ISBN 978-9940-611-04-0

COAST
INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY

I INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY

PROCEEDINGS COAST 2022

FACULTY OF MANAGEMENT HERCEG NOVI

HERCEG NOVI, MONTENEGRO

26-29 MAY 2022

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I INTERNATIONAL CONFERENCE ON
ADVANCES IN SCIENCE AND TECHNOLOGY

PROCEEDINGS COAST 2022

HERCEG NOVI, 26-29 MAY 2022

ANTHRARUFIN AS REVERSE TRANSCRIPTASE (RT) INHIBITOR AND POTENTIAL INHIBITOR OF HIV REPLICATION

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ABSTRACT:

During the 1990s, it was discovered that quinones with one, two, and three aromatic rings are a class of micromolar non-peptidic inhibitors of HIV-1 protease, an enzyme essential for replication of the HIV (Human Immunodeficiency Virus) and an important drug target for AIDS (Acquired immunodeficiency syndrome). It was later observed that simple hydroxyquinones inhibit HIV-1 protease at the micromolar level, which represented a promising goal for the development of HIV drugs. Anthrarufin (1,5-dihydroxy-9,10-anthraquinone) is an anthraquinone already recognized as a molecule with antimalaric and moderate antioxidative activity. In this paper, the molecular interactions between active binding sites of the HIV-1 reverse transcriptase (RT) and anthrarufin were investigated by molecular docking simulations. The binding site of the mentioned protein is defined using AGFR software. The three-dimensional crystal structure of HIV-1 RT is downloaded from the Protein Data Bank (PDB ID: 4RW9). The molecular docking simulations are carried out with (E)-3-(3-chloro-5-(2-(2-(2,4-dioxo-3,4-dihydropyrimidin1(2H)yl)ethoxy)phenoxy)phenyl)acrylonitrile (JLJ532), a non-nucleoside inhibitor, dolutegravir, nevirapine and anthrarufin, as ligands. The molecular docking simulation is performed using the AutoDock 4.0 software. According to the obtained values of free energy of binding (ΔG_{bind}) and inhibition constant (K_i) antrarufin can be considered as a potential inhibitor of HIV-1 RT, since it possesses similar inhibitory potency as examined drugs.

Keywords: Reverse transcriptase (RT), anthrarufin, molecular docking, HIV-1

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1. *INTRODUCTION*

HIV (human immunodeficiency virus) is a virus that attacks the body's immune system. If HIV is not treated, it can lead to AIDS (acquired immunodeficiency syndrome). There is currently no effective cure, but with proper medical care, HIV can be controlled. People with HIV who get effective treatment can live long, healthy and high-quality lives. The first approach to prevent HIV proliferation was aimed at inhibiting viral enzymes, which are

exclusively expressed by the virus and are not present in the human genome. This refers to transcriptase (RT) and integrase (IN). The development of RT inhibitors began with the discovery of antiretroviral activity of the nucleoside analog zidovudine [AZT (azidothymidine)], and soon after other nucleoside analogs such as didanosine (ddI), stavudine (d4T), and lamivudine (3TC). However, treatment with nucleoside induces rapid resistance [1]. So, the two main rules in antiretroviral treatment have become obvious: inhibition of HIV replication is an achievable goal, but virus resistance occurs in a very short time.

Since 1995, the concept of combining several antiretroviral drugs has been used effectively to combat viremia and allows patients to partially reconstitute their immune systems. [2 3]. Since the 2000s, antiretroviral therapy (ART) has been based on a combination of nucleoside reverse transcriptase inhibitors (NRTIs) and one type of non-nucleoside reverse transcriptase inhibitor (NNRTI). The progress in ART in the last 30 years has been remarkable. Treatment has evolved from the initial use of individual agents as monotherapy. The ability to use HIV RNA as a surrogate marker for clinical outcomes enabled faster assessment of new therapies [4]. This has led to the understanding that triple-drug regimens, including the main agent (NNRTI) and two NRTIs, are optimal. These combinations have shown continuous improvement in their efficacy as initial therapies. Recently, integrase chain inhibitors, especially dolutegravir, have shown significantly higher safety and efficacy and have become the main agents of choice in HIV therapy. Dolutegravir is an antiretroviral drug belonging to the class of HIV integrase strand transfer inhibitors (ISTIs) [5]. However, regimens involving only major agents have not been as successful as combination therapy. At least one NRTI appears to be necessary for optimal therapeutic effect [6]. Several studies are currently underway to investigate potential drugs with longer dosing intervals, and at the same time with lower NRTI therapy costs.

During the 1990s, it was discovered that quinones with one, two, and three aromatic rings are a class of micromolar non-peptidic inhibitors of HIV-1 proteinase, an enzyme essential for replication of HIV, and therefore an important drug target for AIDS. It was later observed that simple hydroxyquinones inhibit HIV-1 proteinaze at the micromolar level, which represented a promising goal for the development of HIV drugs. Studies have shown that the presence of hydroxyl groups on one or two rings in the structure of these molecules increases their inhibitory activity. Polyhydroxy anthraquinones can bind to proteins in different ways, both due to hydroxyl groups and due to the polycyclic aromatic π-electron structure. It is believed that those binding interactions are responsible for the detected inhibition of HIV-1 proteinase by anthraquinones [7].

