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EXPERIMENTAL STUDY

In silico and *in vitro* studies of Tyr-Lys-Thr tripeptide against human lung cancer cell line (A549)

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ABSTRACT
OBJECTIVES: The main purpose of this study is to predict the effect of Tyr-Lys-Thr (YKT) tripeptide, recognized for its anticancer properties, on lung cancer through theoretical and experimental analyzes.
BACKGROUND: Peptides are important therapeutic compounds that have been studied for many years. Among these, YKT tripeptide emerges as a significant therapeutic peptide, exhibiting cytotoxic effects on various cancer cell lines.
METHODS: The study investigated the involvement of the PI3K/Akt/mTOR pathway, commonly activated in human cancer, and the pivotal role of caspases in apoptosis. The interactions of YKT tripeptide with mTOR, Akt, PI3K, caspase-3, and caspase-8 were investigated through the molecular docking method. Additionally, MTT test was used to determine the cytotoxic activity of YKT against the A549 cell line across concentrations set at 0.1, 0.25, 0.5, 1, 2.5 and 5 mg/mL for 24 and 48 h.
RESULTS AND CONCLUSION: *In silico* docking studies were conducted with PI3K, Akt1, and mTOR, known to be active in human cancer, as well as caspase-3 and caspase-8, key enzymes in apoptosis. It was determined that YKT exhibited a robust binding tendency with each receptor. YKT tripeptide was also found to have a cytotoxic effect on human lung carcinoma cell line A549 *(Tab. 5, Fig. 11, Ref. 28)*. Text in PDF www.elis.sk
KEY WORDS: peptide, A549, molecular docking, MTT, cytotoxicity.

Introduction

Peptides stand as valuable therapeutic compounds with promising applications across a spectrum of diseases. The therapeutic versatility of peptide structures stems from their ability to initiate diverse biological activities ranging from anticancer to anti-inflammatory effects. Tyrosine, the first amino acid in the sequence of YKT tripeptide is known for its antioxidant properties (1). The subsequent amino acid, lysine, is recognized for its antioxidant, antiviral and anticancer effects (2–5). Threonine, the final constituent incorporated into cellular processes via protein synthesis and cellular signaling mechanisms, plays a vital role in stimulating cell growth, inhibiting apoptosis, and supporting antibody production (6, 7). It has also been reported in the literature that one-dimensional poly (L-lysine)-block-poly (L-threonine) assemblies exhibit anticancer properties (8) while YK dipeptide demonstrates anticancer activity (9). Studies have shown that YKT tripeptide exerts cytotoxic effects on various cancer cell lines, including MCF-7, HeLa and MAT-Lylu (10, 11).

Cancer, one of the leading causes of death worldwide, manifests in form of tumors resulting from uncontrolled cell proliferation within organs or tissue (12). The resistance to drugs, prevalent across various types of cancer, poses a significant treatment obstacle. Addressing this challenge entails recognizing the pivotal role of activated signaling pathways in cancer development and chemotherapeutic resistance. Particularly the studies targeting the phosphatidylinositol-3-kinase (PI3K)-Akt signaling pathway underscore the necessity for drug efficacy against therapeutic resistance (13). It is well-established that the PI3K/Akt/mTOR pathway, pivotal in cellular growth, proliferation and survival, undergoes alterations in cancer (14, 15). Consequently, PI3K, AKT and mTOR have become focal points of investigation in cancer research. Blocking the PI3K/AKT/mTOR pathway is associated with increased antitumor activity (15, 16). Caspases, a group of cysteine-protease enzymes, are fundamental structures regulating apoptosis. The mechanism of caspase activation is recognized as one of the fundamental aspects of apoptosis (17).

In this study, we explored the anticancer properties of YKT tripeptide through both experimental and theoretical approaches. In the theoretical section of the study, the interactions between YKT and receptor groups located in potential pathways were examined via molecular docking technique, Additionally we compared the peptide's interaction profiles with those of molecules known for their anticancer properties in existing literature. In the experi-

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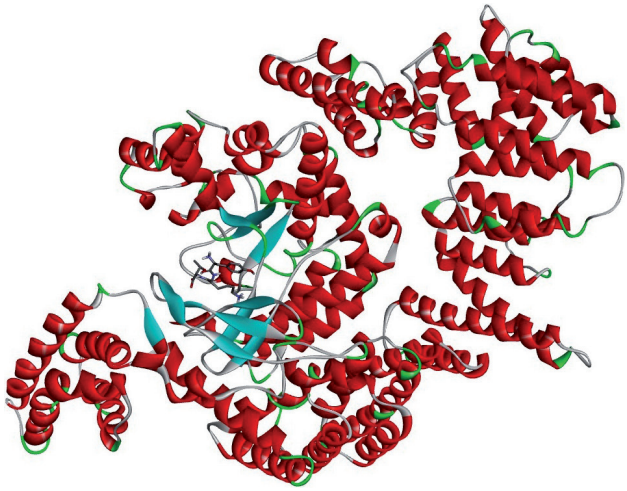

Fig. 1. YKT tripeptide at mTOR active site.

mental segment, we assessed the anticancer effects of YKT by subjecting A549 cells to varying concentrations of the peptide and employing MTT method to determine cell viability. Our findings suggest that different concentrations of YKT tripeptide exerted a cytotoxic effect on A549 cells after both 24-hour and 48-hour experimental periods.

Materials and methods

Materials

The YKT tripeptide (Cas No: 155943-09-2) was purchased from Bachem AG, Bubendorf, Switzerland.

