The main objective of the present study is to investigate the molecular structure and DNA binding interaction of the tyrosyl-lysyl-threonine (YKT) tripeptide, which has anticancer, antioxidant and analgesic properties, using various in silico (MD, QM, molecular docking), spectroscopic (UV, FT-IR, FTIR-ATR, Raman, gel electrophoresis) and in vitro (MCF-7 and HeLa cancer cell lines and BEAS-2B cell line) methods. The optimized geometry, vibrational wavenumbers, molecular electrostatic potential (MEP), natural bond orbital (NBO) and HOMO-LUMO (highest occupied molecular orbital-lowest unoccupied molecular orbital) calculations were carried out with Density Functional Theory (DFT) using B3LYP/6-311+G(d,p) basis set to indicate conformational, vibrational and intramolecular charge transfer characteristics. The assignment of all fundamental theoretical vibration wavenumbers was performed using potential energy distribution analysis (PED). DNA is a significant pharmacological target of drugs in several diseases such as cancer. For this reason, molecular docking calculation was used to elucidate the binding and interaction between YKT tripeptide and DNA at the atomic level. Also, the dynamic behaviors of YKT and DNA was examined using MD simulations. Besides, the interaction of YKT with DNA was experimentally examined by UV titration method and agarose gel electrophoresis method. Experimental results showed that YKT was intercalatively and electrostatically bound to CT-DNA (Calf thymus DNA) and cleavage pBR322 DNA in the presence of H2O2. The pharmacokinetic profile of YKT was also obtained. Cytotoxic effect of YKT was evaluated on MCF-7, HeLa and BEAS-2B cell lines. Hence, these studies about YKT tripeptide may pave the way for the development of various cancer drugs.

1. Introduction
Cancer is one of the major health problems and leading cause of death worldwide, especially in developed countries (Ke & Shen, 2017; Torre et al., 2015). In the cancer treatment, chemotherapy, which is the most preferred treatment method among many treatment methods such as surgery, chemotherapy, radiotherapy, has many side effects that
cannot be ignored and cancer cells may develop resistance to drugs (Longley & Johnston, 2005; Mansoori et al., 2017). Therefore, research is focusing on some new approaches for cancer treatments, such as the use of peptides.

Due to binding to different receptors and also being part of some biochemical pathways, peptides may function as potential diagnostic tools and biomarkers of cancer (Thundimadathil, 2012; Xiao et al., 2015). Therapeutic peptides, which are also approved by the FDA, are used effectively in different disease areas, from cancers to infectious diseases (Fosgerau & Hoffmann, 2015). Multifunctional peptides are also important candidates for drug development studies because they can be used to target multiple symptoms (Agie et al., 2017; Agie & Danquah, 2011; Fosgerau & Hoffmann, 2015; Hancock & Sahil, 2006; Usmani et al., 2017). Together with the modifiable properties of the peptides, strong binding affinity, low immunogenicity, low production cost, high stabilization and safety aspects are important criteria for being a drug molecule (Agie et al., 2018; Craik et al., 2013; Fosgerau & Hoffmann, 2015; Sun, 2013). Moreover, peptides have many benefits such as small size, ability to penetrate the tumor and biocompatibility (Borghouts et al., 2005; Thayer, 2011; Thundimadathil, 2012). They can be used as drug carriers, targeting ligands and protease-responsive substrates in drug delivery (Zhang et al., 2012). Peptides do not accumulate in cells and tissues in mammalian systems, which results in reduced systemic toxicity (Danquah & Agie, 2012). More research on peptides will be very important for cancer treatment in the near future especially peptide-based drugs and vaccines in order to kill tumor cells (Marques et al., 2017; Xiao et al., 2015).

Discovery and characterization of such bioactive peptides is vital to developing tumor therapeutics with more efficient and effective anticancer activities.

YKT (Tyr-Lys-Thr) is a tripeptide that consists of the amino acids tyrosine, lysine and threonine. Tyrosine is a non-essential and antioxidant amino acid with a polar side chain, it is also a neurotransmitter precursor that increases levels of plasma neurotransmitters such as dopamine and noradrenephrine (Chen et al., 1996; Gülçin, 2007; Lieberman et al., 1985; Rasmussen et al., 1983). Lysine amino acid is one of the positively charged and an essential amino acid with antioxidant properties (Chen et al., 1996). It has antiviral effect and features inhibition of the herpes simplex virus (HSV) which causes herpes on the lip (Tankersley, 1964). In addition, poly-lysine structures show antimicrobial and antitumor properties (El-Sersy et al., 2012). Threonine amino acid which plays a role in inhibiting apoptosis (Li et al., 2007) is an essential amino acid included in the class of polar amino acids. It is also effective in the synthesis of mucin and in maintaining the integrity of the intestinal barrier (Bertolo et al., 1998; Wang et al., 2009). Peptides containing threonine have antibacterial properties (Simic et al., 2001) and also support cardiovascular, hepatic, central nervous and immune system functions. It is necessary for the synthesis of glycine and serine amino acids that are required in the production of collagen, elastin and muscle tissue (Bala et al., 2016). This amino acid helps the body maintain protein balance and normal growth and is found in large quantities in most new-born babies. Tyr-Lys dipeptide found in the YKT (Tyr-Lys-Thr) tripeptide which isolated from bovine as1-casein induced necrosis toward various kinds of animal lymphocytes including human leukemic T and B cell lines (Otani & Suzuki, 2003; Sah et al., 2015). The Tyr-Lys dipeptide has also a feature that produces significant and long-term analgesic activity (Chen et al., 1998). Despite all the positive effects of amino acids in YKT tripeptide structure, it has been associated with prostate cancer etiology with Caprolactam and L-Phosphatidic acid (Wiklund, 2013).

In this study, we evaluate the structure of YKT and its potential to be anti-cancer. The molecular structure information of YKT was obtained such as the optimized geometry, vibrational frequencies, HOMO-LUMO, MEP and NBO analyses. Theoretical studies were supported with spectroscopic studies (FT-IR, FTIR-ATR, Raman, UV-Vis). For the experimental determination of the mechanism of interaction of YKT with DNA, which is an important pharmacological target of drugs (Alraqa et al., 2021), DNA binding and cleavage assays were carried out and evaluated together with theoretical studies (such as molecular docking, molecular simulations). Also, the cytotoxic effect of YKT was investigated by *in vitro* studies on different cell lines (MCF-7, HeLa and BEAS-2B cell lines). Pharmacokinetic information of YKT was also obtained with ADME study.

