Molecular Docking, Molecular Dynamics And Reactivity Of Some New Nitrone Derivatives As Anti-HIV

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Abstract. Because nitrones have such a broad range of action, there has been a lot of study into developing new nitrone-based molecules with better biological and spin trapping capabilities. In this study, six new nitrones were synthesized and studied their biological activity as antiviral for two HIV strains HIV-1 and HIV-2, and compared with dideoxyinosine as a reference. The binding and stability of the compound/complexes were further investigated by molecular docking and molecular dynamics. The *in-vitro* results showed that compound **5** has the best antiviral activity against the two HIV strains. The docking scores and MD were in agreement with these results.

Keywords: nitrone, molecular docking, anti,HIV

1 Introduction

Nitrones are highly useful materials for selective organic synthesis and easily accessible substrates. The nitrone chemical structure can be represented in its simplest form as X–CH=NO–Y[1]. The term nitrone was taken from nitrogen ketone[2]. Nitrones can be existed in two geometric isomers trans and cis structure forms due to the double bond of the nitrone group. The most stable is a trans form as shown in Fig. 1 [3].

Fig 1. The most stable is a trans fo rm.

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To generate these organic compounds, oxidation processes, condensation reactions of N-monosubstituted hydroxylamines, and reactions of oximes with electrophiles are often employed [4]. The unique properties of nitrones make them play a significant role in organic transformation reactions, Nitron reactions with nucleophiles or radicals, CH functionalization, and various cycloaddition reactions are among them [5,6].

Nitrones were studied in medicine in various fields. Nitrones with a phenyl group, have recently been recognized as therapeutics due to their widespread anti-cancer activity. This anti-cancer action is not known, but the mechanistic basis of this action may be due to the ability to scavenge radical intermediates that are formed during the processes[1]. Also, nitrones showed moderate antifungal and antibacterial activities[5]. In addition, in a recent study, some nitrones exhibited anti-HIV-1 activity [6].

The main aim of this study is to synthesis and assess new nitrones compounds as anti-HIV.

2 Materials and Methods

2.1 Experimental

2.1.1 Synthesis of N-phenyl Hydroxylamine [7].

In a reaction vessel, 0.47 mol ammonium chloride, 800 ml water, and 0.41 mol nitrobenzene were put. 62 g of zinc dust was added, and the mixture was stirred vigorously using a mechanical stirrer for (15-20) min at 60-65 ˚C. The reaction was complete after 15 minutes of stirring, after which all the zinc dust was applied, and the temperature of the mixture ceased to increase, indicating that the reaction was complete. The precipitate was rinsed in hot water after the hot solution was filtered. After being soaked with salt, the filtrate was chilled to 0°C in an ice-salt bath. The hydroxylamine mixed which crystallized out was filtered and purified by using a mixed solvent (benzene and petroleum ether) to yield (62%) and m.p 80-82 ˚C. Lit. 81˚C [7].

2.1.2 A General Procedure of Synthesis of Nitrones 1-6

Stirred and warmed to 50°C was a solution of N-phenyl hydroxylamine (0.01 mol) in absolute ethanol (15 ml). The mixture was treated with a 0.01 mol aldehyde solution in 100% ethanol (15 ml). After the addition is complete, the reaction mixture was stirred in the dark for 1h then cooled to 0 ˚C and kept out at this temperature overnight except (1). The crude nitrones (2 - 6) were filtered and recrystallized from absolute ethanol [8]. Compound 1 crystallized and recrystallized from ethyl acetate-diethyl ether.

N-((1H-indole-3-yl) methylene) aniline oxide 1

From indol-3-carboxaldehyde 1 (1.45 g). Yellow crystal; m p. 179-181 ˚C; Yield: 88%. FT-IR(KBr): υMax= 3415, 3060, 1610,1501,1180 cm-1; ^{1}H-NMR (300 MHz, DMSO-d6, ppm): 11.39 (1H, s, NH), 8.42 (H-2', s, CH of nitrone), 9.269-9.294 (d, J2, 3=J5, 6=7.5 Hz, H-2, H-6) ,7.17-7.91(m.H-3-H-5, H-2', H-4', H-7'). %: C 75.93; H 5.03; N 11.61. $C_{15}H_{12}N_2O$. Calculated, %: C 76.27; H 5.08; N 11.85. Rf =0.64 TLC (eluent) $CHCl_3$: acetone (9:1). MS(N1) m/z 236.3 [M+].