Molecular docking analyses

To understand anticancer activities of YKT tripeptide, we conducted molecular docking studies targeting specific receptors (mTOR, Akt1, caspase-8, caspase-3, and PI3K). This involved exploring the binding modes of YKT tripeptide with the binding sites of these receptors. Initially, YKT tripeptide was optimized using Gaussian09 program (18) with the DFT method and B3LYP/6-311++G (d,p) basis set. Subsequently, the YKT tripeptide was prepared as a ligand for molecular docking studies using AutoDock Tools 1.5.6. Receptor structures, including mTOR (PDB Code: 4JT6), Akt1 (PDB Code: 4EJN), Caspase-8 (PDB Code: 3KJQ), Caspase-3 (PDB Code: 2XYP) and PI3K (PDB Code: 1E90) were downloaded from Protein Data Bank (https://www.rcsb.org/). Prior to docking, all receptors arranged in preparation steps involving removal of water, ions, and other ligands, followed by addition of polar hydrogens. Following these preparation steps, grid boxes were adjusted accordingly, and molecular docking studies were successfully conducted using AutoDock Vina (19). The best binding affinities were determined and the interaction types of the ligand conformations with the receptor with the highest binding affinities were examined using Discovery Studio Visualizer 2019 (20).

In vitro study

The human lung carcinoma cell line A549 was used for *in vitro* cytotoxicity experiments. A549 cells were cultured in RPMI 1640 medium (Sigma), supplemented with 10% fetal bovine serum (FBS, Gibco Lab) and 100 U/mL penicillin-streptomycin solution (Gibco Lab) and maintained in a humidified atmosphere containing 5% CO_2 at 37°C. For MTT analysis, the cells were seeded at a density of $1x10^4$ cells per well in 96-well plates and incubated for the duration of the experiment. At the end of the incubation period, MTT solution in volume of 40 µl was added, and the cells were incubated for additional 4 hours. DMSO was added to solubilize formazan crystals, and the absorbance was measured using a microplate reader. The YKT tripeptide was dissolved in RPMI 1640 medium to achieve concentrations of 0.1, 0.25, 0.5, 1, 2.5, and 5 mg/ml for the experiment.

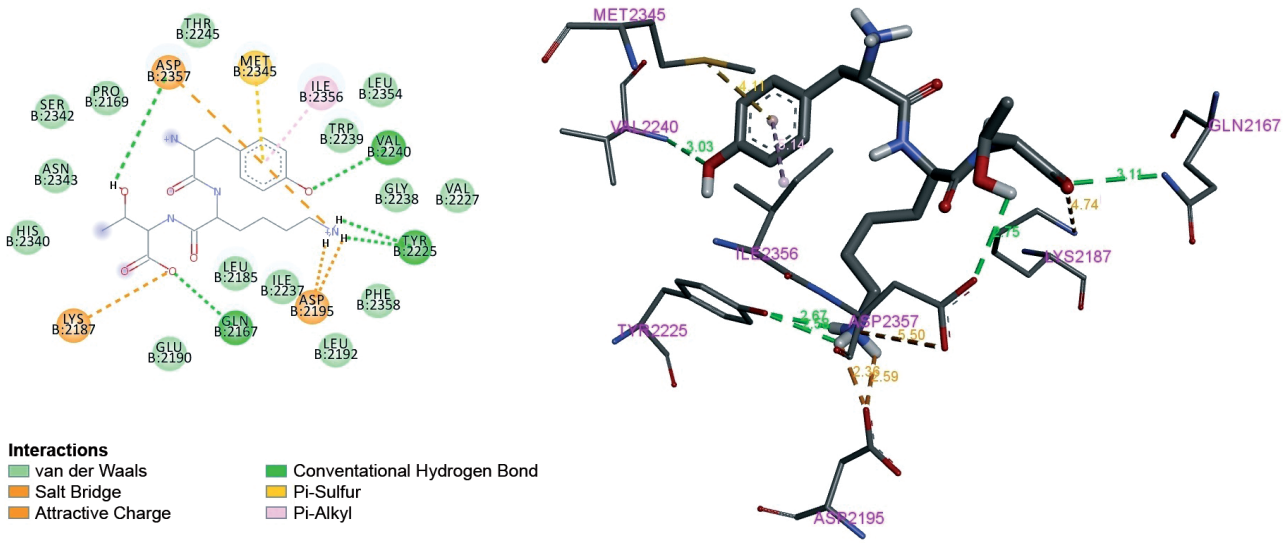

Fig. 2. Close interaction of YKT tripeptide with mTOR.

Tab. 1. Interaction types of YKT-mTOR complex.

Residue (PDB: 4JT6)	Interaction type	Distance
Gln-2167	H-bond	3.11
Tyr-2225	H-bond	2.58 2.67
Val-2240	H-bond	3.03
Asp-2357	H-bond	2.75
	Attractive charge	5.50
Ile-2356	Pi-alkyl	5.14
Met-2345	Pi-sulfur	4.11
Asp-2195	Salt bridge+ attractive charge	2.36 2.59
Lys-2187	Attractive charge	4.74
Pro-2169, Leu-2185, Glu-2190, Leu-2192, Val-2227, Ile-2237, Gly-2238, Trp-2239, Thr-2245, His-2340, Ser-2342, Asn-2343, Leu-2354, Phe-2358	Van der Waals	

Results

The mammalian target of rapamycin (mTOR)

To investigate the binding interactions between the YKT tripeptide and the mTOR receptor (PDB ID: 4JT6) (21), molecular docking using AutoDock Vina program was carried out. The obtained outcomes are shown in Figures 1 and 2, and Table 1. The molecular docking yielded a best binding energy of –7.2 kcal/mol. Notably, the YKT tripeptide formed 5 hydrogen bonds with Gln-2167 (3.11 Å), Tyr-2225 (2.58 and 2.67 Å), Val-2240 (3.03 Å) and Asp-2357 (2.75 Å) residues of mTOR.

