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Docking Studies on the Effect of β-Ring Expansion in Binding of TIBO Derivatives to HIV-1 Reverse Transcriptase

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Designing of new inhibitors to human immunodeficiency virus-1 reverse transcriptase (HIV-1 RT) is one of the important research area in AIDS therapies. Non-nucleoside inhibitors of reverse transcriptase (NNIRT) are attractive drug candidates for their unique binding site to the reverse HIV-1 RT and less adverse side effects. The effect of expansion of diazepine ring from seven to eight in some tetrahydroimidazo [4,5,1-jk][1,4]benzodiazepin-2(1H)-thione (TIBO) derivatives as NNIRT has been investigated by docking procedure. Sixteen conventional TIBO derivatives with known HIV-1 RT inhibitor activity were selected and their β-ring was expanded to eight. The three-dimensional (3D) geometry of the molecules was optimized by AM1 semi-empirical method and then interacted with the HIV-1 RT enzyme using Autodock program. Twelve out of sixteen of the new molecules were docked into the enzyme. The resulted free energies of docking indicated that the newly proposed molecules bond to the enzyme with comparable tendency in relative to their corresponding conventional homologous. It was found that three new compounds bind to the receptor stronger than that of their corresponding 7-membered ring derivatives and can be considered as new candidate for synthesis.

Keywords: HIV-1, Reverse transcriptase, TIBO, Docking, Ring expansion

INTRODUCTION

The acquired immunodeficiency syndrome (AIDS) is caused by the human immunodeficiency virus type I (HIV) [1-4]. Over the past years, many researches have been directed towards the design of drugs that specifically interfere with one of the three enzymes of the HIV-1 virus (reverse transcriptase, integrase, and protease). The reverse transcriptase (RT) performs several critical roles in the replicative cycle of the human immunodeficiency virus and thus considerable efforts have been directed towards this enzyme as a target for therapies treating AIDS. The enzyme is an attractive target for drug therapy not only because it is essential for replication but also it is not required for normal host cell replication [5,6].

The types of inhibitors currently discovered can be divided into two classes: nucleoside inhibitors (NIs) such as azathioprine (AZT, FDA approved drug) [7,8] and non-nucleoside inhibitors (NNIs) such as tetrahydroimidazo [4,5,1-jk][1,4]benzodiazepin-2(1H)-thione (TIBO) derivatives [9-12]. NNIs are interesting drug candidates in that their binding site is unique to the reverse transcriptase of HIV-1, and thus they are less likely to cause adverse side effects by disruption of normal DNA polymerase activity. A serious problem with the NNI inhibitors is the emergence of viral strains that have

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point mutations in the region encoding HIV-1 RT which prevent these drugs from inhibiting the RT enzyme. Therefore, there is a great demand to identify and discover new compounds with high binding affinity in both absence and presence of specific mutations.

Computational methods have developed as useful tools in facilitating new drug discovery [13,14]. By the use of these methods, the biological activity of the candidate molecules can be estimated before experimental trials. Thus, they are simple, non-expensive and expedite to design molecules with desirable biological activity [27]. Quantitative structure-activity relationship (QSAR) [16-23] and docking procedure [24-29] are two mostly used computational methods in drug design. In QSAR methodologies, a mathematical relationship, relating the biological activity to some molecular descriptors is obtained. In docking studies, different search algorithms such as simulated annealing and genetic algorithm in combination with scoring function such as molecular mechanic calculations are used to study the binding of the candidate ligands to an enzyme with known structure. Docking studies involving NNI's and HIV-1 RT have been performed previously [28-36]. The results have shown that most inhibitors can docked into their crystal structure position.

Previously, we designed some new TIBO-like derivatives by contraction of the β-ring (diazepine ring) of the TIBO derivatives from seven to six [37]. Studies of the interactions of these new derivatives with the HIV-1 RT by Autodock program indicated that some of newly proposed molecules bonded to the receptor stronger than the conventional TIBO derivatives. Therefore, we were interested to study the effect of β-ring expansion from seven to eight on the activity of the TIBO derivatives and, in our continuing our works, we will report in this article the results of such studies.

