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Molecular Docking Analysis of Used Drugs for the Treatment of Cancer

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Abstract: In this study, the lowest energy molecular structures were determined by conformational analysis of six drugs commonly used in cancer treatment, in order to use as initial data for docking simulations. Using the AutoDock Vina software, the interaction mechanisms of the 6 FDA approved drugs (Pemetrexed, Irinotecan, Tamoxifen, Gemcitabine, Topotecan and Temozolomide) with DNA were investigated. In addition, MM/PB(GB)SA calculations for the drug-DNA structures under investigation have been performed. The calculated binding affinities and binding free energies of interactions were showed the stability of the structures. It has been found that the active site where these molecules interact with DNA is the same and that their various interactions, primarily hydrogen bond, play an important role in this stability of the structures. Furthermore, the pharmacophoric features of the investigated molecules were determined.The aim of the work is to deeply investigate the binding properties of the title drugs with DNA.

Kanser Tedavisinde Kullanılan İlaçların Moleküler Kenetlenme Analizi

Anahtar Kelimeler
Kanser,
İlaçlar,
Moleküler modelleme,
Konformasyon analizi,
Moleküler kenetlenme

Özet: Bu çalışmada, kenetlenme simülasyonları için başlangıç verileri olarak kullanılmak üzere, kanser tedavisinde yaygın olarak kullanılan altı ilacın konformasyonel analizi ile en düşük enerjili moleküler yapıları belirlenmiştir. AutoDock Vina programı kullanılarak FDA onaylı 6 ilacın (Pemetrekset, Irinotekan, Tamoksifen, Gemsitabin, Topotekan ve Temozolomid) DNA ile etkileşim mekanizmaları araştırılmıştır. Ek olarak, araştırılan ilaç-DNA yapıları için MM/PB(GB)SA hesaplamaları yapılmıştır. Etkileşimlere ait hesaplanan bağlanma afiniteleri ve bağlanma serbest enerjileri yapıların kararlılığını göstermiştir. Bu moleküllerin DNA ile etkileştiği aktif bölgenin aynı olduğu ve başta hidrojen bağı olmak üzere yapmış oldukları çeşitli etkileşimlerin yapıların bu kararlılığında önemli bir rol oynadığı bulunmuştur. Ayrıca, incelenen moleküllerin farmokofor özellikleri belirlenmiştir. Bu çalışmanın amacı, başlıktaki ilaçların DNA ile bağlanma özelliklerini derinlemesine araştırmaktır.

1. Introduction

Cells have management and control mechanisms such as growth, division and apoptosis in order to survive or terminate their lives. Uncontrolled cell proliferation, the ability of cells to metastasize to organs and invasion with surrounding tissues is defined as carcinogenesis [1,2]. Activation of proto-oncogenes, tumor suppressor genes, DNA repair enzymes, and inactivation of apoptosis are mutations that cause malignant transformation [2,3,4]. The mechanism found in every cell and controlling them is DNA. Gene change occurs depending on the change in the molecular structure of DNA. As the cell divides, it begins to produce faulty and different - atypical - cells due to DNA damage. Since these abnormal cells are out of genetic control, they constantly multiply and attack the tissues, and spread to the whole body with blood and lymph [5]. A better understanding of various genes, proteins and their effects at the cellular and molecular levels helps to identify appropriate preventive and diagnostics. In cancer treatment; the building blocks of DNA, purine and pyrimidine have been used as targets for many years and are still in use in these treatments [6].

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Cancer is the uncontrolled division and proliferation of cells. Chemotherapy, radiotherapy, surgical methods as well as hormone therapy and biological methods are used in cancer treatment [7]. Anticancer drugs, alkylating agents that suppress protein production by inhibiting the transcription of DNA [8], corticosteroids in the class of steroid-like drugs used to reduce inflammation and suppress the immune system and suppress cancerous cells [9], anti-metabolites that suppress small molecules responsible for signal transduction in the human body, stimulation or suppression of enzymes [10]. They are divided into several classes as antitumor antibiotics that slow down the growth and division of cancer cells by suppressing DNA / RNA synthesis [11], mitotic inhibitors that interfere with the mitosis phase of the cell [12], and topoisomerase inhibitors that inhibit the transcription of DNA [13].

