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Integrated Computational Exploration of Pyrimidine-2,4-dione Derivatives as Dual HIV-1 RT/RNase H Inhibitor

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Abstract

Introduction. Continuous efforts to uncover new antiviral entities targeting HIV RT RNase H stem from the suffering of affected individuals and the relentless increase in death cases. The present study focused on ten molecules based on the pyrimidine-2,4-dione scaffold, which were discovered to have potent antiviral activity against HIV RT RNase H and acceptable bioavailability and pharmacokinetic properties.

Aim. The main goal of this study is to discover and evaluate promising pyrimidine-2,4-dione-derived molecules as potential inhibitors of HIV.

Materials and methods. A docking protocol was executed to investigate the binding mechanisms of the designed compounds within RNase H's active site using the AutoDock Vina program. The molecular dynamics simulation analysis for 100 ns was carried out using Desmond, and the complexes were constructed using the OPLS3-2005 force field. The frontier molecular orbital energies and related reactivity factors of the best antiviral agents were determined using the DFT-B3LYP/6-31G (d,p) calculations performed with the Gaussian program.

Results and discussion. Two molecules, Pyr06 and Pyr07, displayed a higher docking score (–10.7 kcal/mol) than the control compound (MPD: –9.8 kcal/mol) and the other designed compounds (with affinities ranging from –9.6 to –10.4 kcal/mol). On the other hand, it was noted that these two molecules formed significant hydrogen bonds and hydrophobic contacts with active site residues. Furthermore, our analysis through MD simulations for 100 ns validated the binding stability and conformation of molecules Pyr06 and Pyr07 in complexes with the 5J1E receptor. The DFT findings for both molecules, Pyr06 and Pyr07, also confirmed their docking scores and their ability to form stable complexes with RNase H.

Conclusion. The results obtained could be highly beneficial for designing and developing Pyr06 and Pyr07 as potential therapeutic drugs to combat HIV RT RNase H.

Keywords: pyrimidine-2,4-dione ring, HIV RT RNase H, molecular docking, MD simulation, DFT

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Yassine El Masaoudy, Hafiz Muzzammel Rehman – conceptualization, data curation, writing-original draft. Marwa Alaqarbeh, Hamid Maghat, Tahar Lakhliifi – methodology, investigation, visualization, supervision. Mohammed Bouachrine – validation, writing-review and editing.

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Интегрированное компьютерное исследование производных пиримидин-2,4-диона в качестве двойных ингибиторов РНКазы Н обратной транскриптазы ВИЧ-1

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Резюме

Введение. Постоянные усилия по поиску новых противовирусных средств, нацеленных на РНКазу Н обратной транскриптазы ВИЧ обусловлены высокой заболеваемостью и неуклонным ростом смертности. Настоящее исследование сосредоточено на десяти основанных на пиримидин-2,4-дионе молекулах, которые, как было установлено, обладают мощной противовирусной активностью против РНКазы Н обратной транскриптазы ВИЧ, а также достаточной биодоступностью и фармакокинетическими свойствами.

Цель. Поиск и оценка перспективных производных молекул пиримидин-2,4-диона в качестве потенциальных ингибиторов ВИЧ.

Материалы и методы. Для изучения механизмов связывания спроектированных соединений с активным центром РНКазы Н был использован протокол докинга с применением программы AutoDock Vina. Молекулярно динамическое моделирование продолжительностью 100 нс выполнялось в программе Desmond, комплексы были построены на основе силового поля OPLS3 2005. Энергии фронтальных молекулярных орбиталей и связанные с ними показатели реакционной способности наиболее эффективных антивирусных агентов были рассчитаны с помощью метода DFT B3LYP/6 31G(d,p), реализованного в программе Gaussian

Результаты и обсуждение. Две молекулы, Pyr06 и Pyr07, продемонстрировали более высокий показатель докинга (–10,7 ккал/моль) в сравнении с контрольным соединением (MPD: –9,8 ккал/моль) и с другими спроектированными соединениями (с аффинностями в диапазоне от –9,6 до –10,4 ккал/моль). С другой стороны, было отмечено, что эти две молекулы образуют значительное количество водородных связей и гидрофобных контактов с аминокислотными остатками активного центра. Кроме того, анализ, проведенный с помощью молекулярно динамического моделирования в течение 100 нс, подтвердил устойчивость связывания и конформацию молекул Pyr06 и Pyr07 в комплексах с рецептором 5J1E. Квантово химические расчеты DFT для обоих соединений, Pyr06 и Pyr07, также подтвердили их высокие показатели докинга и способность образовывать стабильные комплексы с РНКазой Н.

Заключение. Полученные результаты могут оказаться крайне полезными для разработки соединений Pyr06 и Pyr07 в качестве потенциальных терапевтических препаратов для борьбы с РНКазой Н обратной транскриптазы ВИЧ.

Ключевые слова: пиримидин-2,4-дионовое кольцо, РНКазы Н обратной транскриптазы ВИЧ, молекулярный докинг, молекулярно-динамическое моделирование, DFT

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. Я. Эль Масауди, Х. М. Рахман – концептуализация, обработка данных, написание исходного текста. М. Алакарбех, Х. Магат, Т. Лахлифи – методология, исследование, визуализация, наблюдение. М. Буахрин – валидация, проверка текста, редактирование.

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INTRODUCTION

The increasing focus on the HIV infection epidemic has been notable since AIDS became a novel public health crisis nearly forty years ago [1, 2]. According to recent reports from UNAIDS¹ and WHO², the number of individuals infected with HIV has surpassed 85.5 million since the beginning of the AIDS epidemic. As of the end of 2022, around 39 million people were grappling with HIV infections, and the disease claimed the lives of nearly 630,000 individuals. HIV strains that have developed resistance to treatment are responsible for these deaths. HIV, categorized as a retrovirus within the lentivirus family, predominantly targets immune system cells that exhibit CD4 receptors on their surface. Precisely, CD4-positive T lymphocytes serve as the foremost target for HIV infection. Integrase, protease, and reverse transcriptase are three viral enzymes enclosed within a membrane called the capsid, which surrounds the RNA genome of the virus [3, 4]. Among these enzymes, RT is essential for the HIV viral life cycle by catalyzing an important process in the cytoplasm [5]. RT is the first viral enzyme to which anti-HIV drugs are directed and represents a major source of the substantial diversity observed among HIV strains in an infected individual [6–8]. HIV RT is a multifunctional enzyme that performs several crucial roles in viral replication, illustrating its flexibility by participating in two distinct enzymatic activities: DNA polymerase (Pol) and ribonuclease H (RNase H). The DNA Pol activity of RT is responsible for copying an RNA or DNA template to generate the corresponding cDNA. This process is called reverse transcription, the mechanism by which the viral RNA genome is transcribed into DNA [9–12]. During reverse transcription, the RNase H (RH) function of RT assumes a key role in breaking down the RNA element within the RNA-DNA hybrid structure. This degradation process is paramount for successfully concluding reverse transcription and forming a DNA duplicate of the viral genome. The cooperative action of the DNA polymerase and RH activity of RT assists the efficient synthesis of cDNA from its viral RNA template. Newly synthesized DNA then integrates into the host cell genome, resulting in chronic infection and viral reservoir establishment [13]. Their profound impact on HIV replication and survival in host cells is of high importance. The RH activity of HIV RT is a new target for the development of antiretroviral agents in treatment against HIV [14]. A class of drugs called RT inhibitors, however, acts to block the enzyme by either slowing it down or inhibiting its activity. This category of drugs is comprised of nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs). They are known as nucleoside analogs and

block the action of HIV reverse transcriptase, which is a form of enzyme necessary for the virus to be able to replicate. They operate by integrating into the growing viral DNA strand, effectively hampering further DNA synthesis. On the other hand, NNRTIs, also referred to as non-nucleoside analogs, target RT specifically by focusing on the enzyme's DNA polymerase-dependent RNA and DNA processes. NNRTIs attach to a specific pocket within the RT enzyme, effectively obstructing its function [15–17]. Despite the effectiveness of these RT inhibitors, resistance can occur, primarily due to mutations in the RT enzyme. The development of resistance poses a challenge in HIV treatment and indicates that it requires continuous research and the discovery of new antiretroviral drugs with novel mechanisms of action.