EXPERIMENTAL

The computational details are the same as our previous paper [37]. Molecular modeling is performed on a Pentium IV personal computer (CPU at 2.8 GHz) with Windows XP operating system. Atomic coordinates for the three dimensional protein models (complex of 8-Cl TIBO with HIV-1 RT) were obtained from the Brookhaven Protein Data Bank [38]. The ligand and all crystallographic waters are removed from the atomic coordinate data files and hydrogen atoms are added to the protein. Meanwhile, the atom coordinates of the ligand are extracted from the RT-inhibitor complex structure PDB files, hydrogen atoms are added and bond orders are corrected. The resulting 3-D structure is saved in a new PDB file.

The structures of the compounds used in this study are listed in Table 1. The molecular structures are drawn in computer utilizing HyperChem software (Hyper Cube, Inc.). Semi empirical (AM1 Hamiltonian) gas phase energy minimization was performed by the software. The optimization of each ligand was repeated many times. In each step, according to the extracted structure of the 8-Cl TIBO, the corresponding angels and dihedrals were varied to obtain a butterfly like geometry with lowest energy. AutoDock version 3.0.5 program was used employing Autodock tools (ADT) [39].

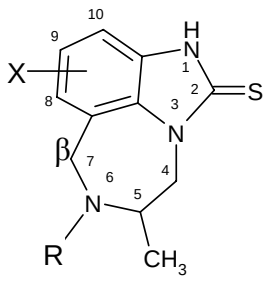
The docking energies were calculated from a set of energy grids centered in the active site of the enzyme. A docking box with a grid consisting of 70 × 70 × 70 and 0.375 Å spacing was used. The protein coordinates were fixed during calculations, while the inhibitor is flexible and moves on the grid. Grid searching was performed by genetic algorithm to locate the ligand in the best binding orientation and conformation based on the binding energy. Ten different docking experiments are performed and, consequently, a population of 10 docked configurations was produced for each inhibitor.

RESULTS AND DISCUSSION

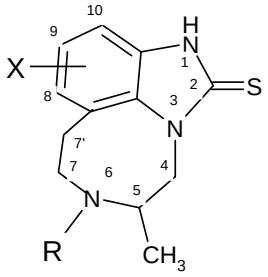
The molecular backbone of the TIBO derivatives consists a 7-membered diazepine ring (β-ring) fused to a bicycle-aromatic moiety (Fig. 1). A dimethylallyl moiety is also attached to the β-ring. TIBO derivatives, like the other non-nucleoside inhibitors, share a common butterfly like shape consisting of two wings; a π-electron-containing moiety and a dimethylallyl moiety. The specific conformation of the 7-membered β-ring of the TIBO derivatives is responsible for producing their butterfly like geometry [29,33-35]. Previous docking studies on the TIBO derivatives revealed that the binding pocket comprises of Leu100, Lys101, Lys103, Val106, Val179, Tyr181, Tyr188, Gly190, Phe227, Trp229,

Table 1. ID and Structural Features of the Compounds Used

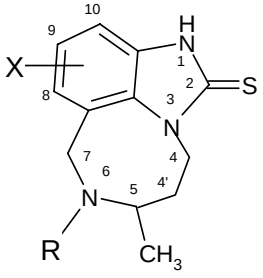
ID of 7MR derivatives	ID of 8MR derivatives	R	X
7MR1	8MR1	$CH_2CH=C(CH_3)_2$	8-Br
7MR2	8MR2	$CH_2CH=C(CH_3)_2$	8-Cl
7MR3	8MR3	$CH_2CH=C(CH_3)_2$	8-F
7MR4	8MR4	$CH_2CH=C(CH_3)_2$	9-F
7MR5	8MR5	$CH_2CH=C(CH_3)_2$	9-Cl
7MR6	8MR6	$CH_2CH=C(CH_3)_2$	8-CH_3
7MR7	8MR7	$CH_2CH=CH_2$	8-Cl
7MR8	8MR8	$CH_2CH=CH_2$	8-CH_3
7MR9	8MR9	$CH_2CH=C(ET)_2$	9-Cl
7MR10	8MR10	CH_2-cyclobutyl	9-Cl
7MR11	8MR11	$CH_2C(CH_3)=CH_2$	H
7MR12	8MR12	$(CH_2)_2$-cyclopropyl	9-Cl
7MR13	8MR13	CH_2-cyclopropyl	9-NO_2
7MR14	8MR14	$CH_2CH_2CH_3$	H
7MR15	8MR15	$CH_2CH=CH_2$	H
7MR16	8MR16	$CH_2CH=C(CH_3)_2$	9-CF_3