N-((pyridine-3-yl) methylene) aniline oxide 2

From pyridine-3-carboxaldehyde 2 (1.07 g). White plate m p.110-112 ˚C; Yield: 85%. FT-IR(KBr): υMax= 3060,1595,1569, 1207cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d6, ppm): 7.908. (H-2', s, CH of nitrone), 7.913-7.917 (d, J2, 3=J 5,6=7.5 Hz, H-2,H-6), 6.885-6.861 (t, J2,3 =J5,6=7.95 Hz, H-3,H-5), 7.122-7.130 (m, H-4, H-4', H-5'). Found, %: 72.50; H 4.91; N 13.98. $C_{12}H_{10}N_2O$. Calculated, %: C 72.65; H 5.04; N 14.12. Rf =0.8 TLC (eluent) $CHCl_3$: acetone (9:1). MS(N2) m/z 198.2 [M+].

N-(2,5-dihydroxybenzylidene) aniline oxide 3

From 2,5-dihydroxy benzaldehyde 3 (1.38 g). Orange powder; mp: 86 -88 ˚C; Yield: 69 %. FT-IR (KBr): υMax= 3542, 3053,1583, 1548,1205 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d6, ppm): 12.659(1H, s, OH), 8.069 (H-2', s, CH of nitrone), 9.274-9.299 (d, J2, 3=J5, 6=7.5 Hz, H-2, H-6),7.948-7.988 (t, J2, 3 =J5, 6=7.5 Hz, H-3, H-5), 6.68-7.49 (m, H-4, H-3', H-4', H-6'). Found, %:C 67.62; H 4.74; N 5.87. $C_{13}H_{11}NO_3$. Calculated, %: C 68.12; H 4.80; N 6.11. Rf =0.65 TLC (eluent) $CHCl_3$: THF (9:1). MS(N3) m/z 229.2 [M+].

N-(2-hydroxy-3-methoxybenzylidene) aniline oxide 4

From 2-hydroxy-3-methoxy benzaldehyde 4 (1.52 g). Deep yellow crystal; m p: 122-124 ˚C; Yield: 72%. FT- IR (KBr): υMax= 3452, 3846, 2925, 3004, 3066, 1571, 1190 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d6, ppm): 12.66 (1H, s, OH), 8.067 (H-2', s, CH of nitrone), 7.56-7.760 (d, J2, 3=J5, 6=7.5 Hz, H-2, H-6), 6.808-7.753 (m, H-3, H-5, H-3', H-4', H-6'), 3.885 (1H, d, CH Aliphatic). Found, %: C 68.91; H 5.26; N 5.49. $C_{14}H_{13}NO_3$. Calculated, %: C 69.13; H 5.34; N 5.76. Rf =0.46 TLC (eluent) $CHCl_3$: acetone (9:1). MS(N4) m/z 243.1 [M+].

N-(2,4-dichlorobenzylidene) aniline oxide 5

From 2,4-dichloro benzaldehyde 5 (1.75 g). Colorless needle; m p: 92-94 ˚C; Yield: 92%. FT-IR(KBr): υMax= 3006, 1575, 1539, 1184, 763 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d6, ppm): 8.372 (H-2', s, CH of nitrone), 9.518-9.543 (d, J2, 3= J5, 6=7.5 Hz, H-2, H-6), 7.767-7.786 (t, J2, 3 =J5, 6=7.95 Hz, H-3, H-5), 7.762-7.511 (m, H-4, H-3', H-5', H-6'). Found, %: C 58.59; H 3.33; N 5.19. $C_{13}H_9NOCl_2$. Calculated, %: C 58.64; H 3.38; N 5.26. Rf =0.69 TLC (eluent) Benzene. MS (N5) m/z 265 [M+].