2. Materials and methods

2.1. Materials

The YKT tripeptide (C₉H₁₅N₄O₆), which has relative molecular mass 410.47 g/mol and Cas no 155943-09-2 was purchased from Bachem. Calf thymus DNA (CT-DNA) and ethidium bromide were purchased from Sigma Aldrich. Tris base, Ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), hydrochloric acid (HCl), sodium hydroxide (NaOH) were from Merck Millipore. PBR322 Plasmid DNA, DNA ladder and EcoRI enzyme were purchased from Thermo scientific. RPMI-1640 Medium, Fetal Bovine Serum, L-glutamin were obtained from Gibco. DMEM, dexomesetone and MTT were purchased from Sigma–Aldrich. Penicillin and streptomycin were obtained from I.E. Ulagay. DMSO was purchased from Merck.

2.2. FT-IR, FTIR-ATR, and Raman analysis

IR spectra using the KBr disk technique and attenuated total reflection (ATR) mode of YKT tripeptide were recorded on a Jasco300E FT-IR Spectrometer with 4000–400 cm⁻¹ spectral region with 2 cm⁻¹ resolutions. The micro-Raman spectrum of the tripeptide was recorded at room temperature under 20x Olympus objective microscope on a Jasco NRS-3100 micro-Raman spectrometer. To filter the Rayleigh scattering, one notch filter was preferred. Using the silicon phonon mode at 520 cm⁻¹, the calibration of the spectrometer was provided. The 532.19 nm (with 1200 lines/mm grating) solid state laser with 33.9 mW laser power was used as an excitation source for the ranges of 3677–2235 cm⁻¹ and 1885–200 cm⁻¹. The exposure time was taken as 1 s and 50 spectra were accumulated.
During the measurement, the spectral slit widths (0.2 × 6 mm) are preferred on each spectrum.

In addition, the software package GRAMS/AI 7.02 (Thermo Electron Corporation), which was used for band component analysis, preferred for visualization, baseline correction, smoothing and fitting procedures of the spectra.

2.3. UV-Vis and HOMO-LUMO analysis

The UV-Vis absorption spectra of the YKT were analyzed by Shimadzu UV 1280 UV Vis spectrometer in the spectral region of 200–700 nm. Absorption spectra were obtained for dissolved YKT in water at room temperature. Theoretical studies were performed within the framework of TD-DFT (Zhang & Musgrave, 2007) calculations with the B3LYP/6-311++G (d, p) method using the Gaussian09 program package. As a result of these studies, excitation energies, absorbance, and oscillator power (f) for the YKT for the optimized geometry was obtained. Using the TD-DFT/B3LYP method and 6-311++G (d, p) basis set, HOMO, LUMO and HOMO-LUMO gap (ΔE) of the YKT tripeptide were calculated. In addition, the ionization potential (I), the electron affinity (A), the absolute electronegativity (χ), softness (S) and the absolute hardness (η) values were obtained.

2.4. MEP analysis

Molecular electrostatic potential (MEP) simultaneously shows the shape, size, and electrostatic potential of the molecule in terms of color assessment. MEP is an important tool used in the interpretation and prediction of chemical reactivity as well as providing information about the charge distribution of the molecule. It is preferred to show the positive, negative and neutral electrostatic potential regions, to identify the molecular structure and to obtain information on hydrogen bonding interactions. In MEP maps, the red color indicates the electrophilic part, i.e. the maximum negative zone, while the blue color shows the nucleophilic part, the maximum positive zone (Govindarajan et al., 2012; Kuruvilla et al., 2018; Raja et al., 2017; Saranya et al., 2018). The molecule is polarized as the differences between the red and blue colors increase. The fact that the surface is in lighter shades shows that the molecule is not polar. Different values of the electrostatic potential on the surface are shown in different colors. The potential increases as red < orange < yellow < green < blue.

2.5. Natural bond orbital (NBO) analysis

In computational studies, localized (natural) orbitals are used to calculate the distribution of electron density in atoms and bonds between atoms. The natural bond orbital is a bond orbital calculated with the highest electron density and gives the information that is important in molecular studies such as charge, bond type and interactions, intramolecular and intermolecular binding, hybridization and charge transfer (Chaitanya, 2012; Glendening et al., 2012; Weinhold & Landis, 2005). For each donor natural bond orbital (i) and acceptor natural bond orbital (j), the stabilization energy E (2) associated with the i-j delocalization is given as follows.

\[ E(2) = \Delta E_{ij} = q_i \frac{F(i, j)^2}{\varepsilon_{ij} - \varepsilon_i} \]  

(1)

Here, \( q_i \) defines donor orbital occupancy, diagonal elements \( \varepsilon_i \) and \( \varepsilon_{ij} \) orbital energies and \( F(i, j) \) non-diagonal NBO Fock matrix element.

2.6. DNA binding assay

UV-Vis absorption titration method was used in order to investigate the interaction between YKT tripeptide and CT-DNA. Experiment was conducted at room temperature, and Tris-HCl/NaCl buffer (pH 7.2) was used. The UV-Vis absorbance of the CT-DNA solution in the Tris-HCl/NaCl buffer gives the 1.8–1.9 ratio, indicating that DNA does not contain protein (Marmur, 1961).

The experiment was carried out by keeping the YKT tripeptide concentration (30 μM) constant and adding increasing amounts of CT-DNA concentration between 0 and 120 μM. Absorption spectra were recorded after incubation of solutions with DNA for 5 min at room temperature. The percentage ratios of changes in absorbance intensity were calculated with Equation (2).

\[ \%H = [(A_i - A_f)/A_i] \times 100 \]  

(2)

In this equation, \( A_i \) indicates the free absorbance intensity of the compound, and \( A_f \) indicates the absorbance intensity of the compound after adding DNA at the maximum concentration. The intrinsic binding constant (Kb) used to investigate the binding strength of the YKT tripeptide to DNA was calculated using Equation (3).

\[ [\text{DNA}]/(\varepsilon_A - \varepsilon_f) = [\text{DNA}]/(\varepsilon_A - \varepsilon_f) + 1/K_b(\varepsilon_A - \varepsilon_f) \]  

(3)

In the formula, \( \varepsilon_A \) extinction coefficient in the measured concentration, \( \varepsilon_f \) Extinction coefficient after binding of all compounds to DNA and \( \varepsilon_f \) is the extinction coefficient of the free compound. In the [DNA]/(\varepsilon_A - \varepsilon_f) versus [DNA] plot, the ratio of the slope to the point of intersection gives \( K_b \).

