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Toward a rational design of selective multi-trypanosomatid inhibitors: A computational docking study

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Abstract—Compound **V7**, a benzothiazole which was recently found as selective inhibitor of trypanosomal TIMs, was docked into TIMs from *Trypanosoma cruzi*, *Trypanosoma brucei*, *Entamoeba histolytica*, *Plasmodium falciparum*, yeast, and human. Structural analyses revealed the importance of the accessibility to the two aromatic clusters located at the dimer's interface for the selective inhibition of trypanosomal TIMs. Thus, it was found that different accessibilities of the protein interface of TIMs plays an important role in the inhibitory activity of benzothiazoles. These findings will contribute to the rational development and improvement of benzothiazoles to be used as multi-trypanosomatid inhibitors.

Trypanosomiasis affects more than 18 million people in Mexico, Central and South America, and Africa. In Latin-America, the protozoan *Trypanosoma cruzi* is the causative agent of the human trypanosomiasis called Chagas' disease. This disease causes about 21,000 deaths/year, and 300,000 new cases are reported every year.[1]

Similarly, the African trypanosomiasis, also known as Sleeping sickness, is caused by two different sub-species of the protozoan *Trypanosoma brucei*: *Trypanosoma brucei rhodesiense* (in East and Southern Africa) and *Trypanosoma brucei gambiense* (mainly in West and Central Africa). The African trypanosomiasis is causing 65,000 deaths per year, and 66,000 new cases appear every year.[1] Unfortunately, the current treatments against trypanosomiasis have not shown satisfactory results. However, recent experimental and theoretical studies have demonstrated that the inhibition of the enzyme triosephosphate isomerase (TIM) represents a promising approach against trypanosomiasis.[2] It has been hypothesized that agents that target the dimer interface would be able to selectively inactivate the enzyme by promoting the destabilization of the quaternary structure of the protein. This approach is based on the fact that the active site is highly conserved along different species, and that TIM is only catalytically active as a dimer.[3] Thus, the selective inhibition of the TIM would lead to the death of the parasite.

For instance, Gomez-Puyou, Perez-Montfort, and coworkers have reported a few compounds, benzothiazoles that were able to selectively inhibit TIMs from *T. cruzi* and *T. brucei*.[4,5] In addition, they were able to experimentally elucidate the three-dimensional structure of a small benzothizole and TIM from *T. cruzi*.[6] The authors showed that these agents were indeed dimer interface-directed, and that these agents bind to the aromatic clusters located at the interface, as previously found by our workgroup.[7,8]

Recently, Olivares-Illana et al. reported a benzothiazole (Fig. 1), which selectively inhibited TIMs from *T. cruzi*, *T. brucei*, and *L. Mexicana* (called *multi-trypanosomatid inhibitor* by the authors).[5] This compound, labeled as **V7** by these authors (Fig. 1), shares a common structure with other benzothiazoles previously reported.[4] It was also found that its activity is similar to that displayed

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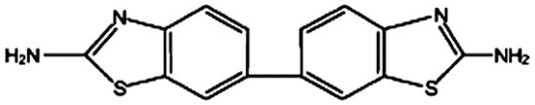

Figure 1. Chemical structure of **V7**.

by other benzothiazoles. Despite the studies previously reported on the selective inhibition of TIM from different species, the rationale on why certain benzothiazoles are potent inhibitors of trypanosomatid TIMs remains unknown.

On this basis, we report a flexible docking study of the benzothiazole **V7** againts TIMs from *T. cruzi*, *T. brucei*, *Entamoeba histolytica*, *Plasmodium falciparum*, yeast, and human. Structural analysis performed on the best docking modes revealed the importance of the accessibility to the interface, leading to an optimal interaction of the compounds with two aromatic clusters for the selective inhibition of trypanosomal TIMs. In this study, we performed a series of combined docking/molecular dynamics simulations in order to determine the factors that play a role in the selectivity of certain benzothiazoles over parasite TIMs. It was found that different accessibilities of the protein's interface of TIMs are a key determinant for the inhibitory activity of benzothiazoles over the enzyme. These findings, which complement previous observations made by our workgroup, will be helpful in the rational development and improvement of benzothiazoles to be used as multi-trypanosomatid inhibitors.