N-(3,4-dichlorobenzylidene) aniline oxide 6

From 3,4-dichloro carboxaldehyde 6 (1.75 g). Yellow fine needle; m.p.: 101-102 ˚C; Yield: 90%. FT- IR (KBr): υMax= 3055, 1581,1548,1203,767 cm^{-1} ; 1H-NMR (300 MHz, DMSO-d6, ppm): 8.069 (H-2', s, CH of nitrone), 9.274-9.299 (d, J2, 3=J5, 6=7.5 Hz, H-2, H-6),7.87 (t, J2, 3 =J5, 6 =7.95 Hz, H-3, H-5), 7.16 -7.48 (m, H-4, H-2', H-5', H-6'). Found, %: C 68.62; H 3.36; N 5.24. $C_{13}H_9NOCl_2$. Calculated, %: C 58.64; H 3.38; N 5.26. Rf = 0.77 TLC (eluent) Benzene. MS(N6) m/z 265 [M+].

2.2 Biological Activity

In vitro Anti-HIV Assay

Compounds 1–6 were evaluated for anti-HIV-1 (strain IIIB) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells using the (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) technique [9], with data for efavirenz [10] and capravirine [11]included for comparison.
The envelope glycoprotein of the human immunodeficiency virus type 1 (HIV-1) contains the gp41 (glycoprotein code 41) component, which plays a significant role in HIV-1 entrance and is a prospective target for the development of HIV-1 entry inhibitors and a new class of anti-HIV medicines [12]. When gp120 attaches to CD4 and a co-receptor, the N-terminal heptad repeat (NHR) and the C-terminal heptad repeat (CHR) form a six-helix bundle, signifying the fusion-active gp41 core, gp41 experiences a conformation change from a native prefusogenic state to a fusogenic state. Any substance that affects the development of the gp41 six-helix bundle can impede HIV-1 entrance into target cells by inhibiting gp41-mediated membrane fusion [13]. The newly synthesized nitrones were designed to interrupt the formation of the gp41 six-helix bundle, resulting in HIV-1 inhibition. In cell culture, compound 5 was discovered to be the only compound in the sequence that inhibited HIV-1 and HIV-2 replication, its EC_{50} value was lower than the CC_{50} value comparing with that of dideoxyinosine. This result encourages us to modify such molecules with another potential group which might fill full the need to achieve our target.

2.3 Computational Details

The chemical structures were fine-tuned using quantum chemistry theory computations. To discover the most stable conformer, optimization computations were performed using Gaussian 09 software [14] and the B3LYP/6-31G basis set [15]. The global reactivity descriptors were calculated using the same way. Maximum force, root-mean-square (RMS) force, maximum displacement, and RMS displacement are all set to convergent values by default. To examine the affinity of ligands, the optimised structures were merged in one database using MOE software [16].

2.3.1 Global Reactivity Descriptors

Electrical characteristics, kinetic stability, optical polarizability, and chemical reactivity descriptors are all determined using global reactivity indices [17]. Hardness (**η**), chemical softness (S), chemical potential (**μ**), Electronegativity (**χ**), global electrophilicity (**ω**), and nucleophilicity (N) are the indices used [18-20]. The energy values for the highest occupied molecular orbital (EHOMO), the energy of the lowest unoccupied molecular orbital (ELUMO), and the energy gap (E) are used to calculate them. All these properties are defined as follows:

$\Delta E = E_{LUMO}-E_{HOMO}$, $\eta = (E_{LUMO}- E_{HOMO})/2$, $S =1/(2\ \eta)$, $\mu = (E_{LUMO}+E_{HOMO})/2$, $\omega = \mu^2/2\eta$

$N = E_{HOMO}$ (Nucleophile) – E_{HOMO} (TCE).

2.3.2 The Molecular Dynamics (MD) Simulation and Molecular Docking

Compounds (1-6) were examined further using molecular docking and molecular dynamics simulations to assess their affinity for the HIV-1 receptor. The RCSB database was used to acquire the crystal structure of HIV-1 reverse transcriptase in association with the bound ligand GWE (PDB ID: 3dlg) [8] (http://www.rcsb.org/pdb).