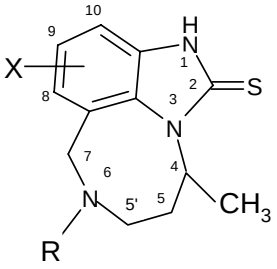
7MR



8MR



8MR'



8MR''

Fig. 1. Basic structure of the conventional (7MR) TIBO derivatives and three types of 8MR derivatives, which can be produced by β-ring expansion.

Leu234, His235, Pro236 and Tyr318, which are mainly hydrophobic and aromatic residues [28-36]. Hydrogen bonds and van der Walls interactions are noticed in the final binding structures. Leu100 is in the active site and makes the active site suitable for a butterfly-shaped inhibitor. NH group of the ligands are forming a hydrogen bond with the backbone carbonyl oxygen of lys101 and the allylic group is interacting with the phenolic ring of Tyr181 and Tyr188. Moreover, the methyl group is trapped in a hydrophobic pocket created by Tyr181 and Leu100. Almost all of the experimental and docking procedures for designing new TIBO derivatives were focused on the variation of the various substituents on the different positions of the TIBO backbone. Here, we will report the results of our study on the effect of expansion of the β-ring from seven to eight.

Designing of New Inhibitors

In order to propose new inhibitors, the 7-membered β-ring (diazepine ring) of the TIBO derivatives is expanded to 8-membered ring by addition of a carbon atom to the β-ring. 7MR and 8MR notations will be used to refer to the 7- and 8-membered ring derivatives, respectively. As shown in Fig. 1, three 8MR derivatives can be produced from each 7MR derivative. Addition of a new carbon atom between N6-C7, C5-N6 and C4-C5 produces new derivatives. These new molecules are referred as 8MR, 8MR' and 8MR'', respectively. As noted previously, a pre-requisite for inhibitory activity of the NNI's is having a butterfly like geometry [29,33-35]. In the 7MR derivatives, the specific conformation of the diazepine ring causes the aromatic ring and R moieties adapt to a butterfly like geometry.

The stereo plots of the AM1-minimized conformations of a 7MR derivative (7MR5) and their corresponding 8MR derivatives (8MR5, 8MR5' and 8MR5'') are represented in Fig. 2. In addition, the overlaid 3D structures of the 7MR and the 8MR derivatives are also represented in this figure. It is clearly seen that the 8MR is adapted to the butterfly conformation while 8MR' and 8MR'' do not show this type of conformation. Indeed, the overlaid structures indicate that there is a close similarity between the 3D structures of 7MR2 and its corresponding 8MR2. Moreover, since anti HIV-1 activity of the TIBO derivatives is depend on the physicochemical and electronic properties of molecules [28-36], comparison between these calculated values for conventional and all types of 8MR derivatives helped us to find out the fact that which kind of ring expansion is more suitable. To do so, different QSAR properties such as physichochemical and electronic descriptors were calculated for each series of molecules (i.e. 7MR, 8MR, 8MR' and 8MR'') and then those of each 8MR series were compared with the corresponding value of 7MR molecules. In a simple manner, the parameters of different types of 8MR derivatives were plotted against those corresponding values of 7MR derivatives [37]. It was found that the similarity between the physicochemical properties of 7MR and 8MR derivatives is much higher than that of 7MR-8MR' and 7MR-8MR''. According to these observations, 8MR were chosen as candidates for docking studies. There are a very large number of TIBO derivatives with known anti-HIV-1 activity [28-36]. Among them, only 16 derivatives representing diverse activities were selected (Table 1) and the effect of β-ring expansion was studied on these molecules.