Heterocycles are organic molecules that have a ring structure and at least one heteroatom. These types of molecules are crucial in drug discovery and development. Thus, considerable efforts have been made to develop facile and green methods for their preparation in satisfactory yield. Compounds derived from pyrimidine-2,4-dione have exhibited a diverse range of pharmacological and biological functions, including antimicrobial, anti-cancer, antitumor, antiproliferative, antiviral, and antiparasitic agents. This study investigates *in silico* methodologies to evaluate the therapeutic viability of new heterocyclic derivatives designed to target HIV RT, driven by the urgent need for new therapeutic measures against HIV RNase H.

The outcomes of our prior QSAR modeling efforts have unveiled a novel category of pyrimidine-2,4-dione derivatives characterized by heightened antiviral efficacy against HIV RT. We observed an increase in RNase H activity [18]. All eleven drug candidates showed significant and promising antiviral activity, ranging from 8.174 to 13.835, among which inhibitors Pyr02 and Pyr08 showed remarkable inhibitory activity, with pIC_{50} values of 10.088 and 13.835, respectively. We also evaluated the Lipinski parameters [19] and the pharmacokinetic (PK) and toxicological properties [20] of the proposed antiviral candidates using an *in silico* ADME/toxicity examination. The results indicated that all of these candidates, except for Pyr05, have oral bioavailability, possess favorable PK characteristics, and demonstrate an absence of AMES toxicity. Our findings in previous work showed that the most active antiviral candidates are a promising scaffold for future anti-HIV RT RNase drug design.

In the present theoretical research, candidates with acceptable bioavailability and favorable ADMET properties were investigated through molecular docking analysis to gain a better handle on the detailed mechanism of proposed binding mode, interactions, and conformation between these chemicals and the target protein of therapeutic interest. We then used molecular dynamics simulation analysis to assess the stability and flexibility of the drug candidates, or ligands, in the active site.

¹ UNAIDS. Available at: <https://www.unaids.org/en> Accessed: 07.11.2025.

² WHO. <https://www.who.int/data/gho/data/themes/hiv-aids> Accessed: 07.11.2025.

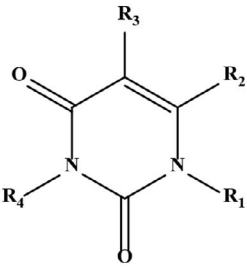
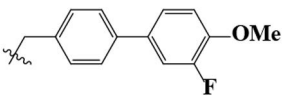
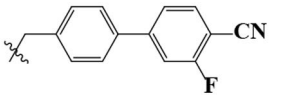
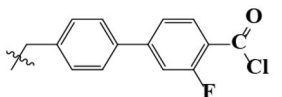
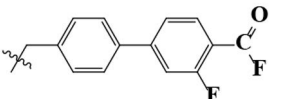
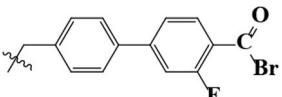
METHODOLOGY

The rapid spread of computational technologies, particularly the Computer Assisted Drug Discovery (CADD) approach, has stimulated the search for potent therapeutic drugs that could act against different diseases. This method has revolutionized drug discovery by accelerating the screening and testing of potential treatment candidates. In our previous work, we reported a series of compounds originated from pyrimidine-2,4-diones that exhibited potent antiviral activity against HIV RT RNase H by using 2D-QSAR, which was developed based on MLR and MNLR. Moreover, we conducted drug similarity and ADMET studies to assess their properties. It was found that all derivatives, except for Pyr05, adhered to Lipinski's rules, exhibited favorable ADME properties, and showed no mutagenicity based on the AMES assay [18]. The anti-HIV RT RNase H enzyme activity, drug-likeness, and ADMET predicted results of MPD and the 11 designed compounds were

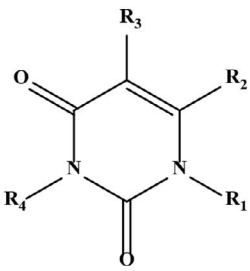
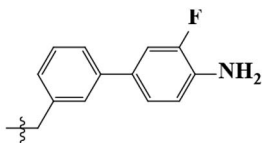
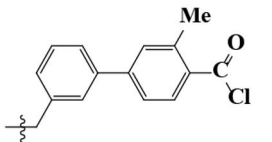
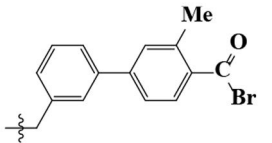
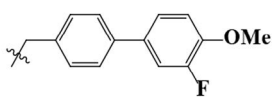
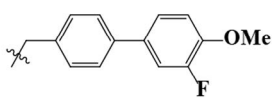
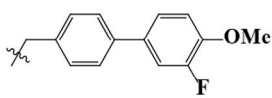
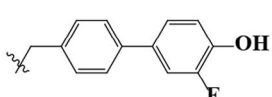
summarized in Table 1. These findings emphasize the encouraging properties of these compounds as potential antiviral agents and deserve further investigation.

In this study, we sought to gain insight into the potential of these derivatives as inhibitors targeting HIV RT RNase H by employing molecular docking based on our recent research and molecular dynamics simulations. Molecular docking is a computational approach that estimates the optimal orientation and binding abilities for small molecules in a protein's active site [21, 22]. In this work, the envisaged interaction and binding modes of the MPD, Pyr01, Pyr02, Pyr03, Pyr04, Pyr06, Pyr07, Pyr08, Pyr09, Pyr10 and Pyr11 compounds with different substituents (e.g., carboxyl [COOH], hydroxyl [OH], amino [NH₂], cyano [CN], methoxy [OMe], and haloformyl [COX] groups) in the active site of RT RNase H were analyzed using the AutoDock Vina program [23]. The 3D crystal structure of RNase H can be identified using the ID code 5J1E [24]. Using AutoDock tools [25],

Table 1. 2D chemical structures, anti-HIV RT RNase H activity, drug-likeness, and ADMET prediction of MPD and 11 proposed compounds

								
Cmpd	R ₁	R ₂	R ₃	R ₄	pIC ₅₀	Acceptable bioavailability	Favorable ADME properties	AMES Toxicity
MPD ^a	H		H	OH	8.119	Yes	Yes	No
Pyr01 ^b	H		H	OH	8.174	Yes	Yes	No
Pyr02	H		H	OH	10.088	Yes	Yes	No
Pyr03	H		H	OH	8.955	Yes	Yes	No
Pyr04	H		H	OH	8.871	Yes	Yes	No

End of Table 1

								
Cmpd	R ₁	R ₂	R ₃	R ₄	pIC ₅₀	Acceptable bioavailability	Favorable ADME properties	AMES Toxicity
Pyr05	H		H	OH	8.952	Yes	Yes	Yes
Pyr06	H		H	OH	8.636	Yes	Yes	No
Pyr07	H		H	OH	8.829	Yes	Yes	No
Pyr08 ^c	H		COCl	OH	13.835	Yes	Yes	No
Pyr09	H		COOH	OH	8.262	Yes	Yes	No
Pyr10	H		Br	OH	8.686	Yes	Yes	No
Pyr11	OH		OH	OH	8.754	Yes	Yes	No

Note. ^a MPD is the most potent derivative in the previous QSAR modeling.

^b Pyr01 is the least active designed compound as HIV RT RNase H inhibitor.

^c Pyr08 is the most active designed compound as HIV RT RNase H inhibitor.

the protein structure was primed for docking by eliminating both H₂O molecules and the co-crystallized inhibitor. Following this, the Gasteiger-Huckel charge was applied to the protein atoms after merging non-polar hydrogens with their corresponding carbon atoms. The

molecular docking process involved optimizing the grid box dimensions to 35 × 26 × 35 Å, with specific center coordinates set at -52.290, 70.325, and -85.394 for the x, y, and z axes, respectively. The best conformation of ligands was selected by considering the lowest bin-

ding energy. A re-docking protocol was carried out to validate the docking method's reliability. This involved superimposing the native co-crystallized molecule's position with its predicted docked pose and calculating the root mean square deviation (RMSD) parameter, which required a value <2 for reliability.