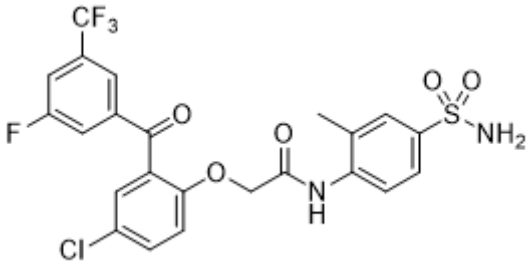


Fig. 2. The Bound Ligand (GWE).

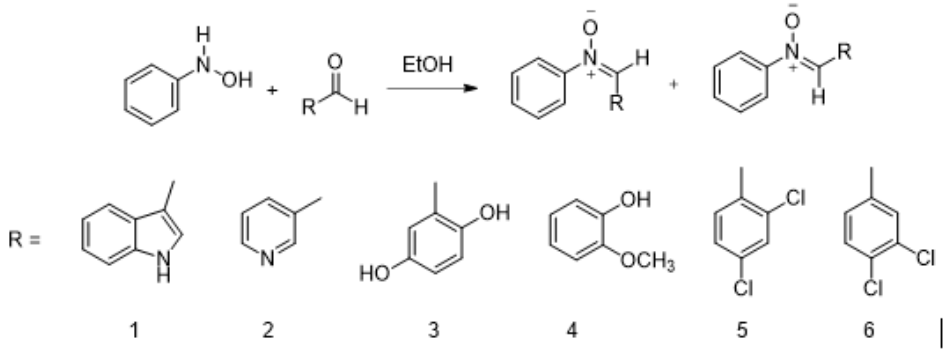
The protein crystal structure has a good quality, which its resolution (2.2) falls in between the valid range 1.5 and 2.5[17,18]. For valid docking results, the best value of RMSD would be near 2 Å with an energy scoreless or equal to -7 kcal/mol [21, 22]. The enzyme was chosen (PDB ID: 3dlg) and hydrogen atoms were corrected, which were broken in X-ray diffraction. The protein structures were optimized with the Amber 10: EHT force field. MOE software was used for all the steps of enzyme preparation. The residues forming the active sites of each enzyme were obtained using a site finder. LEU100, LYS101, LYS102, LYS103, LYS104, SER105, VAL106, VAL179, TYR181, TYR188, VAL189, GLY190, PRO225, PHE227, LEU234, HIS235, PRO236, and TYR318 were among the residues identified.

The MOE software's default parameter settings (Molecular Operating Environment (MOE), 2015) was used to carry out the molecular dynamics (MD) simulation. Nosé-Poincaré-Andesen (NPA) algorithms implemented, the most accurate method was selected to investigate the ligand MD. The MMFF94x force field, sphere form, solvent molecules (H_2O), six margins, and deletion of far existent solvent with a distance larger than four were chosen to optimise the system using MD simulations. The system was equilibrated at 300 K for 100 ps before being operated for 1500 ps with time steps of 0.002 and light bond restrictions.

3 Results and Discussion

3.1 Chemistry

A series of nitrones and isoxazolidines compounds were synthesized by the condensation reaction of aldehyde compounds with N-phenyl hydroxylamine. The latter was synthesized by the reduction of nitrobenzene with zinc dust and in water. Scheme 1 shows the synthetic scheme of the nitrone derivatives in this study.



Scheme 1. The synthetic scheme of the nitrone derivatives.

Also, these nitrones were tested against two strains of HIV, HIV-1, and HIV-2, as presented in Table 1. The outcomes in table 1 show the most active compound against the two strains was nitrone 5 followed 3 and 4, which their CC50 were 0.81, 2.91, and 5.91 respectively to inhibit both types of the virus as shown in Table 1 [23].

Table 1. Nitrone compounds have better antiviral efficacy against HIV-1 than dideoxyinosine.