Automated Docking of the New Derivatives

Different structural data are available for HIV-1 RT in the protein data bank [38]. The PDB file of high-resolution crystal structure of RT/8-Cl TIBO (PDB code pdb1hnv) is used in our docking simulations. In our previous paper, we showed the suitability of the Autodock program to study the interaction between conventional TIBO derivatives and HIV-1 RT, since a good correlation was found between the free energy of docking and the inhibitory activity of the molecules [37].

The Autodock program searches for the best conformation and best place of binding of the ligand within a fixed protein structure. The genetic algorithm is used as searching method. The docking is repeated 10 times with different sets of initial populations. In Fig. 3 are shown the best docked structures of one of 8MR derivatives, with the lowest free energy of docking, alone and overlaid with 7MR7 (8-Cl TIBO). The resulted docked structures are clustered based on total energy of docking. The resulted free energies of docking obtained for 8MR derivatives in this study are listed in Table 2. In addition, the free energy of docking of the 7MR derivatives obtained in our previous work are also included in Table 2, for comparison. As it is seen, some 8MR derivatives (i.e. 8MR5, 8MR6, 8MR7, 8MR8, 8MR12 and 8MR16) are not docked by

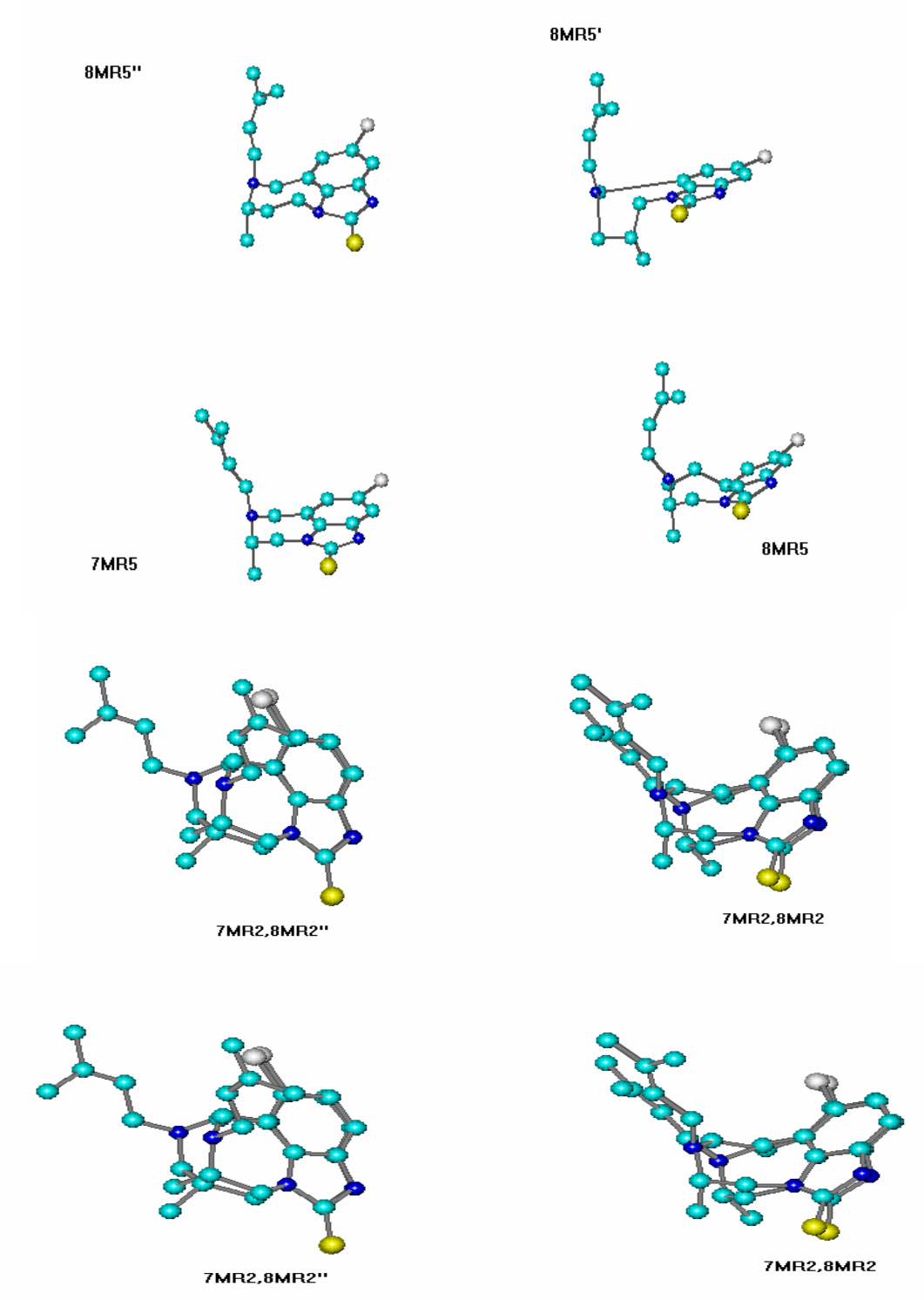


Fig. 2. Stereoplots of AM1-minimized conformations of 7MR5 and its corresponding 8MR homologous.

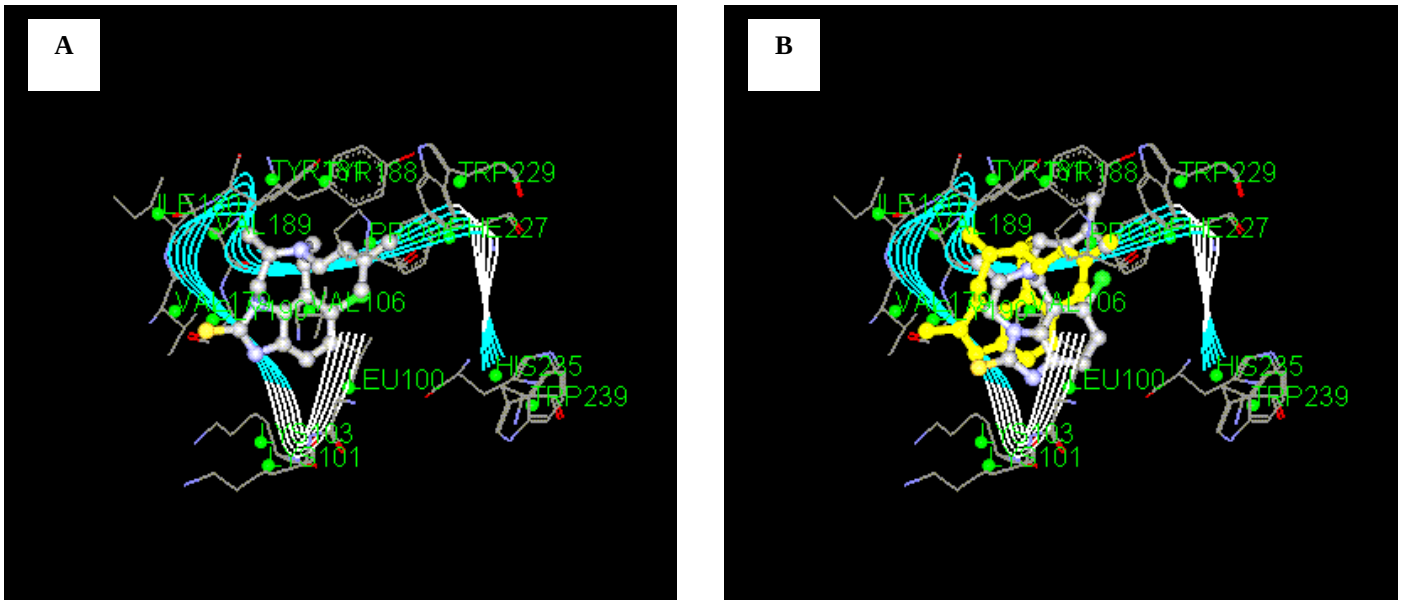


Fig. 3. Superposition of the docked 8MR7 to HIV-1 RT alone (A) and overlaid with 8-Cl TIBO (B).