The ADMET prediction and molecular docking results were confirmed by conducting a 100 ns molecular dynamics simulation analysis for protein-small molecule complexes, emphasizing the binding of the protein (PDB: 5J1E) with the ligands exhibiting the highest stability. The molecular dynamics simulations were carried out using Desmond, and the complexes were constructed using the OPLS3-2005 force field [26]. The explicit solvent model SPC was employed to simulate the solvent, placing the system in an orthorhombic box. The box size was adjusted to ensure that the outermost atom of the complex was distanced by 10 Å from each side of the box. The system was neutralized by adding Na^+ and Cl^- counter-ions during molecular dynamics simulation in order to mimic the physiologically relevant conditions (0.15 M salt concentration or about 0.15 M). The Molecular dynamics simulations were conducted under the NPT ensemble, with temperature and pressure fixed at 300K and 0.987atm, respectively. The evaluation of the simulated systems was based on analyzing several parameters, namely RMSD, RMSF, rGyr, intraHB, MolSA, SASA, and PSA.

Finally, density functional method studies were conducted using the B3LYP hybrid functional to estimate the frontier molecular orbital energies and related quantum chemical factors of the best antiviral agents selected through structure-based Molecular docking and Molecular dynamics simulations. The DFT calculations were executed by the Gaussian program using a 6-31G(d, p) basis set [27]. The objective was to evaluate the electronic properties and reactivity indices of these chemical structures.

RESULTS AND DISCUSSION

Binding modes of MPD and Ligands within the 5J1E receptor

Molecular docking simulations were performed for one reference complex and ten ligand-receptor ones, which in total contributed to obtaining the set of necessary details about the working principle of each pair. The results included affinity-calculated values for the most optimal combination of the enzyme active site with the docked compounds. These affinity values reflect the binding strength between the ligands and the enzyme. Furthermore, the predicted interactions between the docked molecules and essential residues at the active site are illustrated in Figures 1–7. The dotted lines depict the binding interactions between ligands and RT RNase H's active site. At the same time, Table 2 provides a summary of the corresponding favorable interactions and affinity values. The various modes and interactions of ligand-receptor binding among the MPD, Pyr01, Pyr02, Pyr03, Pyr04, Pyr06, Pyr07, Pyr08, Pyr09, Pyr10, and Pyr11 ligands within the RT RNase H's active site were analyzed using the AutoDock Vina program. All the ligands had the same structural feature that was characterized by the biphenyl part, which was joined with a pyrimidine ring containing two carbonyl groups, the pyrimidine-2,4-dione ring. Additionally, it is crucial to focus on some of the critical amino acids that played a key role in hydrogen bonding and hydrophobic interaction formation in the active site of the receptor. These amino acids are the ones that formed the intermolecular hydrogen bonds with compounds. They are as follows: LEU533, GLN507, LYS431, GLU430, TYR532, LYS424, GLN500, TRP266, LEU425, PRO421, and TRP535. The binding affinity among the wide range, from moderate to excellent binding, showed the scores from -9.6 to -10.7 kcal/mol. The ten molecule-receptor complexes possessed a higher negative binding affinity than the reference complex (-9.6 kcal/mol), except for Pyr08 (-9.6 kcal/mol) and Pyr11 (-9.8 kcal/mol).

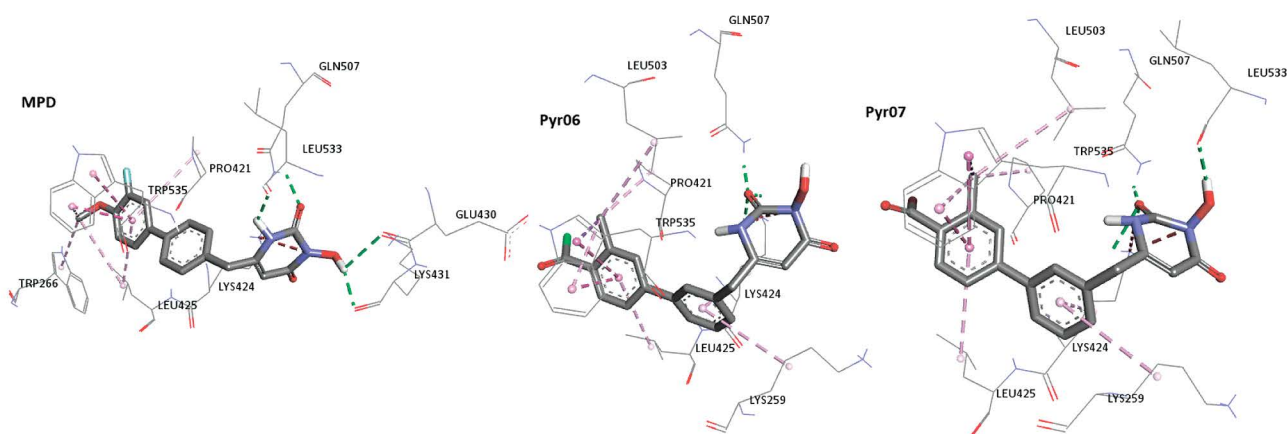


Figure 1. The 3D representation of the predicted binding modes of MPD, Pyr06, and Pyr07 inhibitors in the 5J1E receptor

Table 2. Affinity scores and binding interactions of ligands in the 5J1E receptor in comparison with MPD

Ligand	Affinity (kcal/mol)	Hydrogen bonds interactions		Hydrophobic interactions		
		Conventional H-bond	Carbon H-bond	Pi-Pi stacked	Alkyl/ Pi-alkyl	Pi-sigma
MPD	-9.8	LEU533 5.28 Å GLN507 5.42 Å LYS431 3.69 Å GLU430 5.42 Å	–	TRP535	TRP266 LEU425 PRO421	LEU425
Pyr01	-10.4	TYR532 6.07 Å LYS424 4.21 Å TRP535 5.15 Å GLN500 4.88 Å	TRP266 4.69 Å	–	–	–
Pyr02	-10.3	TYR532 5.76 Å LYS424 4.15 Å TRP535 5.25 Å GLN500 4.62 Å	TRP266 4.62 Å	–	–	LEU425
Pyr03	-10.2	TYR532 5.06 Å LYS424 4.05 Å TRP535 5.55 Å GLN500 4.48 Å	TRP266 4.62 Å	–	–	LEU425
Pyr04	-10.3	LYS424 4.11 Å TRP535 5.33 Å GLN500 4.53 Å	TRP266 4.63 Å	–	–	LEU425
Pyr06	-10.7	LYS424 5.48 Å GLN507 5.61 Å	–	–	PRO421 LEU503 LEU425 LYS259	TRP535
Pyr07	-10.7	LYS424 5.46 Å GLN507 5.63 Å LEU533 5.14 Å	–	–	PRO421 LEU503 LEU425 LYS259	TRP535
Pyr08	-9.6	LYS259 4.44 Å GLN507 5.01 Å LEU533 5.23 Å	–	TRP535	LYS424 PRO421 LEU422 LEU425 TRP266	–
Pyr09	-10.3	GLN507 6.24 Å LYS424 4.94 Å TRP535 5.42 Å	TRP266 4.60 Å GLN269 6.11 Å LYS259 5.05 Å	–	PRO421	–
Pyr10	-10.1	GLN507 5.49 Å LEU533 6.02 Å	–	TRP535	LYS259 LEU425 PRO421	–
Pyr11	-9.8	LYS424 3.62 Å	ALA534 3.73 Å	–	LEU425 PRO421 LEU422	–

The differences in binding affinity values can be attributed to variations in the number of interactions, types of interactions, and distances between each ligand and the amino acid residues.