Compounds	Virus strain	EC50(µ/ml)[c]	CC50(µ/ml)[d]	SI[e]
		HIV-1	MT-4	
1	III_B	>19.21	19.21	<1
	ROD	>19.21	19.21	<1
2	III_B	>33.17	33.17	<1 <1
	ROD	>33.17	33.17	
3	III_B	>2.91	2.91	<1
	ROD	>2.91	2.91	<1
4	III_B	>5.91	5.91	<1
	ROD	>5.91	5.91	<1
5	III_B	>0.81	0.81	<1
	ROD	>0.81	0.81	<1
6	III_B	>8.95	8.95	<1
	ROD	>8.95	8.95	<1
Dideoxyinosine	III_B	2.09	0.68	>24
	ROD	3.78	1.22	>13

3.2 Descriptors of the Global Reactivity

Table 2 shows the chemical reactivity descriptors calculations.

Table 2. Chemical reactivity descriptors determined for nitrones derivatives (measured in eV).

Compounds	HOMO	LUMO	ΔE	η	S	μ	ω)	N
Ref	-6.8771	-3.0656	3.8115	1.9057	194.2700	-4.9714	6.48424	1.77881
1	-4.9846	-1.4779	3.5067	1.7534	211.1538	-3.2312	2.97735	3.67136
2	-5.8412	-2.1957	3.6455	1.8228	203.1156	-4.0184	4.42953	2.81475
3	-5.3250	-1.8204	3.5046	1.7523	211.2850	-3.5727	3.64221	3.33095
4	-5.6069	-1.9984	3.6085	1.8043	205.1987	-3.8027	4.00726	3.04904
5	-5.9911	-2.3919	3.5993	1.7996	205.7261	-4.1915	4.88122	2.66481
6	-6.0083	-2.4164	3.5919	1.7960	206.1469	-4.2123	4.93991	2.64767

The HOMO energy of the reference system (TCE) -8.6559 eV was calculated using DFT/B3LYP 6-31G method.

The energy gap (ΔE) is a useful tool to determine stability, a small gap means a soft molecule, which is linked to reactive molecule. From the results reported in Table 2. Compounds 1 and 3 have the smallest energy gaps (3.5046 eV and 3.5067 eV) respectively. As compared to compound GWE (reference compound), these compounds are stable in the absolute sense because their values are low and approximate (3.8115 eV). Table 2 shows the effects of the global hardness (η), which is related to the compound's reactivity and the system's charge stabilization indicate that compounds 1 (1.7534 eV) and 3 (1.7523 eV) are considered as soft molecules with faster reactions. Also, the results show that compounds 1 and 3 have high softness (S) values. This means that compounds 1 and 3 are more stable and more reactive among the compounds under investigation.

Charge transfer from a system having a greater chemical potential to another system with a lower chemical potential is related to the chemical potential (μ).. The chemical potential offers the global reactivity index. The electronic chemical potential for compound GWE (-4.9714 eV) is higher than the other compounds, this suggests that compound GWE has a higher electron density exchange with the environment. In 1999, a new descriptor was defined by Parr et al. [20] to calculate the compound's global electrophilic power as an electrophilicity index (ω). It is a quantitative classification of the global electrophilic type of a molecule. The electrophilicity index (ω) was developed as a metric for calculating the energy lost due to maximal electron flow between donor and acceptor. GWE has the highest electrophilicity value (6.48424 eV), whereas compound 1 has an electrophilicity value (2.97735 eV).

Chemists were able to classify organic compounds as strong (N > 3 eV), moderate (2.0 eV N 3.0 eV), or marginal (N 2.0 eV) based on the examination of a collection of typical nucleophilic species involved in polar chemical processes. Notably, the nucleophilicity refers to tetracyanoethylene (TCE), which is used as a benchmark because it has the lowest EHOMO among a wide number of compounds previously studied [24]. According to the results in Table 2, GWE (1.77881 eV) can be classified as a marginal nucleophile and compound 1 (3.67136 eV) as a strong nucleophile.

3.3 Molecular Docking Studies

For either small molecule or protein docking, molecular methods are the most practical application of the principle of molecular recognition. To investigate the binding of the six compounds under investigation with the HIV RT binding pocket (NNIBP), molecular docking analysis was performed using MOE (2016) software and compared with the reference ligand GWE.