In this study, the commonly used six anticancer drugs namely Pemetrexed, Irinotecan, Tamoxifen, Gemcitabine, Topotecan and Temozolomide were investigated.

In 1992, Taylor et al. [14] discovered Pemetrexed disodium, which inhibits the proliferation of cancer cells and stops DNA replication. Pemetrexed, non-small cell lung [15, 16], breast [17,18], colorectal [19,20], head and neck [21], stomach [22], bladder [23], cervix [24] and pancreatic cancers [25] has demonstrated single agent activity in various tumor types. Pemetrexed is a new pyrolo (2,3-d) pyrimidine based antifolate. Pemetrexed and its derivatives enable the synthesis of purine and thymidine in DNA and RNA, an inhibitor of multiple enzymes. Folate plays a role in DNA repair and methylation by synthesizing DNA and hemoglobin with a single carbon methyl group [26, 27]. Inhibition of thymidylate synthase (TS), such as 5-fluorouracil (5-FU) and raltitrexed, is also the primary mechanism of action [26, 27]. Thymidylate Synthase (TS) is an enzyme that performs an important function in the synthesis of DNA precursors in all living cells, and overexpression of TS is associated with drug resistance. At the same time, this enzyme catalyzes the conversion of phosphate-based molecules to each other. Prevention of TS function occurs with the help of various inhibitors. It prevents the formation of the Enzyme-Substrate complex, thereby reducing the reaction rate or disappearing completely. Thus, it prevents and destroys rapidly proliferating tumor cells [28,29]. Pemetrexed is a folate antimetabolite chemotherapy drug [30]. It prevents the formation of DNA and RNA, which are necessary for cancer cells to grow and reproduce. To prevent this, it inhibits the formation of purine (adenine and guanine) and pyridine (cytosine and guanine) nucleotides [31].

Irinotecan has the activity to inhibit the camptothecin topoisomerase-I enzyme derived from the bark of the Camptotheca acuminafa tree and is a water-soluble semi-synthetic analogue camptothecin [32]. DNA enables cancer cells to reproduce by interacting with molecules called Topoisomerase. Irinoctecan prevents DNA from being processed by acting on topoisomerase and the cancer cell has to die before it can multiply. This drug is used in the treatment of colorectal cancer. Colorectal cancer is one of the most fatal cancer types in the world [33,34]. CRC metastasis may occur at the beginning of or during treatment when diagnosed early [35,36]. Patients with CRC metastases can be healed by palliative systematic treatment with cytotoxic and biological agents. Chemotherapy treatment with the combination of irinotecan and 5-FU works better than treatment using only 5-FU [37]. Irinotecan's active metabolite converts SN-38 to inactive SN-386 by its hydrolysis. While performing this transformation, topoisomerase-I prevents the reattachment of the DNA strand, resulting in double stranded DNA breakage. Enzyme activity that plays a role in nucleic acid metabolism provides DNA repair [38].

Tamoxifen, as an estrogen receptor, is one of the drugs used by pre- and postmenopausal women in the treatment of breast cancer [39]. It is a hydrophobic anticancer drug that plays an important role in the treatment of breast cancer. The mechanism of action of temoxifen is the estrogen receptor modulator inhibits the growth of breast cancer cells, while it has beneficial effects on bone mineral density and serum lipids [40]. This property may be due to other proteins that interact with DNA and receptors. It helps to stop bone resorption, which is the nightmare of most women after menopause. It shows cholesterol lowering properties. Tamoxifen needs the CYP2D6 enzyme to transform into an active form in the body. It shows activated properties with the help of this enzyme. This enzyme must be metabolized into endoxifene (the first active metabolite) in order to show its activity [41,42]. The drug provides protection for many patients by inhibiting the growth of cancer cells. However, tamoxifen behaves like estrogen bone cells [43,44].