Table 2. Docking Results for Compounds in the Active Site of RT Enzyme

7MR derivatives			8MR derivatives		
ID	pIC50[a]	$\Delta G_{binding}$[b]	ID	$\Delta G_{binding}$[b]	$\Delta(\Delta G_{binding})$[c]
7MR1	8.52	-16.84	8MR1	-12.02	+4.82
7MR2	8.34	-15.50	8MR2	-15.64	-0.14
7MR3	8.24	-15.93	8MR3	-11.04	+4.89
7MR4	7.838	-14.99	8MR4	-10.76	+4.23
7MR5	8.48	-15.84	8MR5	ND	-
7MR6	7.85	-14.49	8MR6	ND	-
7MR7	8.33	-15.70	8MR7	ND	-
7MR8	7.85	-14.30	8MR8	ND	-
7MR9	7.92	-14.80	8MR9	-15.03	-0.23
7MR10	7.88	-14.86	8MR10	-10.09	+4.71
7MR11	7.85	-14.55	8MR11	-13.50	+1.05
7MR12	6.38	-11.22	8MR12	ND	-
7MR13	5.61	-9.27	8MR13	-15.48	-6.21
7MR14	5.78	-7.07	8MR14	-13.66	-6.59
7MR15	4.17	-5.75	8MR15	-14.16	-8.41
7MR16	6.31	-13.25	8MR16	ND	-

[a]Experimental anti-HIV activity complied from references [28-36]. [b]Free energy of binding. [c]Difference between free energy of binding of 8MR derivatives and their corresponding 7MR derivatives ($\Delta G_{bindin}^{8MR} - \Delta G_{binding}^{7MR}$).