3.4 Validation of Molecular Docking

The bound ligand GWE was extracted from the X-ray structure of 3DLG and docked back into the same active site using a variety of docking conditions and recording parameters to verify the reliability of the molecular docking system using MOE software. Between the top and crystallographic poses, the RMSD was 0.19. This means that the chosen docking method and parameters in this study were just a rough approximation for the device. The molecular docking outcomes are presented in Table 3. This illustrates the six compounds gave scores between -5.8562 and -6.5752 (Kcal/mol) with RMSDs range 1.61 – 2.37 Å. Compound 4 hit the lowest molecular docking score (-6.5752 kcal/mol) with RMSD 1.7453 Å. The second-lowest score (-6.4334 kcal/mol) was recorded by compound 5 and RMSD 2.2685 Å. What is interesting about the data in this table is that compounds 1 and 5 had about similar RMSD (2.37 and 2.27) to that of the reference compound GWE (2.33).

Table 3. The docking of nitrones using 3DLG produced the findings.

Compound no.	*Score*	*RMSD*
Ref.	-7.8168	2.3260
1	-6.1794	2.3715
2	-5.8554	2.1181
3	-6.2211	2.4999
4	-6.5752	1.7453
5	-6.4334	2.2685
6	-5.8562	1.6143

For further investigation, interactions were examined for hydrogen bonds and bond length in the binding pocket (NNIP) and were depicted in Fig. 3. The results, as shown in Fig. 3, indicate that all six compounds had significant interactions with HIV RT. The best interactions comparing to the reference were recorded for compound 1 predicted in Fig. 3. This shows the compound 1 had four pi-H interactions with three amino acids LEU100, Val106, and PRO236. Three of these interactions are similar to those reported for the reference compound. Compound 2 had two pi-H interactions with two amino acids LEU100 and PRO 236. Compound 3 had two pi-H interactions and an H-donor interaction with the amino acid LYS103 with LEU100 and PRO236. Both two compounds 4 had and 5 had one pi-H interaction with amino acid VAL106. Compound 6 exhibited two H-pi interactions with the amino acid TRP and a pi-H contact with the amino acid VAL106.