Gemcitabine; It is an antimetabolite chemotherapeutic used in blood cancers such as breast, colon, stomach, bladder, pancreas, non-small cell lung and non-Hodgkin lymphoma [45]. Antimetobolites; They are effective against rapidly proliferating tumors and show their effects mostly during DNA synthesis (S-phase), so they are called phase specific agents. They are either structural analogs of molecules required for cell growth and replication or act on the enzymes required for the synthesis of these molecules. The structure of gemcitabine is diflurodeoxycitidine. It inhibits ribonucleotide reductase and thymidine kinase enzymes, entering the DNA synthesis as pseudo-metabolite disrupts the synthesis and prevents DNA repair. Gemcitabine shows its cytotoxic effect in murine and human tumor cell culture [46]. Anticancer drugs such as gemcitabine are transported

to cells via molecular carriers for nucleosides. The most common molecular carriers for this drug are SLC29A1, SLC28A1 and SLC28A3. In this way, after entering the cell, it is modified with phosphate and becomes monophosphate (dFdCMP). In order for gemcitabine to be pharmacologically active, it can inhibit ribonucleotide reductase into triphosphate (dFdCTP) by adding two more phosphates and is hydrophilic [47].

It is a semi-synthetic, water-soluble analog of the alkaloid camptothecin plant, widely used in the treatment of ovarian and cervical cancers. The intranuclear enzyme inhibits topoisomerase I, resulting in enzyme-dependent DNA cleavage and single-strand breaks [48].

Temozolomide; It is the therapeutic drug targeting brain tumor tissue with this aspect, exerting therapeutic effects that can cross the blood-brain barrier [49,50]. Studies to date have aimed to increase the efficacy of TMZ by restructuring its chemical structure and to reduce its toxic side effects [51,52]. Temozolomide is a second generation monofunctional cytotoxide alkylating agent. It is an imidazole ring joined by a ring system containing 3 nitrogen atoms bonded side by side and a 3-methyl derivative of mitozolomide [53,54]. In combination with radiation therapy, it contributes to prolongation of survival and improves patient quality of life [55]. Temodal is a medicine that contains the active substance temozolomide. In the body, temozolomide converts into a compound called MTIC (Monomethyl trizeno imidazole carboxamide). The cytotoxicity of MTIC results in inhibition of methylation of DNA. Temodal undergoes hydrolysis with the compound 5-imidazole-4 carboxamide (MTIC) and converts to 5-amino imidazole-4 carboxamide [56]. The reactive cation interacts with DNA, causing methylation, leading to cell death. While MTIC proliferates, it stops cell division by binding to the DNA of the cells [57].

While investigating the causes of cancer formation and development, DNA has become the target of anticancer drugs. Because DNA is a critical factor that directs tumorigenesis and ensures its activity. The reasons why it is targeted as the discovery of anticancer drugs to treat cancer is because DNA is a gene mutating substance in tumor cells, its life cycle, tumor cells are more likely to generate extra DNA damage due to DNA replication at a higher rate than normal cells, deficiencies in checkpoint control and DNA repair mechanisms. In addition, the acceptability of cellular DNA replacement as a targeted therapy allows proliferating tumor cells to adhere to DNA integrity more than normal quiescent cells [58].

In this study, conformational analyzes of Pemetrexed, Irinotecan, Tamoxifen, Gemcitabine, Topotecan, and Temozolomide molecules were carried out to examine the energetically possible conformers and to reveal their stability. In order to understand the biological activity and mechanism of these molecules binding DNA, molecular docking studies were carried out with B-DNA and binding modes, and binding affinities were determined.

2. Method

In the study of conformational investigation and determination of the optimized geometry of the six studied molecules (Gemcitabine, Irinotecan, Pemetrexed, Tamoxifen, Temozolomide, Topotecan), the Spartan06 program [59] and the PM3 semi-empirical quantum mechanical process were used. [60-63].