2.7. DNA cleavage

The agarose gel electrophoresis method was used in order to monitor the DNA cleavage ability of YKT. The DNA cleavage properties of YKT tripeptide were examined by using pBR322 DNA both in the presence (oxidative cleavage) and in the absence (hydrolytic cleavage) of the oxidizing agent (H2O2). The positive control was the EcoRI restriction enzyme, and the marker was 1 kb DNA ladder. The experiment was performed by adding pBR322 DNA (0.1 μg/μL), YKT tripeptide and water/H2O2 in Tris-HCl buffer (10 M, pH: 7.2). The incubation of the solutions was performed for 3 h and 37 °C. After then, samples were run in TBE (Tris-Boric acid-EDTA, pH: 8.0) buffer at 60 V for 1 h. Finally, the bands were visualized with UV light.
2.8. Molecular docking analysis

Molecular docking method, which has an important place in the discovery of molecules with the potential of being a drug, allows to reveal the lowest energy binding score between ligand and receptor complex structure by molecular interactions. Tyr-Lys-Thr tripeptide was optimized by Gaussian09 package program with B3LYP/6-311++G(d,p) basis set and removed its non-polar hydrogens by AutoDockTools 1.5.6 before molecular docking analysis was carried out. The structure of a B-DNA Dodecamer (PDB Code: 1BNA) (Drew et al., 1981) having 1.9 Å resolution was selected as receptor and downloaded from protein data bank. All water molecules in pdb file were removed. Polar hydrogen atoms were added to receptor using AutoDockTools 1.5.6 software for docking study. pdb files of ligand and receptor were converted pdbqt file. Grid box was adjusted as 40x40x40 unit with 0.375 Å grid spacing. Molecular docking study was realized by AutoDock Vina 1.1.2 (Trott & Olson, 2010) and obtained binding affinities and RMSD values. The receptor-ligand interactions were visualized by PyMOL (Schrödinger, 2017) and Discovery Studio (Biovia, 2017) software programs with the obtained interaction informations.

2.9. ADME analysis

For the ADME analysis of the YKT tripeptide, the online server Swissadme (Daina et al., 2017), has been used to determine pharmacokinetic profiles.

2.10. Molecular dynamics (MD) simulation analysis

The structure of YKT tripeptide was optimized at DFT/B3LYP level of theory with the 6-311++G(d,p) basis set by the Gaussian 09 software program (Frisch et al., 2009). The structure of the peptide-complex was obtained by molecular docking (see below) of the peptide to a B-DNA. In addition to MD simulations of the complex, we performed an MD simulation of the uncomplexed B-DNA for comparison. The molecular dynamics simulations were carried out in a cubic (TIP3P) water box of 7.4837 nm side length for the DNA and peptide-DNA complex, and 4.433 nm side length for the peptide. Additional Na⁺ and Cl⁻ ions were added so as to neutralize the system and to represent a salt concentration of 150 mmol/liter. We used the Amber14SB force field (Maier et al., 2015) for the peptide and the parmBSC1 force field (Ivani et al., 2016) for the DNA and the GRoningen Machine for Chemical Simulations (GROMACS) software (Van Der Spoel et al., 2005). For all systems, prior to the MD production runs, energy minimization calculations were carried out by using the Steepest Descent method. 20 ps of equilibration in an NVT ensemble, for the solvated system with restraints on the solute, were carried out, followed by 200 ns production run, also in the NVT ensemble. For the free B-DNA and the DNA-YKT complex, three individual such production runs each were performed, started from the same equilibrated structure for DNA and complex, respectively, but with different initial velocities. The V-rescale thermostat (Bussi et al., 2007) has been employed for temperature control. Motion of hydrogen atoms was constrained using the LINCS algorithm (Hess et al., 1997) and the time step for integration was 2 fs.

The DNA conformations were analyzed in terms of base pair and helical parameters using the Curves + programme (Lavery et al., 2009). Analysis of the conformational dynamics of the peptide in terms of its backbone torsion angles, was performed using the tools implemented in GROMACS. Hydrogen bond analyses were performed using the corresponding plugin in VMD and are based on the geometric criteria of 3.5 Angstrom maximal distance between hydrogen-bond donor and acceptor atom and the Donor-hydrogen atom– acceptor angle deviating from linearity by not more than 45°. Obtained graphics were plotted by Xmgrace plotting tool (Turner, 2005) and matplotlib in python (Hunter, 2007). Images of conformational changes i.e. snapshots of the MD simulations were obtained with VMD program (Humphrey et al., 1996).

2.11. Cell cultures and cytotoxicity evaluation with MTT assay

Human breast cancer cell line MCF-7, human cervical cancer cell line HeLa and human normal bronchial epithelial cell line BEAS-2B were used for in vitro cytotoxicity experiments. HeLa and MCF-7 cell lines were maintained in DMEM (Sigma) and BEAS-2B cell line maintained in RPMI-1640 (Gibco), containing penicillin (100 IU/mL, I.E. Ulagay), streptomycin (100 μg/mL, I.E. Ulagay) and 10% fetal bovine serum (FBS, Gibco Lab). All cell lines incubated in a humidified atmosphere containing 5% CO₂ at 37°C. HeLa and MCF-7 cell lines were seeded at density of 1 × 10⁴ per well (Ari et al., 2014) and BEAS-2B cell line was seeded at density of 5 × 10³ per well in a 96-well cell culture plate for the MTT assay.

Cellular viability of different concentrations (0.1, 0.25, 0.5, 1, 2.5 and 5 mg/mL) for 24 and 48 h was evaluated with MTT assay using Equation (4). Therefore, MTT (Sigma) was dissolved in PBS (5 mg/mL, Gibco Lab) and added 40 μL to the each well. Cells were incubated for 4 h at 37°C. 160 μL DMSO (Merck) added to wells and incubated overnight. The measurement was performed using Elisa Reader at 450–690 nm (BioTek, ELx800). The results are expressed relative to the control value.

\[
\%\textit{Viability} = \frac{\text{absorbance value of the sample}}{\text{absorbance value of the control}} \times 100 \tag{4}
\]

2.12. Statistical analysis

To identify significant differences of the cytotoxicity experiments, one-way ANOVA test were used to analyze experimental groups followed by post-hoc Dunnett’s multiple comparison. A P value of <0.05 was considered as statistically significant. All calculations were performed using GraphPad Prism software (Version 7.0, GraphPad Software, Inc., USA).
3. Results and discussion

3.1. Spectroscopy analysis results

In this study, the fundamental vibrational wavenumbers that characterize YKT were determined using both spectroscopic (FT-IR, FTIR-ATR, Raman) and theoretical calculation methods with DFT-RB3LYP/6-311++G(d,p) basis set. First the theoretical wavenumbers were scaled (Balci & Akyuz, 2008; Merrick et al., 2007), and all assignments were made considering the Gar2PED program and potential energy distribution. The assignments of the absorption bands of the YKT tripeptide and the observed FT-IR, FTIR-ATR and Raman spectra were given at Table S1. The recorded FT-IR and FTIR-ATR and Raman spectra of YKT were visualized comparatively in the range of 3300–2600 cm⁻¹, 2000–1000 cm⁻¹ and 1000–400 cm⁻¹ as given in Figure 1.