Predicted binding modes of MPD, Pyr06 and Pyr07 with 5J1E receptor

In the present work, MPD was considered a control compound to identify the binding energy between this new series derived from pyrimidine-2,4-dione and the RNase receptor and study their binding interactions. The affinity and the interactions derived from molecular docking studies with the control compound in the 5J1E receptor are shown in Table 2. The residues of the

5J1E receptor active site that mainly interacted with the docked control compound were LEU533, GLN507, LYS431, GLU430, TRP535, TRP266, LEU425, and PRO421 (Figure 1). Additionally, the pyrimidine-2,4-dione ring of the control compound had H-bond interactions with four residues: LEU533, GLN507, LYS431, and GLU430. These interactions had bond lengths of 5.28, 5.42, 3.69, and 5.42 Å, respectively, indicated by the dotted green line. The hydroxyl group of the control compound forms an H-bond interaction with LYS431 and GLU430. The molecule of interest has a carbonyl group at the C-2 position and an NH group in the pyrimidine-2,4-dione ring; the substituents form H-bond interactions with GLN507 and LEU533, respectively. The biphenyl moiety

exhibited five hydrophobic contacts, such as π - π stacked, alkyl/ π -alkyl, and contact π -sigma interactions with TRP535, TRP266, PRO421, and LEU425.

From Table 2, the binding affinity and interaction records obtained from the molecular docking studies of the docked Pyr06 and Pyr07 ligands in the HIV RT RNase H receptor are displayed. The ligands Pyr06 and Pyr07 displayed the most negative binding affinity of -10.7 kcal/mol in comparison to the other ligands. Additionally, the same two ligands displayed similar interactions in the HIV RT RNase H receptor. Furthermore, the biphenyl moieties of both ligands engaged in a similar hydrophobic contact with amino acid residues LEU503, TRP535, LEU425, and LYS259. The same could be observed for the pyrimidine-2,4-dione ring of Pyr06 and Pyr07, which have the same H-bond interaction for the amino acid residues LYS424 and GLN507. The green dotted lines in Figure 1 illustrate the commonalities of the above interactions. For the carbonyl group located at position C-2 of the pyrimidine-2,4-dione ring of Pyr06 and Pyr07, it engaged in two different H-bond contacts with two amino acid residues. For Pyr06, the H-bond interaction occurred based on LYS424 and GLN507 at a distance of 5.48 Å and 5.61 Å, respectively. Similarly, in Pyr07, H-bond interactions occurred with LYS424 at a distance of 5.46 Å and GLN507 at a distance of 5.63 Å. Compared to Pyr06, there was one additional conventional H-bond engagement for the hydroxyl group in the pyrimidine-2,4-dione ring of Pyr07 and LEU533 at a distance of 5.14 Å.

Predicted binding modes of Pyr01 with 5J1E receptor

The affinity and the amino acid residue are important for predicted binding interactions derived from molecular docking studies with Pyr01 in the 5J1E receptor are shown in Table 2. The residues of the receptor active site that mainly interacted with the docked Pyr01 compound were TYR532, LYS424, TRP535, GLN500, and TRP266 (Figure 2). It is worth noting that although no hydrophobic contacts between the Pyr01 ligand and residues were observed, the Pyr01 ligand exhibited the second-highest negative score affinity of -10.4 kcal/mol after the Pyr06 and Pyr07 ligands. The pyrimidine-2,4-dione ring of the molecule Pyr01 established three H-bond contacts with TYR532, LYS424, and TRP535, with distances of 6.07 , 4.21 , and 5.15 Å, respectively. The hydroxyl group of the docked Pyr01 compound participated in an H-bond with TYR532. Moreover, the carbonyl group located at positions C-2 and C-4 of the pyrimidine-2,4-dione ring formed two H-bonds, one with TRP535 and the other with LYS424. Additionally, the biphenyl moiety is engaged in forming two H-bond interactions. One of the interactions is with GLN500 at a distance of 4.88 Å, and is a conventional H-bond, while the other is with TRP266 at a distance of 4.69 Å and is a carbon H-bond.

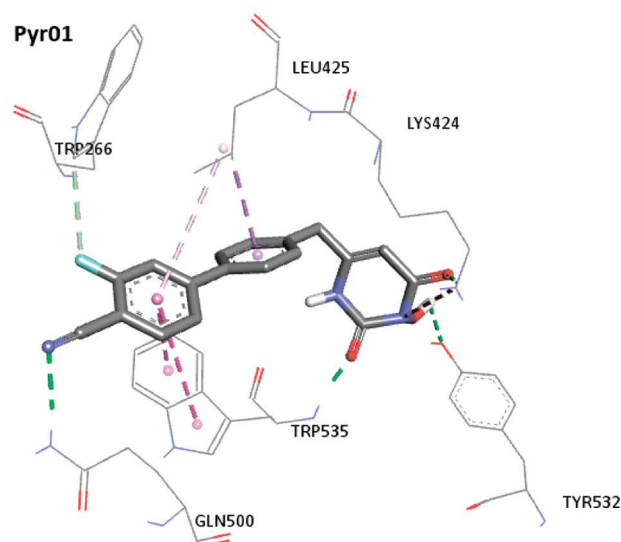


Figure 2. Predicted binding interactions between Pyr01 inhibitor and the 5J1E receptor

Predicted binding modes of Pyr02, Pyr04 and Pyr09 with 5J1E receptor

The affinity and the amino acid residues important for predicted binding interactions derived from molecular docking studies with Pyr02, Pyr04, and Pyr09 in the 5J1E receptor are shown in Table 2. The Pyr02, Pyr04, and Pyr09 ligands displayed the third-highest negative binding affinity of -10.3 kcal/mol among the other ligands. The docking results revealed that the residues of the receptor active site that mainly interacted with the docked compounds were TYR532, LYS424, TRP535, GLN500, TRP266, GLN269, LYS259, LEU425, and PRO421. Four identical residues were engaged in H-bond interactions with the docked compounds (LYS424, TRP535, GLN500, and TRP266).

Additionally, ligand Pyr02 interacts with TYR532, LYS424, TRP535, GLN500, TRP266, and LEU425 residues through H-bond interactions and hydrophobic contacts, as shown in Table 2, and Figure 3. In the case of Pyr02, the pyrimidine-2,4-dione ring interacted with the residue TYR532 through three H-bonding distances of 5.76 and 4.15 Å with LYS424 and a distance of 5.25 Å with TRP535, as shown in Table 2 and Figure 3. The pyrimidine group at N-3 in pyrimidine-2,4-dione engaged in a hydrogen bond with TYR532. In contrast, the carbonyl group located at positions C-2 and C-4 of the same ring participated in two H-bonding contacts, one with TRP535 and the other with LYS424. The biphenyl moiety of the ligand engaged in two H-bonding interactions, one with GLN500 at a distance of 4.62 Å (conventional H-bond) and another (carbon H-bond) with TRP266 at a distance of 4.62 Å. Furthermore, the biphenyl moiety also had a hydrophobic contact (π -sigma interaction) with LEU425. A detailed illustration of the interactions is depicted in Figure 3.