Molecular docking studies were performed on active sites of the target [64] identified using AutoDock-Vina software. The protein database (PDB ID: 1BNA)[65] was used to obtain the three-dimensional crystal structure of DNA. By extracting water molecules and adding polar hydrogen to them, it was prepared to DNA docking procedure. The Kollman charges of DNA were also determined. Gas-phase molecules have been optimized and modified for docking. Using the Geistenger process, the partial charges of molecules were determined. In the molecular docking process, a grid box in 40 Å x 40 Å x 40 Å size was created along the x, y, and z axis. For the grid box the spacing was set at default 1 Å. The binding free energies of ligand-receptor systems were calculated by program developed by Wang [66]. The binding free energy for ligands was calculated using the MM/PB(GB)SA method, which was derived from the Schrödinger suite and Amber package [66].

The molecular interactions can be better explained in terms of the features present in the ligand. This is technically called pharmacophore and is defined as the spatial arrangement of an ensemble of steric and electronic features that are essential for a molecule to interact with a specific target receptor. Pharmacophore modeling is an important strategy followed in rational drug designing. Topotecan's ligand-based pharmacophore model was developed, and a comparison with the other ligands utilized in the docking study was made to better understand the interaction. PharmaGist [67-69] was used to create multiple flexible alignments for the pharmacophore research.

3. Results and Discussion

The Interactions of the Gemcitabine molecule with DNA are as follows (see Figure 1): 2.16 and 3.07 Å length hydrogen bond (H-bond) interactions between the DG10 residue and 2.31 Å length H-bond interaction between the DG16 residue; 3.28 Å length carbon hydrogen bond interaction between the DG12 residue and 3.35 Å length carbon hydrogen bond interaction between the DA17 residue; 2.28 Å length

unfavorable donor-donor interactions between the DG10 residue and the drug. The Gemcitabine molecule shows better binding affinity as -6.5 kcal/mol with DNA.

The Interactions of the Irinotecan molecule with DNA are presented in Figure 2. The results show that 2.41 and 2.49 Å length H-bond interactions between the DG10 residue and 2.77 Å length H-bond interaction between the DG16 residue; 3.52 Å length carbon hydrogen bond interaction between the DG12 residue, 3.51 Å length carbon hydrogen bond interaction between the DG16 residue and 3.45 Å length carbon hydrogen bond interaction between the DA18 residue; 3.56 Å length Pi-Sigma interaction between the DC11 residue; 5.34 and 5.03 Å length Pi-Alkyl interactions between the DA17 residue; 4.54 Å length Pi-Anion interaction between the DA18 residue and the drug. The Irinotecan molecule shows better binding affinity -9.7 kcal/mol with DNA.

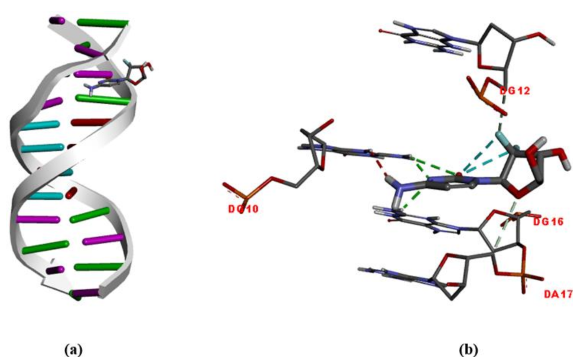

Figure 1. Docking of Gemcitabine with DNA. b) doted lines present the interactions (binding affinity -6.5 kcal/mol)