3.1.1. PED assignment for Tyr-Lys-Thr tripeptide

3.1.1.1. Assigned wavenumbers of Tyr. The ring CH stretching belonging to the benzene ring found in the tyrosine amino acid were recorded in the spectra 3072 cm⁻¹ (FTIR) and 3066 cm⁻¹, 2987 cm⁻¹ (FTIR-ATR) and 3062,3043,3020 cm⁻¹ (Raman) and were found to be compatible with the literature values 3071,3047,3019 cm⁻¹ (FTIR) and 3060,3036,3026 cm⁻¹ (Raman) (Kecel et al., 2011) corresponding to these values (cf. Table S1). Intra-ring CC bond stretching vibrations were recorded at 1615 cm⁻¹ (FTIR-ATR), 1613 cm⁻¹ (FTIR) and 1617 and 1597 cm⁻¹ (Raman), respectively, and these values are compatible with the values of 1623,1594 cm⁻¹ (FTIR) and 1612,1594 cm⁻¹ (Raman) in the literature (cf. Table S1). The wavenumber value calculated as 1450 cm⁻¹ for HCH scissoring motion was observed on the spectra in 1445 cm⁻¹ (FTIR), 1448 cm⁻¹ (FTIR-ATR) and 1447 cm⁻¹ (Raman), respectively. Also, intra-ring CC bond stretching correspond to the appropriate literature values (Kecel et al., 2011) was determined as 1417, 1326, 839 cm⁻¹ (FTIR-ATR), 1424 cm⁻¹ (Raman). The angle bending motion, calculated at 1390 cm⁻¹ and observed at 1398 cm⁻¹ (FTIR), 1395 cm⁻¹ (FTIR-ATR) and 1408 cm⁻¹ (Raman), was assigned as NCH angle bending. The peak, which is assigned as CCH wagging motion and observed in ATR at 1288 cm⁻¹, was calculated at 1288 cm⁻¹. C-O bond stretching of tyrosine was calculated at 1250 cm⁻¹ and observed at 1249 cm⁻¹ in all three spectra (FTIR, ATR and Raman). The wavenumbers assigned as the C-N bond stretching was observed in the FTIR, ATR and Raman spectra, respectively, in 1107 cm⁻¹, 1105 cm⁻¹ and 1104 cm⁻¹, and is in agreement with the value at 1093 cm⁻¹ in the literature (Kecel et al., 2011). CCCH ring torsion motion was recorded and assigned at 925, 827, 804 cm⁻¹ (FTIR), 926, 828, 802 cm⁻¹ (FTIR-ATR) and 928, 832 cm⁻¹ (Raman). C-C ring angle bending, defined in the literature 767 cm⁻¹ and 763 cm⁻¹ (Kecel et al., 2011), was observed experimentally at 760, 646 cm⁻¹ (FTIR-ATR) and 649 cm⁻¹ (Raman) and theoretically calculated at 767 and 640 cm⁻¹, respectively. C-C-C-C torsion motion was calculated at 706 and 506 cm⁻¹ and observed at 692, 509 cm⁻¹ (FTIR) and 708, 494 cm⁻¹ (FTIR-ATR) in accordance with the literature (Kecel et al., 2011).

3.1.1.2. Assigned wavenumbers of Lys. Asymmetric CH bond stretching of the lysine amino acid was observed on the spectra in the range of 2930–2871 cm⁻¹ and were placed corresponding to the appropriate values in the literature (cf. Table S1). Symmetric CH bond stretching vibration was assigned to 2923,2901 cm⁻¹ and 2930 cm⁻¹ at the FTIR-ATR and Raman spectra, respectively. The angle bending vibration defined as the HNH scissoring motion was defined at 1635 cm⁻¹ in the FTIR-ATR in accordance with 1638 cm⁻¹ values in the literature (Marchewka et al., 2003; Paiva et al., 2017). HCH scissoring motion was recorded and assigned to 1457 cm⁻¹ (FTIR), 1489, 1456, 1451 cm⁻¹ (FTIR-ATR) and 1468 cm⁻¹ (Raman). The angle bending vibration, observed in 1368;1345 cm⁻¹ (FTIR) and 1366;1344 cm⁻¹ (Raman) in the literature (Paiva et al., 2017), was assigned as the CCH wagging motion and observed in the FTIR spectrum at 1342 cm⁻¹, in the FTIR-ATR spectrum at 1373; 1349 cm⁻¹, in the Raman spectrum at 1345 cm⁻¹. The peak observed in the FTIR and Raman spectra at 1321 and 1320 cm⁻¹ in the literature was observed in the ATR spectrum at 1320 cm⁻¹ and assigned as a CCH twisting motion, shown in Table S1. NCH angle bending vibration was calculated as 1297 cm⁻¹ and observed at 1296 cm⁻¹ in the FTIR-ATR spectrum in accordance with the literature. CN vibration with CC bond stretching contribution was observed in both ATR and Raman spectra at 1066 cm⁻¹ and calculated at 1065 cm⁻¹ (cf. Table S1). C-C bond stretching was observed at 1049 cm⁻¹ (FTIR), 1056 cm⁻¹ (FTIR-ATR and Raman) and was found to be in agreement with 1052 cm⁻¹ (FTIR) and 1055 cm⁻¹ (Raman) wave numbers in the literature (Paiva et al., 2017). Peaks dominated
by C-C and C-N vibrations were observed at 1028 cm\(^{-1}\) (FTIR-ATR) and 1029 cm\(^{-1}\) (Raman), similar to the literature values (Paiva et al., 2017). C-C-H angle bending vibration was observed at 885 cm\(^{-1}\) in FTIR spectrum and 890 cm\(^{-1}\) in ATR spectrum, and their assignments were made in accordance with the calculated 889 cm\(^{-1}\). C-C-N angle bending vibration of lysine was observed in FTIR and ATR at 425 and 429 cm\(^{-1}\), respectively, and was calculated as 429 cm\(^{-1}\).

3.1.1.4. PED assignment for amide group. Tyrosyl-lysyl-threonine (YKT) tripeptide is composed of three amino acids and two peptide bonds. The peptide bonds are formed by the release of one mol of water between the hydroxyl group of carboxyl group of the previous amino acid and the H atom of amino group of the next amino acid. The peptide group is also defined as the CONH atom group that forms the peptide bond. This group also has its own three types of vibration movements. These vibration movements are named as amide I, II and III. Amide-I band is used for determining protein secondary structure and substantially associated with C=O stretching mode. Amide-I, amide-II, amide-III vibration modes of proteins are observed around 1700 cm\(^{-1}\), 1500 cm\(^{-1}\) and 1200 cm\(^{-1}\) (Hayashi & Mukamel, 2008; Zhao & Wang, 2015).