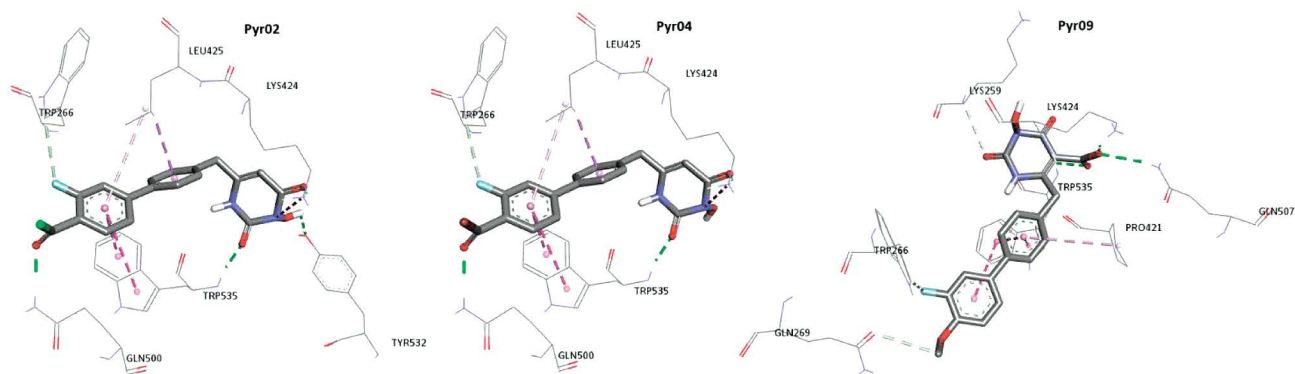


Figure 3. Predicted binding interactions between Pyr02, Pyr04, and Pyr09 inhibitors derived from the pyrimidine-2,4-dione and the 5J1E receptor

Ligand Pyr04 established its presence through H-bond interactions and hydrophobic contacts with LYS424, TRP535, GLN500, TRP266, and LEU425 residues (Figure 3). The carbonyl group positioned at C-2 and C-4 of the pyrimidine-2,4-dione ring also formed two unique hydrogen bond interactions with TRP535 and LYS424 with distances measuring 5.33 and 4.11 Å, respectively. The biphenyl participated in dual H-bonding contacts: one formed a conventional H-bond with GLN500 at a distance of 4.53 Å, while the second produced a carbon bond H-bond with TRP266 at a distance of 4.63 Å. Moreover, a hydrophobic contact was seen with LEU425. It was a Pi-sigma interaction.

Ligand Pyr09 engaged in hydrogen bond interactions and hydrophobic contacts with GLN507, LYS424, TRP535, TRP266, GLN269, LYS259, and PRO421 residues (Figure 3). The carboxylic acid moiety at C-5 of the pyrimidine-2, 4-dione ring had 3 hydrogen bonds with TRP535, LYS424, and GLN507 at distances of 5.42, 4.94, and 6.24 Å, respectively (Figure 3). In addition, a carbon-hydrogen bond was additionally formed by the carbonyl group of the same ring at C-2 with LYS259 at a distance of 5.05 Å. The biphenyl moiety showed two carbon-hydrogen bonds with GLN269 and TRP266 at distances of 6.11 and 4.60 Å, as well as one hydrophobic contact – a pi-alkyl interaction with PRO421.

Predicted binding modes of Pyr03 with 5J1E receptor

Table 2 shows the affinity and important interactions of the molecular docking study with the Pyr03 in the RT RNaseH receptor (Figure 4). Pyr03 with –10.2 kcal/mol has the most negative binding affinity value and is presented in fourth place among the ligands. The docking analysis shows that the docked Pyr03 ligand has an important interaction with specific receptors for the active site. These receptors are TYR532, LYS424, TRP535, GLN500, TRP266, and LEU425. The pyrimidine-2,4-dione ring of the Pyr03 has undergone three separate H-bonds, which are the first H-bond with TYR532 at 5.06 Å, the second H-bond with LYS424 at

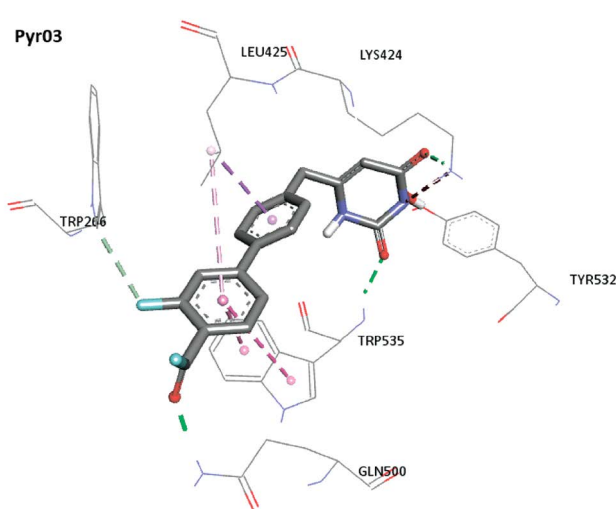


Figure 4. Predicted binding interactions between Pyr03 inhibitor the 5J1E receptor

4.05 Å, and the third H-bond with TRP535 at 5.55 Å. The hydroxyl group at the position N-3 of the pyrimidine-2,4-dione ring was involved in H-bonding with the TYR532 receptor, while a C=O at positions C-2 and C-4 of the same ring benefitted from two H-bonds, one with TRP535 and another one with LYS424. The biphenyl moiety of the Pyr03 made two H-bonds; one is the conventional H-bonding at the distance of 4.48 Å with the GLN500, and another is a sigma interaction between the carbon atom and the hydrogen bond with the distance of 4.62 Å. Further, the biphenyl moiety of the Pyr03 makes a hydrophobic contact with the LEU425.

Predicted binding modes of Pyr10 with 5J1E receptor

Table 2 demonstrates the binding logics and crucial interactions of molecular docking with Pyr10 in the RT RNase H receptor. Pyr10 recorded the fifth-highest binding logic of –10.1 kcal/mol in the ligand evaluation. Docking analysis indicated critical contact residues for

the interaction between the docked compound Pyr10 and the active site 5J1E receptor (Figure 5). The residues include GLN507, LEU533, TRP535, LYS259, LEU425, and PRO421. The Pyr10's pyrimidine-2,4-dione ring made two H-bonds: one with LEU533 at 6.02 Å and one with GLN507 at 5.49 Å. In the pyrimidine ring, the carbonyl group at the C-2 position established a conventional H-bond with GLN507, while the NH-group at the aromatic ring established a conventional hydrogen bond with LEU533. The biphenyl features made extensive contact with the TRP535, LYS259, LEU425, and PRO421 via pi-pi stacked and alkyl/pi-alkyl contacts.

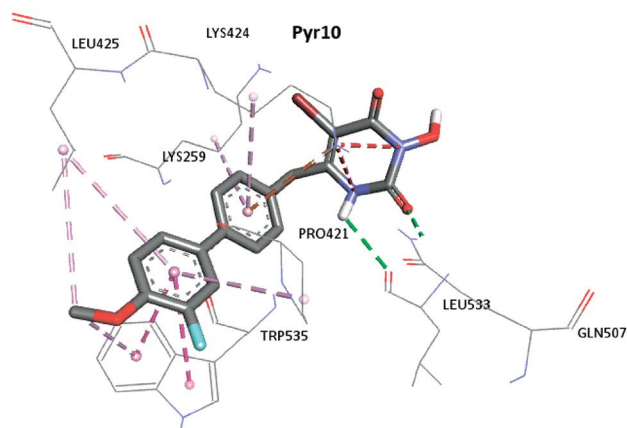


Figure 5. Predicted binding interactions between Pyr10 inhibitor and the 5J1E receptor

Predicted binding modes of Pyr11 with 5J1E receptor

The binding affinity and critical interactions between ligand Pyr11 and specific residues within the active site of the 5J1E receptor are displayed in Table 2. Pyr11 ligand exhibited the sixth-highest negative binding affinity of -9.8 kcal/mol. 5J1E receptor active site residues that mainly interacted with the docked Pyr11 compound were LYS424, ALA534, LEU425, PRO421, and LEU422 (Figure 6). The pyrimidine-2,4-dione ring of the inhibitor Pyr11 established two H-bonding interactions with LYS424 (conventional H-bond) and ALA534 (carbon H-bond), with distances of 3.62 and 3.73 Å, respectively. The carbonyl group located at positions C-2 and C-4 of the pyrimidine-2,4-dione ring formed two H-bonds, one with LYS424 and the other with ALA534. Furthermore, the biphenyl moiety made hydrophobic contacts (Pi-alkyl interactions) with LEU425, PRO421, and LEU422.