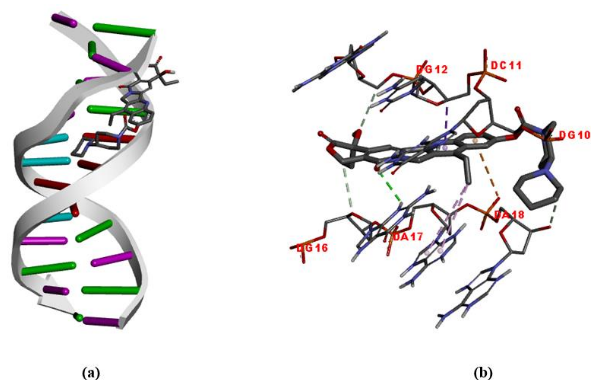

Figure 2. Docking of Irinotecan with DNA. b) doted lines present the interactions (binding affinity -9.7 kcal/mol)

The Interactions of the Pemetrexed molecule with DNA are shown in Figure 3 which shows 2.05 Å long H-bond interaction between the DG2 residue, 2.48 and 2.97 Å long H-bond interactions between the DG4 residue, 2.16 Å long H-bond interaction between the DA5 residue, 1.91 Å long H-bond interaction between the DT20 residue, 3.03 Å long H-bond interaction between the DC21 residue, 2.49 and 3.09 Å long H-bond interactions between the DG22 residue, 2.93 Å long H-bond interaction between the DC23 residue ; 3.49 Å long carbon hydrogen bond interaction between the DG4 residue; 1.72 Å long unfavorable donor-donor between the DA6 residue; 2.76 Å long Pi-Donor hydrogen bond interaction between the DG4 residue and the drug. The Pemetrexed molecule shows better binding affinity -8.7 kcal/mol with DNA.

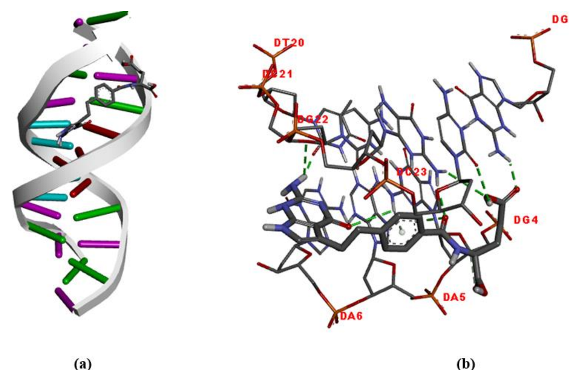

Figure 3. Docking of Pemetrexed with DNA. b) doted lines present the interactions (binding affinity -8.7 kcal/mol)

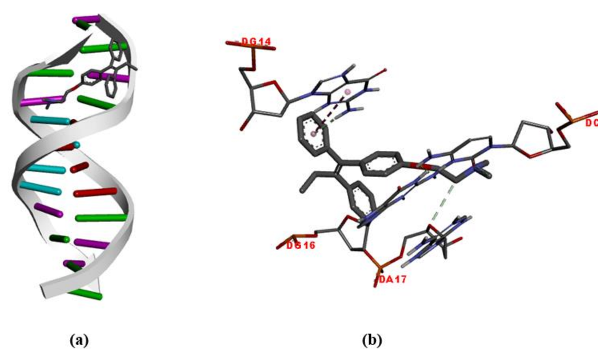

Figure 4. Docking of Tamoxifen with DNA. b) doted lines present the interactions (binding affinity -6.9 kcal/mol)

The Interactions of the Tamoxifen molecule with DNA can be seen in Figure 4. When the results are investigated, it is seen that 2.21 Å long H-bond interaction between the DG16 residue; 3.52 Å long carbon hydrogen bond interaction between the DC9 residue, 3.38 Å long carbon hydrogen bond interaction between the DA17 residue; 2.47 Å long Pi-Donor hydrogen bond interaction between the DG14 residue, 5.68 Å long Pi-Pi T-shaped interaction between the DG14 residue and the drug. The Tamoxifen molecule shows better binding affinity -6.9 kcal/mol with DNA.