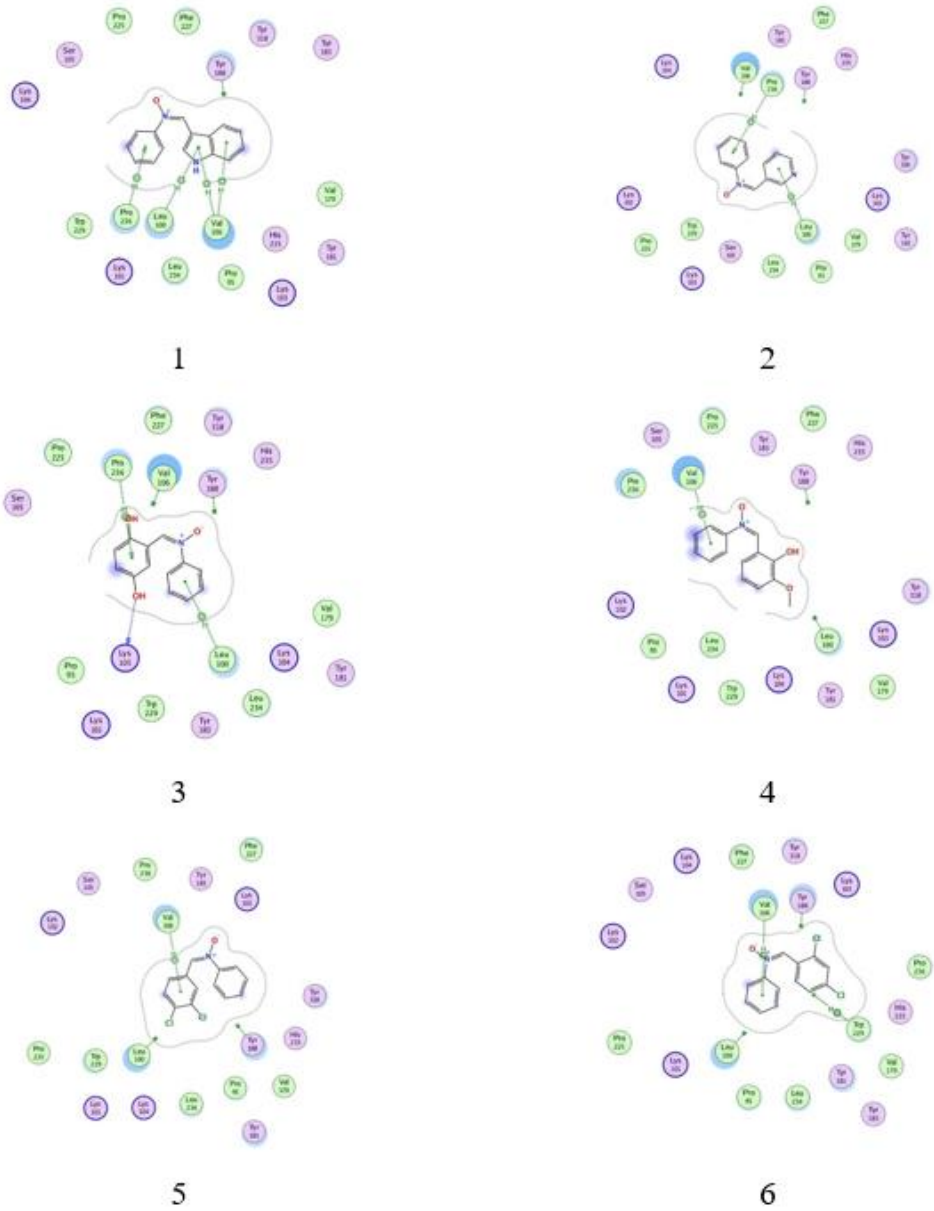


Fig. 3. Nitrones binding with 3DLG.

3.5 Simulations of Molecular Dynamics

Molecular dynamics (MD) and binding energy (MM-GBSA) calculations were performed for 1350 ps on compounds 1 and 3 to determine the conformational flexibility of docked drug-receptor complexes and achieve consistent drug-receptor affinities, and the GWE compound to target 3DLG.. The average binding energies (MM-GBSA) are shown in Table 4. This shows

that the two selected nitrones 1 and 3 exhibited significant binding energies -33 and -28 kcal/mol, respectively. However, these binding energies are higher than that of the reference (-55 kcal/mol).

Table 4. MD simulations for the GWE, 1 and 3, 5 compounds against 3DLG.

Compounds	MM-GBSA (kcal/mol)
GWE	-55
1	-34
3	-28
5	11

During the dynamic analysis measurement in the 3DLG for 1, 3, 5, and GWE, the effects of the atomic potential energy feature are shown in Fig 4. The time-dependent potential energy of the 3DLG/inhibitor complexes was determined during MD trajectories to examine the dynamic stability of the complexes. Complex A (3DLG/1) reached the stabilisation equilibrium about 700 ps, whereas complex B (3DLG/3) reached the stabilisation equilibrium around 600 ps, according to the data in Fig. 4. Around 500 ps, complexes C (3DLG/5) and D (3DLG/GWE) reached equilibrium stability. The largest energy contributions to complex A (0.6 to -2.0 kcal/mol) come mostly from val189, TYR318, TYR188, and LEU234. As seen in Fig. 4, the primary beneficial energy contributions for complex B come from LYS103 and LYS101, which contribute -4.2 and -2.3 kcal/mol, respectively.