The X-ray structures of TIM from different species were obtained from the Protein Data Bank under the following Accession Numbers: 1TCD (*T. cruzi*); 1IIH (*T. brucei*); 1M6J (*E. histolytica*); 1YDV (*P. falciparum*); 1YPI (yeast) and 1WYI (human). Side-chain ionization states were adjusted to a pH of 7.0 using PROPKA.[9] Subsequently, the proteins were embedded in a TIP3P water box. Finally chlorine and sodium counterions were added to reach a net charge of zero and to adjust the ionic strength to that physiologically observed (~0.15 M). All systems were modeled with the CHARMM 22 force field.[10]

In order to produce a set of relaxed structures for each TIM studied here, molecular dynamics simulations were carried out by using the program NAMD 2.5.[11] An NPT ensemble was used, and periodic boundary conditions were imposed on the systems. The non-bonded cutoff was set to 9 Å, and the SHAKE algorithm was used to allow a 2 fs time step. Constant pressure (1 bar) and temperature (300 K) on the system were maintained with an isotropic Langevin barostat and a Langevin thermostat. Thousand steps of conjugate gradient algorithm were used to minimize each system with restraints to protein backbone, followed by 1000 steps without restraints. This procedure was followed by a warming-up period of 60 ps and an equilibration for 100 ps. The simulations finished with a production run of 1.5 ns for each TIM.

For docking purposes, 30 snapshots obtained from the molecular dynamics simulations of the six TIMs were taken. Water molecules and ions were removed from the six structures, and only polar hydrogen atoms within the protein were kept. Grid maps covering the TIM interface were computed by using the program AutoGrid.

The structure of the benzothiazole **V7** was built and optimized with the aid of the program MOE.[12] Docking simulations were performed with the program AutoDock.[13] This program allows full flexibility on the ligand under study. Docking was carried out using the Larmarckian Genetic Algorithm with an initial population of 200 individuals, a maximum number of 10,000,000 energy evaluations, and maximum number of 27,000 generations. For the local search, the pseudo-Solis and Wets algorithm was applied using a maximum number of 300 iterations per local search. Docking simulations consisted of 150 independent runs. Resulting orientations lying within 1.5 Å in the root-mean square deviation were clustered together. The best orientations (i.e., that represented by the lowest free energy of binding) were further subjected to energy minimization by using 1000 steps of conjugate gradient algorithm.

The most representative orientations of **V7** on the six different TIM's are shown in Figure 2. In previous computational studies, it was observed that benzothiazoles, regardless of their size, bind very close to the symmetric aromatic clusters located at the interface of TIM from *T. cruzi*.[7,8] In the present docking study, it was found that **V7** directly interacted with both aromatic clusters located at the interface of the TIM from *T. cruzi*. These aromatic clusters are formed by Phe75 from one monomer, and Tyr102 and Tyr103 from the adjacent monomer. In a similar way, **V7** had direct contact with Tyr101 and Tyr102, which together with Phe74 from the adjacent monomer, constitute the aromatic clusters of TIM from *T. brucei* (Fig. 3). These observations are in good agreement with the previously reported data obtained from docking simulations of related benzothiazoles on TIM from *T. cruzi*.[8] In contrast, it was observed that **V7** did dock into a cavity located at the interface of TIMs from *P. falciparum* and human. It should be noticed that, according to our multiple computational docking experiments, such cavity might not represent an actual binding site for this kind of compounds. This could be attributed, in part, to its location and the dynamic behavior of such region of the dimer. In the first case, the cavity is formed by the following residues (the letters within parentheses indicate the monomeric unit they belong to): Asn65 (A), Glu77 (A), Arg98 (A,B), Phe102 (A,B), Gly103 (A,B), Glu104 (A), Ser105 (A), Leu108 (A), and Tyr 112 (A). In the second case, residues Ser45 (A,B), Gln46 (B), Asn65 (A), Val66 (B), Ser67 (B), Gly76 (A), Glu77 (A,B), Val78 (B), Arg98 (A), and Phe102 (A,B) were found to form the cavity where **V7** binds at the interface of TIM from *P. falciparum*. It was also observed that **V7** had few interactions with some of the residues that surround the aromatic cluster of TIM from *E. histolytica*, although such interactions do not seem to affect the lo-

