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ORIGINAL ARTICLE / ÖZGÜN MAKALE

MOLECULAR DOCKING STUDIES ON SOME BENZAMIDE DERIVATIVES AS TOPOISOMERASE INHIBITORS

TOPOİZOMERAZ İNHİBİTÖRLERİ OLARAK BAZI BENZAMİD TÜREVLERİ ÜZERİNDE MOLEKÜLER DOKİNG ÇALIŞMALARI

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ABSTRACT

Objective: *In order to examine the interactions of some benzamide derivatives, which are thought to exhibit anti-cancer activity, with human Topo I and IIα enzymes at the molecular level, docking studies were carried out on these enzymes.*

Material and Method: *In conducting the docking studies, the protein was selected from the protein data bank for Topo I (1K4T) and for Topo IIα (5GWK). Doking was performed with the CDocker method using the Discovery studio 3.5 program, and the binding energies of benzamide derivatives to enzymes were calculated and their molecular interactions were revealed.*

Result and Discussion: *As a result of the docking process on Topo I and IIα, it was found that benzamide derivative compounds have higher affinity for Topo IIα enzyme. For Topo I compounds 4N6, 5N5; for Topo IIa compounds 5N3, 5N7 have been identified as promising compounds in terms of anticancer activity.*

Keywords: *Anticancer, Benzamide, Docking, Topoisomerase I, Topoisomerase IIα*

ÖZ

Amaç: *Antikanser aktivite göstereceği düşünülen Bazı benzamid türevlerinin insan Topo I ve IIα enzimleri ile moleküler düzeydeki etkileşimlerinin incelenmesi amacıyla bu enzimler üzerinden doking çalışmaları gerçekleştirilmiştir.*

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Gereç ve Yöntem: *Doking çalışmalarının gerçekleştirilmesinde protein veri bankasından Topo I için (1K4T) ve Topo IIα için (5GWK) seçilmiştir, Discovery studio 3.5 programı kullanılarak CDocker yöntemiyle doking işlemi yapılmış ve benzamid türevlerinin enzimlere bağlanma enerjileri hesaplanmış ve moleküler etkileşimleri ortaya çıkartılmıştır.*

Sonuç ve Tartışma: *Topo I ve IIα üzerinden yapılan docking işlemi sonucunda benzamid türevi bileşiklerin Topo IIα enzimine afinitesinin daha yüksek olduğu bulunmuştur. 4N6, 5N5 bileşikleri Topo I; 5N3, 5N7 bileşikleri de Topo IIα inhbitörleri olarak antikanser aktivite göstermesi açısından umut verici bileşikler olarak belirlenmiştir.*

Anahtar Kelimeler: *Antikanser, Benzamid, Doking, Topoizomeraz I, Topoizomeraz IIα*

INTRODUCTION

DNA topoisomerases are the enzymes which play key roles on cellular processes such as replication, transcription, recombination and repair, and chromatin assembly by solving these topological problems of genomic DNA[1-7]. Because of their essential functions in cell cycle, they are significant targets for killing cancer cells or pathogenic bacteria. DNA topoisomerases are classified into two classes as Topo I and Topo II, depending on the number of broken strands of DNA by the enzymes in one reaction cycle. All type of topoisomerases indicates their biochemical functions by catalyzing DNA cleavage and relegation [8].

Topo I functions by generating transient single-stranded cuts in DNA supercoils relaxing torsional strain that has accumulated during DNA replication and transcription [9, 10]. Intracellular levels of Topo I are upraised in some human solid tumors, relative to the corresponding normal tissues, suggesting that variations in Topo I levels are specific to the type of tumor [11-13]. DNA Topo I inhibitors, have recently emerged as a prominent class of anticancer agents with a novel mechanism of action, potent antiproliferative activity on a widespectrum of tumor cells including multidrug-resistant lines, and fascinating activity in xenograft models [14]. At first, camptothecin was discovered as a Topo I inhibitor in 1966, but could not be used in the clinic due to unpredictable and severe myelo suppression, gastrointestinal toxicity, and hemorrhagiccystitis [15]. Afterwards, it was found that the FDA approved anticancer agents topotecan and irinotecan, which are the analogue of camptothecin, inhibited the Topo I activity by intercalating into the cleavage complex and preventing the religation step of the catalytic cycle [16, 17].

