ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL

THE COMPUTATIONAL STUDY ON THE ELUCIDATION OF THE BINDING INTERACTIONS AND MECHANISM OF ANTI-NEOPLASTIC PURINE DERIVATIVE DRUGS WITH DNA: MOLECULAR DOCKING AND MD SIMULATION STUDIES

Ph.D. THESIS

Soykan AĞAR

Department of Chemistry

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JUNE 2022

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Soykan AĞAR
(509162216)

Department of Chemistry

Chemistry Programme

Thesis Advisor: Prof. Dr. Mine YURTSEVER

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

ANTİ-NEOPLASTİK PÜRİN TÜREVİ İLAÇLARIN DNA ETKİLEŞİMLERİNİN VE BAĞLANMA MEKANİZMALARININ MOLEKÜLER KENETLENME VE MD SİMULASYONLARI İLE İNCELENMESİ

DOKTORA TEZİ

Soykan AĞAR
(509162216)

Kimya Anabilim Dalı

Kimya Programı

Tez Danışmanı: Prof. Dr. Mine YURTSEVER

HAZİRAN 2022

Soykan AĞAR, a Ph.D. student of ITU Graduate School student ID 509162216, successfully defended the thesis/dissertation entitled "THE COMPUTATIONAL STUDY ON THE ELUCIDATION OF THE BINDING INTERACTIONS AND MECHANISM OF ANTI-NEOPLASTIC PURINE DERIVATIVE DRUGS WITH DNA: MOLECULAR DOCKING AND MD SIMULATION STUDIES", which he prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor	**Prof. Dr. Mine YURTSEVER** Istanbul Technical University	……..
Jury Members:	**Prof. Dr. Ayşegül GÖLCÜ** Istanbul Technical University	……..
	Prof. Dr. Serdar DURDAĞI Bahcesehir University	……..
	Prof. Dr. Nurcan TÜZÜN Istanbul Technical University	……..
	Asst. Prof. Dr. Özge ŞENSOY Medipol University	……..

Date of Submission : 11 May 2022
Date of Defense : 22 June 2022

To my mom, dad and buddies,

FOREWORD

I'd like to thank and state that I'm much obliged to my cognoscenti and esteemed professor and thesis advisor Professor Mine YURTSEVER who provided me with all kinds of help and support since the beginning of this study, and guided me with valuable advice during my studies, did not spare her help, opened my horizons as an exemplary academician and suggested different perspectives and paths.

My Ph.D. committee members Professor Aysegul GOLCU's and Professor Serdar DURDAGI's suggestions were invaluable in the support of our research paper publications and Ph.D. thesis concerning theoretical/simulation results with experimental laboratory studies and making valuable ideas and contributions to our molecular docking and molecular dynamics simulations during my Ph.D. process and therefore I'd like to express my gratitude to them. I would like to thank my previous cognoscenti professor, Professor Ersin YURTSEVER for his invaluable teachings in the science of chemistry since my undergraduate degree at Koc University.

I also express my gratitude and infinite love to my dear mother and father who were with me at all times during my doctorate, to my Lawyer Ph.D. friend Umut Ozan ERGINER who gave me academic motivation and moral support when I said I was most tired, and my spiritual sister Associate Professor Arzucan OZGUR TURKMEN who aided me with nice conversations regarding the dynamics of academic life, to Specialist Medical Doctor Rahime OZGUR who was with me at all times when I got sick, to former Deputy Minister of Justice and now the Judge of the Constitutional Court, Lawyer Yildiz SEFERINOGLU for the times he taught invaluable life lessons to me, and Kahraman KELES who stood behind me as a true friend with his wisdom and genius and showed the true path that I should follow, and my Teaching Fellow Ph.D. friend Barbaros AKKURT who aided me in many aspects within the academic life and my dear friend Associate Professor Omer Tahir GUNKARA for sharing great fun times together regarding many aspects of the chemical science.

June 2022

Soykan AGAR
(Chemical and Biological Engineer)

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ABBREVIATIONS

1BNA	:B-Deoxyribonucleic acid with the dodecamer of (5'D(*CP*GP*CP*GP*AP*AP*TP*TP*CP*GP*CP*G)-3')
A, T, C, G	:Adenine, Thymine, Cytosine, Guanine nucleotides of DNA respectively
d(Ado)	:The voltammetric signal of Adenine
FDA	:U.S. Food and Drug Administration
FSDSDNA	:Fish Sperm Double Helical Deoxyribonucleic acid
GNP	:Gold nanoparticle
GPCR	:G-protein coupled receptor
d(Guo)	:The voltammetric signal of Guanine
H-Bond	:Hydrogen Bond
HC50	:The hazardous concentration (kg/m^3) of a chemical at which 50% of the target population (cells) dies
Kcal/mol	:kilocalories per mole
MD	:Molecular Dynamics
NPT	:Normal Temperature and Pressure
OPLS-2005, OPLS-3	:Optimized Potentials for Liquid Simulations version 2005 and the most recent 3
PDB	:Protein Data Bank
Ph.D.	:Doctor of Philosophy
RMSD	:Root mean square deviation
RMSF	:Root mean square fluctuation
TIP3P	:3-site rigid water molecule with charges and Lennard-Jones parameters assigned to each of the 3 atoms

SYMBOLS

Å **:** Angstrom = 0.1 nanometer

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THE COMPUTATIONAL STUDY ON THE ELUCIDATION OF THE BINDING INTERACTIONS AND MECHANISM OF ANTI-NEOPLASTIC PURINE DERIVATIVE DRUGS WITH DNA: MOLECULAR DOCKING AND MD SIMULATION STUDIES

SUMMARY

This Ph.D. thesis has begun to discover how to defeat cancer by causing apoptosis via studying the DNA binding mechanism of anticancer (antineoplastic) drugs. To do that, mechanisms should have been revealed regarding the silencing of specific nucleotides within the genes and/or breaking the double helix of cancerous cell DNA via performing molecular docking and molecular dynamics simulations to understand how chemical interactions and functional groups play roles in the binding of anticancer drugs to DNA. Fludarabine, Pemetrexed, Cladribine, Clofarabine, and Azacitidine, are very well-known marketed, significant anticancer medicinal drugs falling under the antimetabolite Purine analog drug class. Although these drugs were used in the past with FDA approval for treating various cancer types, their main indications for DNA binding, nucleotide regioselectivity, and mode of binding on DNA have not been discovered in detail yet in the scientific literature. Therefore, how they can be further developed from the perspective of altering the functional groups according to the laws and tools of pharmaceutical chemistry, organic chemistry, analytical chemistry, and computational *in-silico* drug design were deciphered with this Ph.D. thesis and supported with the research articles published along with it.

The above-mentioned Purine analog drugs are generally considered to be a group of agents with similar structures, possessing slightly different mechanisms of action, indications, and side effects. These agents are nucleoside analogs and are considered antimetabolites that interfere with or compete with nucleoside triphosphates in the synthesis of DNA or RNA or both. The substances are analogs of adenine or guanine and generally have excellent activity against chronic lymphocytic leukemia, acute lymphoblastic leukemia, acute myeloid-lymphoma leukemias, and small lymphocytic leukemia due to their preferential uptake, activation, and action in lymphoid tissue.

These drugs act by interacting with the cancerous cell DNA by turning off its function and protein expression. Hence, the binding interactions of these drugs with DNA in the chemical and pharmaceutical industry are significant topics for pharmaceutical, medical, bio/genetic engineering, and biochemical studies aimed at designing better DNA binding drugs. The discovery of how these drugs dock onto which region and nucleotide of DNA and the mechanism by which drugs bind to DNA are essential in discovering how to synthesize new drugs and learn how to make their indications perfect. A systematic general scheme and model of how drugs bind to DNA and which functional groups in drugs bind to which DNA nucleotide with which affinities were formed with this thesis, thus the contribution to the pathways of new drug design discovery and drug use methodology was made. Before the drugs go into production, it will now be possible to know how the chemical structures of other drugs and new

pharmaceutical drugs on the market should be in terms of studied functional groups, and what the DNA binding mechanism of the designed drugs might be, thanks to this schematic inferred information.

All over the world, the pharmaceutical industry spends hundreds of billions of dollars on new research settings that can synthesize efficiently, high-yield, non-toxic drugs possessing correct indications. Each antineoplastic drug requires an average of two to three billion dollars in research and development costs and at least 10 to 15 years for its indication to be approved by the FDA. Synthesizing specific antineoplastic drugs for the receptor target plays a crucial role in terms of saving time and money. Studying human DNA and antineoplastic drugs targeting it, which is the most significant of these receptors, is a very essential research topic on a global scale.

Thanks to this Ph.D. thesis and the published research articles produced from the subject of the thesis, a methodological system that can determine the indication mechanism, nucleotide regioselectivity, and binding type before synthesizing the drug had been created, thus as all the scientists aim in the future, a contribution may be made with this study on revoking the animal and human drug trial phases and can reduce the time loss that causes billions of dollars and immeasurable years spent in the research of antineoplastic drugs. Finding such a way to predict the mechanisms of these drugs, using various computational and simulation techniques, would greatly contribute to the national and world drug economy and scientific knowledge library. Since this is a very comprehensive theoretical study also involving the comparison of experimental results, it is believed that it will pioneer and shed light on future studies in this area.

The DNA interactions of the aforementioned 5 important cancer drugs have not been investigated in detail yet in the scientific literature. Therefore, this Ph.D. thesis study focuses on the binding properties of drugs with molecular dynamics (MD) simulations as well as molecular docking studies. Therefore, it was determined which region of the drug attacks which nucleotide of the cancer cell DNA and destroys the cancer cell DNA.

The unique value of this Ph.D. thesis was the discovery of the ability and capacity of the cancer drugs we studied to break down cancerous cell DNA by interacting directly with it. How this happens and what the actual mechanisms are for each drug have been examined in detail with both theoretical and experimental results in order to reveal them. The mechanisms of action of DNA fragmentation of such 5 important drugs in the pharmaceutical and chemistry world were not known in such detail, and in addition, it was not known which part of the DNA of the groups in the chemical structures of these drugs and how they interact. With the aid of such research, how these drugs can be developed further for more efficiency and how cancer cell DNA can be destroyed with more selectivity with the aid of studied functional groups have been now revealed through my work. It will be possible to accurately predict the DNA binding pattern, indication, activity, stability, and degree of DNA denaturation of any drug produced or to be produced in the future, just by checking its chemical structure.

The main objective of this thesis was to research which drug matches which binding pattern and therefore to determine the DNA binding affinity and efficiency of the drugs of interest. This knowledge, computed theoretically and compared with the results of multispectroscopic experimental studies, has revealed the capabilities of hitherto unknown mechanisms of antineoplastic drugs. To achieve such a goal, the effectiveness of interactions appears to depend on various factors, including the

affinity of the drug's external groups to DNA, and the topology of the binding. The mechanisms of these interactions are basically divided into four groups. The first is gene silencing by direct covalent binding of the drug to the backbone and nucleotides of the DNA, the second is gene silencing by non-covalent chemical interactions, and the third is the intercalation mechanism in which the drug gets stuck between the base pairs of the DNA and breaks down the DNA like a zipper, and the fourth is the groove binding mechanism to silence the DNA gene expression via minor and/or major groove binding that can shut down DNA completely.

By simulating and computing the theoretical stability and binding energies of such drugs docked onto DNA, there made be a comparison with multispectroscopic experimental findings to ensure the binding mode and efficacy. Finding out which drug has which mode of binding reveals the effectiveness of systematical silencing/cleavage (by intercalation or groove binding) of the DNA in the cancer cell. By doing so, it can be predicted that an antineoplastic drug can attack which part of the DNA in the cancerous cell and lead the cancerous cell to apoptosis, even by theoretically drawing its chemical structure at the design stage.

It is found with this Ph.D. thesis that Fludarabine suppresses the DNA via major groove binding, while Pemetrexed, Cladribine, and Clofarabine do it by minor groove binding whereas Azacitidine is an intercalator agent and unzips the helical structure of DNA completely. It is drawn from the pharmaceutical chemistry analysis of such massive data that when a Fluorine replacement takes place in the Purine ring of the aforementioned drugs, adenylation type of nucleotide-binding occurs whereas when Carboxylation, Methylation, and Hydroxylation take place in the Purine ring, poly-adenylation occurs. When a Chlorine replacement takes place in the Ribose ring, Guanidinylation ends and Adenylation occurs. Also, Chlorine replacement in the Ribose ring causes the mode of binding alteration from minor to major groove.

ANTİ-NEOPLASTİK PÜRİN TÜREVİ İLAÇLARIN DNA ETKİLEŞİMLERİNİN VE BAĞLANMA MEKANİZMALARININ MOLEKÜLER KENETLENME VE MD SİMULASYONLARI İLE İNCELENMESİ

ÖZET

Bu doktora tezinde, antikanser (antineoplastik) ilaçların DNA bağlama mekanizmaları incelenerek, apoptoza (hücre ölümüne) neden olan kanserin nasıl yenilebileceğine yönelik çalışılmıştır. Kimyasal etkileşimlerin ve fonksiyonel grupların bağlanmada nasıl rol oynadığını anlamak için, moleküler kenetlenme ve moleküler dinamik simülasyonları gerçekleştirilmiş ve genlerdeki spesifik nükleotitlerin susturulması ve/veya kanserli hücre DNA'sının çift sarmalının kırılması ile ilgili mekanizmalar ortaya çıkarılmıştır. Piyasada çok iyi bilinen FDA onaylı purin analogları sınıfına giren antineoplastik ilaçlar olan Fludarabin, Pemetrekset, Kladribin, Klofarabin ve Azasitidin, antimetabolit sınıfında oldukça önemli ilaçlardır. Bu ilaçlar geçmişte çeşitli kanser türlerini tedavi etmek için FDA onayı ile kullanılmış olsa da, bunların DNA bağlanması, nükleotit bölgesel seçiciliği ve DNA'ya bağlanma şekli için ana endikasyonları henüz bilimsel literatürde detaylı keşfedilmemiştir. Bu nedenle, farmasötik kimya, organik kimya, analitik kimya ve hesaplamalı in-siliko ilaç tasarımının yasa ve araçlarına göre fonksiyonel grupların değiştirilmesi perspektifinden, ilaçların nasıl geliştirilebilecekleri bu doktora tezi ile gösterilmiş ve birlikte yayınlanan araştırma makaleleriyle de bilimsel olarak desteklenmiştir.

Yukarıda sözü edilen Purin analog ilaçları, genellikle benzer yapılara sahip, biraz farklı etki mekanizmalarına, endikasyonlara ve yan etkilere sahip olan bir grup ilaç ajanı olarak kabul edilir. Bu ajanlar nükleosit analoglarıdır ve DNA veya RNA veya her ikisinin sentezinde nükleosit trifosfatlara müdahale eden ve/veya bunlarla rekabet eden antimetabolitler olarak kabul edilir. Bu ilaçlar, adenin veya guanin analoglarıdır ve tercihli alımları, aktivasyonları ve lenfoid dokudaki etkileri nedeniyle genellikle kronik lenfositik lösemi, akut lenfoblastik lösemi, akut miyeloid lenfoma lösemileri ve küçük lenfositik lösemiye karşı mükemmel aktiviteye sahiptirler.

Bu ilaçlar, kanserli hücre DNA'sı ile etkileşerek onun işlevini ve protein ekspresyonunu kapatarak etki gösterirler. Bu nedenle, kimya ve ilaç endüstrisinde bu ilaçların DNA ile bağlanma etkileşimleri, daha iyi DNA bağlayıcı ilaçlar tasarlamayı amaçlayan farmasötik, tıbbi, biyo/genetik mühendisliği ve biyokimyasal çalışmalar için önemli konulardır. Bu ilaçların, DNA'nın hangi bölgesine ve nükleotitine nasıl kenetlendiğinin ve ilaçların DNA'ya hangi mekanizmayla bağlandığının keşfedilmesi, yeni ilaçların nasıl sentezleneceğinin keşfedilmesi ve endikasyonlarının nasıl daha kusursuz hale getirileceğinin öğrenilmesi açısından yol gösterebilecektir. Bu tez ile ilaçların DNA'ya nasıl bağlandığına ve ilaçlardaki hangi fonksiyonel grupların hangi DNA nükleotitlerine nasıl afinitelerle bağlandığına dair sistematik bir genel şema ve model oluşturularak, yeni ilaç tasarımı keşif ve ilaç kullanım metodolojisinin yollarına katkı sağlanmıştır. İlaçlar üretime geçmeden önce, piyasada bulunan diğer ilaçların ve yeni farmasötik ilaçların, kimyasal yapılarının çalışılan fonksiyonel gruplar açısından

nasıl olması gerektiğinin ve tasarlanan ilaçların DNA bağlanma mekanizmasının ne olduğunun bilinmesinin, bu şematik çıkarım bilgileri sayesinde bilinebileceği gösterilmiştir.

Tüm dünyada ilaç endüstrisi, doğru endikasyonlara sahip, verimli, yüksek sentetik majör ürün odaklı, toksik olmayan ilaçları sentezleyebilen yeni araştırma ortamlarına yüz milyarlarca dolar harcamaktadır. Her bir antineoplastik ilaç, araştırma ve geliştirme maliyetlerinde ortalama iki ile üç milyar dolar ve endikasyonunun FDA tarafından onaylanması için en az 10 ile 15 yıl gerektirmektedir. Reseptör hedefi için spesifik antineoplastik ilaçların sentezlenmesi, zaman ve para tasarrufu açısından çok önemli bir rol oynamaktadır. Bu reseptörlerin en önemlisi olan insan DNA'sı ve onu hedefleyen antineoplastik ilaçların araştırılması, küresel ölçekte çok önemli bir araştırma konusudur.

Bu doktora sayesinde tez ve tez konusu ile ilgili olarak üretilen yayınlanmış araştırma makaleleri, ilacın sentezlenmesinden önce endikasyon mekanizmasını, nükleotit bölge seçiciliğini ve bağlanma tipini belirleyebilen bir metodolojik sistem oluşturmuştur. Böylece bilim insanlarının hedeflediği üzere, gelecekteki hayvan ve insan deney evrelerini ortadan kaldırılmasına katkıda bulunulabilecek ve antineoplastik ilaçların araştırılmasında harcanan milyarlarca dolara neden olan zaman kaybı azaltılabilecektir. Çeşitli hesaplama ve simülasyon teknikleri kullanılarak bu ilaçların mekanizmalarını tahmin etmenin bir yolunu bulmak, ulusal ve dünya ilaç ekonomisine ve bilimsel bilgi kütüphanesine büyük katkı sağlayacaktır. Bu tez, deneysel sonuçların karşılaştırılmasını da içeren oldukça kapsamlı bir teorik çalışma olduğundan, bu alanda ileride yapılacak çalışmalara öncülük edeceği ve ışık tutacağı düşünülmektedir.

Bahsedilen 5 önemli kanser ilacının DNA etkileşimleri bu zamana kadar henüz bilimsel literatürde detaylıca araştırılmadığı görüldüğünden, bu nedenle, bu doktora tez çalışması, moleküler dinamik (MD) simülasyonları ve moleküler kenetlenme çalışmaları ile ilaçların bağlanma özelliklerine odaklanmaktadır. Böylece ilaçların hangi fonksiyonel bölgesinin, kanser hücresi DNA'sının hangi nükleotitine saldırdığı ve bu yolla kanser hücresi DNA'sını yok ettiği belirlenmiştir.