The Interactions of the Temozolomide molecule with DNA are given in Figure 5. The figure shows the results to be 1.85 Å long H-bond interaction between the DG10 residue; 2.80 and 2.94 Å long H-bond interactions between the DG14 residue, 2.52 Å long H-bond interaction between the DC15 residue; 2.74 Å long unfavorable donor-donor interaction between the DG10 residue, 2.13 Å long unfavorable donor-donor interaction between the DG16 residue; 5.60 Å long Pi-Pi T-stacked interaction between the DC11 residue and the drug. The Temozolomide molecule shows better binding affinity -7.6 kcal/mol with DNA.

The Interactions of the Topotecan molecule with DNA are as follows (see Figure 6): 2.77 Å long H-bond

interaction between the DG10 residue, 2.89 Å long H-bond interactions between the DG16 residue, 2.82 Å long H-bond interaction between the DA17 residue; 3.56 Å long carbon hydrogen bonds interaction between the DG12 residue and the drug. The Topotecan molecule shows better binding affinity -9.2 kcal/mol with DNA.

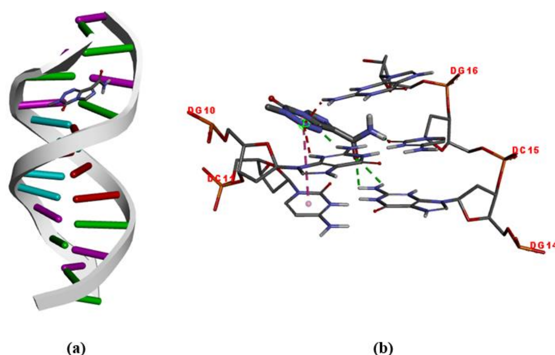

Figure 5. Docking of Temozolomide with DNA. b) doted lines present the interactions (binding affinity -7.6 kcal/mol)

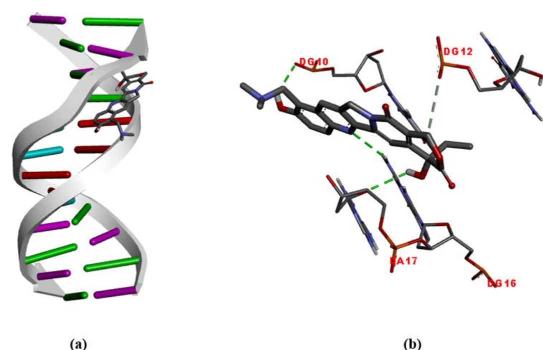

Figure 6. Docking of Topotecan with DNA. b) doted lines present the interactions (binding affinity -9.2 kcal/mol)

Table 1. AutodockVina predicted the binding affinity values of the title compounds (kcal/mol).

	B-DNA
Gemcitabine	-6.5
Irinotecan	-9.7
Pemetrexed	-8.7
Tamoxifen	-6.9
Temozolomide	-7.6
Topotecan	-9.2

If we compare the binding affinities of the molecules with DNA, it is seen that the Irinotecan molecule exhibits the strongest binding affinity with -9.7 kcal/mol towards DNA (see Table 1). The binding free energies of DNA-Temozolomide and DNA-Gemcitabine were calculated to be -5.09 and -16.53 kcal/mol by using the MM/PB(GB)SA method and the GB6 procedure [66]. We could not perform the calculations for all investigated complexes due to the insufficiency of the program. The pharmacophore properties of the investigated molecules are listed in Table 2.

To examine the common pharmacophoric characteristics shared by Topotecan and other ligands, a pairwise alignment of ligands was performed (see Table 3). The matching features are reflected in the pairwise alignment's score. This pharmacophore data will aid in the development of lead drugs for the target [70]. Topotecan and the investigated ligands have pharmacophoric characteristics, which is evidence of a similar kind of interaction with the target proteins.