Topo II cuts both strands of DNA by the enzymes in one reaction circle. Human Topo II have two available isoforms as α and β. Both of them sharing a similar tertiary structure and primary sequence, and perform similar functions but their levels differ depending on the replicative activity and type of tissue [18-20]. They also show various cellular functions, Topo IIα overexpressed in proliferating cells and generally located in the nuclearplasma. Topo IIβ plays apparent roles in transcriptional regulation, cell development, and differentiation, but not essential for cell

proliferation and survival. Although human Topo IIα relaxes negatively supercoiled plasmid slower than positively supercoiled plasmids, but Topo IIβ is not. Thus selective Topo IIα inhibitors have been of particular interest in cancer therapy, as they may represent a more targeted approach to highly proliferative cells [21-24]. Doxorubicin and Etoposide, classified as DNA Topo II inhibitors, have recently emerged as a prominent class of anticancer agents. Topo II inhibitors prevents re-ligation of the DNA strands, and breaks the DNA strands. Cancer cells depend on this enzyme more than healthy cells, for that they divide more rapidly. Therefore, this generates errors in DNA synthesis and promotes apoptosis of the cancer cell [10, 25].

Recently, amide derivatives received significant attention for their antitumor properties, especially the compounds which containing benzamide pharmacophore. The benzamide derivatives have been reported for their wide range of pharmacological activities including antitumor [26], histone deacetylase inhibition [27] and CYP24A1 inhibitory activity [28]. In addition to these activities some benzamide derivatives were used as HDAC inhibitors [29], glucokinase activators [30], antiprion agents [31] and topoisomerase inhibitors [32, 33] etc.

Recent developments in the field of cell biology want to introduce selective anticancer agents with low side effects to the pharmaceutical market, and the promising bioactive diversity of benzamide derivatives made us think that these derivatives will act as topoisomerase inhibitors, and in this study, the docking studies were performed to elucidate the interactions between various previously synthesized benzamide derivatives [34] and human Topo I and IIα enzymes and were aimed to identify a new type of anticancer drug candidates which have suitable properties to be promising oral human Topo I and IIα inhibitors.

MATERIAL AND METHOD

Preparation of the enzyme

Human Topo I has monomer structure and composed of 765 amino acids and human Topo IIα has a homo dimer structure and its monomer is composed of 1531 amino acids including four sections DNA-gate, Ngate, C-gate, and CTD [35]. The X-ray crystallographic structure of Topo I (PDB: 1K4T) and Topo IIα (PDB: 5GWK) are available in Protein Data Bank and further modified for docking calculations [36]. For preparation of protein Discovery Studio 3.5 software [37] was used. The target proteins were taken, hydrogens were added and their positions were optimized using the all atom CHARMm [38] force field and the Adopted Basis set Newton Raphson (ABNR) method [39] available in the D.S 3.5 protocol until the root mean square deviation (RMSD) gradient was <0.05 kcal/mol Å2. The minimized protein was defined as the receptor using the binding site module.

The binding site was defined from current selection around the ligand inside. The binding sphere were selected for 1K4T 6.12, 47.51, 26.54, 14.67 **(Figure 1A)** and for 5GWK 31.34, -23.16, -57.75, 10.32 **(Figure 1B)** from the active site using the binding site tools.

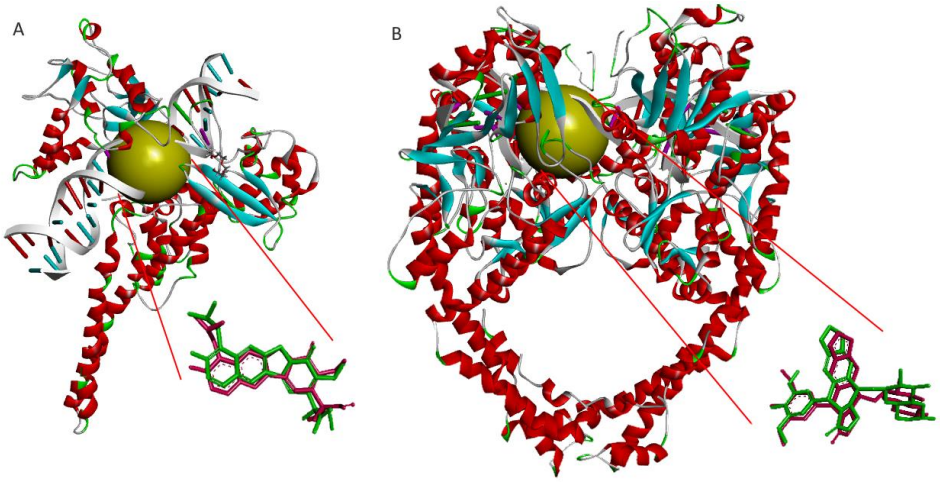
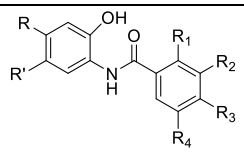


Figure 1. A. Topo I (pdb:1K4T) enzyme, the active site is located inside the sphere marked in yellow, superimpose position of Camptothecin with RMSD:1.2424**. B.** Topo IIα (pdb:5GWK) enzyme, the active site is located inside the sphere marked in yellow, superimpose position of Etoposide with RMSD:1.7219.

