of targeted therapy for cancer. Current Opinion in Pharmacology, 53, 41-48.

Cuadrado, A., & Nebreda, A. R. (2010). Mechanisms and functions of p38 MAPK signalling. Biochemical Journal, 429(3), 403-417.

Diasio, R. B. (2018). Cancer chemotherapy: Principles and practice. In R. C. Bast Jr, D. W. Kufe, R. R. Weichselbaum, J. F. Holland, & E. Frei III (Eds.), Cancer Medicine (pp. 222-238). Springer International Publishing. https://doi.org/10.1007/978-3-319-60060-3_14

Fitzmaurice, C., Akinyemiju, T. F., Al Lami, F. H., et al. (2018). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2016: A systematic analysis for the Global Burden of Disease Study (vol 68, pg 394, 2018). JAMA Oncology, 4(9), 1288.

Gallo, K. A., & Johnson, G. L. (2002). Mixed-lineage kinase control of JNK and p38 MAPK pathways. Nature Reviews Molecular Cell Biology, 3(9), 663-672.

Global Burden of Disease Cancer Collaboration. (2019). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: A systematic analysis for the Global Burden of Disease Study. JAMA Oncology, 5(12), 1749-1768.

Hoesel, B., & Schmid, J. A. (2013). The complexity of NF-κB signaling in inflammation and cancer. Molecular Cancer, 12, 86.

Irfan Dar, M., Qureshi, M. I., Zahiruddin, S., Abass, S., Jan, B., Sultan, A., & Ahmad, S. (2022). In Silico Analysis of PTP1B Inhibitors and TLC-MS Bioautography-Based Identification of Free Radical Scavenging and α-Amylase Inhibitory Compounds from Heartwood Extract of Pterocarpus marsupium. ACS omega, 7(50), 46156–46173.

Jha, P., Singh, P., Arora, S., Sultan, A., Nayek, A., Ponnusamy, K., Syed, M. A., Dohare, R., & Chopra, M. (2022). Integrative multiomics and in silico analysis revealed the role of ARHGEF1 and its screened antagonist in mild and severe COVID-19 patients. Journal of cellular biochemistry, 123(3), 673–690.

Johnson, G. L., & Nakamura, K. (2007). The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. Biochimica et Biophysica Acta - Molecular Basis of Disease, 1772(8), 1097-1111.

Kapoor, K., Singh, B., & Dewan, R. (2019). Precision medicine in cancer: An overview. Cancer Res Stat Treat, 2(2), 223-231.

Kyriakis, J. M., & Avruch, J. (2012). Mammalian MAPK signal transduction pathways activated by stress and inflammation: A 10-year update. Physiol Rev, 92(2), 689-737.

Liu, Y., Wu, C., Lyu, Y., & Chen, Q. (2019). Optimization of the MTT assay for in vitro drug sensitivity testing of tumor cells. J Huazhong Univ Sci Technolog Med Sci, 39(5), 750-755.

Lu, Z., Xu, S., & ER, X. (2019). Targeting MAPK signaling pathways in breast cancer: A review of targeted therapies. Zhong Nan Da Xue Xue Bao Yi Xue Ban, 44(6), 638-644.

McDonald, P. C., & Dedhar, S. (2022). New perspectives on the role of integrin-linked kinase (ILK) signaling in cancer metastasis. Cancers, 14(13), 3209.

Pinzi, L., & Rastelli, G. (2019). Molecular docking: Shifting paradigms in drug discovery. Int J Mol Sci, 20(18), 4331.

Rule, C. S., Patrick, M., & Sandkvist, M. (2016). Measuring In Vitro ATPase Activity for Enzymatic Characterization. J Vis Exp, (114), 54305.

Sabapathy, K., & Jochum, W. (2018). ΔJunD and the stress response in cancer. Biochim Biophys Acta Rev Cancer, 1870(2), 285-296.

Shrestha, S., Hamanaka, R. B., & Martin, A. (2020). Mixed lineage kinase inhibitors: A patent review. Expert Opin Ther Pat, 30(4), 251-261.

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. CA Cancer J Clin, 70(1), 7-30.

Singh, P., Gurung, R., Sultan, A. et al. Understanding the role of adipokines and adipogenesis family in hepatocellular carcinoma. Egypt J Med Hum Genet 24, 17 (2023).