RAC (Rho family)-alpha serine/threonine-protein kinase (Akt1)

The molecular docking of YKT tripeptide was carried out with RAC-alpha serine/threonine-protein kinase (PDB ID: 4EJN) using AutoDock Vina program to determine its interaction profile. The interaction profiles of YKT tripeptide are illustrated in Figures 3 and 4, and Table 2. The docking analysis yielded a best binding energy of –8.1 kcal/mol and revealed that YKT tripeptide made robust hydrogen bonds with RAC-alpha serine/threonine-protein kinase.

Tab. 2. Interaction types of YKT-Akt1 complex.

Residue (PDB: 4EJN)	Interaction type	Distance
Asn-54	H-bond	2.83
Trp-80	H-bond	3.00
	Carbon H-bond	3.66
Thr-82	H-bond	2.10
Ser-205	H-bond	3.14
Asp-292	H-bond	2.19 2.47
Leu-210	Pi-alkyl	4.85
Leu-264	Pi-sigma	3.66
Asn-53, Gln-79, Thr-81, Ile-84, Asn-204, Thr-211, Val-270, Val-271, Tyr-272, Thr-291, Gly-294	Van der Waals	

Phosphoinositide 3-kinase (PI3K)

To investigate the interaction between YKT tripeptide and PI3K, the molecular docking study was carried out using AutoDock Vina program (Fig. 5). The best binding energy was –7.0 kcal/mol. YKT formed hydrogen bonds with Lys-833, Val-882, Thr-887 and Asp-964 residues. The Thr-887 residue particularly stands out due to its involvement in multiple hydrogen bonds with YKT. Additionally, the docking study revealed numerous other interactions such as pi interactions and salt bridges, further highlighting the comprehensive interaction profile of YKT (Fig. 6, Tab. 3).

Caspase-3

The interaction profile and binding energy of YKT-caspase-3 complex were determined using AutoDock Vina program (Fig. 7). The best binding energy was –6.1 kcal/mol. YKT tripeptide formed 3 hydrogen bonds along with pi-pi stacked and van der Waals interactions. YKT formed hydrogen bonds with Arg-207 and Glu-

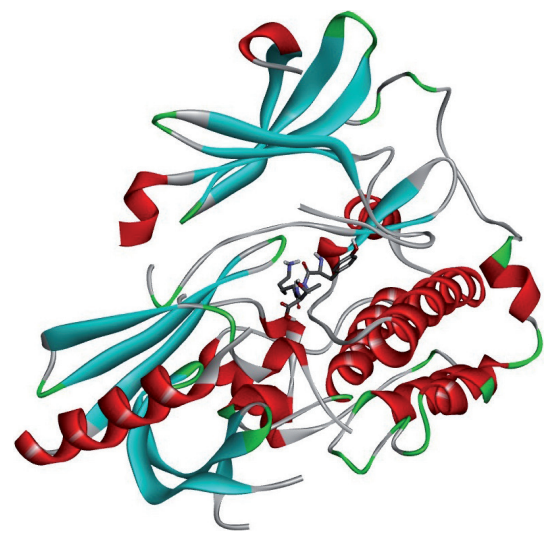

Fig. 3. YKT tripeptide at Akt1 active site.

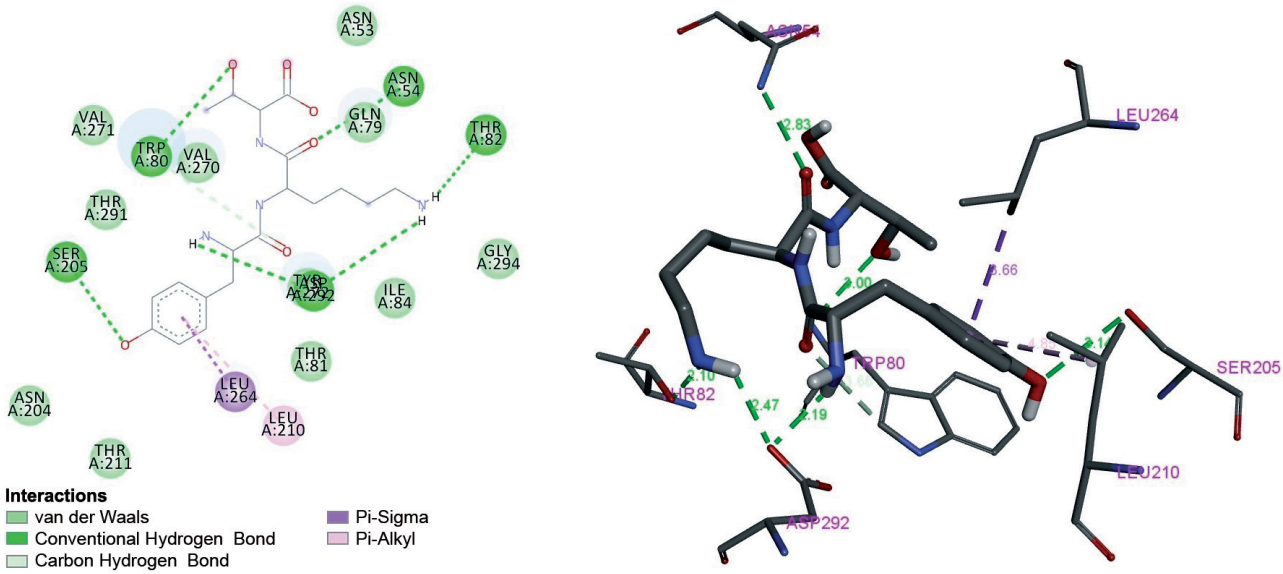

Fig. 4. Close interaction of YKT tripeptide with Akt1.