Bu doktora tezinin önemi ise, üzerinde çalışılan kanser ilaçlarının, kanserli hücre DNA'sı ile doğrudan etkileşerek parçalama yeteneğinin ve kapasitesinin keşfidir. Her ilaç için bunun nasıl olduğu ve gerçek mekanizmasının ne olduğu konusu hem teorik hem de deneysel sonuçlarla ayrıntılı olarak incelenmiştir. Farmasötik ve kimya dünyasında bu kadar önemli 5 ilacın DNA parçalanmasının etki mekanizmaları bu kadar detaylı bilinmiyordu ve ayrıca bu ilaçların kimyasal yapılarındaki grupların, DNA'nın hangi kısmıyla nasıl etkileşime girdikleri bilinmiyordu. Bu araştırma sayesinde, bu ilaçların nasıl geliştirilebileceği ve kanser hücresi DNA'sının nasıl daha verimli bir şekilde belli fonksiyonel gruplar tarafından yok edilebileceği, çalışmalarım sayesinde daha net ortaya çıkarıldı. Üretilen veya gelecekte üretilebilecek herhangi bir ilacın, DNA bağlanma modeli, etkileşimi, aktivitesi, stabilitesi ve DNA denatürasyon derecesini bu sayede sadece o ilacın kimyasal yapısını kontrol ederek doğru bir şekilde tahmin etmek mümkün olacaktır.

Bu tezin temel amacı, hangi ilacın hangi bağlanma modeline uyduğunu araştırmak ve dolayısıyla ilgili ilaçların DNA bağlanma afinitesini ve etkinliğini belirlemektir. Teorik olarak hesaplanan ve multispektroskopik deneysel çalışmaların sonuçlarıyla karşılaştırılan bu bilgiler, antineoplastik ilaçların şimdiye kadar bilinmeyen bir açıdan mekanizmalarının yeteneklerini ortaya çıkarmıştır. Böyle bir amaca ulaşmak için bağlanmanın etkinliğini sağlayan önemli olan faktörler, ilacın dış gruplarının DNA'ya

olan afinitesi ve bağlanmanın topolojik şekli olarak görülmüştür. Bu etkileşimlerin mekanizmaları temel olarak dört gruba ayrılır. Birincisi ilacın DNA'nın fosfodiester omurgasına ve nükleotitlerine doğrudan kovalent bağlanmasıyla gen susturulması, ikincisi kovalent olmayan kimyasal etkileşimlerle gen susturulması ve üçüncüsü ilacın baz çiftleri arasında sıkışıp kaldığı ve DNA'nın çift sarmalını parçaladığı interkalasyon mekanizmasıdır ve son olarak dördüncüsü ise, DNA'yı tamamen kapatabilen minör ve/veya majör oluk bağlanma yoluyla DNA gen ekspresyonunu susturan oluk bağlama mekanizmasıdır.

DNA'ya bağlanan bu tür ilaçların teorik stabilitesi, bağlanma enerjileri, bağlanma modu ve etkinlikleri, bilgisayarda simüle edilerek ve hesaplanarak ve bunun yanısıra multispektroskopik deneysel bulgularla karşılaştırılarak elde edilmiştir. Hangi ilacın hangi bağlanma moduna sahip olduğunu bulmak, kanser hücresindeki DNA'nın sistematik susturulmasının/parçalanmasının (interkalasyon veya oluk bağlanması yoluyla) etkinliğini ortaya çıkarmada yardımcı olmuştur. Bu sayede antineoplastik bir ilacın, teorik olarak tasarım aşamasında kimyasal yapısını çizerek bile kanserli hücredeki DNA'nın hangi kısmına saldırabileceği ve kanserli hücreyi apoptoza götürebileceği tahmin edilebilir hale gelmiştir.

Fludarabinin DNA'yı majör oluk bağlanma yoluyla baskıladığı ve gen ekspresyonunu susturduğu, Pemetrekset, Kladribin ve Klofarabin'nin minör oluk bağlanma yoluyla yine bunu yaptığı, Azasitidin'in ise bir interkalatör ajan olduğu ve DNA'nın sarmal yapısını tamamen parçaladığı bu tezle ortaya konulmuştur. Farmasötik kimyasal bağlanma şeması oluşturularak elde edilen büyük veri analizinden ortaya çıktığı üzere, yukarıda bahsedilen ilaçların Purin halkasında bir Flor yer değiştirmesi gerçekleştiğinde, Adenilasyon tipi nükleotit bağlanması meydana gelirken, Purin halkasında bir Karboksilasyon, Metilasyon ve Hidroksilasyon meydana geldiğinde, hiper-Adenilasyon meydana geldiği, ortaya çıkarılmıştır. Riboz halkasında bir Klor değişimi gerçekleştiğinde, Guanidinilasyon sona erer ve Adenilasyon meydana gelir. Ayrıca, Riboz halkasındaki Klor yer değiştirmesi, minör oluk bağlanmasını majör oluk bağlanması moduna değiştirir.

1. INTRODUCTION

1.1 Purpose of Thesis

In this Ph.D. thesis, the DNA binding of antineoplastic medicinal drugs, Fludarabine, Pemetrexed, Cladribine, Clofarabine, and Azacitidine is discussed thoroughly in terms of existing experimental and theoretical studies explaining the multispectroscopic utilization and theoretical nucleotide affinities and toxicities towards DNA under which conditions and parameters from the perspectives of computational chemistry, genetic engineering, drug engineering, pharmaceutical chemistry, analytical chemistry, physical chemistry, and organic chemistry. These antineoplastic pharmaceutical drugs are chemotherapeutics that are popularly used in oncological treatments for many years. Therefore, by deciphering, comparing, and discussing their DNA binding mechanisms, their true regioselectivity towards nucleotides and the indication effects on patients can be well understood. Thus, their toxicity values might be elucidated with DNA binding mechanisms and suggest a way to what can be done regarding the alteration in the pharmaceutical groups of the whole drugs for drug discovery and drug development purposes. The research papers published throughout this Ph.D. thesis study, consist of academicians who already work in the research of these above-mentioned parameters of DNA binding oncological drugs. Hence, this thesis contributes a significant overall guide and knowledge for a possible future de novo drug design from the scratch without needing to experimentally synthesize an antineoplastic drug that would cost billions of dollars resulting in 10 - 15 years of research time for the formulation. With his thesis, the pharmaceutical chemistry domains of drugs are mapped and their affinities towards DNAs specific sites are well understood and depending on this knowledge, can be designed solely using the computational tools. This would contribute a great addition to the shelves of chemistry, genetic/drug engineering, medicinal biology, pharmacy, and oncology.

1.2 Literature Review

1.2.1 The necessity of cancer research targeting DNAs

Continuously, the expansion of cancer disease's spread with the confirmed numbers of 2016 pass 1,685,210 patients for the types including breast, lung/bronchus, kidney/renal pelvis, prostate, non-Hodgkin lymphoma, bladder, melanoma (skin), colon/rectum, leukemia, thyroid, endometrium, and pancreas that has been quite a major problem that needs to be solved immediately with the help of some scientific areas including experimental analytical chemistry, genetic/drug engineering, medical sciences, pharmaceutical chemistry, and computational chemistry/biology. Worldwide, cancer is one of the leading causes of mortality due to the fact that cell cycles become intervened causing uncontrollable cell growth and cell differentiation. These malignant cells occur in any organ or tissue at any time even in healthy humans. Therefore, this drew the attention of the scientists to find a once and for all treatment. In terms of technical definition, cancer is the uncontrolled cell proliferation where the cancerous cells occur depending on the heavily non-repaired DNA damages in the nucleotides and a failure to stop the cell cycle before the damaged DNA is transmitted to daughter cells. Pyrimidine and Purine, the nucleic acids of DNA, are the essential building blocks forming the helical DNA structure where any inhibition of their synthesis has been a major focus for anticancer therapy research. A wide range of carcinogens, UV radiation, and oxidation continuously attack the helical structure of DNA and affect the hydrogens bonds of the nucleotides. This causes lesions in most cases where strands break or form DNA adducts or even may lead to base mismatches, insertions, and deletions. Even though the fact that some neurodegenerative diseases may occur due to these reasons, most of the cases end up with cancerous cell formation. Our healthy cells evolved a mechanism to be able to detect such DNA damages and signal for repair via protein machinery. The design or repurposing of a medicinal drug that can help this mechanism either by involving in this protein pathway (methylation agents, DNA polymerases, etc) to repair DNA or directly eliminate the helical or euchromatin structures of cancerous DNAs is the crucial concept of the fight against cancer since there were unsuccessful attempts in the past regarding the inhibition of purines and pyrimidines efficiently. Since the first fight with cancer, there occurred some essential main mechanisms to disrupt the cancerous cells from the beginning of the first methodology found in the 1970s. The first approach is the ‘’cytotoxic

therapy'' which is also known as the ''DNA damaging therapeutics'', the second is ''specific drug targeting class'' and the third is ''enhancing the immune system agents''. Anticancer drugs fall under the following categories, where ''Hormones and hormone Antagonists'' supress the hormones that cancerous cells causing secretion, ''mitotic inhibitors'' interrupt cancerous cell divisions, ''alkylating agents'' alkylate DNA base pairs to cause impaired DNA synthesis, and thus DNA polymerase cannot dock onto DNA hexamers and cannot perform its repair task properly, while ''anti-metabolites (anti-folates, pyrimidine (T,C) and purine (A,G) analogues)'' incorporate as false analogues into the DNA structure, and the last is miscellanous anti-cancer drugs category.

12 drugs of interest, the ligands (5 drugs targeting hDNA and the other 7 their hypothetical derivatives) were studied comprehensively via molecular docking and molecular dynamics (MD) simulation techniques as can be seen in Figure 1.1. The five main drugs of this thesis (Fludarabine, Pemetrexed, Cladribine, Clofarabine and Azacitidine) fall under the category of chemotherapeutic DNA damager agents which are small cytostatic molecules that often bind to double-stranded DNA resulting in modifications of their structural and nanomechanical properties and thus interfering with the cell proliferation process of cancer cells. These cytotoxic drugs are used for their interference in various ways with cell division. The drugs cannot distinguish between normal and malignant cells usually except for rare cases, but inhibit the process of cell division with the objective to kill cancer before the host. To be able to reverse the disease or at least slow down its proliferation in the pace of cancerous diseases, the pharmaceutical drug design and repurposing that targets cancerous cell DNAs has become one of the major strategies. Even though there were some quite many antineoplastic drug usage so far, the reported incidents in cancerous diseases worldwide have expanded rapidly. Thus, the great efforts within the academic and industrial community regarding the synthesis and applications of antineoplastic drugs were not enough. It was realized that computational chemistry and computational biology/genetics have begun playing essential roles in terms of deciphering the mechanisms, binding modes, and possible toxic sites of these drugs, rather than instead of solely focusing on the synthesis of such drugs within the scope of organic chemistry, pharmaceutical chemistry, chemical/genetic engineering, and pharmacology. This leads us to the point where the drug repurposing and drug discovery settings are now

in a new direction. Therefore, the pharmaceutical drug designs that specifically target cancer cell DNAs have earned a crucial title in the chemical and pharmaceutical sciences along with the increasing indication efficiency in the cure of such a widely expanding health issue. The pharmaceutical drugs in the oncological field keep growing in numbers, especially the ones that can irreversibly destroy DNA double-helical structure completely via intercalation or bind to DNA to shut it down by either inhibiting its protein expression ability via external binding or groove binding. The drugs that need to be repurposed or *de novo* designed should have high-yield, non-toxic effects, immediately indicating, effective and long-lasting half-life activity. So it should be noted that DNA is one of the key and main targets in any type of cellular system for cytotoxic and therapeutic drugs, deciphering and knowing the insight of the mechanism of how an antineoplastic drug binds to DNA with which type of bonding and which domains play a significant role in this binding is quite a weighty knowledge to reveal the true potential of such antineoplastic drugs [1-26].

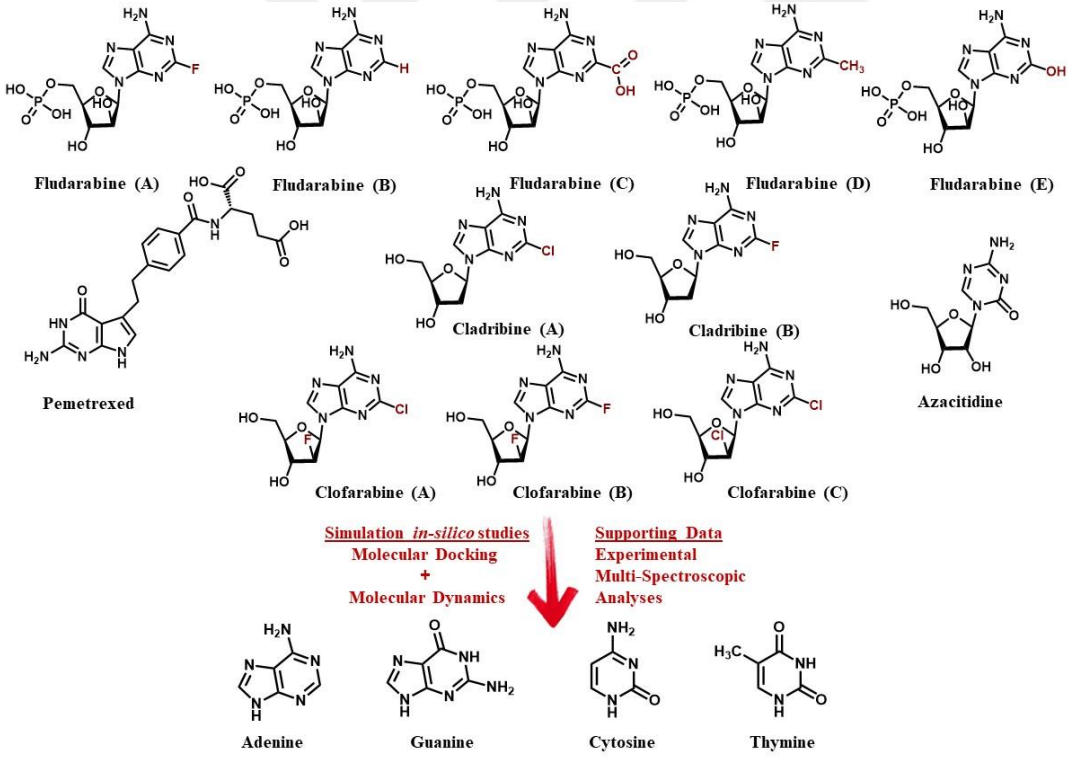

Figure 1.1 : The chemical structures of all medicinal antineoplastic drugs and DNA nucleotides studied in the Ph.D. thesis.

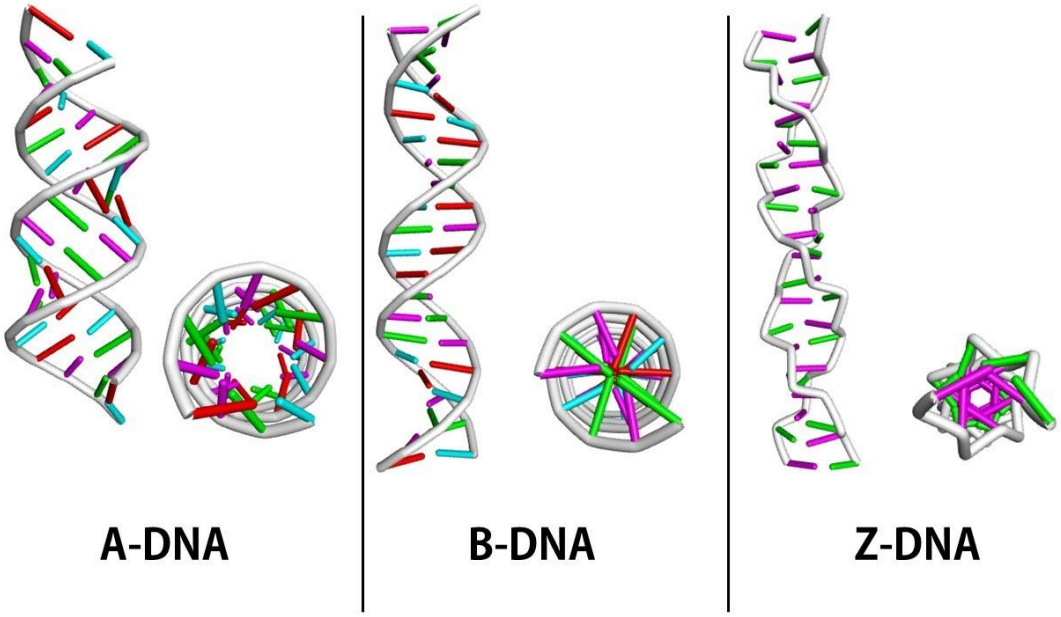

Figure 1.2 : DNA types: A-DNA, B-DNA and Z-DNA from left to right.

There are three types of DNA structures, A-DNA, B-DNA, Z-DNA. The most common and natural one is B-DNA, which is the essential right-handed double helix with strong hydrogen bonds between the nucleotides residing on opposite strands, found almost in all organisms and the ideal target/receptor for this thesis due to this reason. The second one is A-DNA, which is also a right-handed double helix similar to B-DNA. However, it is more compact, shorter with its base pairs and these base pairs are not perpendicular to the helix-axis as in B-DNA. When a crystallization occurs under dehydrating conditions, B-DNA turns into A-DNA. The third one is Z-DNA, which is the left-handed double helix form of B-DNA, can form *in vitro* from B-DNA under low salt conditions or by raising negative super helical stress. 1-BNA is the most common double helical structure found in nature and as well as humans. The double helix is right-handed with about 10 - 10.5 base pairs per turn with the sequence of d(CGCGAATTCGCG) dodecamer. It is commonly used structure for hDNA in prestigious, high impact factored research papers. If the heteroatom oxygen within the sugar domain of a nucleotide looking upwards, then that section of DNA is a major groove. If it looks downwards, it is minor groove then. The major groove is slightly known to be deeper than the minor groove, minimum of 8.5 Angstroms depth compared to 7.5 Angstroms of the minor groove [27-29].

1.2.2 The Drug-DNA interactions and binding mechanisms

The drugs of interest were not studied within the scientific literature regarding the determination of their binding mode and the above-mentioned mechanism types via both analytical methods and/or molecular docking and molecular dynamics studies. DNA binding antineoplastic drugs penetrate the cellular membrane and nuclear membrane via either carrier proteins or diffusion and carry themselves into the nuclear cytoplasm. After this stage, they begin interacting with the euchromatin and choose intron regions of the genes for the correct interaction and the shutdown of cancerous cell DNA to inhibit its proliferation any further.

Fludarabine, Pemetrexed, Cladribine, Clofarabine and Azacitidine have low molecular weight and less branched out chemical structures compared to other antineoplastic types in the scientific literature and tend to get into the cell nucleus and exert direct interaction with DNA easily compared to some bigger and bulkier structured drugs. Molecules such as Cytarabine, Cisplatin, Arsenic Trioxide, Fludarabine, Pemetrexed, Cladribine, Clofarabine, and Azacitidine are the tinier structured drugs where they can dock onto the DNA's double helices directly with very strong hydrogens bondings or cleave the nucleotides via intercalation mechanism with significantly high efficiency. The tendency of an antineoplastic drug to reach DNA in the nucleus within the cellular environment without any alteration in its structure is a significant issue that the pharmaceutical drug designers deal with daily and, therefore finding out what sort of mechanisms are involved in such binding to DNA's varying domains is also another significant question that needs to be answered. Therefore, deciphering the affinity strength and the domain choice of the drug to DNA, make this Ph.D. thesis paper unique in its own way [30-36].