Predicted binding modes of Pyr08 with 5J1E receptor

The Pyr08 ligand interacted with several residues through H-bonds and hydrophobic contacts (Figure 7). Particularly, it made contacts with LYS259, GLN507, LEU533, TRP535, LYS424, PRO421, LEU422, LEU425, and TRP266. The pyrimidine-2,4-dione ring of inhibitor Pyr8

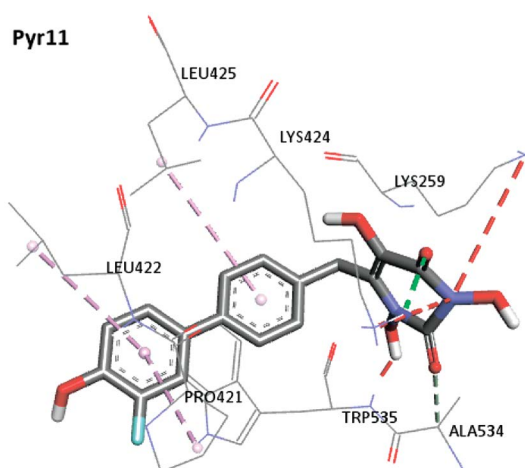


Figure 6. Predicted binding interactions between Pyr11 inhibitor and the 5J1E receptor

formed three conventional hydrogen bond contacts with LYS259, GLN507, and LEU533 at distances of 4.44, 5.01, and 5.23 Å, respectively. The carbonyl of the position C-2 of the pyrimidine-2,4-dione ring formed an H-bond with GLN507, and the NH of the pyrimidine-2,4-dione ring made an H-bond with LEU533. Furthermore, the oxygen of the COCl group formed an H-bond with LYS259. Lastly, the biphenyl moiety of Pyr8 made hydrophobic contacts with TRP535, LYS424, PRO421, LEU422, LEU425, and TRP266.

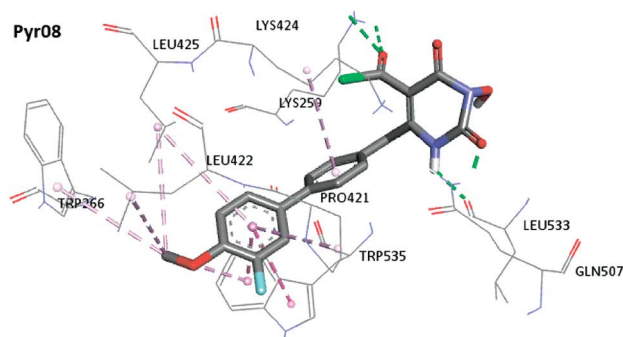


Figure 7. Predicted binding interactions between Pyr08 inhibitor and the 5J1E receptor

The superposition method

The precision of the results from the molecular docking protocol was confirmed by utilizing the superposition approach, as the docking technique underwent validation through a redocking procedure into the HIV RT RNase H crystal structure (PDB code 5J1E). This validation process revealed a noteworthy success rate when comparing the redocking outcomes with the co-crystallized conformation, resulting in an RMSD value of 1.053 Å. The alignment of the docked small molecule's orientation was found to be highly accurate and perfectly matching the bioactive conformation of the co-crystallized small molecule, as depicted in Figure 8.

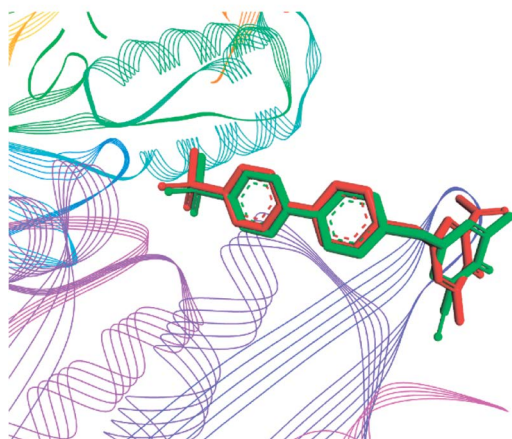


Figure 8. The superposition of the native small molecule (red) and the redocked small molecule (green)

Molecular dynamics simulation

The Molecular analysis results indicate that, except for Pyr08, all the ligands studied showed more excellent stability in the receptor (PDB code: 5J1E) than the control ligand (MPD: -9.8 kcal/mol). Pyr06 and Pyr07 were the most stable compounds in the receptor's binding site, suggesting that they have a more substantial binding energy for the protein and are more likely to interact regularly and stably with the receptor than the other ligands, including the control ligand. To examine the interactions of the best-binding docked compounds (Pyr06 and Pyr07) within the receptor's binding pocket and to evaluate the conformational stability of both anchored complexes formed by 5J1E receptor and Pyr06 and Pyr07 molecules (5J1E-Pyr06 and 5J1E-Pyr07), molecular dynamics simulations were conducted.

RMS deviation (RMSD) tracks how the overall conformation of the system evolves, highlighting changes from the initial structure. On the other hand, the RMS fluctuation (RMSF) parameter was considered to articulate the system's stability, the mobility of individual atoms, and the flexibility of residues during protein-compound interactions. The peaks in the RMSF plot indicate the fluctuating regions of the protein during the molecular dynamics simulation analysis.

The recorded RMSD values of the 5J1E-Pyr06-complex and 5J1E-Pyr07-complex are shown in Figures 9 and 10, respectively. As can be seen from Figure 9, the RMS deviation parameter of the 5J1E-Pyr06 led to an initial deviation ranging from 3–6 Å until nearly 40 ns, after which the system attained equilibrium, and the deviation stabilized between 6–7 Å. Similarly, the RMS deviation of the 5J1E-Pyr07 displayed a deviation from 2.4 to 4.0 Å until 35 ns, following which the RMSD stabilized, and the system equilibrated until 100 ns (Figure 10). After looking at the trajectories, it was found that both systems remained stable, with ligands staying within the binding pockets, engaging in significant interactions, and maintaining consistent backbones. The

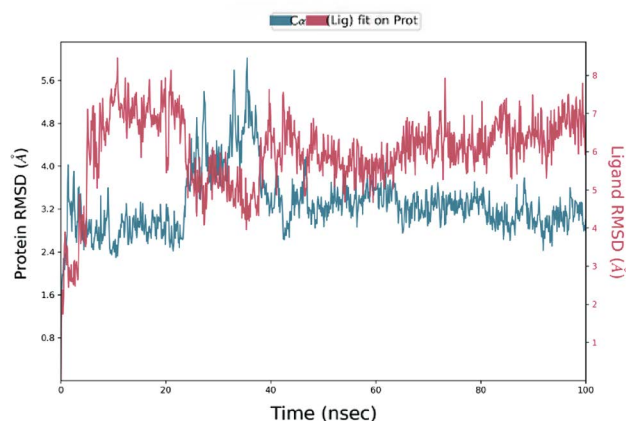


Figure 9. RMS deviation plot of 5J1E-Pyr06-Complex

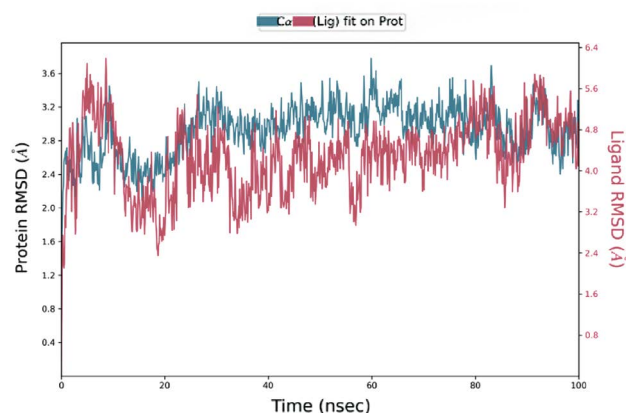


Figure 10. RMS deviation plot of 5J1E-Pyr07-Complex

observed deviation could be attributed to conformational changes. Likewise, recorded RMSF values below 3 Å revealed remarkable stability of both complexes. There was no significant fluctuation in regions where the ligand interacted with the 5J1E receptor, as depicted by the green lines in Figures 11 and 12. The examination focused on the ligand in the complex to discern its impact on the overall protein.