248 residues. YKT formed two hydrogen bonds with Arg-207, measuring 3.11 Å and 3.18 Å in length, respectively. Additionally, YKT formed a hydrogen bond with Glu-248, measuring 2.17 Å in length (Fig. 8, Tab. 4).

Caspase-8

The molecular docking was carried out for YKT tripeptide and caspase-8 via AutoDock Vina program. The best binding energy obtained was –6.7 kcal/mol (Fig. 9). YKT tripeptide formed 6 hydrogen bonds with 6 different residues of caspase-8 (Fig. 10). The prominent residue in hydrogen bond interactions was Arg-413. In addition to the hydrogen bond with Arg-413, YKT tripeptide formed a salt bridge and unfavorable donor-donor interactions (Fig. 10, Tab. 5), as well as a hydrogen bond with Arg-260 residue, measuring 3.01 Å in length.

Cytotoxic effect

The cell viability of the A549 cells treated with YKT after 24-hour and 48-hour incubation periods are shown in Figure 11. In the 24-hour experimental group, the cell viability was measured at 89.2%, 83.3%, 75.8%, 77.5%, 70% and 51.7%, respectively. Significantly lower cell viability was observed in the experimental groups compared to the control group ($p<0.01$). In the 48-hour experimental group, a further decrease in cell viability was observed depending on applied concentrations of YKT. Cell viability was measured at 64.6%, 60.3%, 57.6%,

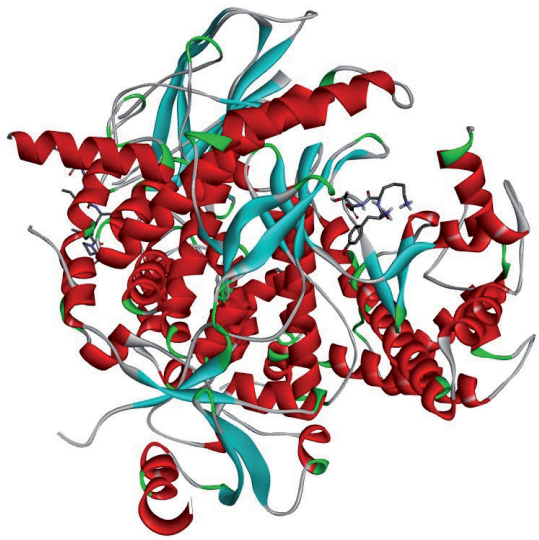

Fig. 5. YKT tripeptide at PI3K active site.

Tab. 3. Interaction types of YKT-PI3K complex.

Residue (PDB: 1E90)	Interaction type	Distance
Lys-833	H-bond Salt bridge	3.10 3.97
Val-882	H-bond	3.01
Thr-887	H-bond	2.35 2.75 2.95
Asp-964	H-bond	2.92
Ile-881	Pi-sigma	3.91
Met-953	Pi-sulfur	4.00
Asp-841	Unfavorable negative-negative	4.75
Lys-890	Unfavorable positive-positive	4.62 4.80
Asp-950	Salt Bridge+ Charge-Charge	1.99
Met-804, Ser-806, Lys-807, Pro-810, Trp-812, Ile-831, Tyr-867, Ile-879, Glu-880, Phe-961, Ile-963	Van der Waals	