Singh, P., Mohsin, M., Sultan, A., Jha, P., Khan, M. M., Syed, M. A., Chopra, M., Serajuddin, M., Rahmani, A. H., Almatroodi, S. A., Alrumaihi, F., & Dohare, R. (2023). Combined Multiomics and In Silico Approach Uncovers PRKAR1A as a Putative Therapeutic Target in Multi-Organ Dysfunction Syndrome. *ACS omega*, *8*(10), 9555–9568.

Sultan, A., Choudhary, V., & Parganiha, A. (2017). Worsening of rest-activity circadian rhythm and quality of life in female breast cancer patients along progression of chemotherapy cycles. Chronobiol Int, 34(5), 609-623.

Sultan, A., Pati, A. K., Choudhary, V., & Parganiha, A. (2018). Hospitalization-induced exacerbation of the ill effects of chemotherapy on rest-activity rhythm and quality of life of breast cancer patients: A prospective and comparative cross-sectional follow-up study. Chronobiol Int, 35(11), 1513-1532.

Sultan, A., Pati, A. K., Choudhary, V., & Parganiha, A. (2020). Repeated chemotherapy cycles produced progressively worse and enduring impairments in the sleep–wake profile of hospitalized breast cancer patients. Biological Rhythm Research, 51(8), 1166-1181.

Sultan, A., Taj, S., Choudhary, V., & Parganiha, A. (2022). Predictive role of socio-demographic and chronotype on health-related quality of life of cancer patients from southeastern India. Biological Rhythm Research, 53(4), 592-607.

Sultan, A., Ali, R., Sultan, T., Ali, S., Khan, N. J., & Parganiha, A. (2021). Circadian clock modulating small molecules repurposing as inhibitors of SARS-CoV-2 Mpro for pharmacological interventions in COVID-19 pandemic. Chronobiology International, 38(7), 971-985.

Sultan, A., Ali, R., Ishrat, R., & Ali, S. (2022). Anti-HIV and anti-HCV small molecule protease inhibitors in-silico repurposing against SARS-CoV-2 Mpro for the treatment of COVID-19. Journal of Biomolecular Structure and Dynamics, 40(23), 12848-12862.

Viala, M., Charrier, L., & Tesson, B. (2018). NF-κB in cancer: from innocent bystander to major culprit. International Journal of Biochemistry and Cell Biology, 95, 52-54.

Wada, T., & Penninger, J. M. (2019). Mitogen-activated protein kinases in apoptosis regulation. Oncogene, 38(13), 2333-2345.

Wang, X., Wang, Q., Ives, K. L., Evers, B. M., & Zhang, W. (2003). The role of NF-kappaB in the regulation of

cell stress responses. International Journal of Cancer, 107(6), 877-886.

Wagner, E. F., & Nebreda, A. R. (2009). Signal integration by JNK and p38 MAPK pathways in cancer development. Nature Reviews Cancer, 9(8), 537-549.

Wu, P., Nielsen, T. E., & Clausen, M. H. (2009). Small-molecule kinase inhibitors: an analysis of FDA-approved drugs. Drug Discovery Today, 14(5), 291-298.

Xie, J., & Guo, Q. (2018). The role of mixed lineage kinases in the regulation of cell proliferation and cancer metastasis. Seminars in Cancer Biology, 54, 84-91.

Zhang, L., Chen, J., Fu, H., Zhou, L., & Zhao, Y. (2019). Mixed lineage kinase 3 (MLK3) is involved in lipopolysaccharide (LPS)-induced activation of NF-κB pathway in goat mammary epithelial cells. Microbial Pathogenesis, 128, 348-355.

Zhang, X., Zhang, J., Wang, L., Wei, Y., Du, G., & Tao, X. (2018). Mixed lineage kinase inhibitors as potential therapies for cancer. Journal of Hematology and Oncology, 11(1), 1-10.

Zhou, H., Yang, Y., & Zhang, X. (2019). Recent development of small-molecule inhibitors targeting MLKs in cancer therapy. Future Medicinal Chemistry, 11(14), 1709-1729.

Zou, H., Li, Y., & Liu, X. (2019). NF-κB pathway in cancer therapy. Aging and Disease, 10(4), 863-879.

.