Anthrarufin is a compound with three aromatic rings and two hydroxyl groups (Fig.1.) and it is structurally similar to compounds already used as RT inhibitors.

In this paper, the interactions between the active binding site of the HIV-1 RT and anthrarufin (1,5-dihydroxy-9,10-anthraquinone) are investigated by molecular docking simulations.

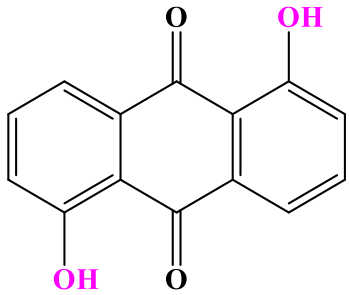

Fig. 1. The structural formula of anthrarufin

2. METHODOLOGY SECTION

The geometry optimization of molecules of anthrarufin, dolutegravir, and nevirapine is done using Gaussian 09 software package [8], with the purpose of preparation of ligands for molecular docking simulations. The calculations are performed at the b3lyp/6-311+G(d,p) level of theory. The three-dimensional crystal structure of HIV-1 RT is downloaded from the Protein Data Bank (PDB IDs: 4RW9) [9]. The molecular docking simulations are performed using the AutoDock 4.0 software [10]. The Discovery Studio 4.0 [11] is applied for the preparation of protein for molecular docking simulations. The potential binding sites of the 4RW9 are defined using AGFR (AutoGridFR) [12]. A binding site with the lowest binding energy is used for molecular docking simulations. The grid box center with dimensions 212.466Å x -40.173Å x 36.592Å in -x, -y, and -z directions of 4RW9, is used to cover the protein binding site and accommodate ligand to move freely. A grid point spacing of 0.375 Å is used for auto grid runs. The preparation of molecular docking simulation includes setting the ligand to be flexible, and the bonds in the ligand are set to be rotatable. The protein remains standing as a rigid structure. The Lamarckian Genetic Algorithm (LGA) is used in these cases of protein-ligand flexible docking simulations. The molecular docking simulation is performed at a temperature of 298.15 K. Analysis of docking results is performed by using BIOVIA Discovery Studio.

3. *RESULTS AND DISCUSSION*

Dolutegravir and nevirapine are antiretroviral medications used, together with other medications, to treat HIV/AIDS. The mechanisms of action of both of those drugs include inhibition of the corresponding protein, and they prevent the proliferation of virus cells. These two molecules are non-nucleoside compounds, with a polycyclic structure, which in some parts has π-delocalized electrons. Therefore the inhibition potency of these molecules is comparable with the inhibition activity of the investigated compound, anthrarufin.

The inhibitory potency of preferred compounds can be estimated based on the thermodynamical parameters obtained from molecular docking simulations: free energy of binding (ΔG_{bind}) and inhibition constant (K_i). The pockets and binding sites of RT are determined using AGFR. The results of the binding pockets analysis revealed the most probable location for binding (Fig. 2.). The docking simulations are performed, and as ligands

molecules are set inhibitor consists of the crystal structure of RT (*(E)-3-(3-chloro-5-(2-(2-(2,4-dioxo-3,4-dihydropyrimidin1(2H)yl)ethoxy)phenoxy)phenyl)acrylonitril (JLJ532)*), anthrarufin, dolutegravir, and nevirapine, and obtained results are given in Table 1.

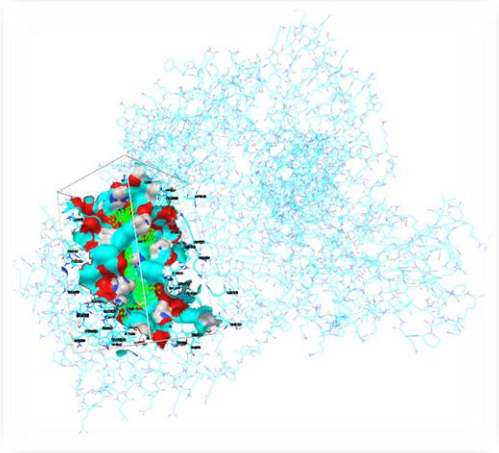

Fig. 2. The location of the most probable binding site of RT

The low ΔG_{bind} energy values indicate the easiness of binding inhibitor molecules to the protein. At the same time, low values of the K_i indicate that low concentrations of inhibitors need to be used to inhibit the targeted protein. As it can be assumed, the analysis of results presented in Table 1 indicates that the best binding affinity and inhibitory potency possess the JLJ532 inhibitor. The lowest values of ΔG_{bind} and K_i are obtained exactly for this inhibitor. The other examined compounds show lower inhibitory potency. It seems that dolutegravir is the best potential inhibitor of RT, after the original inhibitor of RT, JLJ532. It can be said that anthrarufin can be bound to the RT, and in that way accomplish the inhibition effect (ΔG_{bind} is -8.49 kcal/mol, while K_i is 601.49 nM). The highest values of ΔG_{bind} and K_i are calculated for nevirapine. This indicates that nevirapine is the most undesirable compound for inhibition of RT among here observed molecules.