As a result of the theoretical and spectroscopic analysis for the YKT tripeptide, amide I mode, which was theoretically calculated at 1694 cm\(^{-1}\) and represented by a 72 percent dominant C=O stretching motion of peptide group in PED analysis was observed 1698 cm\(^{-1}\) (FTIR) that assigned according to the band component analysis result as seen in Figure 2a. In the PED analysis, the second amide I mode, in which the C=O vibration was dominant with a 66 percent contribution, calculated at 1679 cm\(^{-1}\) and experimentally observed in the FTIR and FTIR-ATR spectra accordance with the
literature (Koleva et al., 2007) at 1655 cm$^{-1}$ and 1652 cm$^{-1}$, respectively was also shown in Figure 2b. The Amide II mode recorded in the FTIR and FTIR-ATR spectra in 1516 cm$^{-1}$ and 1519;1507 cm$^{-1}$, respectively and defined by the CNH angle bending vibration of the peptide group in PED analysis, was determined 1520 cm$^{-1}$ and 1507 cm$^{-1}$ as a result of the band component analysis that performed in the wavenumber range of 1545-1505 cm$^{-1}$ as seen in Figure 2c. Amide III modes, identified by CNH angle bending contribution together with CN bond stretching vibration in the peptide group, calculated at 1222 cm$^{-1}$ and 1184 cm$^{-1}$, respectively, observed at 1214 cm$^{-1}$ (FTIR), 1181 cm$^{-1}$ (FTIR-ATR) and 1221 cm$^{-1}$ (Raman), were revealed at 1225 cm$^{-1}$ at band component analysis, shown in Figure 2d.

### 3.2. UV-Vis and HOMO-LUMO analysis results

The experimentally obtained absorption wavelength $\lambda$ (nm), excitation energies $E$ (eV) and absorbance values of the YKT tripeptide dissolved in distilled water were listed in the Table 1. As stated in the literature, two peaks were observed in the experimental UV spectrum of the peptide in the 200–230 nm range and in the 240–300 nm range due to the tyrosine amino acid (see Figure S3(a)). In the literature, the peak of tyrosine at 275 nm was observed at 274.5 nm in this study and it was determined that it is in full agreement with the literature (Schmid, 2001). The theoretically obtained absorption wavelength $\lambda$ (nm), excitation energies $E$ (eV) and oscillator strengths (f) were listed together with the major contribution values of YKT tripeptide in Table 2. The theoretical and experimental wavenumbers values were given in Figure S1.

HOMO (H) and LUMO (L) provide important information about the electrical properties and chemical activity of the molecule (Shafieyoon et al., 2019). HOMO and LUMO are related to ionization potential and electron affinity (Sarac, 2017). Also, the $\Delta E$ energy difference determines the energy required to move from the most stable state to an excited state in the molecule (Tanış, 2017). Information on chemical hardness and potentials is also determined with the help of HOMO and LUMO energies. Based on the data obtained using the GaussSUM program (O’boyle et al., 2008) in Table 2, the major contribution to molecular orbitals is from HOMO-1 to LUMO transitions with 59 percent contribution. Using the TD-DFT method with the B3LYP/6-311++G (d, p) basis set (Sevvanthi et al., 2020), the HOMO-LUMO values and HOMO-LUMO energy difference of the YKT tripeptide were obtained and given in Table S2 and Figure 3. The calculated band gap ($\Delta E_{\text{HOMO-LUMO}}$) value was determined as 5.54051 eV. According to the analysis, HOMO was located on the lysine amino acid, however, LUMO was settled on the tyrosine and threonine amino acids in the YKT tripeptide. The HOMO-LUMO transition showed that the charge transfer in the molecule occurs from the lysine amino acid to

| Table 1. Experimentally obtained absorption wavelength $\lambda$ (nm), excitation energies $E$ (eV) and absorbance values of YKT tripeptide. |
|-----|-----|-----|
| E (eV) | $\lambda$ (nm) | Abs. |
| dH$_2$O | 4.5167 | 274.5 | 2.189 |

| Table 2. Calculated absorption wavelengths $\lambda$ (nm), excitation energies E (eV) and oscillator strengths (f) of YKT along with transition levels and assignments. |
|-----|-----|-----|-----|
| E (eV) | $\lambda$ (nm) | f | Major contribution |
| Gas | 4.8648 | 254.8598 | 0.0283 | H-1$\rightarrow$L (59%) |
| | 5.0221 | 246.8772 | 0.0094 | H-1$\rightarrow$L + 1 (23%) |
| | 5.1038 | 242.9253 | 0.0044 | H-1$\rightarrow$L + 2 (24%) |

Figure 3. Patterns of principle frontier molecular orbitals of YKT tripeptide obtained with TD-DFT/B3LYP/6-311++G (d,p).
tyrosine and threonine amino acids in the YKT. Since DNA is generally electron donor, the molecular structure with which it interacts is an electron acceptor. The high HOMO energy of the DNA molecule and the low LUMO energy of the molecule it interacts with is more advantageous for the interaction between them (Sama et al., 2019). So, the tyrosine and threonine amino acids in the peptide structure, which are the LUMO region of the molecule, were prominent places in MD and docking studies. In the realized MD and DNA-peptide docking studies, especially, LUMO areas of YKT were important interaction places. In addition, calculated values such as ionization potential, electron affinity, electronegativity, chemical hardness, and chemical softness values of YKT tripeptide were also given in the Table 3. The ionization potential and electron affinity values of YKT tripeptide were obtained as 6.23249 a.u and 0.69198 a.u, respectively. So, these values gave us that YKT is able to electron donor and electron acceptor capabilities. Looking at the MD and molecular docking study, it is possible to see that the electron donor, acceptor features of YKT and the lysine group of YKT where HOMO is localized has interactions that cannot be ignored in docking work.

### 3.3. MEP Analysis results

Map was obtained as a result of molecular electrostatic potential (MEP) calculations, as shown in Figure S2. The Table 3. The calculated values of ionization potential, electron affinity, electronegativity, chemical hardness, chemical softness and HOMO-LUMO gaps for YKT tripeptide.

| Gas         | TD-DFT/6311 + i-G(d,p) | Energy (a.u.) | Energy (eV) |
|-------------|------------------------|---------------|-------------|
| HOMO energy | $E_{\text{HOMO}}$     | -0.22904      | -6.23249    |
| LUMO energy | $E_{\text{LUMO}}$     | -0.02543      | -0.69198    |
| Ionization potential | $I = -E_{\text{HOMO}}$ | 0.22904       | 6.23249     |
| Electron affinity | $A = -E_{\text{LUMO}}$ | 0.02543       | 0.69198     |
| Electronegativity | $\chi = (I + A)/2$ | 0.12724       | 3.46238     |
| Chemical potential | $\mu = -(I + A)/2$ | -0.12724      | -3.46238    |
| Chemical hardness | $\eta = (I - A)/2$ | 0.10181       | 2.77039     |
| $\Delta E$ (gap) | $E_{\text{LUMO}} - E_{\text{HOMO}}$ | 0.20361       | 5.54051      |