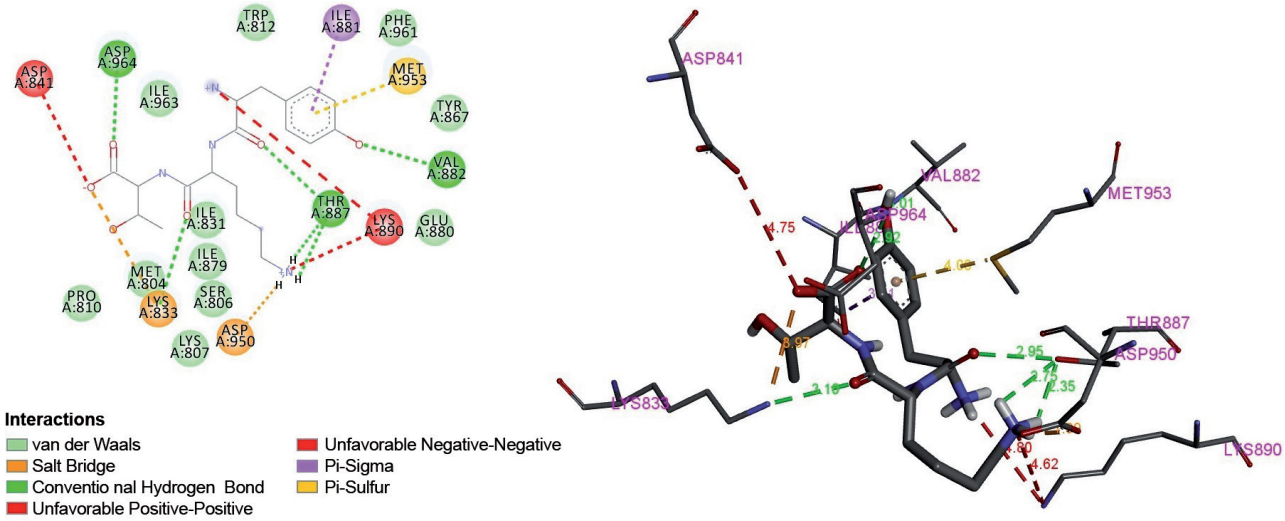

Fig. 6. Close interaction of YKT tripeptide with PI3K.

56.3%, 41.1% and 29.3%, respectively. Similar to the 24-hour group, a significant difference was noted compared with the control group ($p<0.01$). The comparison between the 24-hour and 48-hour experimental periods revealed a relatively less toxic effect in the 24-hour experimental group. The increasing cytotoxic effects over time suggest the activation of cell death pathways.

Discussion

This study is focused on the PI3K/Akt/mTOR pathway, widely implicated in human cancer, as well as on caspases, pivotal in apoptosis. Molecular docking was used to assess the interaction profile of the YKT tripeptide with PI3K, Akt, mTOR, caspase-3 and caspase-8. Furthermore, the cytotoxic effect of YKT tripeptide was investigated through *in vitro* studies, using A549 cells. In the YKT-mTOR docking study, comparison of the interaction profiles between the reference ligand, X6K, and YKT tripeptide revealed similar interactions with mTOR residues. Notably, YKT tripeptide formed more hydrogen bonds with mTOR compared to X6K. While the X6K ligand formed hydrogen bonds with Val-2240, Tyr-2225 and Asp-2195, YKT established hydrogen bonds with Val-2240 and Tyr-2225, along with salt bridge interaction with Asp-2195. Both X6K and YKT ligands were found to engage in van-der-Waals (vdW) interac-

Tab. 4. Interaction types of YKT-caspase-3 complex.

Residue (PDB: 2XYP)	Interaction type	Distance
Arg-207	H-bond	3.11 3.18
Glu-248	H-bond	2.17
Phe-256	Pi-Pi stacked	3.91
Tyr-204, Trp206, Asn-208, Ser-209, Trp-214, Ser-249, Phe-250, Ser-251, Asp-253	Van der Waals	

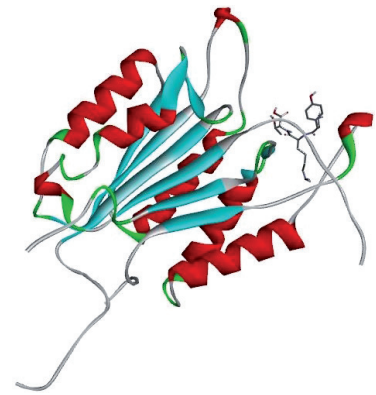

Fig. 7. YKT tripeptide at caspase-3 active site.

Tab. 5. The interaction types of YKT-Caspase 8 complex.

Residue (PDB: 3KJQ)	Interaction type	Distance
Lys-253	H-bond	2.65
Ser-256	H-bond	2.44
Arg-260	H-bond	3.04
His-317	H-bond	3.01
Gly-318	H-bond	1.95
Arg-413	H-bond	3.18
Leu-254	Pi-alkyl	3.15
Ile-257	Pi-alkyl	4.75
Arg-258	Unfavorable positive-positive	5.36
Ser-411	Unfavorable acceptor-acceptor	2.86
Arg-413	Unfavorable donor-donor	2.17
	Salt bridge	3.01
Arg-260	Attractive charge	
Asp-319	Attractive charge	
Tyr-324, Ala-359, Cys-360, Gly-362, Asp-363, Val-410, Tyr-412, Thr-419	Van der Waals	