Fludarabine is an anti-neoplastic drug under the purine derivative category targeting cancerous cell DNA and its nucleotides to shut it down where it is still in the utilization in the treatments of Non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and acute myeloid leukemia, small lymphocytic lymphoma. Finding out its mode of binding mechanism is a significant subject for pharmaceutical and biochemical studies aiming at designing better DNA binding drugs. With its commercial name called Fludarabine Phosphate, it is used as a commercial chemotherapy medicinal drug in the treatment of hematological cancers. Rather than being able to decipher its nucleotide-binding mechanism, in the scientific literature, it is most popular with interference in

DNA synthesis. Although Fludarabine is known to possess strong counter-indications varying from lymphopenia to severe autoimmune hemolytic anemia, its targeted drug delivery enhances the therapeutic effects of other drugs by improving both the amount and the selectivity of the delivered drug to the targeted tissues. Researchers actively look for new cancer treatment methods involving more than one chemotherapeutic at once. Besides also antibody treatments falling under immunology is a good choice representing one of the most effective ways for targeted treatment. However, due to the lack of tumor-specific antibodies and their high costs that cannot be paid by patients, small compounds such as Folic Acid, are also commonly used along with Fludarabine since Folate receptors are highly expressed extensively in cancerous cells. In one of the clinical applications, in order to both enhance the drug indication and reduce counter-indication, gold nanoparticles (GNPs) are used to covalently be bound to Folate and Fludarabine all together forming the structure of GNP-F/f. The GNPs' role here is to become a vector, a carrier molecule targeting cancerous cells. In such dual (GNP-Fludarabine) or even trio-linked (GNP-F/f) chemotherapeutic application, the efficiency for targeting increases drastically. The higher cell-killing ability has been formed with such application where the selectivity of the drug increased very much in terms of pharmacological results. This means that the indication of the drug increased (EC50 value efficiency) while the toxicity (HC50 value) decreased. Nearly no cytotoxicity levels were observed. So, all in all, Fludarabine has been studied recently regarding the determination of its binding mode, and its deciphered mechanism [37-43, 96].

Pemetrexed is a folate analog that is widely active and utilized in a wide variety of solid tumors, including non-small cell lung cancer (NSCLC), mesothelioma, breast, bladder, head and neck, and ovarian cancers. The combination studies of Pemetrexed with Gemcitabine, Carboplatin, Cisplatin, Oxaliplatin, and Vinorelbine demonstrate promising antitumor activity. Pemetrexed's efficiency is accepted to be very good and it is generally well-tolerated as first-line therapy when in combination with Cisplatin and as second-line or in monotherapy for advanced non-squamous NSCLC. Pemetrexed is a significant selection for use in NSCLC, especially in terms of target therapy in patients with non-squamous histology. The combination of Pemetrexed and Gemcitabine in Phase III clinical trials yielded very good results, and as well as the same goes for the combination of Pemetrexed and Cisplatin. Within the context of this

thesis, the knowledge of the binding interaction of Pemetrexed with dsFSDNA and the binding domains were deciphered in detail to explore all possible affinities of nucleotide domains and Pemetrexed's groups. Thus, a contribution can be made to the fields of oncology, immunology, chemistry, pharmacy, bioengineering, biochemistry, and medicinal biology. Within the theoretical chemistry field, the computational chemistry and computational genetic tools greatly enhanced the understanding of the true potential of antineoplastic drugs that the scientists aim at designing better DNA binding drugs. Although, some of the DNA mode of binding discoveries of some antineoplastic drugs have been studied, the DNA interaction of Pemetrexed has not been studied in detail. Very recently, the elucidation of the experimental and theoretical investigation of Pemetrexed binding mechanism via multispectroscopic techniques including Ultra-Violet (UV) absorption spectroscopy, thermal denaturation, fluorescence, and Fourier-Transform Infrared Spectroscopy (FTIR) spectroscopy, electrochemical and viscosity measurement methods as well as with molecular docking studies and molecular dynamics studies were investigated thoroughly. The cluster analyses and the lowest energy (highest affinity) docking poses of molecular dockings showed that Pemetrexed binds to DNA via minor groove binding mode. Pemetrexed falls under the category of antimetabolites that inhibits the activity of Purines' and Pyrmidines' synthesis in the scientific literature as well even though the fact that direct attack on the nucleotides was not studied. Pemetrexed has wide activity in epithelial solid tumors and vitamin supplementation is advised in clinical applications where it diminishes the toxic effects of Pemetrexed. Great feasibility and activity were observed regarding Pemetrexed when in combination with Platinum compounds and Gemcitabine. Daily folate supplementation, when taken orally, can reduce the incidence of hematologic toxicities while preserving the antitumor activity of Pemetrexed. In Phase III malignant mesothelioma patients, the efficacy of Pemetrexed was found to be very good [44-47].

Cladribine and Clofarabine are purine derivative anti-neoplastic drugs that are used in the treatments of varying cancers including hairy cell leukemia, acute lymphoblastic leukemia, and acute myeloid leukemia respectively. They achieve their functionality by interacting directly with DNA. Therefore, the binding interactions of such drugs with DNA are significant subjects for pharmaceutical and biochemical studies aiming at designing better DNA binding drugs. Cladribine was first synthesized in the 1970s

and its use in the antineoplastic role was discovered at the beginning of the 1990s where it is a very well tolerated antineoplastic drug with very good selectivity (activity/toxicity ratio) in hairy cell leukemia. Cladribine showed significant efficiency in several other hematological malignancies, including chronic lymphocytic leukemia, low-grade malignant lymphoma, and Waldenstrom macroglobulinemia. The drug's nonhematological toxicity, such as alopecia, nausea, vomiting, stomatitis, diarrhea, and organ toxicity is mild or absent. Also, it is very well known that Cladribine is used in the treatment of autoimmune disease, Multiple Sclerosis. Therefore, the scientific literature backs up the data that while fighting cancer, if the patients have Multiple Sclerosis as well, the drug doesn't increase the expansion of any cancer cell while suppressing the damage in the myelin that Multiple Sclerosis caused since it is also an immunosuppressive drug. Cladribine's impressive activity in other lymphoproliferative disorders was generally underappreciated in the scientific literature. Therefore, studying its mechanism and possible uses in the prevention of cancer via theoretical/computational studies is crucial. Cladribine was observed to possess particular activity among lymphoid disorders specifically in chronic lymphocytic leukemia, lymphoplasmacytic lymphoma, marginal zone lymphoma, and mantle cell lymphoma. Cladribine has a remarkable selectivity in hairy cell leukemia (HCL). It produces an overall response rate (ORR) of 98% with 91% complete responses (CRs), and extremely prolonged remissions after only a single 7-day course with a very favorable toxicity profile [48-60].

Clofarabine is an intentionally designed, second-generation purine nucleoside analog. It was designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of both Cladribine and Fludarabine which were studied previously by our group. Clofarabine also demonstrated high selectivity when both utilized as a single agent and/or in combination with other agents as front-line therapy and refractory-relapsed adult myeloid leukemias. Clofarabine possesses three main mechanisms, the first is the inhibition of ribonucleotide reductase, where the second is the induction of apoptosis and the third is docking into DNA. One of the leading reasons emphasizing the significance of this medicinal drug is that the development of new antineoplastics in children was quite left behind as historically compared to the medicinal anti-cancerous drug usage of adults. However, unlike its predecessors, in the year 2004, Clofarabine was approved by FDA (United States Food and Drug

Administration) and Clofarabine is quite active in adults within the usage of hematologic malignancies including acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) and also it is used intravenously and proved its efficacy in the pediatric patients who have advanced leukemias and refractory-relapsed acute lymphoblastic leukemia (ALL). Concerning the above-mentioned mechanisms of action of Clofarabine, it was predicted that it acts synergistically with other chemotherapeutic agents such as platinum-based medicinal drugs, purine nucleoside analogs, and DNA damaging/cross-linking agents such as anthracyclines. In adults, there is scientific literature claiming that the remission of this medicinal drug may be low when it is used as a single agent. Also, in some case studies, it was observed that it causes hepatotoxicity in leukemia patients. However, when Clofarabine was used with other chemotherapeutic agents such as Cyclophosphamide and Etoposide, although some pancreatitis and lipase elevation symptoms were observed in minor cases, all of the patients were treated to reach full health without going over the dose-limiting toxicity levels. Cytarabine is also another antineoplastic with which was used in combination with Clofarabine. The dual effect in this such case was not quite beneficial according to the pharmacological and chemical literature [61-79].

Azacitidine is a pyrimidine nucleoside analog used to treat certain subtypes of myelodysplastic syndrome. It is a nucleoside analog of Cytidine that inhibits DNA methylation by trapping DNA methyltransferases. It was first designed as a cytotoxic agent and an application to the FDA requesting its approval was turned down more than nearly 40 years ago. Its hypomethylating agent role and DNA hypermethylation effect in cancer in the 1980s emphasized its value to be reevaluated and eventually led to its approval by FDA a decade ago. Azacitidine's mechanism involves hypomethylation of DNA's nucleotides and consequent reactivation of silenced genes including tumor-suppressor genes. It is also thought that it intercalates into RNA. In terms of clinical trials, one of the leading studies states that a randomized, open-label, controlled trial compared the safety and efficacy of subcutaneous azacitidine plus supportive care with supportive care alone (observation group) with any of the five subtypes of myelodysplastic syndromes and acute myeloid leukemia of 200 patients, Azacitidine was administered at a subcutaneous dose of 75 mg daily for a week in every month; and then the dose was increased to 100 mg when there is no beneficial indication was observed after two treatment cycles. 15.7% of the patients were

remitted. The median duration of clinical response took 330 days where 75% of the responding patients were still in partial remission or fully recovered. Also, in terms of combination studies, Azacitidine and its deoxy derivative, Decitabine, can work together to form a better hypermethylation function in DNA causing growth arrest and apoptosis in tumor cells. These antineoplastic drugs proved that the clinical activity in the treatment of certain hematologic malignancies were gene hypermethylations and were shown to be doing their job successfully. The demethylating agent Azacitidine and Decitabine, are powerful inhibitors of DNA methylation. In the experimental studies, it was observed that after its phosphorylation, Azacitidine incorporates into RNA where it suppresses RNA synthesis and creates cytotoxicity in the cancerous cell [80, 98-99].

The effectiveness of the interactions depends on some varying factors including the affinity of the drug's external groups and the mode of binding. The mechanisms of these interactions are categorized into four main groups. The first one is the covalent bonding of the drug directly with DNA, whereas the second main group consists of the non-covalent interactions which also have three sub-groups. The first sub-group is the intercalation mechanism where the drug is inserted between the base pairs of DNA via strong inter-molecular forces causing a rupture in the hydrogen bondings of DNA bases irreversibly, the second is the groove binding mode which can be named either as a minor groove or major groove binders depending on the drug's location within the DNA's minor or major groove and the third is the external binders where the drug is located outside of the DNA and mostly forms hydrogen bonds with the phosphodiester backbone. The third main group contains DNA cleavage agents which can break DNA strands directly via the sugar-phosphate backbone whereas the fourth group includes the alkylators where their strong electrophilic nature forms an irreversible covalent bond with the nucleophilic domains of DNA, resulting in transcription inhibition [81-84].

1.2.3 The medium of cancerous cells

It is quite clearly stated in the scientific literature that the pH values of the cell cytoplasm, nucleus cytoplasm (intracellular matrix in general), and the intercellular matrix drop in cancerous cells. The mechanism behind this is as follows; All cells rely on glycolysis and neoglucogenesis pathways to generate Adenine Triphosphate (ATP) so that the nurturing, maintenance, and proliferation can go on as usual. In terms of

definition, Pasteur-effect is the anaerobic pathway where solely 2 ATP gets produced instead of 38 ATP production in the aerobic pathway. While in the anaerobic pathway, pyruvate is formed and it is further converted to lactic acid. This is normally known as the anaerobic condition of someone who is utilizing all the oxygen in the skeletal muscles during an exercise. Due to the oxygen limitations, the body produces much less ATP than it used to be depending on the formation of lactic acid. Just like in that situation the cancer cells undergo a similar situation and cancer cells metabolize glucose to lactate even when there is enough oxygen which is called Warburg effect in the scientific literature. After the accumulation of lactic acid along with the reduced efficiency in ATP conversion, the cell's biological system requires increased glucose flux and the up-regulation of glycolysis. This creates a loop where the acidic microenvironment should form non-stop in the cancerous cells. Therefore, cancerous cells alter their metabolic pathway from the Pasteur-effected one into the Warburg-effected one. This alteration creates an acidic microenvironment within the tumor and becomes the driving force for a positive carcinogenesis feedback loop. Hence, the tumor acidity causes the tumor microenvironment to select certain cell phenotypes that will be able to survive in such a caustic environment where the other cell types that will not be able to adapt to this will perish. So in fact, what tumor cells do is that they lower their own fitness by creating an acidic environment which further causes chaos for the adjacent healthy cells and forces them to make a choice regarding whether to become a cancerous cell or die. Solid tumors become acidic due to increased glucose production and varying above-mentioned enzymatic accumulations which cause fermentative metabolism and poor perfusion. It has been found that this pH drop causes a significant increase in protons which enhance local invasive growth and metastasis. The excess protons formed due to fermentation within the cell cytoplasm diffuse through the phospholipid bilayer to reach the intercellular matrix where adjacent healthy cells reside and are nurtured. Within this proximal tumor microenvironment, the healthy cells are heavily damaged by the excess proton assault. The tendency of the cancerous cells to invade the adjacent healthy tissues results in local tumor growth which is the beginning of possible metastasis. Sometimes even targeted sodium bisphosphate type bases are sent to these microenvironments to stop or slow down the growth of a possible metastasis. Since there is an increased glucose production in a cancerous cell, both proton production and excretion to the outside increase drastically.

In malignant tumors, pH drops to 6.5 – 6.9 compared to the healthy tissue cells under physiological conditions where they have the pH range of 7.2 – 7.4 [85-93].

Since solid tumors are high in acidity and known to possess excess presence of hydrogen ions, reducing the acidity can lead to tumor tissues for cancer treatment. Among many antineoplastics, the Acridine chromophore compounds are able to intercalate DNA's bases to lead the cells into apoptosis. The carboxylic groups neutralize the negative charge caused by the acidity of the tumor. Acridine-9-carboxylic does not intercalate healthy cell DNA nucleotides. Hence, this is a target selective method in cancer therapy and knowing the mode of binding in this context is crucial. That is why structure optimization and drug repurposing are essential in this oncological field for long-term antineoplastic drugs such as Fludarabine, Pemetrexed, Cladribine, Clofarabine, and Azacitidine since their mode of bindings and the extents of their nucleotide affinities are not known in the scientific literature [94, 95].

1.3 Hypothesis

Initially, from the perspective of scientific literature, the factors that satisfy how antineoplastics can easily interact with DNA and with which domains they interact were not very well known. However, the research of this Ph.D. thesis will shed light upon this topic. The hypothesis is to find out the relation between the size, ESP charge, modes of binding, binding tendencies of functional groups and stabilities of ligands (Fludarabine, Pemetrexed, Cladribine, Clofarabine, and Azacitidine and their respective derivatives) towards the nucleotides of DNA so that in the near future depending on this correlation knowledge, a de novo drug design of an antineoplastic drug can be made that has great indiction that suppressing a specific nucleotide of cancerous cell DNAs without needing to go into experimental studies, spending billions of dollars and decades of organic and analytical chemistry research and thus this will allow the computational chemistry science, pharmaceutical chemistry, and genetic engineering to achieve a great goal in guessing the behaviours of drugs while they are being designed.

2. MAIN BODY TEXT: METHODS, RESULTS & DISCUSSION

2.1 Methods

2.1.1 Molecular docking simulations

2.1.1.1 Target preparation

A common human DNA fragment as 1BNA (PDB NDB ID: BDL001) was selected for docking protocols and its X-ray crystal structure was downloaded from the PDB databank. Water molecules inside the crystal structure were deleted and hydrogen atoms were added. A grid box of 60, 60, and 110 points in x, y, z directions was built around the center of the DNA fragment, so the whole structure is enclosed in the box.

2.1.1.2 Ligand preparation

3-D structures of Fludarabine (ZINC ID: 3927870), Pemetrexed (ZINC ID: 1851132), Cladribine(ZINC ID: 3798064), Clofarabine (ZINC ID: 3798247), Azacitidine (ZINC ID: 3861768) were downloaded from ZINC database. Polar hydrogen atoms were added as default. The protonation state of Azacitidine molecules was adjusted according to the data in the ZINC database. Using Avogadro software, Gasteiger-Marsili charges were established and geometrically optimized and then fed into Autodock Vina 1.1.2 and Desmond program for molecular docking and molecular dynamics simulations.

2.1.1.3 Docking procedure

All docking calculations were performed with AutoDock Vina 1.1.2. ranging from 50 posed simulations to 100 posed simulations, totaling 2000 poses for each drug, where all of the drugs were run illustrating the interaction of the drug and the DNA fragment. The docking scores obtained were expressed as Gibbs free binding energy in units of kcal/mol. The docking pose with the most favorable binding energy within the best-clustered data was chosen to be used as the initial structure for MD simulations in each drug.

2.1.2 Molecular dynamics (MD) simulations

Utilizing Desmond Program, all the ligands were run for molecular dynamics (MD) simulations with 50 nanoseconds, 100 nanoseconds, and 200 nanoseconds time intervals each including 5000 poses with 10, 20, and 40 ps time intervals respectively. Each molecular dynamics were also redone 10 times with different seed numbers to make sure the simulation parameters and the structure of the nucleotide-bound ligand complexes are correct. A minimum of 50,000 poses of MD data was created for each drug for evaluation. MD simulations were performed to evaluate the dynamic properties of the 1BNA-Azacitidine complex over time. The box in which the system was created was set to $30 \times 30 \times 30$ $Å^3$ and the box shape was determined as orthorhombic. TIP3P type water molecules were placed in the box and 0.15 M NaCl ions were also added to neutralize the system. The system box prepared for MD simulations contains approximately 75,000 atoms. The docking poses having the best binding energy according to the docking results were used as the starting structure for the MD simulations. NPT ensemble at 310 K with Nose-Hoover temperature coupling and the constant pressure of 1.01 bar via Martyna Tobias−Klein pressure coupling was employed. There were no constraints on the systems and the initial velocity values were used as default. In the study, MD simulations of both 1BNA-ligand complexes and non-ligand bound DNA fragments were performed. RMSD and RMSF curves of the 1BNA-ligand complexes were produced via trajectory analyses. The dynamic profile of the interactions of the antineoplastic drugs of interest with 1BNA was deciphered based on these curves. Hydrogen bonds made by the antineoplastic drugs with DNA nucleotides in the 1BNA chain were also investigated.

2.2 Results

2.2.1 Antineoplastic drug: Fludarabine (A) parent drug

Fludarabine's binding mode and affinity were revealed through a series of wet laboratory analytical observations, molecular docking, and MD studies in order to assure the scientific community regarding its binding mechanism to DNA. Since Fludarabine possesses a monophosphate domain and a Fluorine atom in the Adenine ring compared to its other derivatives such as Cladribine, Clofarabine, or Pemetrexed within the scientific literature, its mode of binding should be assessed carefully due to the fact that it has the potential to yield different interesting results. The family of Fludarabine derivatives was reported to have an intercalation mode of binding to DNA

within the scientific literature. However, it has not been reported what sort of a binding mode Fludarabine has in any research so far. Hence, I carried out the binding studies of Fludarabine on 1BNA along with the analytical group collaborator on dsFSDNA (double strain fish sperm deoxyribonucleic acid). The binding mechanism and the binding site on DNA with which the drug interacts efficiently are investigated via experimental and simulation tools.