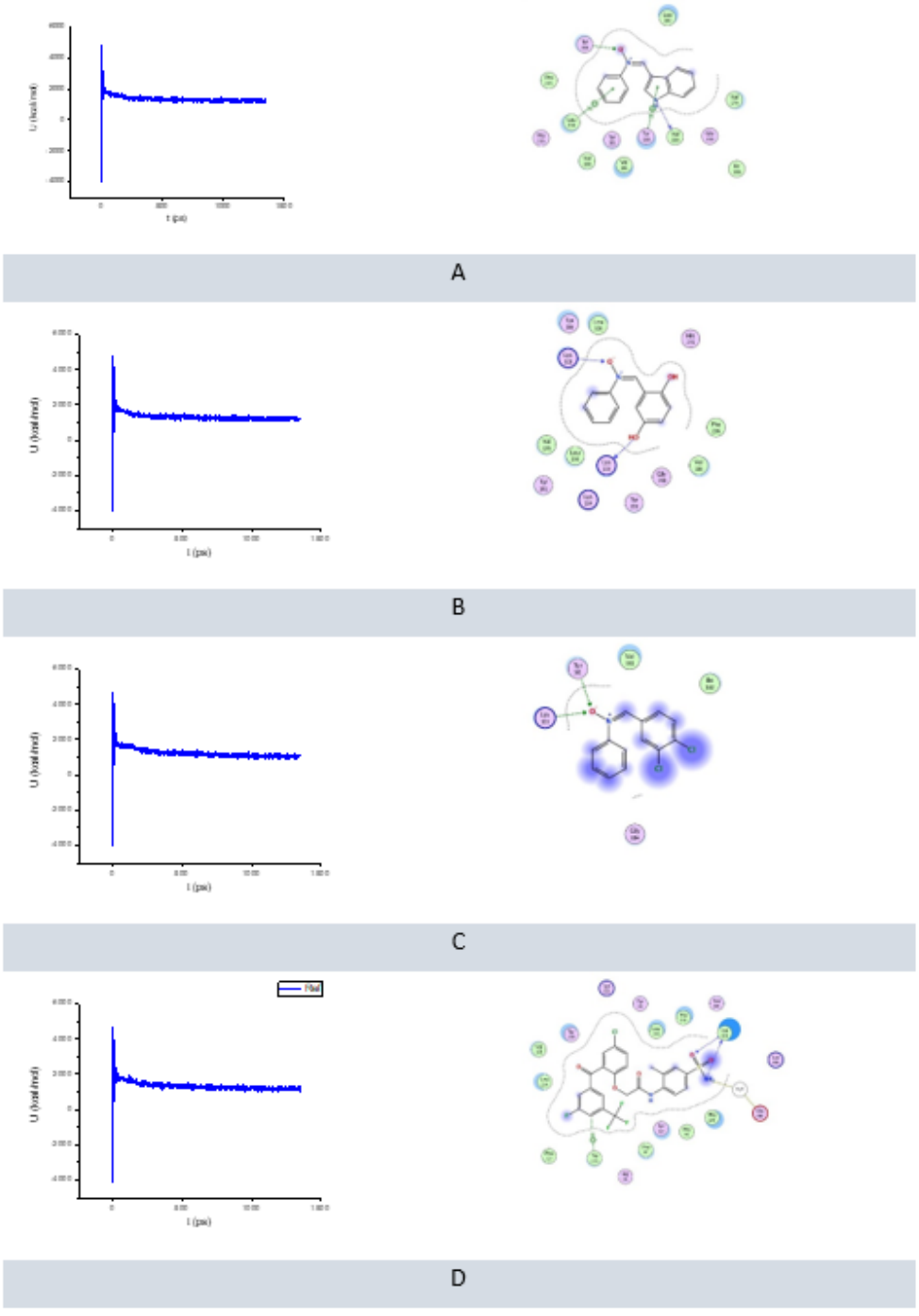


Fig. 4. The assessment of a complex's potential energy (A) compound 1, (B) compound 3, (C) compound 5, and (D) GWE with 3DLG receptor as a function of time.

4 Conclusion

In this investigation, the aim was to assess six new nitrones as anti-HIV. This study has shown that compound 5 gave the best in-vitro antiviral agent action against the two selected strains of HIV, HIV-1, and HIV-2. In molecular docking and molecular dynamics, the compound gave good binding with the receptor and its complex stability was as same as the reference compound. The research has also shown that compounds 1 and 3 gave good binding with the receptor and good stability of their complexes with the receptor.

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