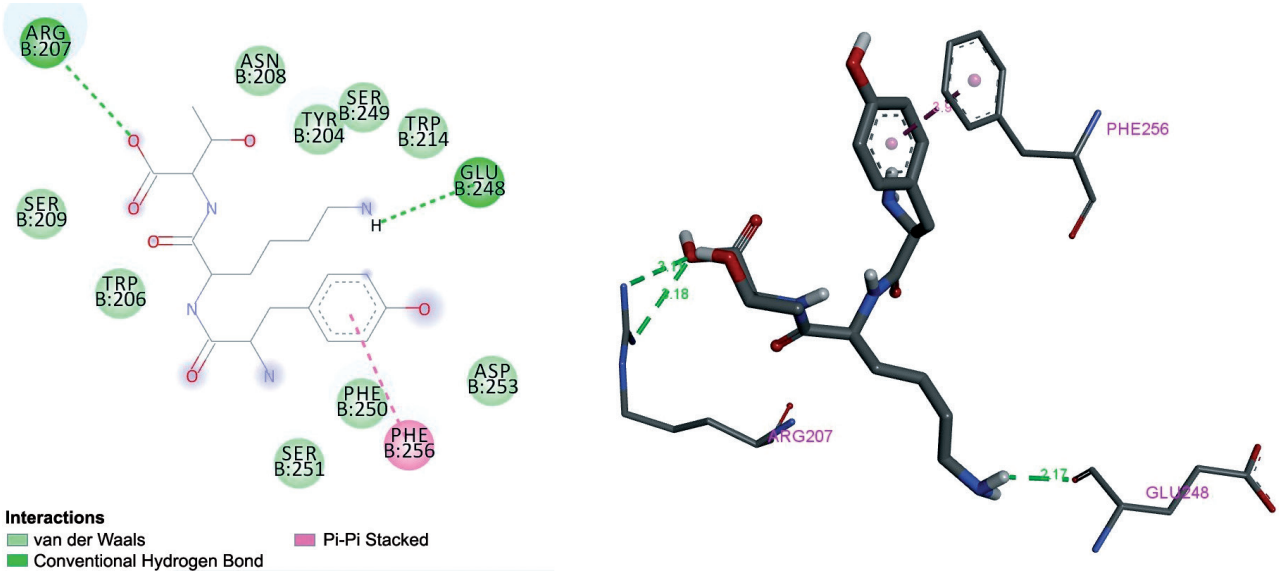

Fig. 8. Close interaction of YKT tripeptide with caspase-3.

tions with residues Pro-2169, Glu-2190, Leu-2192, Gly-2238, Leu-2354 and Phe-2358 in mTOR. In docking studies reported in the literature for Akt1, it was observed that the ligands described in the .pdb file and existing literature did not have as many hydrogen bonds as YKT. This study clearly demonstrates the significant contribution of hydrogen bond interactions to the binding energy. Notably, the observed interaction profile of YKT resembled that of the ligand in 4EJN (22). The 0R4 ligand is known to form hydrogen bonds with Asn-54 and Trp-80, while additional literature reports indicate that kaempferol also forms hydrogen bonds with Asn-54 (23). Examination of these residues revealed that YKT indeed formed hydrogen bonds with these residues. It was determined that residues Leu-210, Leu-264 and Ile-84, involved in pi interactions with the 0R4 ligand,

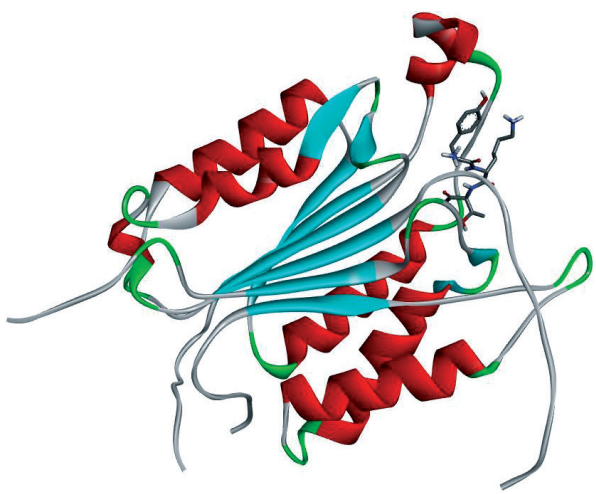

Fig. 9. YKT tripeptide at caspase-8 active site.

also engaged in pi interactions and vdW interactions with YKT tripeptide. YKT formed vdW interactions with Asn-53, Val-271 and Thr-291 residues such as 0R4 (22). In the third docking study, examining the interactions of the reference compound myricetin with phosphatidylinositol 3-kinase, it was observed that YKT, like myricetin, established hydrogen bonds with residues Val-882 and Lys-833. This study also revealed unfavorable negative-negative and vdW interactions between YKT and other residues binding with myricetin through hydrogen bonds, particularly Asp-841 and Tyr-867. Also, like myricetin, the YKT tripeptide engaged in pi interactions with residues Met-953 and Ile-881 (24). Notably, similar residues have been found to be involved in the interaction profiles observed in molecular docking studies of *Lonchocarpus* flavonoids associated with cancer (25). Other docking analyzes in this study were focused on interactions with caspases. While numerous caspase-3-docking studies have been described in the literature, the comparison of the findings obtained in the docking of YKT with those observed in the study using quercetin and rutin molecules reveals that YKT exhibited a more similar interaction profile to the rutin molecule (26). In the caspase-3-docking study carried out by Herawati et al., residues such as Trp-206, Arg-207, Asn-208, Ser-209 and Ser-249 were found to be prominent. It was determined that YKT also engages in hydrogen bonds and vdw interactions with these compounds (27). Furthermore, Ac-IETD-aldehyde, the inhibitor of caspase-8, was observed to form salt bridges with Arg-413 and Arg-260 (28). Arg413 stands as a residue playing a crucial role in connecting the main chain and peptide inhibitor chain. In a published study, it was reported that Risin-A and Arg-413 can enter the binding site of caspase-8 because the hydrogen bond formed between them is able to break the salt bridge between Arg413 and an aspartate residue (27). Based on this study, it can be inferred that YKT may readily access the binding site of