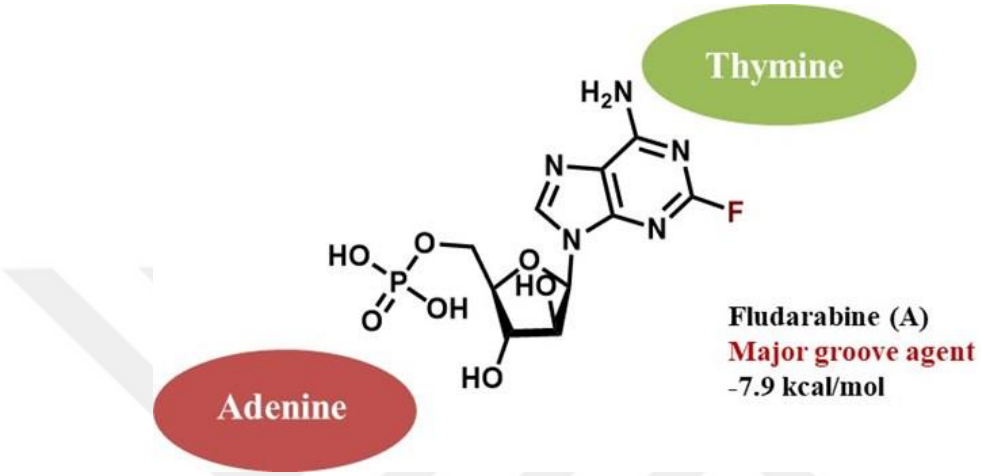

Figure 2.1 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Fludarabine (A) to DNA's nucleotides.

In the molecular docking studies, neither external binding nor intercalation was observed. Unlike the other purine derivatives such as 6-mercaptopurine and 6-thioguanine which are known to be intercalating drugs, Fludarabine is found to be a major groove binder drug because of the nonplanar and extended structure in Figures 2.1 and 2.2. Besides, the out-of-plane hydroxyl groups of the Ribose ring and tetrahedral phosphate group hinder the intercalating of the molecule between the base pairs and thus favor the major groove binding to maintain stability. Because of the structural similarity, Cytarabine also prefers groove binding to A-T regions of DNA as reported in my published Fludarabine research paper [96].

To better understand the functional group behaviour towards DNA nucleotides, some hypothetical Fludarabine derivatives were also studied as stated in Figure 1.1 before via molecular docking and molecular dynamics. The results were quite significant. Normally, Fludarabine (A) which is the parent molecule that is being currently used in the market, binds to Adenine and Thymine.

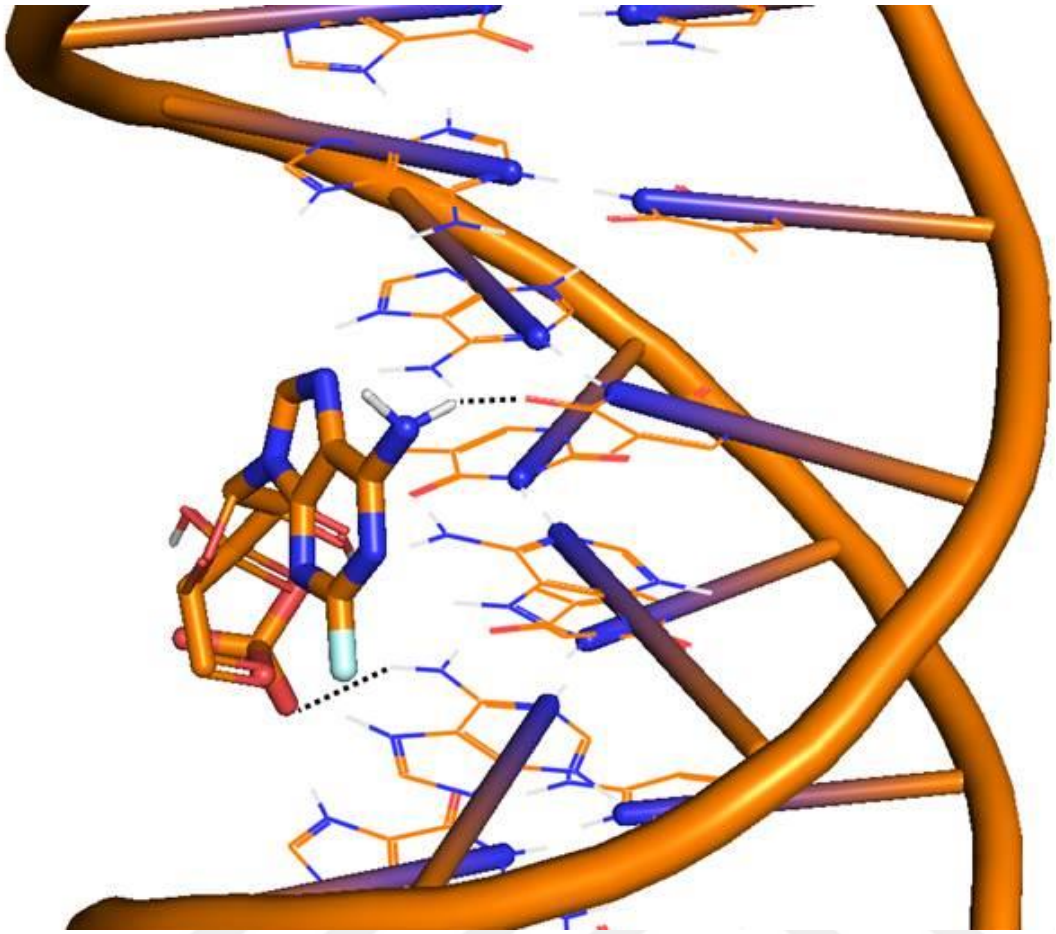

Figure 2.2 : The equilibrium pose of Fludarabine (A) as a major groove agent.

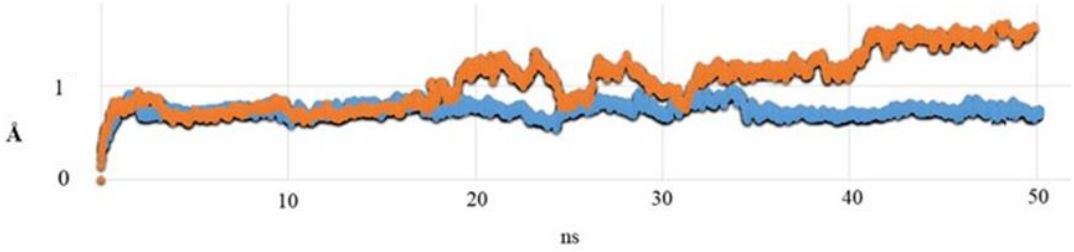

Figure 2.3 : The RMSD of Fludarabine-BNA complex (blue curve) and 1-BNA (orange curve).

Table 2.1 : Fludarabine (A) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine **Guanine** **Cytosine**	45.4	**Ribose Phosphate**	-7.7	2.08
Thymine **Phosphodiester**	49.7	**Purine Ring**	-8.1	2.27

According to Figure 2.3 and Table 2.1, the RMSD 50 ns was worked under OPLS-3 forcefield as in all of the drugs of interest. The RMSD data illustrates the Fludarabine (A) complex is stabilized after 15 ns and the drug-DNA complex has less than 1 Å oscillation under the OPLS-3 force field. The contact analysis yields the data that Fludarabine (A) binds to Adenine and Thymine via Ribose Phosphate and Purine Ring respectively. In the experimental findings with our analytical collaborator group verifying the results of theoretical studies of Fludarabine that was published [96] helped me to justify the following conclusions; to begin with, in UV absorption spectra, the significant hypochromic shifts whereas the maximum absorption wavelengths remained the same. The binding constant K_b value was calculated to be 2.24×10^4 ±0.09 L. mol^{-1} for Fludarabine and is lower than the known value for the basic intercalator such as ethidium bromide (10^7 L. mol^{-1}), but this K_b value was coherent with one previously reported for groove binding drugs such as moxifloxacin (9.4×10^4 L. mol^{-1}), methotrexate (1×10^3 L. mol^{-1}) and spermidine (8.22×10^4 L. mol^{-1}). The consistency between the K_b values above is a clear indication of the groove binding of Fludarabine to DNA. The second supportive data was the incorporation of Fludarabine not yielding any increase in the T_m value of DNA solution. The molecules change their T_m values depending on their binding affinity to DNA. In the absence of Fludarabine, the T_m value of 120 μM dsFSDNA was measured as 72.20°C whereas the T_m value of dsFSDNA solution was not significantly increased by the addition of Fludarabine (at a given concentration). As an example, the T_m value of dsFSDNA was 76.00°C and the temperature difference was 3.80°C, a pretty low increase in DNA melting temperature, suggesting that the introduction of this drug doesn't decrease the

stability of DNA's double-helical conformation stating that there is no intercalation and most likely a groove binding occurs since for a molecule to be an intercalator, the difference should be more than 8 to 10 ^{o}C. Upon the addition of Fludarabine to DNA solution in the presence of a strong intercalating agent, EtBr, there was no significant change in the fluorescence spectrum (half of the fluorescence intensity) observed since EtBr was not replaced by Fludarabine even though when the drug concentration is increased by ten times. As a fourth supportive data, there was also no significant change in the viscosity values in the drug-containing DNA solutions indicating that not any DNA strand was broken apart due to intercalation and again a clear sign of groove binding for my simulation studies exists. On top of that, in all electrochemical measurements, the observed decrease in the current, proved the binding of the drug to DNA whereas nucleotide regioselectivity was confirmed with the comparison of theoretical and experimental FTIR data. The disappearance of the characteristic peaks at the binding sites, as supported by the simulation studies yielded the same results. The disappearance of characteristic Fludarabine's amine band at 1732 cm^{-1} and the carbonyl band of DNA's Thymine base at 1640 cm^{-1} indicate the involvement of Thymine in the binding. Secondly, the disappearance of N-H stretching band at 3272 cm^{-1} of amine group imidazole moiety of Adenine indicates that Fludarabine binding also involves Adenine.

2.2.2 Antineoplastic drug: Fludarabine (B) derivative

In the case of Fludarabine (B), as can be seen in Figures 2.4, 2.5, and Table 2.2, it is a major groove agent that binds to Adenine and Thymine with a relatively reduced binding energy compared to Fludarabine (A).

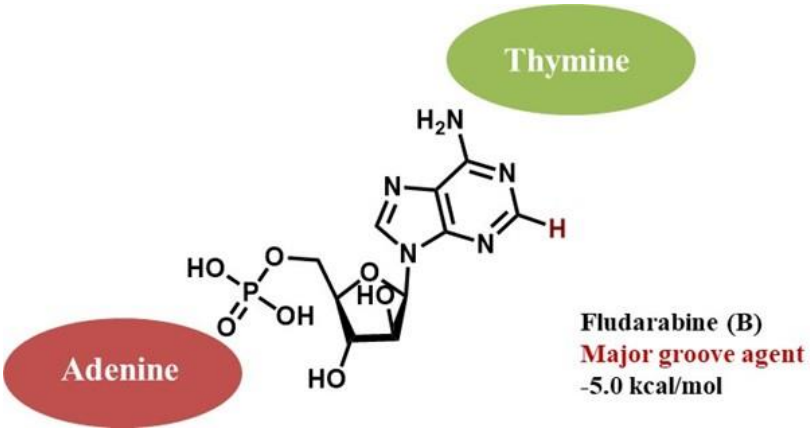

Figure 2.4 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Fludarabine (B) to DNA's nucleotides.

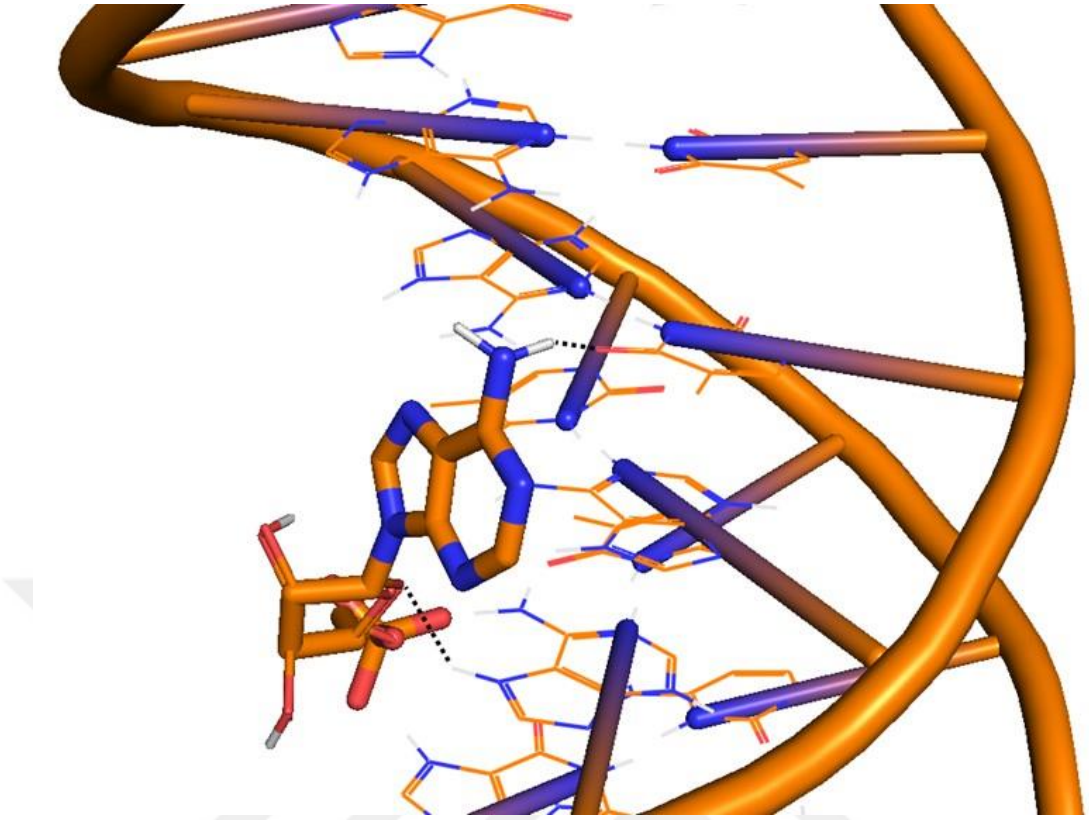

Figure 2.5 : The equilibrium pose of Fludarabine (B) as a major groove agent.

Table 2.2 : Fludarabine (B) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	42.3	**Ribose Oxygen**	-4.6	1.73
Guanine				
Cytosine				
Thymine	44.6	**Purine Ring**	-5.2	1.79
Phosphodiester				

The binding energies of Fludarabine (B) are relatively low compared to Fludarabine but the Hydrogen Bond distances are much closer. The drug domains' binding to nucleotides remains the same.

2.2.3 Antineoplastic drug: Fludarabine (C) derivative

In the case of the second derivative of Fludarabine, Fludarabine (C), there is poly-adenylation almost via all active domains when carboxylate is present as the Purine functional group.

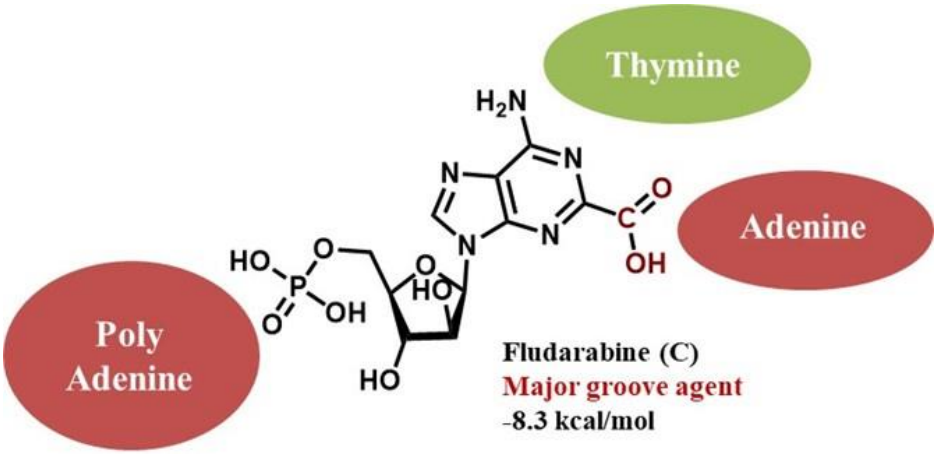

Figure 2.6 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Fludarabine (C) to DNA's nucleotides.

Fludarabine-C is a major groove agent as can be seen in Figures 2.6, 2.7, and Table 2.3 within its MD poses, it mostly binds to Adenine and rarely Thymine.

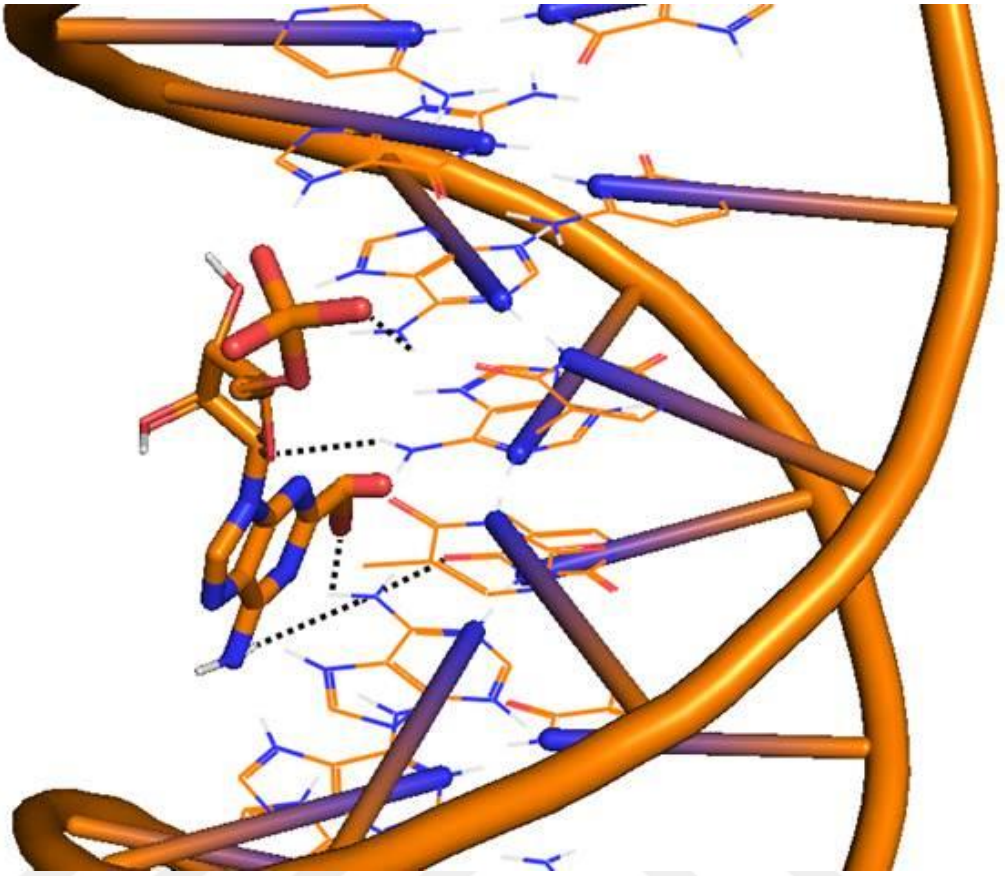

Figure 2.7 : The equilibrium pose of Fludarabine (C) as a major groove agent.

Table 2.3 : Fludarabine (C) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	64.3	**Purine Carboxylate**	-8.5	1.87
		Ribose Phosphate	-8.7	1.72
		Ribose Oxygen	-8.4	2.14
Guanine				
Cytosine				
Thymine	30.8	**Purine Ring**	-8.1	2.08
Phosphodiester				

Hydrogen contact mapping yields 64.3% Adenine binding heavily based on carboxylate and oxygen groups as well as Ribose's phosphate.