Preparation of ligands

Benzamide derivatives [34] given in **Table 1**, selected inhibitors Camptothecin and Etoposide were sketched with ChemDraw Professional; all-atom CHARMm force field parameterization was assigned and then minimized using the ABNR method as described above.

Table 1. Benzamide derivatives tested in molecular docking process



COMPOUND	R	R'	R_1	R_2	R_3	R_4
4N1	NO_2	H	H	H	C_4H_9	H
4N2	NO_2	H	H	H	$C(CH_3)_3$	H
4N3	NO_2	H	H	H	OC_2H_5	H
4N4	NO_2	H	H	H	OC_4H_9	H
4N5	NO_2	H	H	CH_3	H	CH_3
4N6	NO_2	H	H	OCH_3	H	OCH_3
5N1	H	NO_2	H	H	C_2H_5	H
5N2	H	NO_2	H	H	$C(CH_3)_3$	H
5N3	H	NO_2	H	H	OC_2H_5	H
5N4	H	NO_2	H	H	OC_4H_9	H
5N5	H	NO_2	CH_3	H	CH_3	H
5N6	H	NO_2	H	CH_3	H	CH_3
5N7	H	NO_2	H	OCH_3	H	OCH_3

Validation of Docking Process

In order to validate the accuracy of the process, docking studies were performed using the CDOCKER method [40] to the region determined on the proteins of the ligands carried by the enzymes. RMSD values were calculated by overlapping the obtained poses with the ligand found in the X-ray crystallography of the protein. The RMSD values expressing the difference between the optimal conformation of the ligand and X-ray crystallography were found to be 1. 2424 **(Figure 1A)** and 1.7219 **(Figure 1B)** for 1K4T and 5GWK, respectively.

Molecular Docking

Docking process was performed using the CDOCKER method in which the ligand moves flexibly while keeping the receptor stable. Ligands were interacted in 3000 different conformations in the active site of the enzyme. After the validation step, docking processes of benzamide derivatives and selected inhibitors were performed. Among the poses obtained as a result of these processes, the most suitable ones were determined, and their binding energies were calculated.

RESULT AND DISCUSSION

The interactions of benzamide derivatives with Topo I and IIα enzymes have been elucidated by applying molecular docking processes, and it has been found that the compounds generally show a better interaction with the Topo IIα enzyme. When benzamide derivatives and Camptothecin were docked in the active site of the 1K4T enzyme selected from pdb as the Topo I enzyme, they show various interactions with residues DT10, DG12, DA113, DC112 and TGP11 of DNA and amino acids ASN352, GLU356, ARG364, TRP416, LYS425 and THR718 of enzyme as given in **Table 2** and the binding energies of these compounds also range between -57,7457 and 97.388 kcal/mol. The interactions of Camptothecin, Topo I enzyme inhibitor, were examined, it was observed that it binds to the enzyme with -16,5852 kcal/mol binding energy and interacted with LYS425 amino acid and DT10, DA113, DC112, TGP11 residues, as given **Figure 2A**. Compounds 4N6, 5N5, 4N2, 5N4, 4N4, 4N3 and 5N3 were interacted with lower binding energies than Camptothecin to the enzyme respectively, while other compounds exhibited positive binding energies. The compound 4N6 gave the best binding energy (-57,7457 kcal/mol) with Topo I enzyme and interacted with GLU356, TGP11, DC112, DA113 residues through phenyl and hydroxyl groups in the molecule, as given **Figure 2B**. The compound 5N5 showed a good interaction with Topo I enzyme with its binding energy of -50,3612 kcal/mol and made H bond to DA113 residue with its hydroxyl group and showed

pi interactions between phenyl rings and DT10, TGP11, DC112, DA113 residues, as given **Figure 2C**.