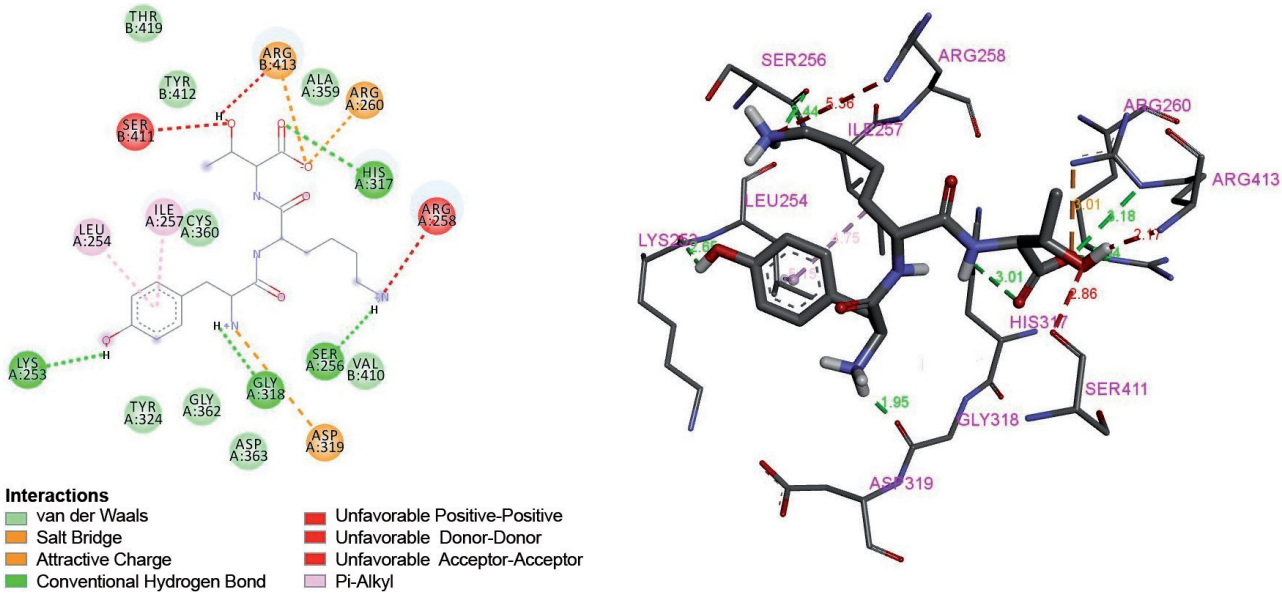

Fig. 10. Close interaction of YKT tripeptide with caspase-8.

caspase-8. After conducting the docking studies of YKT, experimental studies were performed to explore the cytotoxicity of this tripeptide on A549 cells . The integration of *in vitro* findings with theoretical study outcomes suggests that YKT tripeptide may activate the apoptosis pathway via caspase-3 and caspase-8. Previous studies (10, 11), conducted to demonstrate the anticancer activity of YKT molecule, revealed its cytotoxic effects on MAT-Lylu, HeLa and MCF-7 cancer cell lines. Notably, the cytotoxic effects were observed only at high concentrations (2.5 mg/ml and 5 mg/ml) in the healthy cell line BEAS-2B. Comparing our study results with these findings, it was determined that the highest cytotoxic effect was observed in MAT-Lylu cells, followed by A549 cells used in our study. This cytotoxic effect of the YKT molecule in a wide range of cell lines suggests that this tripeptide is suitable for further evaluation as a potential anticancer agent.

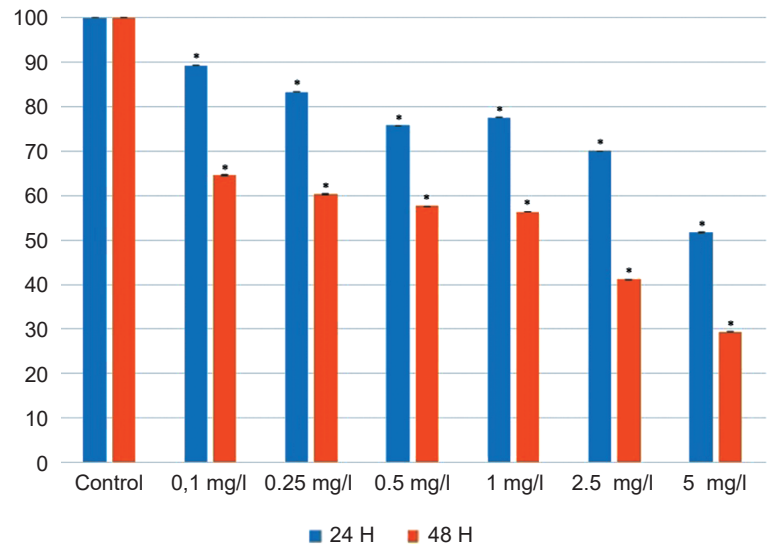

Fig. 11. Cell viability results of YKT on A549 cell lines at 24h and 48 h (* $p < 0.01$).

Conclusion

In conclusion, the effect of YKT tripeptide on human lung cancer cell line (A549) was investigated through both *in silico* and *in vitro* studies. The *in silico* docking studies were conducted with PI3K, Akt1, and mTOR, key targets in human cancer, as well as with caspase-3 and caspase-8, crucial agents in apoptosis, revealing a strong binding tendency of YKT with each receptor. Additionally, the YKT tripeptide, known for its cytotoxic effect on various cancer cell lines (MCF-7, HeLa and MAT-Lylu) demonstrated cytotoxicity against human A549 cells, representative of human lung carcinoma. The comparative analysis shows that A549 cells exhibit the most cytotoxic effect after MAT-Lylu compared with other cell lines. However, further molecular investigation is required to elucidate the underlying mechanisms of these biological activities.

References

1. Gülçin I. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids 2007; 32: 431–48.

2. Chen H-M, Muramoto K, Yamauchi F, Nokihara K. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. J Agricult Food Chem 1996; 44 (9): 2619–2623.