2.2.4 Antineoplastic drug: Fludarabine (D) derivative

In Fludarabine (D), even though the binding energy diminishes compared to the carboxylic acid derivative (Fludarabine(C)), poly-adenylation still is the chosen nucleotide regioselectivity when the methyl is the functional group in the Purine ring.

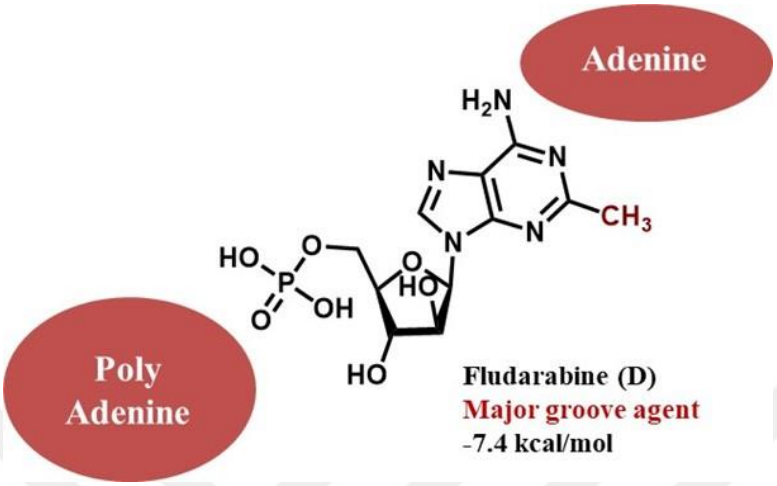

Figure 2.8 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Fludarabine (D) to DNA's nucleotides.

Table 2.4 : Fludarabine (D) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	79.3	**Ribose Phosphate**	-7.4	1.91
		Ribose Hydroxyl	-7.4	2.17
		Purine Ring	-7.2	2.25
Guanine				
Cytosine				
Thymine				
Phosphodiester				

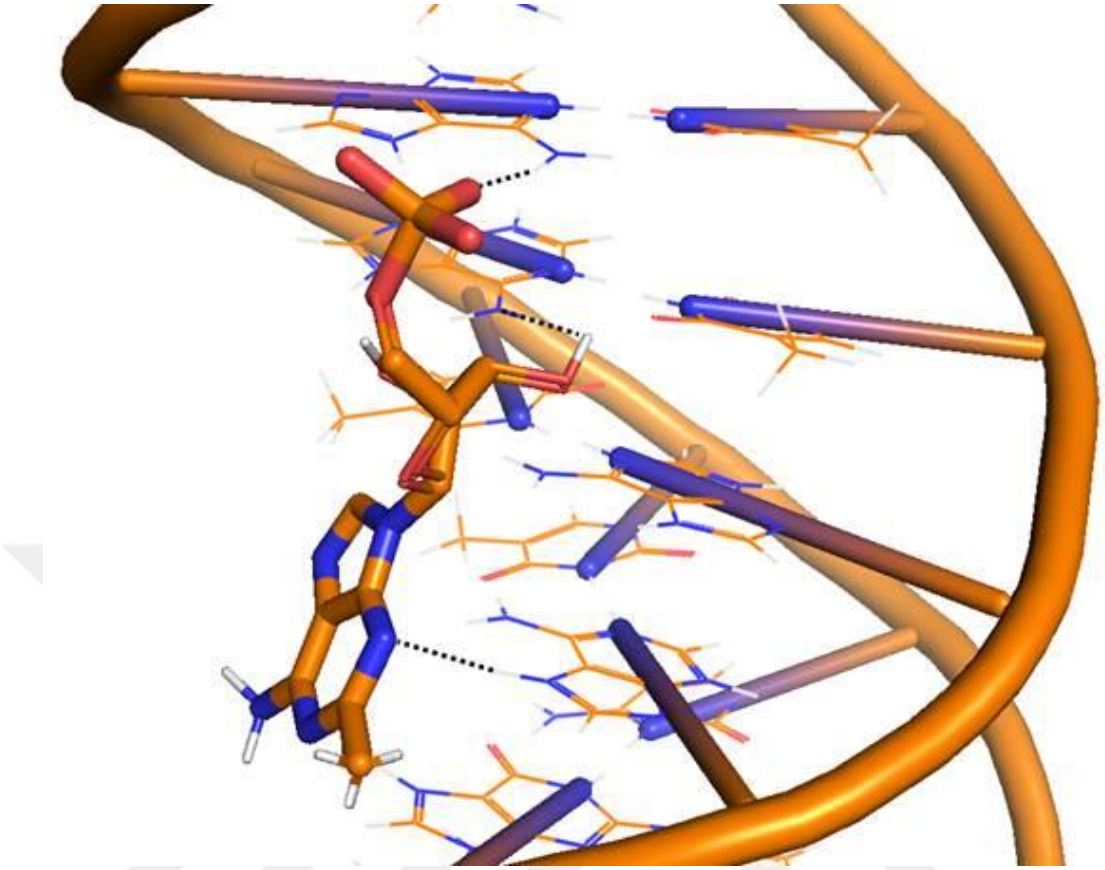

Figure 2.9 : The equilibrium pose of Fludarabine (D) as a major groove agent.

In Figures 2.8, 2.9, and Table 2.4, poly-adenylation occurs in Fludarabine (D) in its MD poses. The methylation of the Purine causes Thymine affinity loss and there remains solely Adenine binding.

2.2.5 Antineoplastic drug: Fludarabine (E) derivative

In the final derivative of Fludarabine, Fludarabine (E), according to the data provided in Figures 2.10, 2.11, and Table 2.5, the hydroxylation of the Purine ring also causes poly-adenylation.

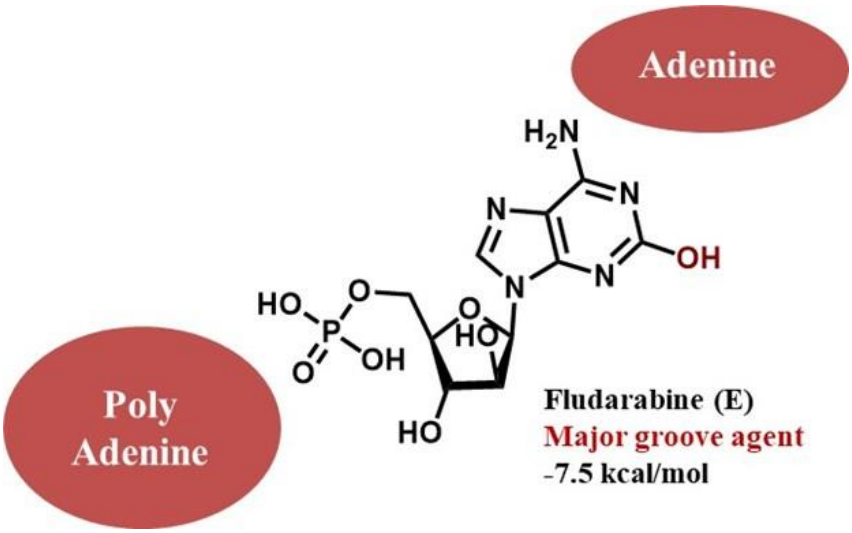

Figure 2.10 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Fludarabine (E) to DNA's nucleotides.

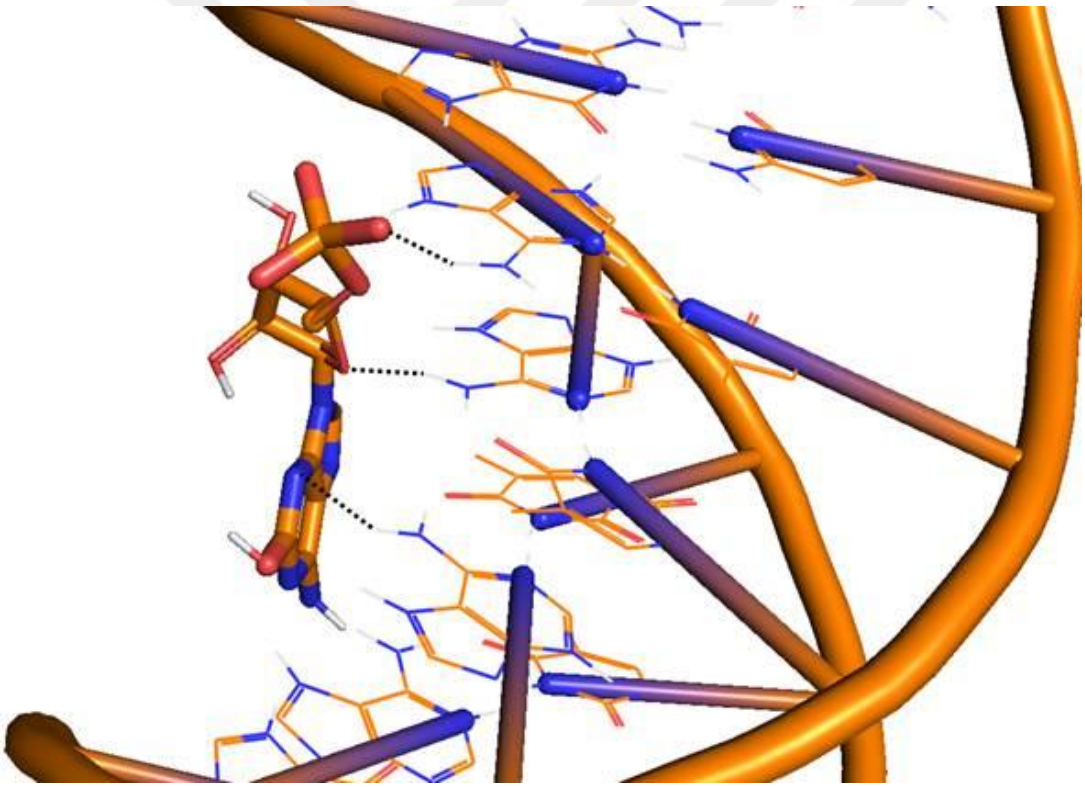

Figure 2.11 : The equilibrium pose of Fludarabine (E) as a major groove agent.

Table 2.5 : Fludarabine (E) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	63.6	**Ribose Oxygen**	-7.5	2.09
		Ribose Phophate	-7.6	2.27
		Purine Ring	-7.1	2.11
Guanine				
Cytosine				
Thymine				
Phosphodiester				

The hydroxylation of the Purine causes also Thymine affinity loss and there again remains solely Adenine binding via three centers.

2.2.6 Antineoplastic drug: Pemetrexed

The binding mode and the H-bond interactions of the Pemetrexed drug with Guanine and Adenine of DNA are found. This strong H-bonding clearly indicates that the binding of the drug is reasonably strong. The mode of binding is clearly a strong minor groove one in which the lowest docking scored results were chosen over ten times of trials with the same parameters. I believe that with such a flexible structure, intercalation would be very unlikely since drugs such as Methotrexate or Ethidium Bromide have distinct planar geometries for such a binding to occur. Pemetrexed was shown to bind to Adenine and Guanine via docking and MD results as it was published in our research paper [97] as well as in Figures 2.12, 2.13, and 2.14, and Table 2.6. The drug was studied with the control group dockings blindly and MDs where it freely chose its minor groove binding site and nucleotides.

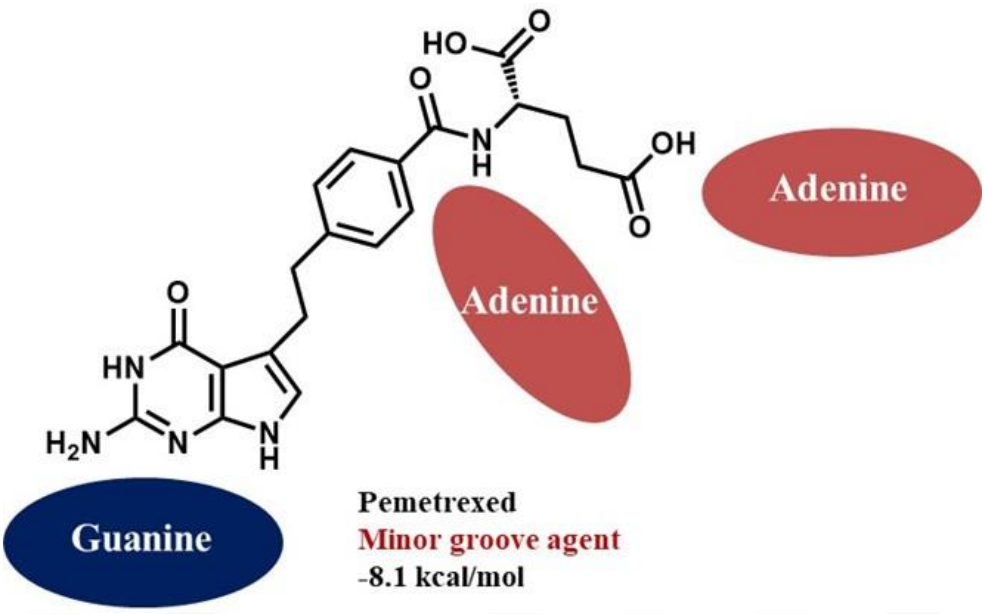

Figure 2.12 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Pemetrexed to DNA's nucleotides.

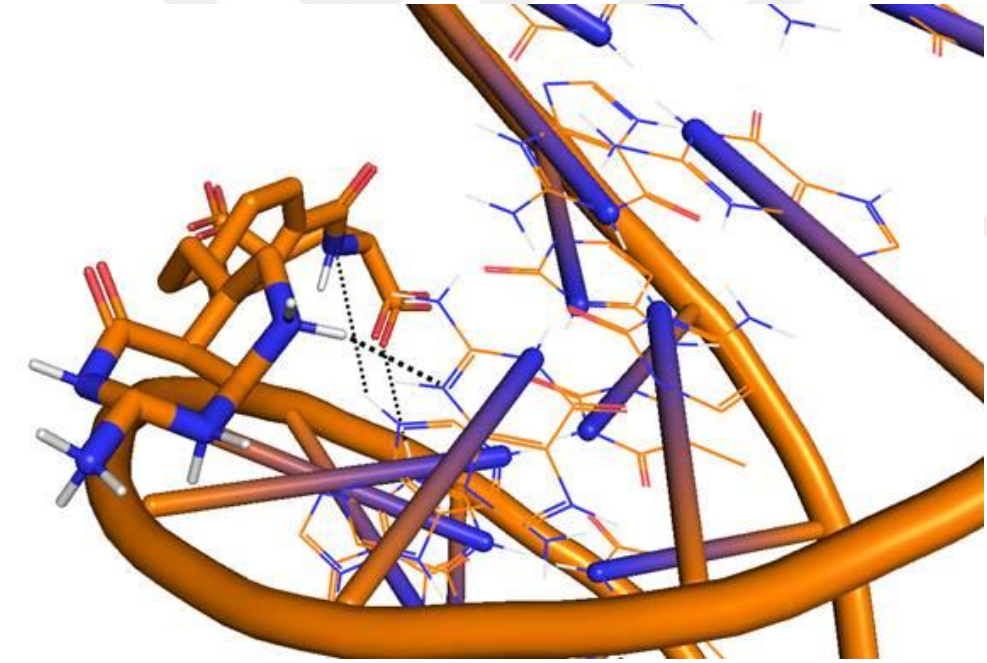

Figure 2.13 : The equilibrium pose of Pemetrexed as a minor groove agent.

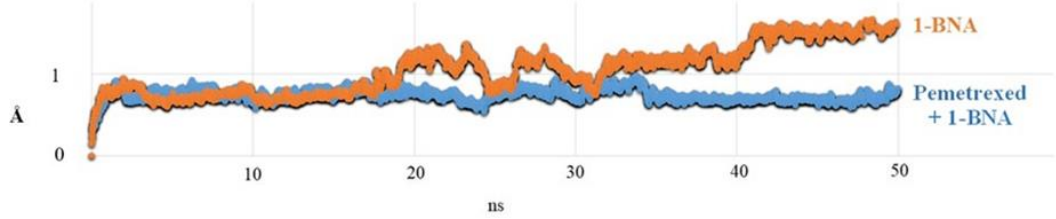

Figure 2.14 : The RMSD of Pemetrexed-BNA complex (blue curve) and 1-BNA (orange curve).

Table 2.6 : Pemetrexed post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	48.3	**Carboxylate**	-8.5	1.74
		Benzamide	-8.1	1.95
Guanine	41.6	**Pyrrolo Pyrimidine Ring**	-8.2	2.11
Cytosine				
Thymine				
Phosphodiester				

The RMSD of Pemetrexed illustrates the stability of the Pemetrexed-DNA complex. This vindicates that the interactions between the ligand and the DNA chain continue throughout the simulation, and the binding of the ligand keeps the DNA chain more stable compared to the unbound form. Under the latest force field version of Desmond Maestro, OPLS 3 works very well and the RMSD graph yields very limited oscillation for DNA's double-strand motion. From one pose to another hydrogen-bonded strands move rapidly and intensely. To compare and contrast the findings of simulation studies, our collaborator group yielded multi-spectroscopic analyses for Pemetrexed which its research paper was published [97] vindicates all of the results as follows; in UV absorption spectra, the significant hypochromic shifts whereas the maximum absorption wavelengths remained the same. The K_b value was calculated to be $1.72\times10^4 \pm 0.06$ L. mol^{-1} for Pemetrexed and is lower than the known value for a basic intercalator such as ethidium bromide (10^7 L. mol^{-1}), but this K_b value was coherent with one previously reported for groove binding drugs such as moxifloxacin (9.4×10^4 L. mol^{-1}), methotrexate (1×10^3 L. mol^{-1}) and spermidine (8.22×10^4 L. mol^{-1}). Pemetrexed was bound to dsDNA via the groove binding mechanism. The second supportive data regarding the T_m value of DNA solution states that in the absence of Pemetrexed, the T_m value of 120 μM dsFSDNA was measured as 72.20ºC whereas the T_m value of dsFSDNA solution was not significantly increased by the addition of Pemetrexed. The T_m value of dsFSDNA was 77.00ºC and this suggests the fact that a slight increase in temperature (4.8ºC) reveals that the introduction of Pemetrexed doesn't result in loss of stability of DNA's double-helical topology, again a clear

indication of groove binding was yielded from this data. Upon the addition of Pemetrexed to DNA solution in the presence of a strong intercalating agent, EtBr, there was no significant change in the fluorescence spectrum (half of the fluorescence intensity) observed since EtBr was not replaced by Pemetrexed even when the drug concentration is increased by ten times, but it replaced Rhodamine B which is a minor groove agent expressing that Pemetrexed is a minor groove agent. As further supportive data it was observed in the viscosity studies that the increasing amount of the Pemetrexed addition had almost no effect on the relative viscosity of dsDNA. The viscosity remained practically the same as the drug concentration was constantly increased. These insignificant changes in the relative viscosity demonstrated that the binding mode of Pemetrexed with dsDNA is groove binding. In all electrochemical measurements, the observed decrease in the current, proved the binding of the drug to DNA whereas nucleotide regioselectivity was confirmed with the comparison of theoretical and experimental FTIR data. The carbonyl oxygen in Pemetrexed interacts with the Adenine and Guanine bases of dsDNA by making H-bonds.

2.2.7 Antineoplastic drug: Cladribine (A) parent drug

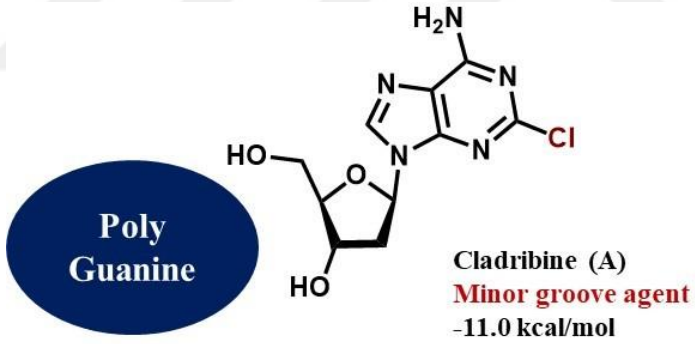

Figure 2.15 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Cladribine (A) to DNA's nucleotides.