Table 2. Interaction properties of benzamide derivatives with Topo I

Compound	binding energy (kcal/mol)	conventional Hydrogen Bond	carbon Hydrogen bond	Pi Interactions
4N1	97.388	THR718, TGP11	DG12	DC112, DA113
4N2	-37,6963	-	DA113	TGP11, DA113, LYS425
4N3	-23,5498	ASN352	DT10	DA113, TGP11,
4N4	-32,0942	ASN352	DT10	DA113
4N5	30,0267	DC112, DA113, ARG364	TGP11	DT10
4N6	-57,7457	-	GLU356, DC112, DA113	TGP11
5N1	-19,2227	TGP11	-	TRP416, LYS425
5N2	0,04961	TGP11	-	DA113, TRP416, LYS425
5N3	-22,824	DT10	-	DC112, DA113
5N4	-35,4994	ASN352	DT10	GLU356, TGP11, DC112, DA113
5N5	-50,3612	DA113	-	DT10, TGP11, DC112
5N6	15,7991	-	TGP11	TGP11, DC112, DA113
5N7	2,02614	ASN352	DT10, DA113, TGP11	-
Camptothecine	-16,5852	-	LYS425	DT10, TGP11, DC112, DA113

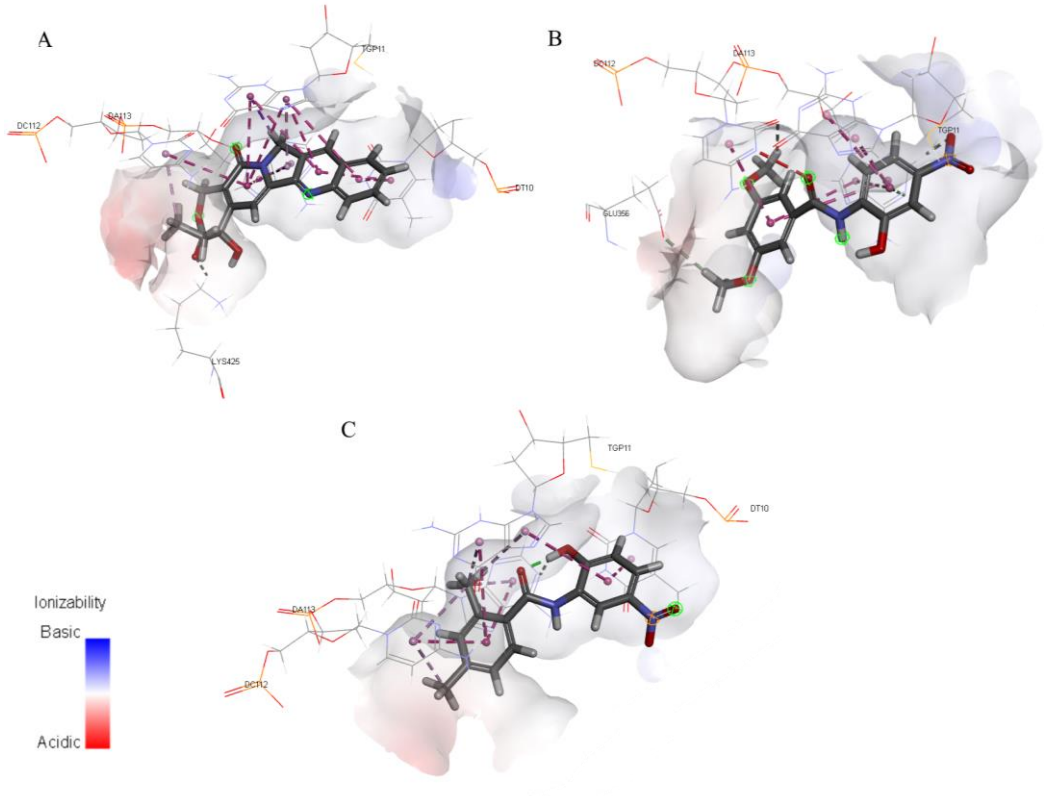


Figure 2. Molecular interactions of the Topo I enzyme **A.** Docked pose of Camptothecine, **B.** Docked pose of compound 4N6, **C.** Docked pose of compound 5N5.

When benzamide derivatives and Etoposide were docked in the active site of the 5GWK enzyme selected from pdb as the Topo IIα enzyme, they show various interactions with residues DC8, DT9, DG10, DC11, DA12, DG13 and DC14 of DNA and amino acids GLY462, ARG487, GLY760, MET762 and TYR805 of enzyme as given in **Table 3** and the binding energies of these compounds also range between -114,71 and -60,1444 kcal/mol. The interactions of Etoposide, Topo IIα enzyme inhibitor, were examined, it was observed that it binds to the enzyme with -114,71 kcal/mol binding energy and made H bond to DG13 residue with its hydroxyl group. It also interacted with LYS440, ARG487 amino acids and DT9, DA12, DG13, ARG487 residues, as given **Figure 3A**. The binding energies of benzamide derivatives were higher than etoposide, but it was observed that all molecules interacted with the enzyme with low binding energies. Compounds 5N3 and 5N7 indicated good interactions with the Topo IIα enzyme with binding energies of -94,3762 and -92,0598 kcal/mol, respectively. The compound 5N3 made H bond to DG13 residue with its nitrogen atom in amide group and also interacted with ARG487, DC8, DT9 residues, as given **Figure 3B**. The compound 5N7 made H bond to ARG487 with its methoxy group and showed pi interactions with DC8, DT9, DA12, DG13 residues, as given **Figure 3C**.