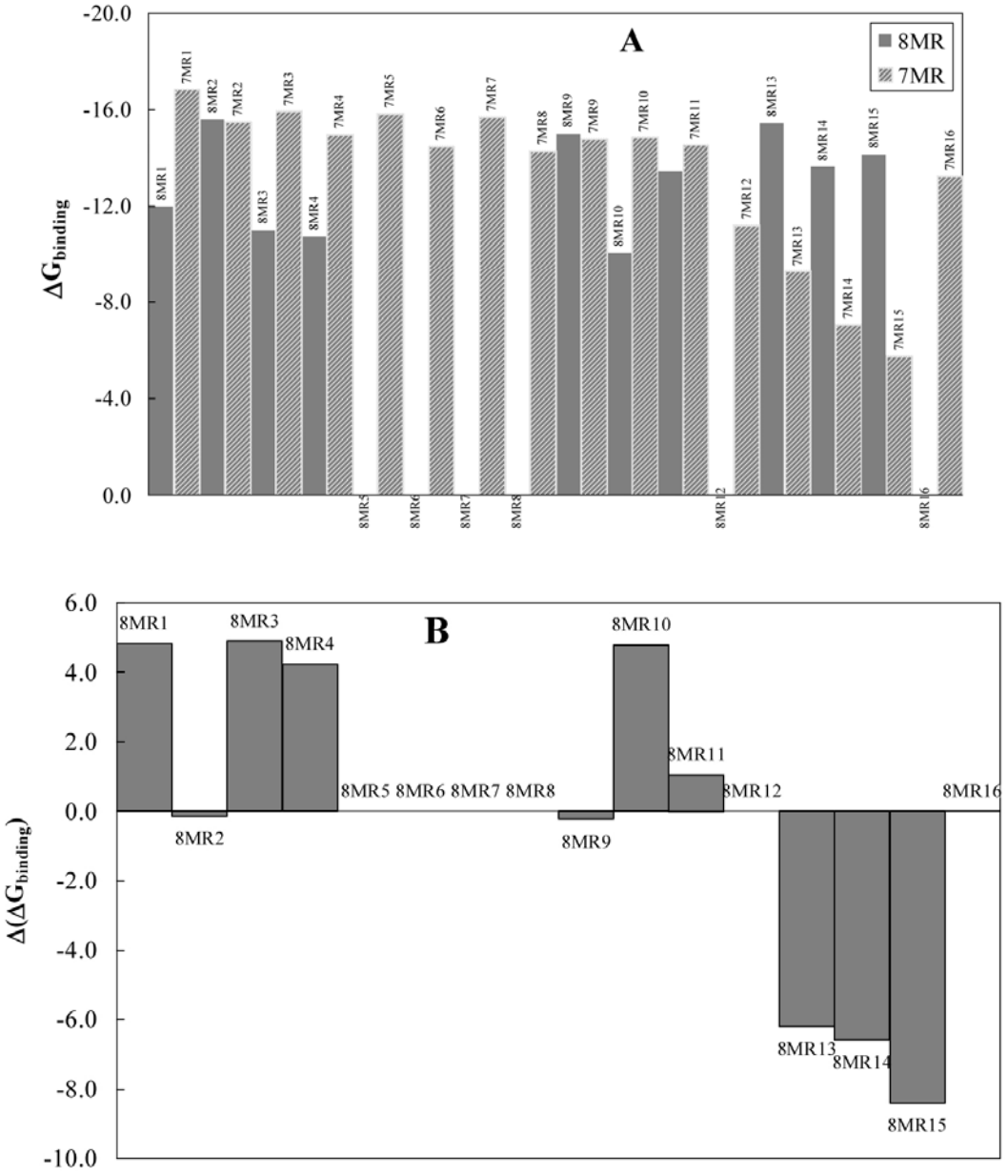


Fig. 4. Binding free energy of the 7MR and 8MR derivatives (A) and differences between binding free energy of the 7MR molecules and their corresponding 7MR inhibitors (B).

the automated docking procedure. Docking procedure is repeated many times for these molecules and, in all docking trials, it was found that the molecules are not entered into the binding site of the receptor. A probable reason for such surprising result is a slight conformational difference between these derivatives and the others. For more clarification, the binding free energy data of Table 2 are also plotted in Fig. 4. Comparison of the resulted binding free energies ($\Delta G_{binding}$) of the 8MR and 7MR derivatives indicates that the new derivatives produced comparable binding affinity toward the HIV-1 RT. Among the 8MR derivatives, 8MR2 and 8MR13 produce the most negative binding energies which are

somehow lower than that of all 7MR molecules.

The binding affinity of the 8MR derivatives was also compared with that of their 7MR homologues by subtracting the $\Delta G_{binding}$ of the 7MR derivatives from the $\Delta G_{binding}$ of the corresponding 8MR derivatives (i.e. $\Delta(\Delta G_{binding}) = \Delta G_{binding}$ (8MR) - $\Delta G_{binding}$ (7MR)). As it is shown in Fig. 4B, the binding of 8MR13, 8MR14 and 8MR15 to the HIV-1 RT enzyme is stronger than their corresponding 7MR derivatives while the tendency of 8MR1, 8MR2, 8MR4, 8MR10 and 8MR11 toward the HIV-1 RT is lower than that of their corresponding 7MR homologue.

As it was mentioned in the beginning of section 3, the amino acids in the binding pocket of RT enzyme are mainly lipophilic with aromatic residues. Therefore, by addition of a carbon atom to the β-ring of conventional TIBO derivatives, the liphophilic interaction will increases. However, in this case, the size of β-ring and consequently the steric hindrance for entering the molecule to binding pocket will also increase. Therefore, it is not surprising that 8MR derivatives do not represent a much higher binding free energy to the HIV1-RT enzyme comparing with the conventional TIBO derivatives. Previously, for some TIBO derivatives, we found that β-ring contraction produces derivatives that could bind to HIV-1 RT much higher than conventional derivatives [37]. Thus, it can be concluded that, in binding of TIBO derivatives to HIV-1 RT, the steric effect is more important than liphophilic interaction.

CONCLUSIONS

Some new nonnucleoside reverse transcriptase inhibitors were designed by expansion of the diazepine ring in the TIBO derivatives from seven to eight. The flexible docking of the new ligands as well as the conventional new derivatives was performed utilizing Autodock program. Docking analysis of the new derivatives revealed that 10 out of 16 molecules are docked into the receptor. The binding of two new derivatives to the receptor found to be stronger than their conventional TIBO homologous, and can be considered as candidate for synthesis.

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