Cladribine was studied with a Fluorine derivative of its own to further test the binding mode alteration due to the structural halogenation difference. Cladribine chooses Guanine strictly in the cluster analyses of docking and MD results in Figures 2.15, 2.16, and 2.17, and Table 2.7.

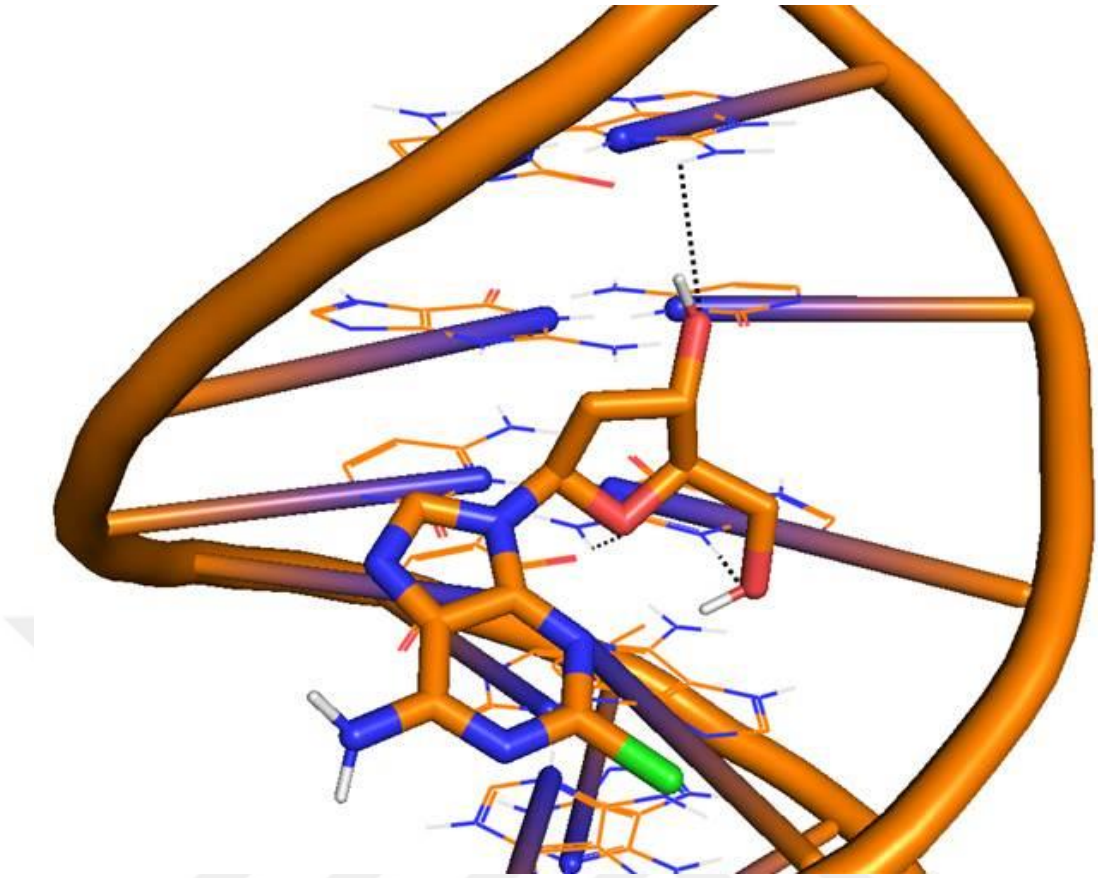

Figure 2.16 : The equilibrium pose of Cladribine (A) as a minor groove agent.

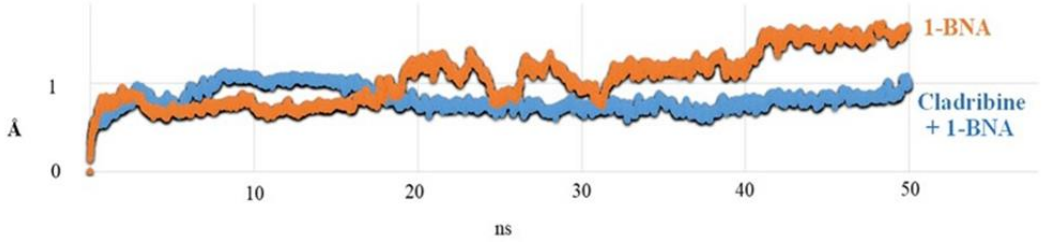

Figure 2.17 : The RMSD of Cladribine-BNA complex (blue curve) and 1-BNA (orange curve).

Table 2.7 : Cladribine (A) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine				
Guanine	71.6	**Ribose Oxygen** **Ribose Hydroxyls**	-11.0 -10.9, -10.9	1.65 2.07, 2.34
Cytosine				
Thymine				
Phosphodiester				

Cladribine's RMSD is a stable one just like the previous drugs and its tendency is heavily based on Guanine via Oxygen and Hydroxyl groups of Ribose.

2.2.8 Antineoplastic drug: Cladribine (B) derivative

In Cladribine-B, Fluorine replacement causes poly-adenylation and revokes Guanine tendency in Figures 2.18, 2.19, and Table 2.8.

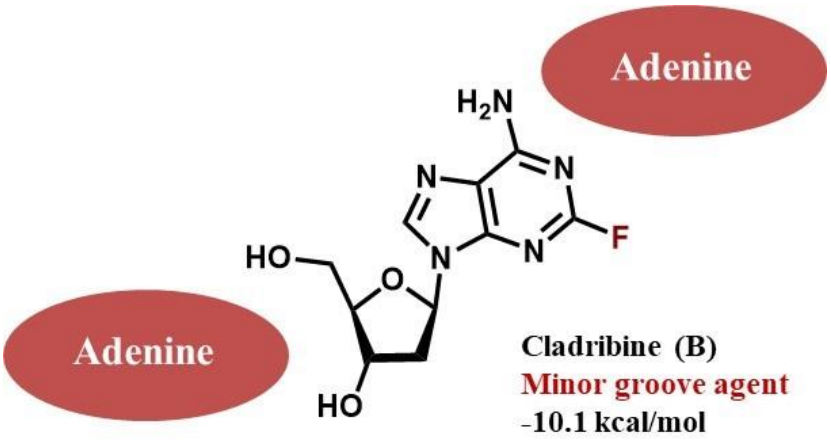

Figure 2.18 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Cladribine (B) to DNA's nucleotides.

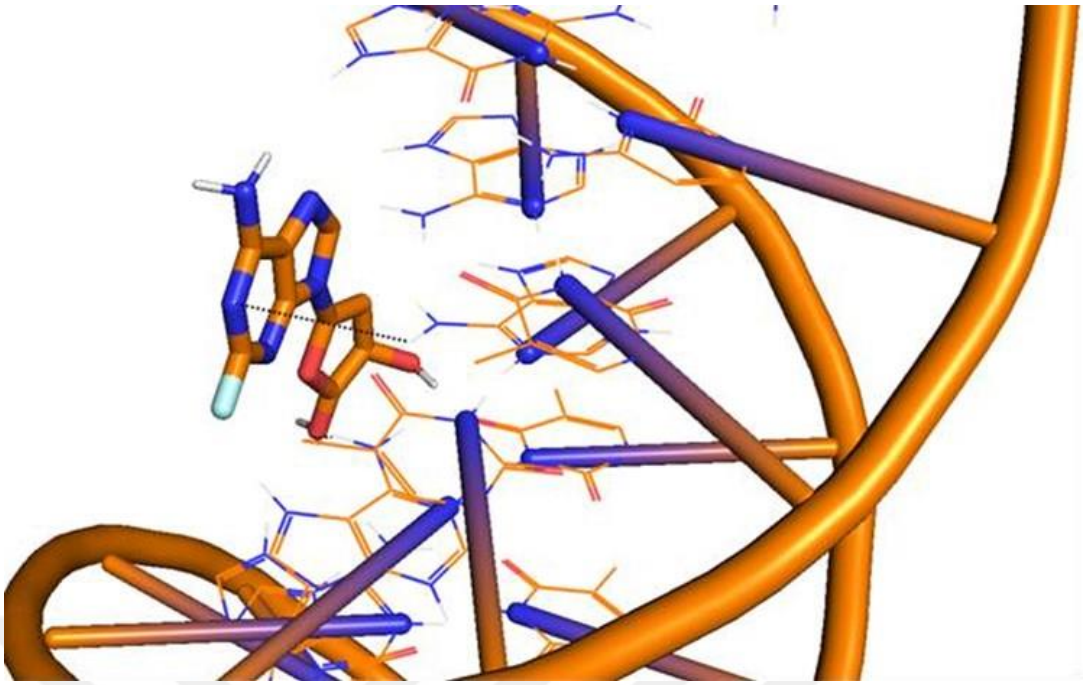

Figure 2.19 : The equilibrium pose of Cladribine (B) as a minor groove agent.

Table 2.8 : Cladribine (B) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	52.1	**Ribose Hydroxyl** **Purine Ring**	-10.3 -9.8	1.89 2.13
Guanine				
Cytosine				
Thymine				
Phosphodiester				

Guanine binding of Cladribine (A) drastically alters to Adenine binding in the case of Fluorine introduction to Purine Ring of Cladribine (B).

2.2.9 Antineoplastic drug: Clofarabine (A) parent drug

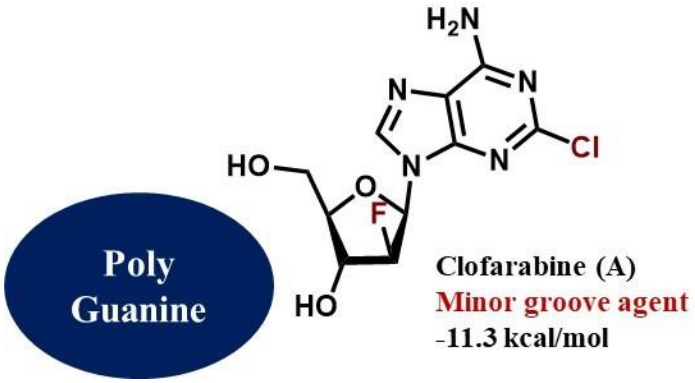

Figure 2.20 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Clofarabine (A) to DNA's nucleotides.

As can be seen in Figures 2.20 and 2.21, Clofarabine (A) binds quite efficiently to Guanine in the presence of Chlorine in the Purine ring. Clofarabine possesses extremely high binding efficiency and resides in the minor groove area according to the MD poses.

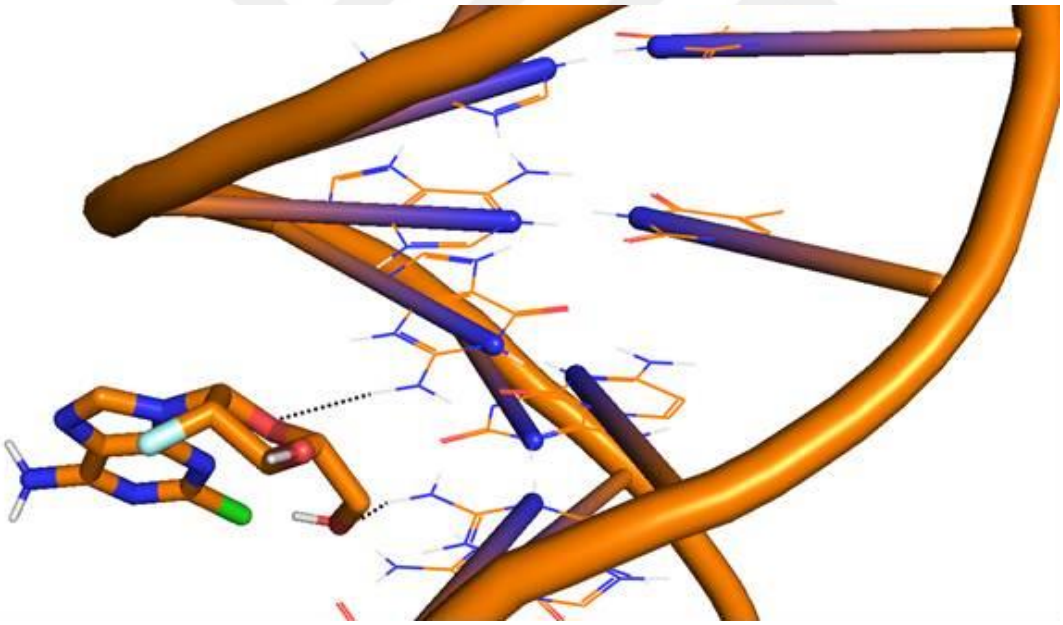

Figure 2.21 : The equilibrium pose of Clofarabine (A) as a minor groove agent.

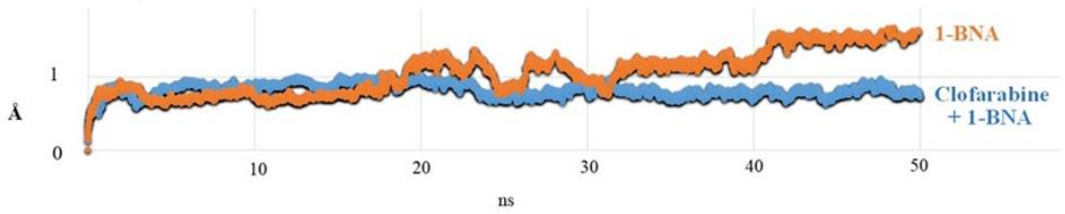

Figure 2.22 : The RMSD of Clofarabine-BNA complex (blue curve) and 1-BNA (orange curve).

Table 2.9 : Clofarabine (A) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine				
Guanine	58.9	**Ribose Hydroxyl**	-11.3	1.87
		Ribose Oxygen	-10.6	1.97
Cytosine				
Thymine				
Phosphodiester				

Clofarabine's Ribose ring binds strictly to Guanine concerning the Post-Molecular Dynamics Hydrogen bond contact analysis.

2.2.10 Antineoplastic drug: Clofarabine (B)

In Clofarabine (B), the Fluorinated Clofarabine undergoes Adenylation in the purine ring while retaining Guanine binding as can be seen in Figures 2.23, 2.24, and Table 2.10. It keeps on residing in the minor groove.

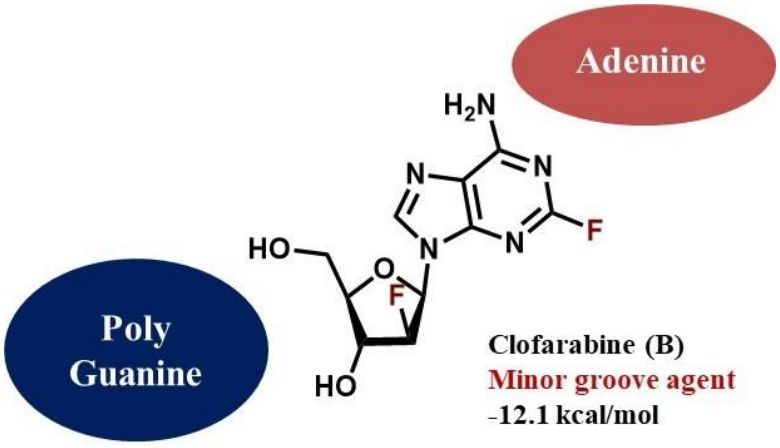

Figure 2.23 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Clofarabine (B) to DNA's nucleotides.

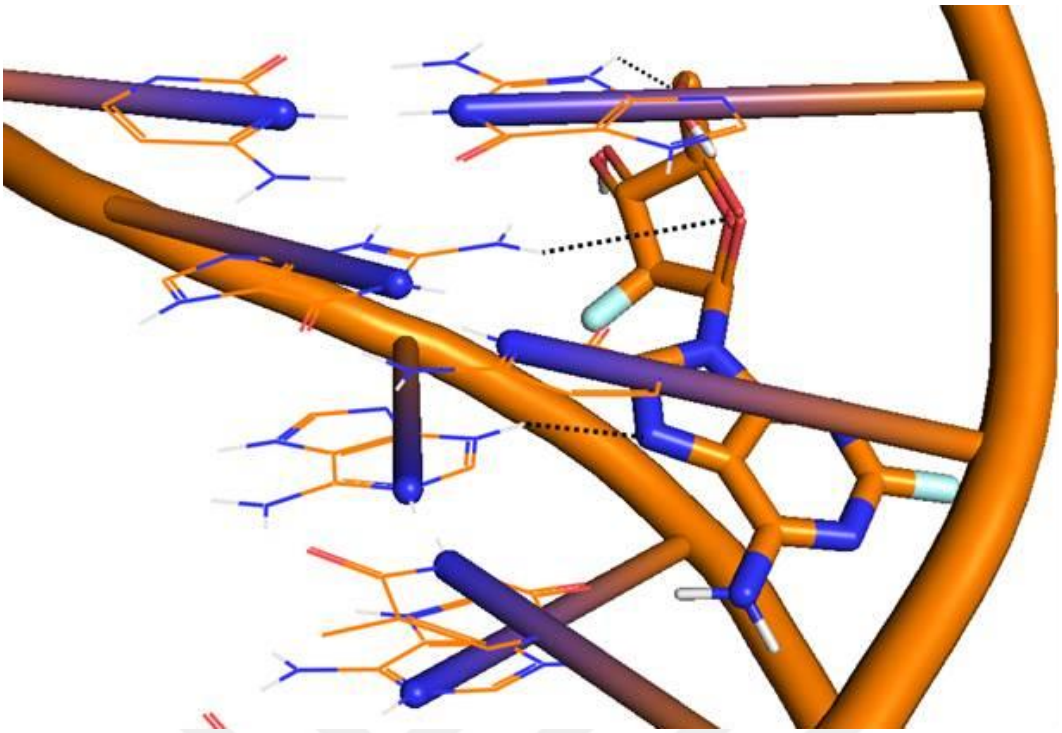

Figure 2.24 : The equilibrium pose of Clofarabine (B) as a minor groove agent.

Table 2.10 : Clofarabine (B) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	37.2	**Purine Ring**	-12.1	1.71
Guanine	42.3	**Ribose Hydroxyl**	-12.2	1.94
		Ribose Oxygen	-12.3	2.22
Cytosine				
Thymine				
Phosphodiester				

In the Clofarabine (B) derivative, Fluorination brings the nucleotide regioselectivity capability of Adenylation while the Ribose ring remains the same with Guanine binding.

2.2.11 Antineoplastic drug: Clofarabine (C)

In Clofarabine (C), Chlorinated structure (double halogenation with Chlorine) causes poly-adenylation and this situation revokes the Guanine binding. Poly-adenylation takes place with very strong binding energies and proximity hydrogen bonds for Ribose and Purine rings that can be seen in Figures 2.25, 2.26, and Table 2.11.