Table 1. The important thermodynamical parameters from molecular docking simulations between HIV-1 reverse transcriptase (RT) (PDB ID: 4rw9) and selected compounds

	ΔG_{bind} (kcal/mol)	**Ki (nM)**
JLJ532	-12.00	1.59
anthrarufin	-8.49	601.49
dolutegravir	-9.62	88.49
nevirapine	-7.88	1670

In Figure 3. are presented interactions achieved in all molecular docking simulations. The obtained interactions are of different types of interactions. The most represented of them are formations of hydrogen bonds. Two types of hydrogen bonds are present, conventional and carbon-hydrogen bonds. Conventional hydrogen bonds are formed between RT and all compounds set as ligands, while the carbon-hydrogen bonds are formed only with dolutegravir

and nevirapine. The other types of established interactions are π - σ, π - sulfur, halogen, amide-π stacked, π- π stacked, alkyl, and π – alkyl. It is interesting to notice that dolutegravir formed one unfavorable acceptor–acceptor interaction with Lys104 of RT. The JLJ532 inhibitor and dolutegravir established the highest number of interactions (Fig. 3.), which is in correlation with the obtained values of ΔG_{bind} and K_i. Namely, the number of interactions has a significant effect on binding and inhibition processes. The higher number of interactions affects the stability of the formed protein-ligand complex and decreases the values of ΔG_{bind} and K_i.

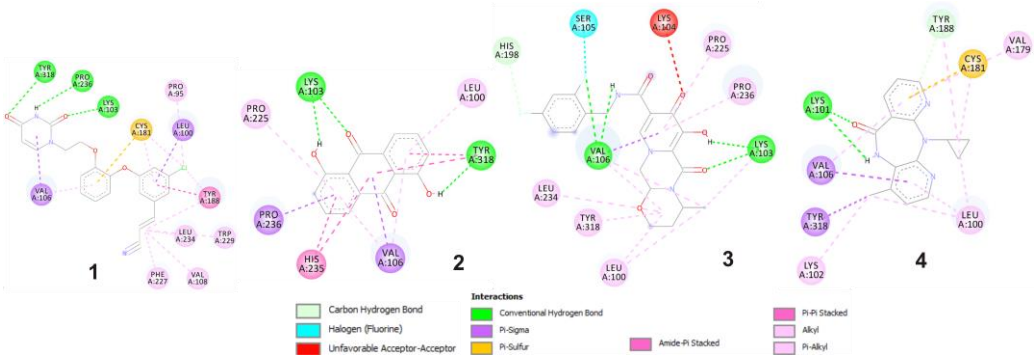

Fig. 3. The obtained interactions between RT and inhibitor consist of the crystal structure of RT, anthrarufin, dolutegravir, and nevirapine presented in pictures 1, 2, 3, and 4 respectively

4. *CONCLUSION*

Most of the treatment of HIV is based on the application of drugs that inhibit the proliferation of the virus cells. One of the most important proteins in the virus cell's proliferation is the HIV-1 reverse transcriptase (RT). The role of RT is to enable the conversion of the virus RNA genomes into DNA and thus enable retroviral replication. Therefore finding effective inhibitors of the RT would lead to the most efficient HIV treatment. The inhibition activity of anthrarufin, a molecule with proven biological activity is estimated for that purpose. It is compared with the inhibition activity of dolutegravir and nevirapine, molecules that are already used in HIV treatment, as with the inhibition activity of (E)-3-(3-chloro-5-(2-(2-(2,4-dioxo-3,4-dihydropyrimidin1 (2H)yl)ethoxy)phenoxy) phenyl)acrylonitrile (JLJ532). The results obtained by molecular docking simulations indicated that all four investigated compounds generate numerous interactions with RT. Among them, the most important are hydrogen bonds, as interactions that involve π-electrons of ligands. The obtained values of free energy of binding and inhibition constant indicate that the highest inhibitory potency possesses JLJ532, while nevirapine has the lowest inhibitory potency. Anthrarufin is a molecule with moderate inhibitory activity. Therefore, the inhibitory potency of anthrarufin is between the inhibitory potency of dolutegravir and nevirapine, already used as RT inhibitors in HIV treatment. Bearing it in mind, anthrarufin can be selected for further consideration as an RT inhibitor.

Acknowledgments: Authors acknowledge the Ministry of Education, Science and Technological Development of the Republic of Serbia for the support through Contracts No. 451-03-68/2022-14/200378 and 451-03-68/2022-14/200252.

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