Table 2. Pharmacophoric features of the ligands

Molecule	F	S	R	H	D	A	N	P
Topotecan	15	13	3	3	2	7	0	0
Pemetrexed	21	20	3	3	5	6	2	2
Temozolomide	10	10	2	1	1	5	0	1
Gemcitabine	10	8	2	0	4	4	0	0
Tamoxifen	14	14	3	9	0	2	0	0
Irinotecan	18	17	3	6	1	8	0	0

F: Features, S: Spatial features, R: Aromatic, H: Hydrophobic, D: Donors, A: Acceptors, N: Negatives, P: Positives

The docking data further demonstrate the role of ligand acceptor characteristics in hydrogen bond formation and aromatic features in non-bonded interactions with active site residues, which proves the consistency of the pharmacophore finding and docking results. The superposition of pharmacophore models for all six ligands was also performed in order to identify common characteristics shared by the various structures (see Figure 7). The scores reflect the characteristics that ligands must possess in order to elicit the required biological activity against the specified targets. One aromatic feature and two acceptor features are common to all ligands (see Table 3).

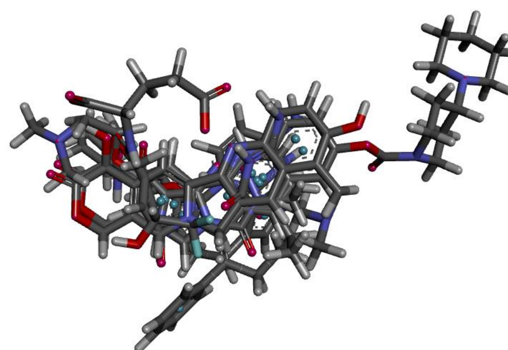
Figure 7. Superposition of all six ligand pharmacophoric models

Table 3. The best pairwise alignment of Topotecan and other ligands

Score	F	S	R	H	D	A	N	P	Molecules
12.0273	5	5	3	0	0	2	0	0	Topotecan and Pemetrexed
9.0302	5	5	1	0	1	3	0	0	Topotecan and Temozolomide
10.5311	5	4	2	0	1	2	0	0	Topotecan and Gemcitabine
6.01952	3	3	1	0	0	2	0	0	Topotecan and Tamoxifen
20.1661	12	11	3	2	1	6	0	0	Topotecan and Irinotecan

F: Features, S: Spatial features, R: Aromatic, H: Hydrophobic, D: Donors, A: Acceptors, N: Negatives, P: Positives

Pharmacophore-based scoring method results support Docking analysis results. The Irinotecan molecule, which shows the highest binding affinity in DNA docking analysis (-9.7 kcal/mol), is also found to be the best ligand according to the pharmacophoric features.

According to the best alignment score (19.843) of the six investigated ligands, Irinotecan is the first, and Gemcitabine is the sixth molecule, indicating that among the 6 ligands, Irinotecan is the best and Gemcitabine is the worse ligand, according to the pharmacophoric properties (see Table 4). The result is also consistent with the docking analysis, as seen in Table 1, Gemcitabine was found to have the lowest binding affinity (-6.5 kcal/mol) to DNA, among the 6 ligands.

Table 4. Best alignment of the six investigated ligands

Score	Molecules
19.843	Irinotecan Topotecan Temozolomide Tamoxifen Pemetrexed Gemcitabine

4. Conclusion

In this study, the possible interaction mechanisms of the 6 FDA-approved drugs (Pemetrexed, Irinotecan, Tamoxifen, Gemcitabine, Topotecan, and Temozolomide) with DNA were determined. Molecular docking studies revealed a strong interaction between the investigated drugs and DNA. Irinotecan exhibited the strongest binding affinity towards DNA (-9.7 kcal/mol), it was followed by Topotecan (-9.2 kcal/mol) and Pemetrexed (-8.7 kcal/mol). The results reveal that the target DNA had a more stable interaction with irinotecan compared to the other DNA-ligand complexes. The binding free energies of DNA-Temozolomide and DNA-Gemcitabine were estimated be -5.09 and -16.53 kcal/mol, respectively, by using MM/PB(GB)SA method and the GB6 procedure. However, the calculations with the other drugs were not be performed due to insufficiency of the program. Nevertheless, the calculated binding free energies of both complexes showed the stability of the structures. The pharmacophore analyses of the examined molecules were carried out and the structure-activity relationships of the molecules were presented comparatively.

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Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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