Table 3. Interaction properties of benzamide derivatives with Topo IIα

Compound	binding energy (kcal/mol)	conventional Hydrogen Bond	carbon Hydrogen bond	Pi Interactions
4N1	-74,4069	DT9, ARG487	DG13	DC8, DA12
4N2	-74,456	TYR805	GLY462	ARG487, DG13
4N3	-65,2691	DT9	DG13	ARG487, MET762, DG8, DG13
4N4	-88,0887	DT9	DG13	DC8, DA12
4N5	-62,9599	DG13	GLY760	DA12
4N6	-65,1616	DT9	DG13	DC8, DA12
5N1	-75,7866	DT9, ARG487		MET762, DC8, DT9, DG13
5N2	-61,5032			DC8, DT9
5N3	-94,3762	DG13	ARG487	DC8, DT9
5N4	-90,2323	DT9, DC14		DC8, DT9, ARG487
5N5	-60,1444	DG13	GLY760	ARG487, DC8, DT9
5N6	-87,8215	DG13		DC8, DT9
5N7	-92,0598	ARG487		DC8, DT9, DA12, DG13
Etoposide	-114,71	DG13	DG10, DC11, DA12, LYS440, ARG487	DT9, DA12, DG13, ARG487

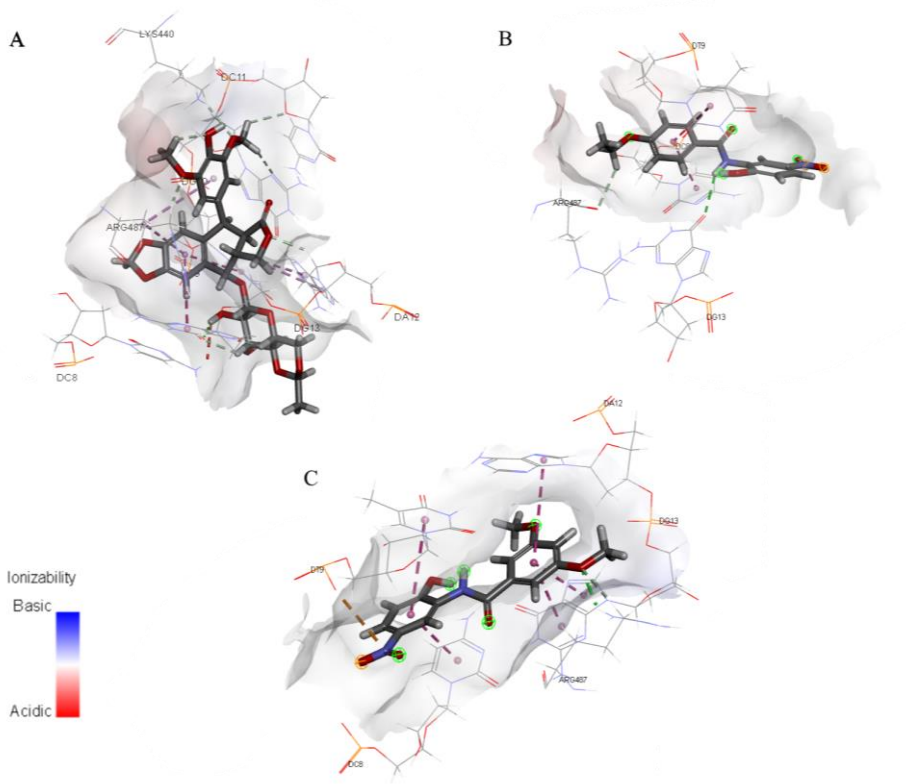


Figure 3. Molecular interactions of the Topo IIα enzyme **A.** Docked pose of Etoposide, **B.** Docked pose of compound 5N3, **C.** Docked pose of compound 5N7.

As a result of the docking studies on Topo I and IIα enzymes of benzamide derivatives, which are thought to have anticancer activity as topoisomerase inhibitors, it has been shown that the compounds have higher affinity for the Topo IIα enzyme, but have a lower effect than the reference compound. However, most of the compounds docked on Topo I enzyme were performed better results than the reference molecule. The performed docking studies should be supported by experimental results, but its clear that the accompanying results represent that compounds are promising inhibitors for Topo I and IIα enzymes.

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