The purpose of MEP analysis is to determine the electron rich and poor regions in the molecule. The MEP, which is associated with electronic density, is also a useful descriptor for determining hydrogen bond interaction regions. Different values of the electrostatic potential at the surface were represented by different colors from red to blue. Red represents the lowest electrostatic potential energy value and blue indicates the highest electrostatic potential energy value. In the mapping for YKT tripeptide, the electron rich region which shown in red color has $-0.06189$ a.u value, and the blue region which is poor by electrons, has a value of 0.06189 a.u. The poorest regions by electrons were concentrated on the OH groups in the side and carboxyl groups of the threonine amino acid and the OH group at the end of the ring group of the tyrosine amino acid. The $H_{55}$ atom in the OH group in the side chain of threonine amino acid has a value of 0.0566714 a.u. energy value, and the $H_{99}$ atom in the carboxyl group has also 0.0613608 a.u. Besides, the $H_{20}$ atom in the OH group which attached the ring in the amino acid tyrosine has 0.0593732 a.u. energy value. It was seen that the regions of red color, which are rich regions by electrons, were concentrated on the end of the side chain of lysine and on the amino group of tyrosine amino acids and on the peptide bond that connects tyrosine and lysine. The region where the electrons were most concentrated is the $N_{39}$ atom in the lysine and with $-0.0564202$ a.u. energy value. Other groups are the $N_{2}$ atom in the amino group in the tyrosine amino acid and the $O_{22}$ atom in the peptide group, and their energy values are $-0.0445616$ a.u and $-0.0402084$ a.u, respectively. In molecular docking studies, the value of the electrostatic potential of a molecule is important in the binding site, since the receptor and the ligand recognize each other from their molecular surface (Mary et al., 2015). When compared with molecular docking study, it was seen that region rich in electrons as donors and poor regions as acceptors interact with DNA.

![Figure 4](image-url)
3.4. Natural bond orbital (NBO) analysis results

With natural bond orbital analysis, important information in molecular studies such as interaction between bonds and charge transfer can be obtained (Chaitanya, 2012; Glendening et al., 2012). It is known that in aromatic ring groups, \( \pi \) orbitals and \( \sigma \) orbitals produce some strong intra-molecular interactions with the effect of the orbital overlap within themselves (Esme & Sagdic, 2017). In this study, natural bond orbital analysis for YKT (Tyr-Lys-Thr) tripeptide was performed using the DFT/B3LYP/6-311++G (d, p) basis set in the Gaussian09 package program. The donor-acceptor interactions were estimated with the second order Fock-matrix. As a result of the studies carried out, the highest stabilization energy in our molecule belonged to the transition from \( \pi^* (C_{56}-O_{58}) \) to \( \pi^* (C_{56}-O_{50}) \) anti-bond orbital and from the electron pair \( n_1 (O_{58}) \) to the \( \sigma^* (C_{56}-O_{58}) \) anti-bond orbital, with 43.57 kcal/mol and 33.62 kcal/mol perturbation energy, respectively. Another important transition belonged to the side chain of the tyrosine amino acid. This transition was from electron pair \( n_2 (O_{19}) \) to the \( \pi^* (C_{12}-O_{14}) \) anti-bond orbital and had 26.9 kcal/mol energy. In addition, it is also known that the peaks observed in the UV spectrum of YKT tripeptide between 200 and 230 nm and 274 nm are caused by \( n-\pi^* \) and \( \pi-\pi^* \) transitions in the structure (Erdik, 1993).

| Mode | Affinity (kcal/mol) | RMSD l.b. | RMSD u.b. |
|------|---------------------|-----------|-----------|
| 1    | −4.5                | 0.000     | 0.000     |
| 2    | −4.2                | 2.430     | 3.915     |
| 3    | −4.2                | 1.412     | 1.958     |
| 4    | −4.2                | 1.763     | 3.427     |
| 5    | −4.0                | 2.297     | 3.689     |
| 6    | −4.0                | 1.652     | 2.136     |
| 7    | −3.9                | 1.738     | 2.416     |
| 8    | −3.9                | 2.583     | 3.537     |
| 9    | −3.9                | 1.971     | 3.157     |

Table 4. Binding affinities and RMSD values of YKT tripeptide.

Table 5. The information of hydrogen bonds between YKT tripeptide and B-DNA Dodecamer.

| Acc-Don Bond length (Å) |
|-------------------------|
| DT7_O2P-TYR1_OH         |
| DT9_N4-TYR1_O           |
| DC9_N4-TYR1_O           |
| DG16_O6-LYS2_NZ         |
| DT8_O4-LYS2_NZ          |
| DC15_N4-THR3_N          |
| DC15_N4-THR3_O          |
| DC15_N4-THR3_O          |
| DG14_N7-THR3_O          |

3.5. DNA Binding results and discussion

DNA is one of the most important targets for drugs used in cancer therapy (Gaur & Mishra, 2013; Houk et al., 1999). It is well known that drug molecules bind to DNA in different ways (Chen et al., 2011; Novoa & Mota, 2000). UV-Vis spectroscopy is a useful technique for determining the binding mode of drugs to DNA (Shah et al., 2008). By UV-Vis analysis, binding of a drug molecule to DNA is observed as a hyperchromic or hypochromic effect with the red or blue shift. While hypochromism and redshift are associated with...
intercalation, hyperchromism and blue shift are associated with electrostatic interaction and groove binding (Arif et al., 2020). Also, in the literature (Arif et al., 2020), it was stated that $\pi-\pi^*$ interactions between a molecule and DNA are associated with hypochromism and redshift. In another literature study, it was stated that the observed bathochromic shift may occur in the absorption band derived from the $\pi-\pi$-stacking in case of a decrease in the HOMO-LUMO gap (Seo et al., 2015). In Figure 4a, the UV absorption spectra of YKT tripeptide in the absence of CT-DNA and in the presence of CT-DNA were given. Along with the increase in CT-DNA concentration, the 37% hypochromism and 2 nm redshift (bathochromic shift) at 199 nm, 90% hyperchromism and 16 nm blue shift (hypsochromic shift) were observed at 268 nm in the spectrum of YKT tripeptide. It could be stated that YKT peptide bounded to the DNA groove or intercalated into the DNA helix, due to the hypochromic effect and bathochromic shift (Shahabadi, Mohammadi, et al., 2011). Although intercalating agents do not have DNA sequence selectivity, studies have shown that they bind more to G-C rich regions (Geierstanger & Wemmer, 1995). Also, in silico molecular docking study showed that YKT tripeptide bound to the G and C bases of DNA, and the binding type could have been intercalative. Moreover, the hyperchromic effect in the DNA binding mode showed that there was an electrostatic interaction (He et al., 2017; Qiao et al., 2011; Shi et al., 2018). The DNA-double helix possesses many hydrogen bonding domains accessible in the minor and major grooves. The amine group of the YKT tripeptide could form hydrogen bonds with DNA that could contribute to hyperchromism (Liu et al., 1999; Shahabadi, Kashanian, et al., 2011). In addition, the hyperchromic effect may be due to the electrostatic interaction between the negatively charged phosphate backbone around the CT-DNA (Xiao & Zhan, 2002). According to the in silico molecular docking study, it was theoretically determined that the YKT tripeptide DNA interacted electrostatically with phosphate groups. The calculated $K_b$ values for the YKT tripeptide were $3.3 \times 10^4 \text{M}^{-1}$ for the hypochromic effect, and $1.6 \times 10^5 \text{M}^{-1}$ for the hyperchromic effect. From these results obtained, it was understood that YKT tripeptide had a greater hyperchromic effect on the binding affinity to CT-DNA. Although calculated results were low compared to the classical intercalator (Ethidium bromide $K_b = 7 \times 10^7 \text{M}^{-1}$) (Waring, 1965), similar results have been reported in the studies in the literature (Abu-Dief et al., 2020; Alpaslan et al., 2019; Coban et al., 2016; Cusumano et al., 2006; Iqbal et al., 2020).