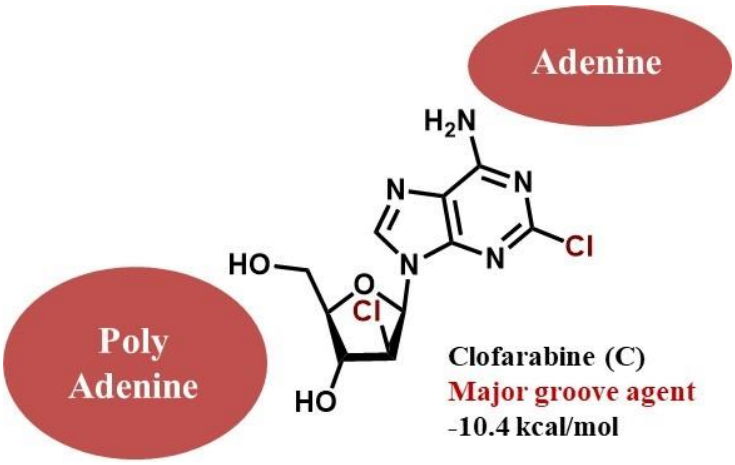

Figure 2.25 : The representative chemical structure of nucleotide regioselectivity of the functional binding groups of Clofarabine (C) to DNA's nucleotides.

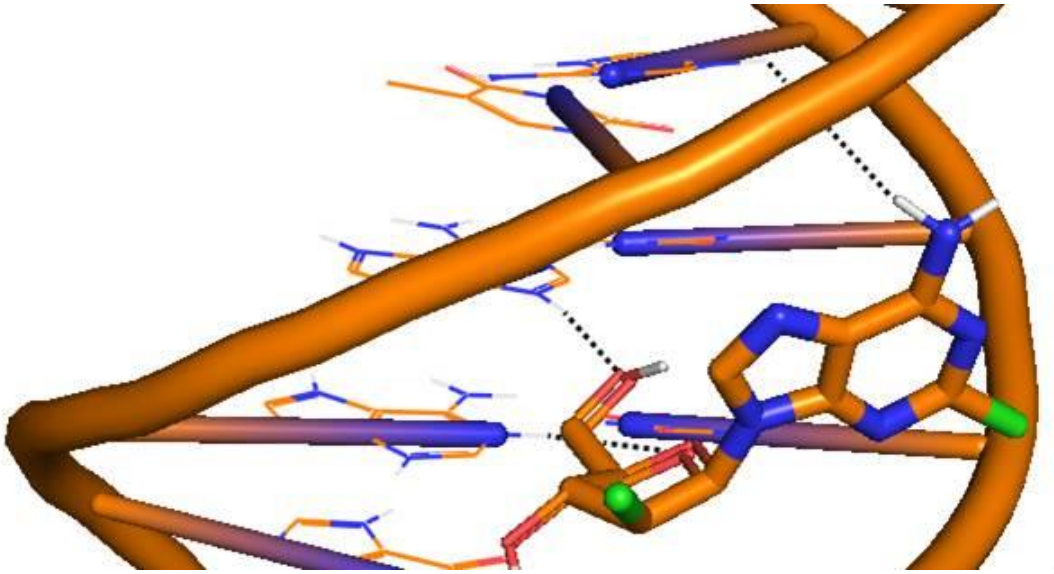

Figure 2.26 : The equilibrium pose of Clofarabine (C) as a minor groove agent.

Table 2.11 : Clofarabine (C) post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine	65.9	**Ribose Oxygen**	-10.5	1.83
		& Hydroxyl	-10.5	2.10
		Purine Ring	-10.2	2.20
Guanine				
Cytosine				
Thymine				
Phosphodiester				

Not only Guanine binding was revoked here, but also an alteration in the mode of binding occurs in the presence of double Chlorine within the drug's structure.

2.2.12 Antineoplastic drug: Azacitidine

Azacitidine is an intercalating agent which unzips DNA double-strand by primarily infiltrating the hydrogens bonds among the Guanine and Cytosine as can be seen in Figures 2.27, 2.28, 2.29, and Table 2.12. It was found that not only does RNA intercalation on Azacitidine as in the scientific literature but DNA intercalation plays a crucial role in the inhibition of DNA and its expression. Azacitidine definitely chooses major groove area nucleotides and intercalates between the stacks of Guanine and Cytosine which our research paper regarding this, is in the press for publication [98].

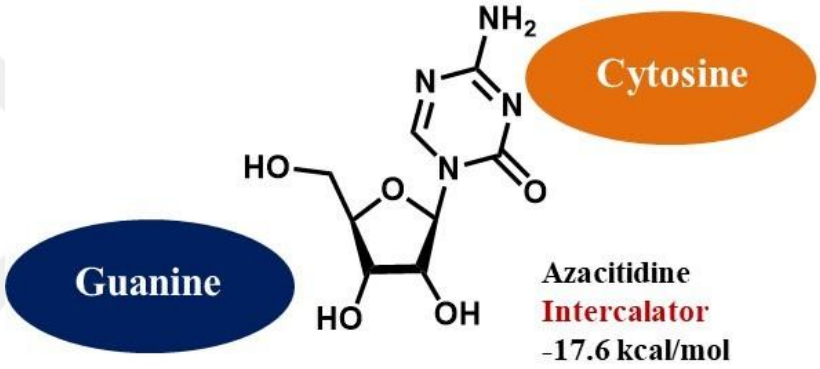

Figure 2.27 : The representative chemical structure of nucleotide regioselectivity during the intercalation of the functional binding groups of Azacitidine to DNA's nucleotides.

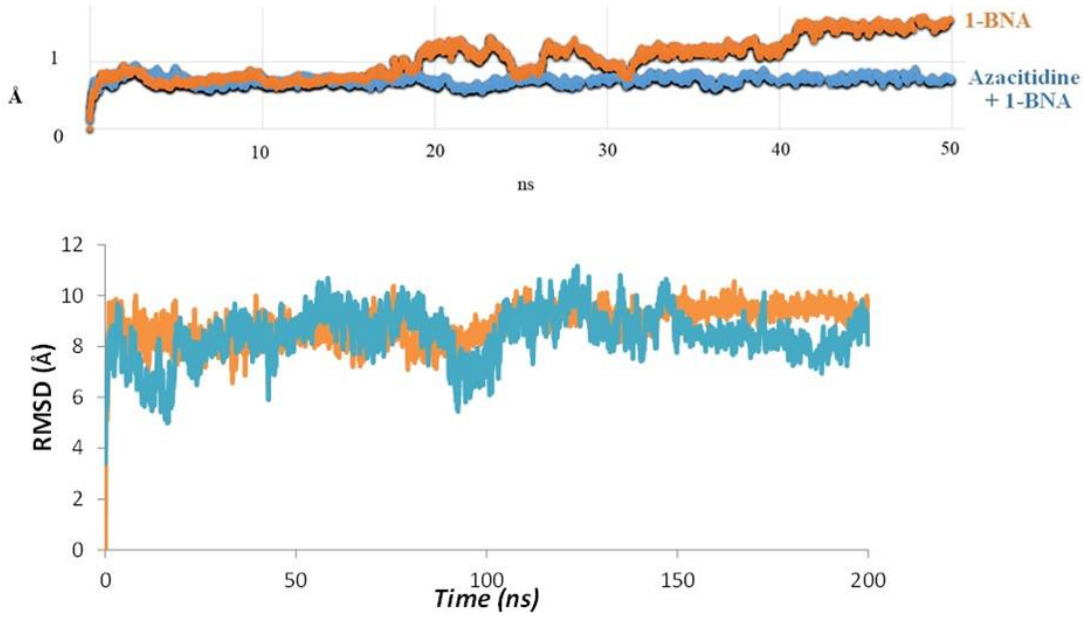

Figure 2.28 : The RMSD of Azacitidine-BNA complex (blue curve) and 1-BNA (orange curve).

Guanine and Cytosine are heavily preferred by Azacitidine for the DNA helical unzipping. Therefore there is nearly no phosphodiester bonding.

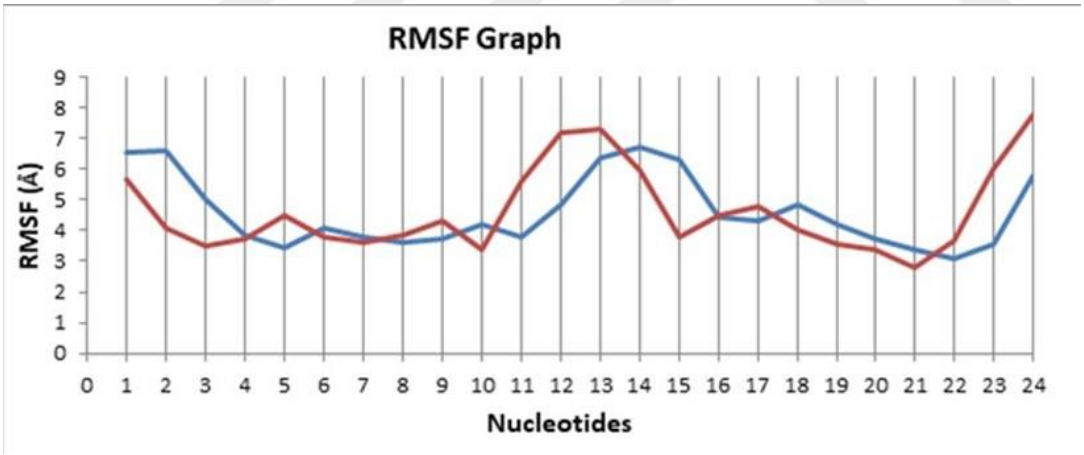

Figure 2.29 : The RMSF graph of the 1BNA-Azacitidine complex in red and non-ligand bound DNA chain in blue.

Table 2.12 : Azacitidine post-MD hydrogen bond contact analysis.

DNA location	% of H-Bonds Drug	Drug Domains	Binding Energy (kcal/mol)	H-Bond Distances (Å)
Adenine				
Guanine	54.9	**Ribose -> Intercalation**	-18.1	1.68
Cytosine	29.2	**Amino triazine ring -> Intercalation**	-16.5	1.84
Thymine				
Phosphodiester				

Guanine and Cytosine are heavily preferred by Azacitidine for the DNA helical unzipping. Therefore there is nearly no phosphodiester bonding. The dynamic properties of the Azacitidine molecule and DNA chain were investigated via RMSD graphs belonging to ligand and 1BNA chain obtained from MD simulations. In addition, RMSF plots of 1BNA and the types, variations, and percentages of hydrogen bonds that occurred between the ligand and the DNA nucleotides were also taken into account. Using this information, the overall interactions of the Azacitidine molecule with the nucleotides of 1BNA are deciphered in detail. The figures above illustrate the RMSD curves in different time intervals (50 ns and 200 ns) of the 1BNA-Azacitidine complex and DNA chain to which the ligand is not bound. As can be seen from the figures, MD simulations for the 1BNA chain with no ligand bound system reached equilibrium. However, the 1BNA-Azacitidine complex could not reach such stability in the same time interval. This result may seem strange at first glance, but when we look at the docking results and the trajectory of the MD simulations, it is understood that the Azacitidine molecule is not attached to the major or minor groove parts of the DNA helix, since it interacted with both helices to make intercalation type of binding. Even when only the trajectory frames are examined, it is well understood that the studied ligand is located between the DNA strands and tries to settle between the stacks and unzips the DNA strands. The trajectory analyses and docking results are compatible with each other vindicating the same result.

If there would be such a major or minor groove binding case of Azacitidine, the RMSD values of the 1BNA-Azacitidine complex would be lower than that of the unbound DNA chain. However, the situation is the opposite where a clear intercalation mechanism occurs. Since the Azacitidine molecule locates itself mostly within Guanine nucleotides stacks (and within a little bit of Adenine and Cytosine from time to time as can be seen in the Figure 2.29), it tries to unzip these chains from each other, thus RMSD values do not exhibit a stable profile, instead, oscillatory behavior was observed due to destructions of the strands. The naked Azacitidine molecule maintained its position as well-fined stabilized throughout the MD simulation. This result indicates that the ligand-DNA interaction is stable within the DNA strands and it preserves its location throughout the simulations. The RMSD plots of 1BNA, Azacitidine, and their complex showed the same results.

The RMSF values of the 1BNA nucleotides were also investigated to better perceive the mechanistic detail with which nucleotide Azacitidine molecule interacts specifically to shut down DNA. To achieve this, the RMSF values of the nucleotides of the DNA chains to which both bound and non-bound ligand results were combined in the same graph. Therefore, the nucleotides with which Azacitidine interacts via hydrogen bonding interactions were explored.

We see that the RMSF values of the 11th, 12th, and 13th nucleotides increase compared to the non-ligand bound structure. This increase is attributed to the tendency of the Azacitidine molecule to position itself in proximity to the aforementioned nucleotides by separating the DNA strands. When trajectory frames of MD simulations are followed, the Azacitidine intercalation is clearly observed between the nucleotides 10-15. Followed by the intercalation, the other nucleotides at the ligand's coordination site move away from each other, and therefore, a noticeable increase in RMSF values of the 1BNA-Azacitidine complex is observed as the interactions within the vicinity of the 10th and 15th nucleotides are weakened. The RMSF results are consistent with the RMSD data.

In this study, we have carried out MD simulations to examine the hydrogen bonds formed between the Azacitidine and specific 1BNA nucleotides, which facilitate the drug's incorporation or intercalation into the DNA. In each MD simulation, 5000 frames were stored for further elucidation of the place and distance of the H-bonds. It was observed that a total of 12,416 hydrogen bonds were formed during the 200 ns

simulation. It should be noted that the Azacitidine can make an H-bond with the organic base of a nucleotide as well as with the sugar or phosphate backbone portion of the same nucleotide. In Table 1, the detailed analysis of percentages of the H-bonds that the drug makes is given. The statistically significant number of H-bonds that the drug molecule establishes were with Guanine and Cytosine nucleotides.

The Hydrogen bonds occurring in MD simulations were formed with the nucleotides as sequenced A17 - A18 - G10 - G12 - G14 - G16 - C9 - C11 - C13 - C15.

When writing down these nucleotides stated in Table 12, H-bonds formed with all of the chemical domains of the nucleotides (organic base, sugar, phosphate) were taken into consideration. When the RMSF graph is examined, it is observed that the RMSF values of the C11, G12, and C13 nucleotides compared to the unbound DNA chain increase with the ligand binding. This may show us that the Azacitidine molecule could not contact these nucleotides (C11, G12, and C13) quite efficiently during the simulation. When the H-bonds obtained from the MD simulation were examined in detail depending on these above-mentioned nucleotides, it was seen that the number of H-bonds formed with these nucleotides was much less than the other H-bonding nucleotides (A17 - A18 - G10 - G14 - G16 - C9 - C15).

In these detailed investigations, it was found that G10 nucleotide had the highest number of H-bonds with Azacitidine and thus is more stable since it is lower than the unbound DNA chain in Table 12. However, the G14 nucleotide also made contact with the studied ligand with a large number of H-bonds. When these conclusions are examined carefully, the results obtained from the analysis of H-bonds are compatible with those obtained from the RMSF curves. Nucleotides that had large numbers of H-bonds with the Azacitidine molecule, did not show any increase in RMSF values, on the contrary, as can be seen in the graphic, a slight decrease compared to DNA can be noticed since they become more stabilized when bound to the ligand. To sum up, a thorough computational study was performed to decipher the mode of binding and nucleotide-binding tendencies of the medicinal drug Azacitidine. The DNA inhibition mechanism which is of significant interest for further oncological studies was determined. Azacitidine interacts mostly and strongly with Guanine and Cytosine nucleotides within the human 1BNA double-stranded chain via H-bonds. Especially, G10 and G14 nucleotides play an important role in the intercalation of Azacitidine. This drug’s intercalation induces the weakening of the interchain interactions and

hence results in the separation of DNA helices. We showed that Azacitidine is an important intercalating agent for DNA and it functions as a DNA inhibitor since its intercalation breaks down the DNA structure. This behavior may open up more opportunities in the anti-cancer studies where the DNA is targeted. The analytical wet-lab studies also confirm my molecular docking and MD findings where the change in the signal of electroactive bases (guanine (dGuo) or adenine (dAdo) or both) in the dsDNA structure is measured before and after the interaction and interpreted according to these changes. Guanine and Adenine binding is definetly present in which Adenine, Guanine and Cytosine based bindings were also proven in post-MD RMSF analysis. According to the scientific literature, when small molecules intercalate with dsDNA, the viscosity of the dsDNA solution increases, since dsDNA base pairs are separated. So, to further support the mode of binding, the viscosity studies yield us the data that with increasing Azacitidine addition, there was an increasing effect on the relative viscosity of dsDNA. These changes in relative viscosity, but no real change within this relativity, is the evidence that the binding mode of Azacitidine with dsDNA is truly intercalation.

2.2.13 The comparison of the results of all drugs

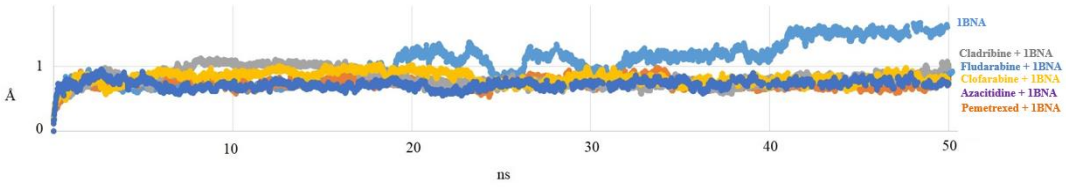

Figure 2.30 : RMSD data of all antineoplastic drugs.

Depending on the data discussed in this Ph.D. thesis, more research and review papers will be published and one of them is currently in preparation [99], the results for each drug are as follows;

In the cases of Fludarabine (A) (Fludarabine-Fluorine) and Fludarabine (B) (Fludarabine-Hydrogen) cases, the Carboxylate has a short H-bond distance and has an affinity preferring the Adenine binding. Purine ring normally chooses Thymine since it is ‘’Adenosine’’. The Oxygens in the Ribose ring choose Adenine as well.

While in the case of Fludarabine (C) (Fludarabine-Carboxyl) and Fludarabine (D) (Fludarabine-Methyl) and Fludarabine (E) (Fludarabine-Hydroxyl), the carboxyl group has one acceptor and two donors for H-bonding. The introduction of carboxylate

and the increase in the active binding sites cause poly-adenylation since Adenine nucleotides' amine seeks Hydrogen donors. Binding energy is better when a bulky group such as Chlorine or Carboxyl is covalently attached to the Purine ring. Sterically tiny groups such as Methyl and Hydroxyl cause Thymine binding loss while Adenine binding remains. The binding energy is better when a bulky group such as Chlorine or Carboxyl is covalently attached to the Purine ring.

In Pemetrexed's result, it can be said that Carboxylate has a shorter H-bond distance and due to its less steric hindrance compared to Pyrrolo Pyrimidine, it has an affinity to prefer Adenine binding. The bulkier the structures become, the more tendency to choose Guanine in the case of Pyrrolo Pyrimidine.

In the results of Cladribine (A) (Cladribine-Chlorine) and its derivative Cladribine (B) (Chladribine-Fluorine), the higher the polarizability of the functional group such as Chlorine, the higher the binding energy becomes.

When it comes to Clofarabine (A) (Clofarabine – Chlorine, and Fluorine) and its two derivatives Clofarabine (B) (Clofarabine – Fluorine, and Fluorine) and Clofarabine (C) (Clofarabine – Chlorine and Chlorine), the strength of binding energy decreases as the H-bond distance increase due to double halogenation. The halogenating synchronization may probably cause a different mode of binding and alter the site of binding to the major groove in the case of Chlorine-Chlorine replacement. Chlorine-Chlorine has a higher affinity and shorter bond distance to Ribose compared to Fluorine causing nucleotide affinity towards Adenine instead of Guanine in the Ribose ring.

In the results of the final antineoplastic drug, Azacitidine, the smaller the compound and geometrically flat the chemical structure is, the more tendency to become an intercalator along with the aid of the triazine domain. That's why Azacitidine acts as a strong intercalator.