The six analyzed parameters (RMSD, rGyr, intraHB, MolSA, SASA, and PSA) allow us to assess the stability of ligands Pyr06 and Pyr07 in the receptor's binding pocket during the 100 ns of simulation. Thus, ligand RMSD is a metric that quantifies the amount of structural variance of a compound from a reference conformation, frequently their initial structure. It provides information on how much the ligands have moved from the initial conformation during the simulation. In general, lower RMSD means the ligands keep the structure closer to the reference structure, which means intraHB bonds within ligand pieces are of a more specific type sensitive to different ligand parts' heat. These IntraHBs are responsible for the molecule's shape and activity. The existence of intraHBs can affect multiple molecular properties like structure, reactivity, function, etc. MolSA is the total area of the surface of a molecule that is being

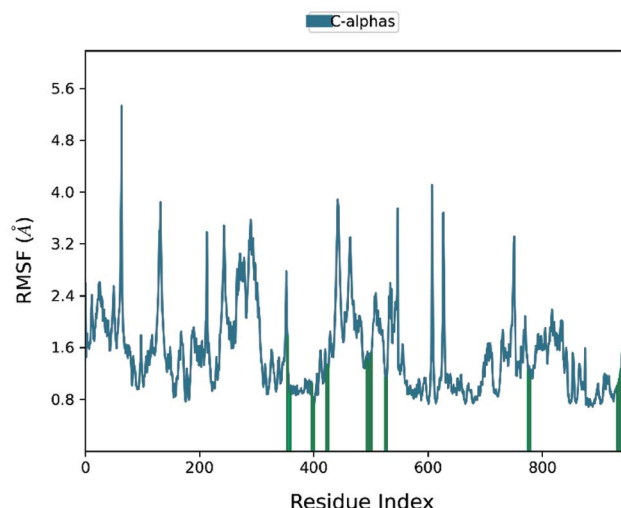


Figure 11. RMS fluctuation plot of 5J1E-Pyr06-Complex

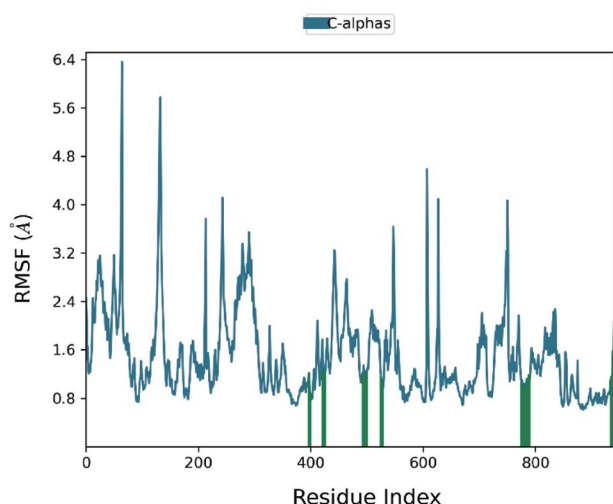


Figure 12. RMS fluctuation plot of 5J1E-Pyr07-Complex

allowed or denied access to its environment. MolSA is often taken as a theoretical calculation using minimum probe radius and based on the probe-accessible surface. The value of MolSA gives an idea about the molecule's openness to the environment and the interactions possible with the neighboring molecules. SASA is a measurement of the percent of a molecule's surface that is solvent-accessible. This parameter will give the idea of whether the part of a molecule is exposed or hidden within the environment. Since understanding the specific areas of a molecule is more common, changes in SASA will indicate potential interactions, particularly in the binding site. PSA is to measure the solvent-accessible superficial area of a compound composed of polar atoms. It concentrates on regions conducive to polar interactions, including hydrogen bonding. Plots of the six properties mentioned below of the ligands Pyr06 and Pyr07 are depicted in Figures 13 and 14, respectively. As shown in Figure 13, the RMSD of the mo-

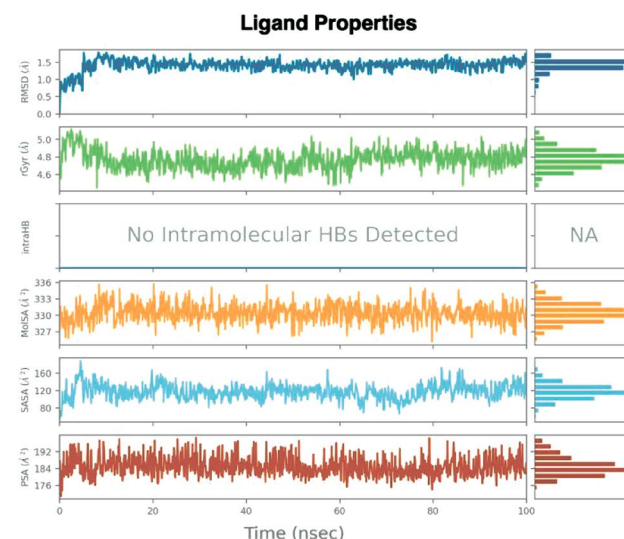


Figure 13. Variation in the small molecule's properties in 5J1E-Pyr06-complex as a function of time during a 100 ns MD simulation

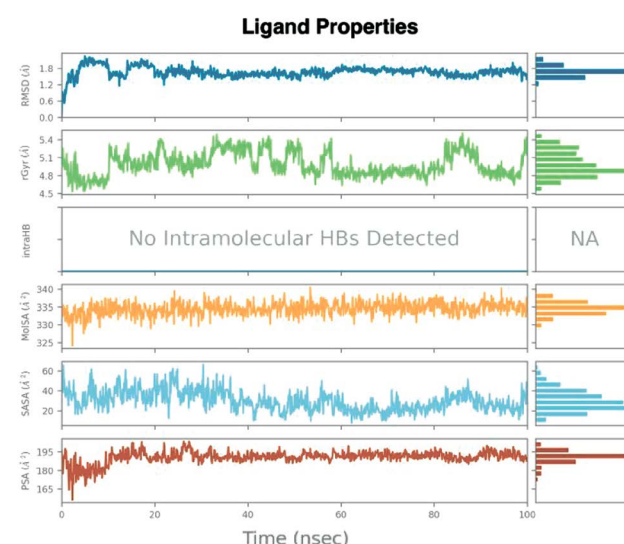


Figure 14. Variation in the small molecule's properties in 5J1E-Pyr07-complex as a function of time during a 100 ns MD simulation

lecule Pyr06 remained nearly 1.5 Å and maintained consistency throughout the 100 ns duration. The constant rGyr values of the molecule Pyr06, ranging from 4.6 to 4.8 Å, indicate stability in the spatial distribution of the molecule, suggesting a stable behavior in its interaction with the 5J1E receptor. Additionally, the graphs for the MolSA, SASA, and PSA parameters reveal the coherence of the Pyr06 molecule across the entire MD analysis, with curves remaining steady and smooth across the 100 ns period, indicating the ligand's stability inside the binding pocket throughout the trajectory of the protocol of MD analysis. Along the same lines, for the 5J1E-Pyr07 complex, the ligand's RMSD was consistent-

ly maintained at 1.8 Å throughout the simulation. The estimated rGyr values of the Pyr07 molecule ranged from 4.8 to 5.4 Å until the simulation's conclusion, indicating constant values reflective of its stable behavior in interaction with the 5J1E receptor. It is also important to notice that during the simulation the Pyr07 molecule remained stable, which is apparent from the MolSA, SASA, and PSA parameter plots. As seen from Figure 14, there are small fluctuations in the Pyr07 line over all three parameters, and the values remained invariable during the whole simulation.