3. Szende B, Szökán G, Tyihá E, Pál K, Gáborjányi R, Almás M et al. Antitumor effect of lysine-isopeptides. Cancer Cell Internat 2002; 2: 1–7.

4. Tankersley Jr RW. Amino acid requirements of herpes simplex virus in human cells. J Bacteriol 1964; 87 (3): 609–613.

5. Wang C, Dong S, Zhang L, Zhao Y, Huang L, Gong X et al. Cell surface binding, uptaking and anticancer activity of L-K6, a lysine/leucine-rich peptide, on human breast cancer MCF-7 cells. Scientific Reports 2017; 7 (1): 8293.

6. Duval D, Demangel C, Munier-Jolain K, Miossec S, Geahel I. Factors controlling cell proliferation and antibody production in mouse hybridoma cells: I. Influence of the amino acid supply. Biotechnology Bioengineering 1991; 38 (6): 561–570.

7. Li P, Yin Y-L, Li D, Kim SW, Wu G. Amino acids and immune function. Brit J Nutrition 2007; 98 (2): 237–252.

8. Chen Y-F, Shiau A-L, Chang S-J, Fan N-S, Wang C-T, Wu C-L et al. One-dimensional poly (L-lysine)-block-poly (L-threonine) assemblies exhibit potent anticancer activity by enhancing membranolysis. Acta Biomaterial 2017; 55: 283–295.

9. Chalamaiah M, Yu W, Wu J. Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. Food Chem 2018; 245: 205–222.

10. Bicak B, Budama-Kilinc Y, Kecel-Gunduz S, Zorlud T, Akman G. Peptide based nano-drug candidate for cancer treatment: Preparation, characterization, in vitro and in silico evaluation. J Mol Struct 2021; 1240: 130573.

11. Bicak B, Kecel Gunduz S, Budama Kilinc Y, Imhof P, Gok B, Akman G et al. Structural, spectroscopic, in silico, in vitro and DNA binding evaluations of tyrosyl-lysyl-threonine. J Biomol Struct Dynamics 2021: 1–17.

12. Franks LM, Teich NM. Introduction to the cellular and molecular biology of cancer: Oxford University Press, USA; 1997.

13. Falasca M. PI3K/Akt signalling pathway specific inhibitors: a novel strategy to sensitize cancer cells to anti-cancer drugs. Curr Pharm Design 2010; 16 (12): 1410–1416.

14. Markman B, Dienstmann R, Tabernero J. Targeting the PI3K/Akt/mTOR pathway–beyond rapalogs. Oncotarget 2010; 1 (7): 530.

15. Yadav RP, Chatterjee S, Chatterjee A, Pal DK, Ghosh S, Acharya K et al. Identification of novel mycocompounds as inhibitors of PI3K/AKT/mTOR pathway against RCC. J Receptors Signal Transduction 2022; 42 (6): 599–607.

16. Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. Frontiers in oncology 2014; 4: 64.

17. Canan K, Çalişkan Y, Yönden Z. Apoptozis. Mustafa Kemal Üniversitesi Tıp Dergisi 2012; 3 (11): 26–37.

18. Frisch M, Trucks G, Schlegel H, Scuseria G, Robb M, Cheeseman J et al. GAUSSIAN09. Gaussian Inc., Wallingford, CT, USA 2009.

19. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Computat Chem 2010; 31 (2): 455–461.

20. Biovia DS. Discovery studio visualizer. San Diego, CA, USA 2017; 936.

21. Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ, Pavletich NP. mTOR kinase structure, mechanism and regulation. Nature 2013; 497 (7448): 217–223.

22. Ashwell MA, Lapierre J-M, Brassard C, Bresciano K, Bull C, Cornell-Kennon S et al. Discovery and optimization of a series of 3-(3-Phenyl-3 H-imidazo [4, 5-b] pyridin-2-yl) pyridin-2-amines: orally bioavailable, selective, and potent ATP-independent Akt inhibitors. J Med Chem 2012; 55 (11): 5291–5310.

23. Li Z-H, Yu D, Huang N-N, Wu J-K, Du X-W, Wang X-J. Immunoregulatory mechanism studies of ginseng leaves on lung cancer based on network pharmacology and molecular docking. Sci Reports 2021; 11 (1): 18201.

24. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP et al. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. Mol Cell 2000; 6 (4): 909–919.

25. Cassidy CE, Setzer WN. Cancer-relevant biochemical targets of cytotoxic Lonchocarpus flavonoids: a molecular docking analysis. J Mol Modeling 2010; 16: 311–326.

26. Khan MA, Singh R, Siddiqui S, Ahmad I, Ahmad R, Upadhyay S et al. Anticancer potential of Phoenix dactylifera L. seed extract in human cancer cells and pro-apoptotic effects mediated through caspase-3 dependent pathway in human breast cancer MDA-MB-231 cells: An in vitro and in silico investigation. BMC Complementary Med Ther 2022; 22 (1): 1–19.

27. Herawati I, Lesmana R, Levita J, Subarnas A. Molecular Interaction Of Ricin-A With Caspase-3, Caspase-8, Caspase-9 And Autophagy-Related Gene5 (ATG5) To Understand Its Role As Anticancer Agent, Rasayan J Chem 2021; 14 (3): 1790–1794.

28. Watt W, Koeplinger KA, Mildner AM, Heinrikson RL, Tomasselli AG, Watenpaugh KD. The atomic-resolution structure of human caspase-8, a key activator of apoptosis. Structure 1999; 7 (9): 1135–1143.

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