3.6. DNA Cleavage results and discussion

The main targets in DNA cleavage activity are the phosphodiester bond, deoxyribose sugar or nucleobases, which are the main components of DNA. The cleavage of these main targets can be either by hydrolytic or oxidative means. In hydrolytic cleavage activity, chain breaks occur as a result of the hydrolysis of phosphodiester bonds in DNA. It is formed by the oxidation of deoxyribose sugar or nucleobases in oxidative cleavage activity (Breen & Murphy, 1995). The potential of the YKT peptide to cleavage DNA was examined by agarose gel electrophoresis using supercoiled pBR322 DNA in TAE (Tris-Acetic acid-EDTA) buffer (pH 8.0). Circular plasmid DNA migrates relatively quickly in gel electrophoresis, and intact supercoiled structure (Form I). If the cleavage occurs in the single chain of the plasmid DNA, the excessive coiled state of the plasmid relaxes, and transforms into Form II. If both strands are cleaved, Form III, a linear form migrating between Forms I and Forms II, is formed (Alpaslan et al., 2019; Barton & Raphael, 1984). The cleavage efficiency of the YKT peptide was evaluated in the presence of H$_2$O (hydrolytic cleavage) and H$_2$O$_2$ (oxidative cleavage). It has been
shown in the studies that DNA cleavage was not observed in control experiments using only DNA and H$_2$O$_2$ (Aboafia et al., 2018). The YKT peptide showed cleavage activity only in the presence of H$_2$O$_2$ compared to the control (Figure 4b). Oxidative cleavage reduced some of the supercoiled DNA to the open circular form (Form II). Based on the DNA shearing activity results, it can be concluded that oxidative cleavage of DNA is facilitated in the presence of YKT.

3.7. Molecular docking results

Molecular docking study provides to obtain possible binding modes and binding affinities of the drug candidate molecule. Binding affinities and RMSD values of Tyr-Lys-Thr tripeptide were examined by AutoDock Vina software program, shown in Table 4 and Figure 5. According to Table 4, it was seen that the best binding affinity have $-4.5$ kcal/mol energy value and RMSD values are generally under 2 Å.

YKT made 12 hydrogen bonds with B-DNA Dodecamer in result of docking study. YKT tripeptide made 3 hydrogen bonds from tyrosine residue, 4 hydrogen bonds from lysine residue and 5 hydrogen bonds from threonine residue, with DNA. Additionally, Charge-Charge interaction was observed between YKT and DNA. In the docking study, it was observed that important hydrogen bonds are concentrated especially in guanine and cytosine. In the experimental study, it was reported that the important bonds contributing to the hyperchromic effect came from the amine groups of YKT and the phosphate backbone around the DNA (see Section 3.5). According to this; hydrogen bound to oxygen in side chain of Tyr side in YKT tripeptide made hydrogen bond having 2.6 Å with OP2 of DT7, hydrogens bound to nitrogen in the side chain of Lys side in YKT tripeptide made hydrogen bonds having 2.3 Å, 2.6 Å and 3.0 Å with O6 of DG16 (chain B) and O4 of DT8 (chain A) in DNA, and nitrogen in the amino group of Thr side in YKT tripeptide made one hydrogen bond having 2.8 Å with H42 of DC15 (chain B) in DNA. Additionally, nitrogen, in the side chain of Lys side in YKT tripeptide and ring of DC15 at the chain B form a pi-cation...
interaction with a distance having 4.88 Å, shown in Figure 5c. In the literature, it was stated that positively charged amino acid residues such as lysine can interact with negatively charged centers of pi ring systems in aromatic residues and may have pi-cation interactions (Harris et al., 2013). All information about hydrogen bonds were listed in Table 5 and Figure 6. According to all these results, it was observed that YKT tripeptide mainly made hydrogen bonds with DNA with Guanines and Cytosines. In experimental studies, it is understood from batochromic and hypsochromic shifts that DNA and YKT interact.

3.8. ADME results of YKT

Lipinski’s 5 rules provide information on whether a drug candidate has the ability to become an orally active drug. Lipinski determined four simple physicochemical parameters depending on 90% of the active drugs taken orally that have reached phase II clinical stage. Octanol-water partition coefficient logP is important at drug design studies to predict the hydrophobicity of a molecule. Octanol-water partition coefficient logP values was predicted using different server systems. Polar Surface Area (PSA) is used to characterize the transportation process of drug molecules like octanol-water partition coefficient and PSA can be attributed to several reasons for drug absorption (Gunduz et al., 2021). Polar surface area is described as a sum of surfaces of polar atoms and attached hydrogens in a molecule. PSA parameter is connected with human intestinal absorption, Caco-2 monolayers permeability, and blood-brain barrier penetration. In ADME calculation studies, it was calculated in the SwissADME server that YKT tripeptide has a molecular weight of 411.4 and this value complies with the Lipinski rule. It was determined that the octanol/water coefficient did not exceed 5 and the general acceptance of all log P o/w calculations was -2.62. Considering the conditions in the Lipinski rule (Lipinski et al., 1997), it was stated that YKT can comply with the Lipinski rule in the study on SwissADME server system. The polar surface area value was determined as 163.68 Å² and this value is high to use as a substitute for BBB penetration (Cusumano et al., 2006; Hitchcock & Pennington, 2006; Kubinyi & Folkers, 2008; Pajouhesh & Lenz, 2005). CYP2D6 inhibition experiments are required in drug discovery studies, as inhibition of CYP2D6 by a drug can cause serious drug-drug interactions (de la Nuez & Rodriguez, 2008; Szumlak et al., 2016). In this study, CYP2D6 inhibition of YKT was evaluated and YKT was classified as non-inhibitor. All obtained ADME predictions were shown in Table S4.