DFT analysis

In this theoretical work, we estimated the quantum reactivity parameters of the best antiviral agents (Pyr06 and Pyr07) using the DFT-B3LYP/6-31G(d,p) calculation. The obtained parameters are fundamental for determining the strength of the interactions of the specified agents in the 5J1E receptor binding pocket. This means that the larger the E_{LUMO} value, the better the molecule is to act as an acceptor, whereas the larger the E_{HOMO} value, the better the molecule is to act as a donor. Also, there is the difference between LUMO and HOMO, which is denoted as ΔE figuit is the energy gap between the mentioned orbitals. The lower the difference between the two, the more reactive the compound. The electronegativity, which is denoted as χ , describes the entities' ability to attract electrons to themselves. Ionization potential, which is denoted as I , is about the energy required to remove an electron from a certain compound, and electron affinity, denoted as A , is about the energy released when a molecule gains electrons. The corresponding formula for them is $\chi = (I + A)/2$. Hardness, denoted as η , which is not a well-used term, is the ability of a molecule to resist deformation during a reaction. One can define it as $\eta = (I - A)/2$. Electrophilicity, denoted as ω , is a measure of a molecule's affinity to pair a bond – one can define it as $\omega = \chi^2/2\eta$. Dipole moment, denoted as DM, is practically the polarity measurement of an entity, and q is the charge separation,

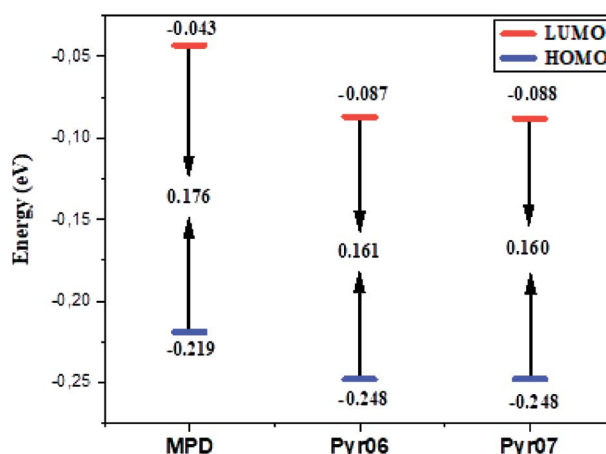


Figure 15. The FMO energy values of the studied compounds (MPD, Pyr06, and Pyr07)

and d is the distance between the charges. A higher DM indicates a higher polarity and a stronger interaction with electric fields. Figures 15 and 16 display the energy values and the contour plots of the FMOs of the tested agents, respectively, calculated at the B3LYP/6-31G(d,p) level. Table 3 presents the results of the DFT calculation.

As can be observed in Figure 16 and Table 3, the frontier molecular orbital energies for the studied compounds are MPD: $E_{\text{LUMO}} = -0.043$ eV, $E_{\text{HOMO}} = -0.219$ eV; Pyr6: $E_{\text{LUMO}} = -0.087$ eV, $E_{\text{HOMO}} = -0.248$ eV; and Pyr07: $E_{\text{LUMO}} = -0.088$ eV, $E_{\text{HOMO}} = -0.248$ eV, respectively. Additionally, the gap values for the MPD, Pyr06, and Pyr07 were also found to be high, i.e., $\Delta E = 0.176$ eV, 0.160 eV, and 0.161 eV, as presented in Table 3. FMO energies and their gap are key parameters used to judge molecule stability. Therefore, the negative characteristics of LUMO and HOMO energies show that the molecules' energy is minimized. The HOMO-LUMO energy gap reveals the electronic structure, with a decrease indicating a more active molecule. As shown in Table 3, results

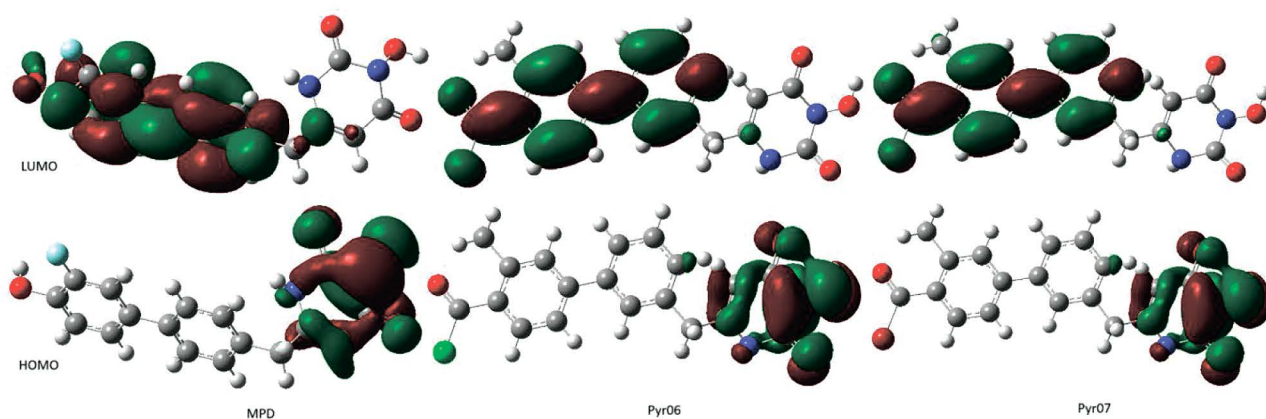


Figure 16. Contour plots of the E_{HOMO} and E_{LUMO} for the studied compounds MDB, Pyr06, and Pyr07

Table 3. The results of the DFT calculation of the quantum chemical parameters of the compounds MPD, Pyr06, and Pyr07

Cmpd	DM ^d	E ^e _{LUMO}	A ^e	E ^e _{HOMO}	I ^e	ΔE ^e	η ^e	χ ^e	ω ^e
MPD	6.124	-0.043	0.043	-0.219	0.219	0.176	0.088	0.131	0.098
Pyr06	6.186	-0.087	0.087	-0.248	0.248	0.161	0.081	0.168	0.174
Pyr07	6.891	-0.088	0.088	-0.248	0.248	0.160	0.080	0.168	0.176

Note. ^d MPD The values of the dipole moment in debye (D).

^e The values of the FMO energies and global reactivity indices in electronvolt (eV).

indicate that Pyr06 and Pyr07 exhibit less chemical reactivity and thus are more stable. The highest dipole moment from Pyr07, 6.891 D, is high, followed by Pyr06, 6.186 D, and MPD, 6.124 D. These results can mean that Pyr07 has the most polarity. Table 3 also shows the quantum chemical parameters acquired from FMOE, for instance, the mathematical formulas above, namely, electronegativity, electrophilicity, and hardness, which Pyr06 and Pyr07 have. Table 3 also demonstrates that the Pyr06 and Pyr07 electrode and electronegativity assessments are higher than those of MPD, indicating that they have the potential to attract or acquire electrons from other molecules. The results obtained by the DFT calculation of the electronic properties and reactivity indices are consistent with the outcomes of the structure-based computational methods employed in this study.

CONCLUSION

In summary, we successfully conducted structure-based computational evaluations on ten therapeutic compounds derived from pyrimidine-2,4-dione with enhanced antiviral activity and favorable ADMET properties, including molecular docking and dynamics simulations. Our molecular docking investigations indicated that the ten ligand-5J1E complexes possessed a higher negative binding affinity than the reference complex (-9.6 kcal/mol), except for the Pyr08-5J1E complex. These studies revealed that two compounds, Pyr06 and Pyr07, exhibited the most negative binding affinity, displaying -10.7 kcal/mol compared to the other ligands. The selected compounds strongly bind to the 5J1E receptor's active site, forming hydrogen bonds and engaging in hydrophobic contacts with crucial amino acid residues (LYS424, GLN507, PRO421, LEU503, LEU425, and LYS259). These interactions are vital for inhibiting the enzyme's activity. Moreover, the MD simulation showed that Pyr06 and Pyr07 were remarkably more stable when bound to the enzyme's active site. Finally, the results obtained from the FMO energies and associated reactivity parameters, using the DFT method, were consistent with the findings of the structure-based computational evaluations used in this research. Hence, our findings imply that the Pyr06 and Pyr07 compounds from pyrimidine-2,4-dione provide an essential structure-based pharmacophore that can be explored to design better drug agents for the HIV RT RNase H receptor.

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