2.2.14 Receptor and ligand parameter trials

There have been examined as many possibilities as could regarding the molecular dockings and MDs. Each docking for the whole drug was redone with 100 posed cluster docking simulations in Autodock Vina 1.1.2; totaling 2000 poses for each medicinal drug. The results of clusters, the binding modes, and the nucleotide-binding types can be explained and the parameters that were looked into are as follows;

First off, using the Avogadro pH selection system, in the ligand preparation step of Autodock Vina, under both physiological 7.4 and acidic pH 4.8, ligands went under the docking in Autodock Vina. It was seen that there was not any factor affecting the ligands' regioselectivity towards the nucleotides and their binding mode due to pH difference.

Secondly, using the Kollman charge calculation of Autodock Vina which is simply based on electrostatic potential using quantum mechanics, it was observed that the ligand-DNA complex yields interaction problems in terms of lacking overall charges in the process of MD Desmond program preparation. Therefore, this method has been eliminated and went on with the Gasteiger charge process in the ligand prep.

Finally, utilizing the Gasteiger-Marsili empirical atomic partial charges based on electronegativity equilibration of Avogadro and Autodock, the geometrically optimized ligands in the ligand prep went under the docking process in large clusters of 50 and 100. This method proved to be useful, since in terms of technical knowledge depending on biostatistics, clusters that have a population over 30, yield the correct distribution of the docking poses and throughout the research of the thesis, it did not occur any problem in the cluster binding modes and MD Desmond preparation process. Hence, in terms of statistical trials, it was observed that after the magical number of 30, the clusters began to converge into a few clusters or time to time solely two clusters, which is the expected outcome in the scientific papers, since 30 is both statistically a correct number for yielding the distribution data expected in ligand-DNA dockings. Thus, the best data are gathered in the clusters of 50 and 100 as far as observed.

2.3 Discussion

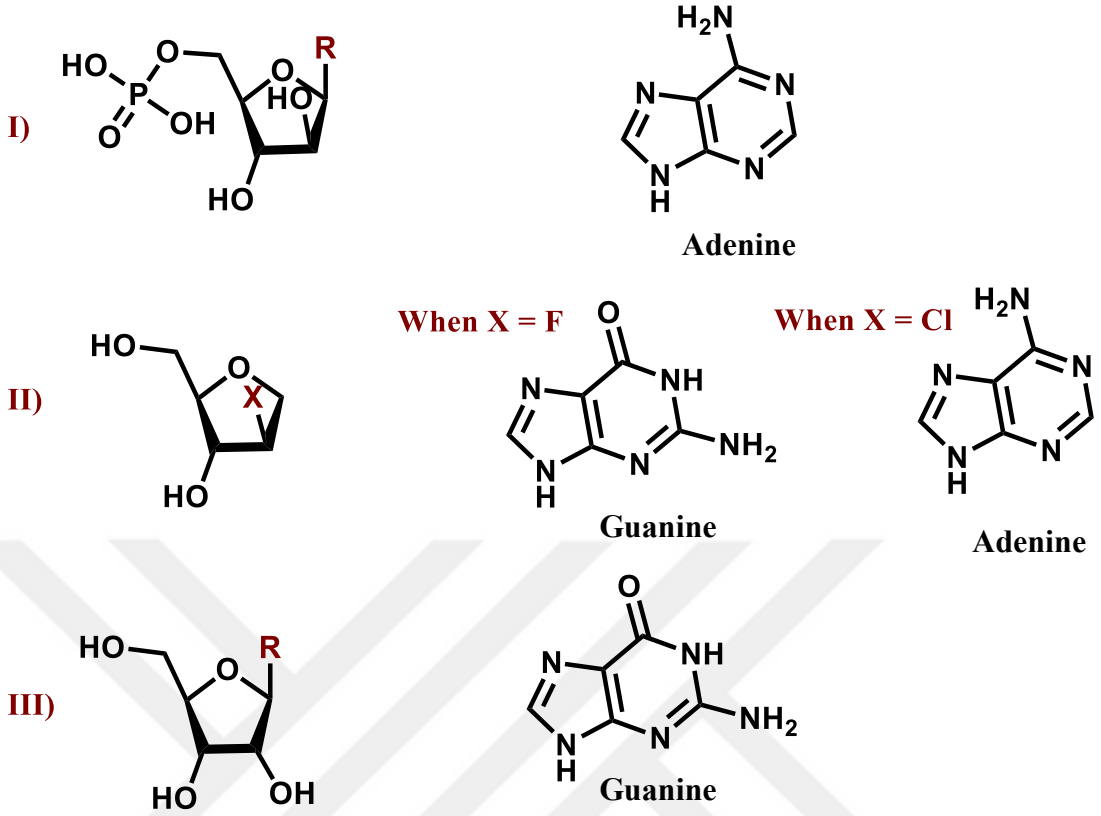

Figure 2.31 : The regioselectivity of Ribose functional groups.

In Figure 2.31, Phosphated Ribose ring must bind to an Adenine due to its R group is Adenosine which has to bind to Thymine. Therefore, the sole nearest available nucleotide becomes Adenine for the Phosphated Ribose rings for any DNA sequence. Chlorine has a higher affinity and shorter bond distance to Ribose compared to Fluorine causing nucleotide affinity towards Adenine instead of Guanine in the Ribose ring due to electron pull from the closest Hydroxyl that forms the H-bond with the nucleotide since Adenine nucleotides seek free H-donors. ‘’Aromatic Triazine electron clouds in the R group’’ donate the electrons to the Ribose ring with the positive inductive effect. Therefore, fewer H-donor sites leave out in the Hydroxyls of Ribose structures. Guanine nucleotides look for more H-accepting sites compared to Adenine and therefore solely Guanine binding occurs.

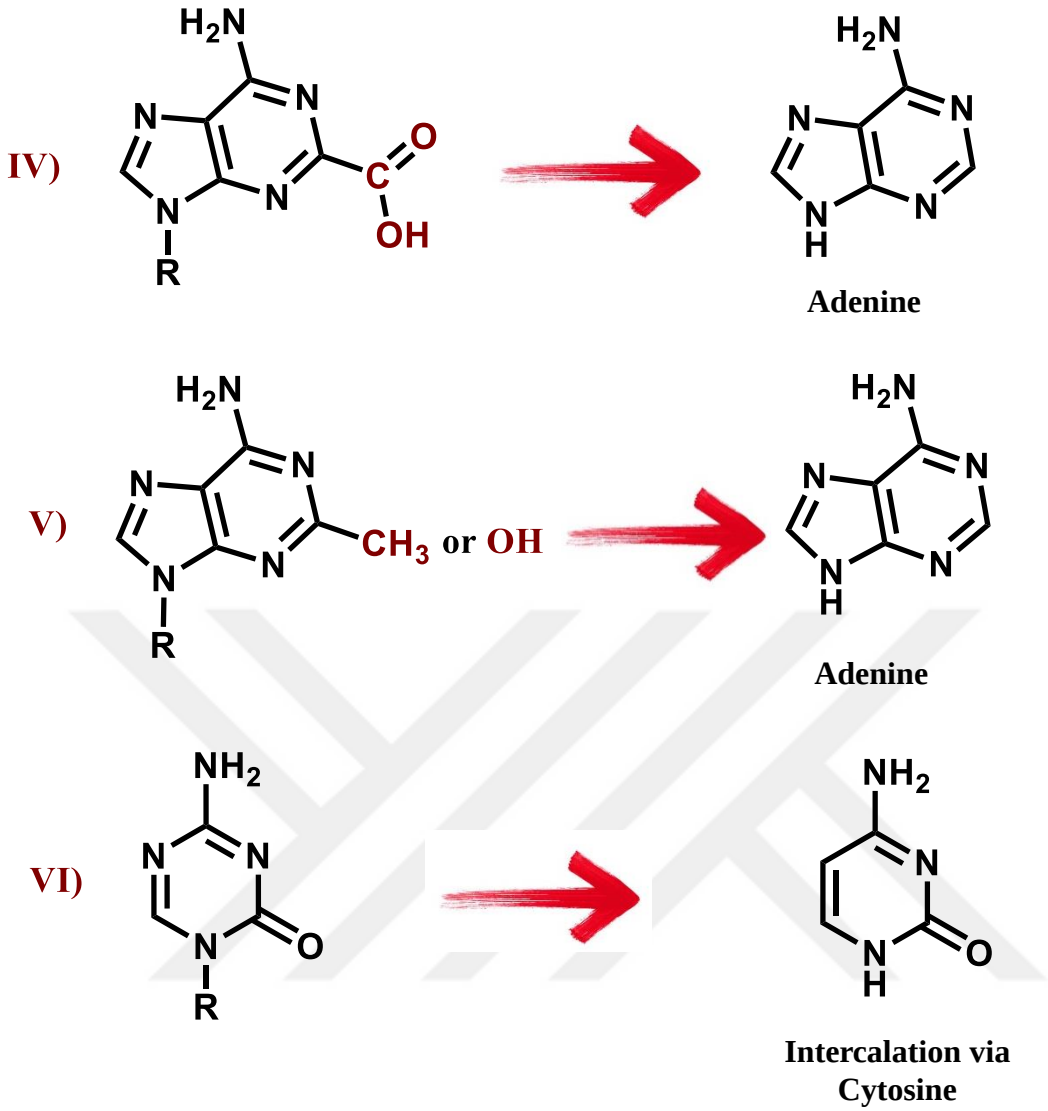

Figure 2.32 : The regioselectivity of Purine and Triazine rings.

In Figure 2.32, the docking and MD stated in IV, the Carboxyl group has one acceptor and two donors for H-bonding. The introduction of carboxylic acid and the increase in the active binding sites cause poly-adenylation since Adenine nucleotides' amine seeks Hydrogen donors. In V and VI, Sterically tiny groups such as Methyl and Hydroxyl cause Thymine binding loss while Adenine binding remains. Since Ribose rings bind to Guanine both in groove binding and intercalation situations, the rest of the drug which is the ''Amino Triazine'' structure must bind to the Cytosine since it is the pair of Guanine in the DNA sequence and Azacitidine is fairly small, restricting its reach to any other nucleotide.

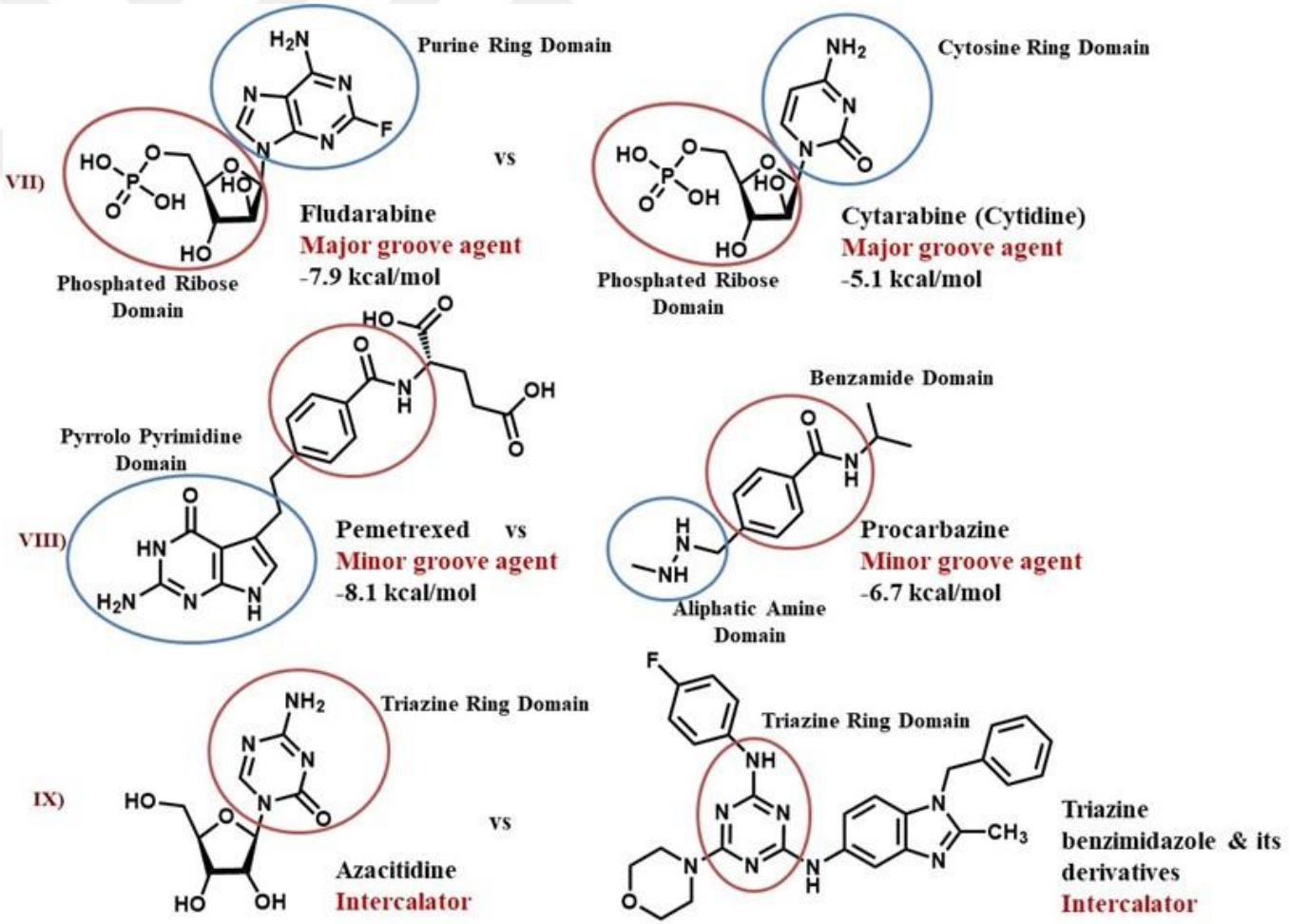

Figure 2.33 : The mode of binding and nucleotide regioselectivity comparison.

The scientific literature supporting our drugs' MD results for major groove agents includes the Cytarabine research where its phosphate metabolized structure Cytidine docks onto the DNA via major groove area and suppresses the diseases of acute and myeloid leukemia and lymphomas. Cytarabine is found to be in Cytidine form since it is converted to the triphosphate form within the cell and enters the nucleus to dock into DNA. The Ribose domain is assumed to be in charge of this mode of binding. In the comparison of VIII of Figure 2.33, Procarbazine suppresses DNA via the minor groove area, it is used along with other anti-cancer drugs for the treatment of stage III and stage IV of Hodgkin's lymphoma disease. Benzamide domains choose Adenine binding in both drugs which vindicate the same mode of binding and regioselectivity. While Aromatic Pyrrolo Pyrimidine chooses Guanine and Aliphatic Amine groups choose Thymine binding. Triazine derivatives are found to be anti-tumor agents and intercalators according to the scientific literature. Therefore, such studies in the literature confirm the intercalation mode of binding of Azacitidine via the triazine domain [100-102].

3. CONCLUSION AND RECOMMENDATIONS

In this Ph.D. thesis, the DNA binding of antineoplastic drugs Fludarabine, Pemetrexed, Cladribine, Clofarabine, and Azacitidine and their respective derivatives were studied thoroughly, employing theoretical molecular docking and molecular dynamics as well as the experimental studies along with the collaborated analytical chemistry group. Our findings proved that in terms of mode of binding structural affinities, Fludarabine is a major groove binder to DNA, Pemetrexed, Cladribine, and Clofarabine are minor groove agents and Azacitidine is an intercalator agent. These interactions with DNA through fairly strong hydrogen bond interactions yield us the conclusion that Fludarabine suppresses the DNA via major groove binding, while Pemetrexed, Cladribine, and Clofarabine do it by minor groove binding whereas Azacitidine is an intercalator agent and unzips the helical structure of DNA completely. The hypothetical derivatives served the true purpose to understand the nucleotide regioselectivity and mode of binding alterations when a variety of functional groups were introduced to the drugs. It is drawn from the pharmaceutical chemistry grand analysis of such massive data that when a Fluorine replacement takes place in the Purine ring of the aforementioned drugs, Adenylation type of nucleotide-binding occurs whereas when Carboxylation, Methylation, and Hydroxylation take place in the Purine ring, poly-adenylation occurs. When a Chlorine replacement takes place in the Ribose ring, Guanidinylation ends and Adenylation occurs. Also, Chlorine replacement in the Ribose ring causes the mode of binding alteration from minor to major groove.

Molecular docking and molecular dynamics simulations of all 5 drugs and their hypothetical derivatives were made clear how they interact with DNA and break it down where these results were also supported by experimental analytical chemistry characterizations. Therefore, the actual mechanism of action of these drugs in a very comprehensive study was found and how they can be improved further is now known and revealed through studied functional groups. Hence, what the true chemical structure of antineoplastic drugs will be which can defeat certain types of cancer is now better known and can be designed from the scratch for a better future.

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CURRICULUM VITAE

Name Surname : Soykan AĞAR

EDUCATION

B.Sc.Eng. : 2011, Koc University, Faculty of Engineering, Department of Chemical and Biological Engineering

M.Sc. : 2015, Istanbul Technical University, Faculty of Science and Letters, Department of Chemistry

PROFESSIONAL EXPERIENCE AND REWARDS:

EXPERIENCE

- Vice General Manager
  Dr. Agar Medical Clinic & Software & Consultancy · 2021 - Present

- Teaching Fellow, R&D Designer in Pharmacy
  Istinye University · 2020 - 2021

- TUBITAK Research Scholarship in Chemistry
  Istanbul Technical University · 2018 - 2020

- Medical Manager
  Medesform Corp. · 2017 - 2020

- Teaching Fellow, Chemistry Department
  The University of British Columbia, Canada · 2016 - 2017

- Project Coordinator - Chemical Engineering
  Politecnico di Torino, Italy · 2015 - 2016

- R&D Consultant of Factory
  Turktex Chemical & Textile Company · 2011 - 2015

- Undergrad. Teaching Assistant - Chemical Engineer & Biological Engineering
  Koc University · Apr 2008 - Jan 2011
- Chemical Engineer in Crude Oil & Fluid Catalytic Cracking Units
  TUPRAS · 2009

- Bio-Engineer in Bio-materials Dpt. & Physics of Complex Fluids Dpt.
  Mesa+ Institute - Twente University, Netherlands · 2009

REWARDS

- Neuromuscular Diseases Certificate
  Issued by Istinye University · Feb 2020

- Pathology & Biochemical Hazardous Waste Management Certificate
  Issued by Istinye University · Feb 2020

- 7th International BAU Drug Design Congress - Conference Poster & Certificate
  Issued by Bahcesehir University · Dec 2019

- Distinguished Tübitak Research Scholarship
  Issued by Tübitak · Jan 2019

- Holistical Medicine Bio-Resonance Certificate
  Issued by Riccon Academy - Switzerland · Oct 2018

- Canada Research Grant - The bio-analytical investigation of glycans (Part 2)
  Issued by University of British Columbia · Apr 2016

- Work Health & Safety Specialist Award
  Issued by University of British Columbia · Feb 2016

- Canada Research Grant - The bio-analytical investigation of glycans (Part 1)
  Issued by University of British Columbia · Jan 2016

- Graduate Dean's Entrance Scholarship Award
  Issued by University of British Columbia · Jan 2016

- High Honor Graduation Certificate
  Issued by Istanbul Technical University · Dec 2014

- BAP Project Grant
  Issued by Istanbul Technical University · Jan 2013

- Motor Neuron Biology & Neurodegeneration Certificate
  Issued by Suna & Inan Kirac Foundation · Nov 2012

- High Honor Graduation Certificate
  Issued by Koç University · May 2011

- Vehbi Koç Scholar Award - Crystal Plaque
  Issued by Koç University · May 2010

- Dean's Honor Roll Award
  May 2009 at Koc University

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

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