3.9. MD simulation results

In the MD simulation of YKT tripeptide, as can be seen in Figure S3, the YKT peptide exhibits a rich conformational dynamic, its backbone torsion angles, phi and psi, sampling all areas, that is alpha-helix, beta-sheet, and left-handed helix, in the course of the 500 ns long MD simulation. MD simulations of the peptide-DNA complex show the peptide in contact to the DNA almost throughout the simulations,
albeit not at the same position. Figure S4 shows snapshots of the peptide-DNA complex, taken from the three MD runs with 10 ns spacing in between them. Consequently, there is no unique, or at least dominating, hydrogen-bond pattern as would be expected for a distinct binding site of the peptide.

The changing distances of the peptide to the phosphorous atoms in the DNA backbone, together with the snapshots, taken from the three MD simulation runs illustrate the change of binding position of the peptide on the DNA. The peptide does not stay at the initial, i.e. docked position in any of the three simulations. Rather, it leaves the DNA twice in two of the runs, as manifested by the long distances to all P-atoms (Figure 7). In the remainder of the simulation time, the peptide stays at the DNA, albeit with varying interactions as can be seen from the snapshots. It is therefore unclear, whether the peptide would reside on the DNA sites it is bound to towards the end of the simulations for longer times or rather dissociate again from the DNA, possibly to rebind again elsewhere, etc.

Hydrogen-bonded contacts are formed by each amino acids in the tripeptide (see Table 6). The probability of hydrogen bonds between DNA and peptide, computed from the last 100 ns simulation time varies accordingly. Neither on the DNA nor in the peptide, a highly probable hydrogen bond donor/acceptor can be nominated, both backbone and side chains contribute, to varying degree, in the hydrogen bonds observed.

Analysis of the base pair parameters reveals no significant difference between the uncomplexed B-DNA and the DNA bound to the YKT peptide. The difference in axis bend, that is still negligible within error bars, is due to more extensive fraying of the terminal base pairs in the simulation of the free DNA and thus an artifact (Figure 8).

Taken together, the MD simulation data suggest the YKT peptide to have a certain affinity to bind to the DNA, as supported by the frequent observations of the peptide on the DNA, even after dissociation, but without a preferred binding site (on the DNA), or binding mode (by the peptide). It is therefore unclear, how the shifts observed in the UV absorption spectra of the peptide in presence of DNA can be rationalized. One possibility is a binding position and mode of the peptide on the DNA that indeed intercalates the Tyrosine ring of the peptide, perhaps even between C and G bases, that has not been observed/sufficiently sampled in the present MD simulations. Alternatively, loose and unspecific binding to the DNA, even without intercalating, may be enough to shift the UV absorption of the YKT peptide due to the presence of the negatively charged phosphate backbone.

### 3.10. Cytotoxicity results

The positive net charge of the anticancer peptide is important in targeting and killing cancer cells (Harris et al., 2013). YKT is a tripeptide that contains a positively charged lysine residue. Literature studies have shown that lysine has anticancer properties on its own (El-Sersy et al., 2012). In this study, MTT assay was performed to determine the cytotoxic

**Table 8.** % viability values of Hela, MCF-7 and BEAS-2B cells at 48 h.

| Groups          | HeLa   | MCF-7   | BEAS-2B |
|-----------------|--------|---------|---------|
| Control         | 100    | 100     | 100     |
| 0.1 mg/mL (0.244 mM) | 98.5   | 69.72** | 94.19   |
| 0.25 mg/mL (0.609 mM) | 95.75  | 54.93** | 91.98   |
| 0.5 mg/mL (1.218 mM) | 93.25** | 51.05** | 95.8    |
| 1 mg/mL (2.43 mM)   | 92.75* | 50.34** | 93.33   |
| 2.5 mg/mL (6.09 mM) | 70.75**| 48.77** | 88.02** |
| 5 mg/mL (12.181 mM) | 67.75**| 47**    | 69.5**  |

*p < 0.05, **p < 0.01.
activity of YKT for 24 and 48 h on HeLa, MCF-7 and BEAS-2B cell lines at 0.1, 0.25, 0.5, 1, 2.5 and 5 mg/mL concentrations. As seen in Figures 9 and 10; Tables 7 and 8, YKT showed cytotoxic effect on HeLa and MCF-7 cell lines compared to control groups (*p < 0.05, **p < 0.01) on both 24 and 48 h experimental groups. While HeLa cells showed the highest toxic activity in the experimental group for 24 h, MFC-7 cells showed the highest toxic activity in the experimental group for 48 h. The average IC50 value was determined as 1 mg/mL for MCF-7 cell line on 48 h according to MTT assay. This corresponds to 2.43 mM. In addition, in experiments with the normal cell line BEAS-2B, a statistically significant difference was determined compared to the control at only 2.5 mg/mL and 5 mg/mL concentrations (*: p < 0.05, ** p < 0.01). Considering the % viability values, the BEAS-2B cell line shows higher viability than the HeLa and MCF-7 cell lines in the 24 and 48 h experimental groups. In the studies, it was reported that the IC50 concentration was 0.5 mg/mL in MAL-My-Lu cells treated with YKT peptide (Bicak et al., 2021). Also, cytotoxicity studies with different peptides on HeLa and normal human embryonic lung (HEL) cells have shown, sp22 peptide was used at increasing concentrations in the range of 0-100 mg/L caused a decrease in HeLa cells, in contrast increasing concentrations had no effect on HEL cells (Li et al., 2012). In study performed by Maher & McClean have shown that IC50 values of magainin I, magainin II, nisin and melittin peptides on HT29 cells were found 65, 81, 89.9 and 1.2 μM, on Caco-2 cells were found 66.3, 79.9, 115 and 1.8 respectively (Maher & McClean, 2006).

4. Conclusions
The molecular structure of YKT tripeptide was successfully characterized using various experimental (UV, FT-IR, FTIR-ATR, Raman, gel electrophoresis) and theoretical techniques, and its interaction with DNA was performed by UV titration method, molecular docking and MD analyses, and thus DNA binding affinity and close interaction properties were revealed. Also, DNA Cleavage ability of YKT was investigated using agarose gel electrophoresis method and it was obtained that YKT peptide showed cleavage activity only in the presence of H2O2. In addition, the dynamic behavior of YKT, the energetically most favorable geometric structure was examined, and all fundamental vibration wavenumbers were determined and assigned for the first time in this study. Using HeLa and MCF-7 cancer cell lines and BEAS-2B normal cell line the cytotoxic activities of the YKT tripeptide were determined for the first time after 24 and 48 h of incubation with absorbance and % viability values. It was determined that YKT has more cytotoxicity on MCF-7 than on HeLa. And normal cell line BEAS-2B showed less cytotoxicity than HeLa and MCF-7 cell lines. The pharmacokinetic information of YKT, such as ADME properties, which contribute significantly to its development as a drug candidate, has also been calculated.

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Disclosure statement
The authors have no conflicts of interest to declare.

Ethical approval
No animals were used in this study. Ethics approval was not required.

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