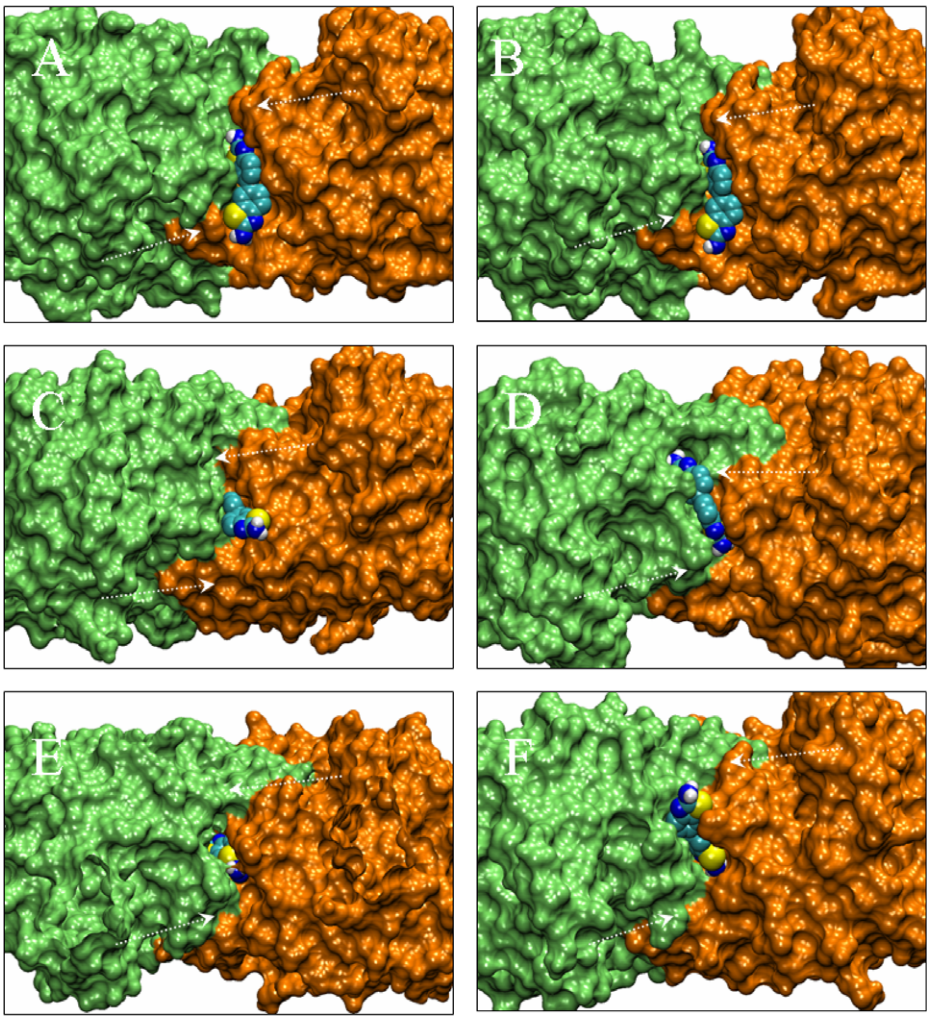

Figure 2. Binding modes of **V7** on TIMs from (A) *Trypanosoma cruzi*, (B) *Trypanosoma brucei*, (C) human, (D) *Entamoeba histolytica*, (E) *Plasmodium falciparum*, and (F) yeast. TIM is rendered as a surface and **V7** as van der Waals spheres. White arrows indicate the location of the two aromatic clusters at the interface.

cal dynamics of the aromatic clusters. These residues are Trp75 (B), Thr76 (B), Phe109 (A,B), His110 (A,B), Gln115 (B), and Val116 (B). Similarly, the residues of TIM from yeast that made contacts with **V7** are Asn65 (A), Glu77 (A), Arg98 (A), Phe102 (A,B), His103 (A), Glu104 (B), Phe108 (A,B), and Lys112 (A).

In a recent study, Olivares-Illana et al. found that **V7** selectively inhibits trypanosomal TIM's.[5] In their study, the authors estimated that the half-maximal inactivation on TIM by **V7** was 21 and 35 μM for *T. cruzi* and *T. brucei*, respectively.[5] In contrast, high concentrations of this agent were not unable to affect the catalytic activity of TIM from *P. falciparum*, *E. histolytica*, yeast and human. By comparing the structure of the interfaces of the six structures studied here, it was observed that the packing of the aromatic clusters of TIM from *T. cruzi* and *T. brucei* is very similar and that the overall accessible surface of the interfaces provides a wide cavity, thus yielding better binding sites for trypanosomatid agents. This observation is supported by the fact that **V7** displayed a very similar binding mode on both TIMs, directly interacting with the aromatic clusters, which were found to be critical for the stability of the dimer. In addition, the ratio of the experimental half-maximal inactivation between the two species was low (~0.6).

In contrast, the reduced accessible surface of the TIM's interfaces and the packing of the aromatic clusters of *E. histolytica*, *P. falciparum*, yeast, and human did not allow the formation of a well-defined binding site for **V7**. Moreover, it was observed that despite the fact that **V7** was found to bind at the interface of TIM's from *P. falciparum*, yeast, and human, it was not able to interact with the aromatic clusters. Thus, its inhibitory activity on such species is considerably reduced, as experimentally observed by Olivares-Illana et al.[5]

The docking modes of **V7** on the six different structures of TIM revealed some interesting features of different TIMs that can be exploited in the design of multi-trypanosomatid inhibitors. First, it was observed that the binding site of **V7** at the interface of TIMs from *T. cruzi* and *T. brucei* is very similar to that observed in previous studies for other benzothiazoles. In principle, it could mean that the mode of action of this agent is somehow

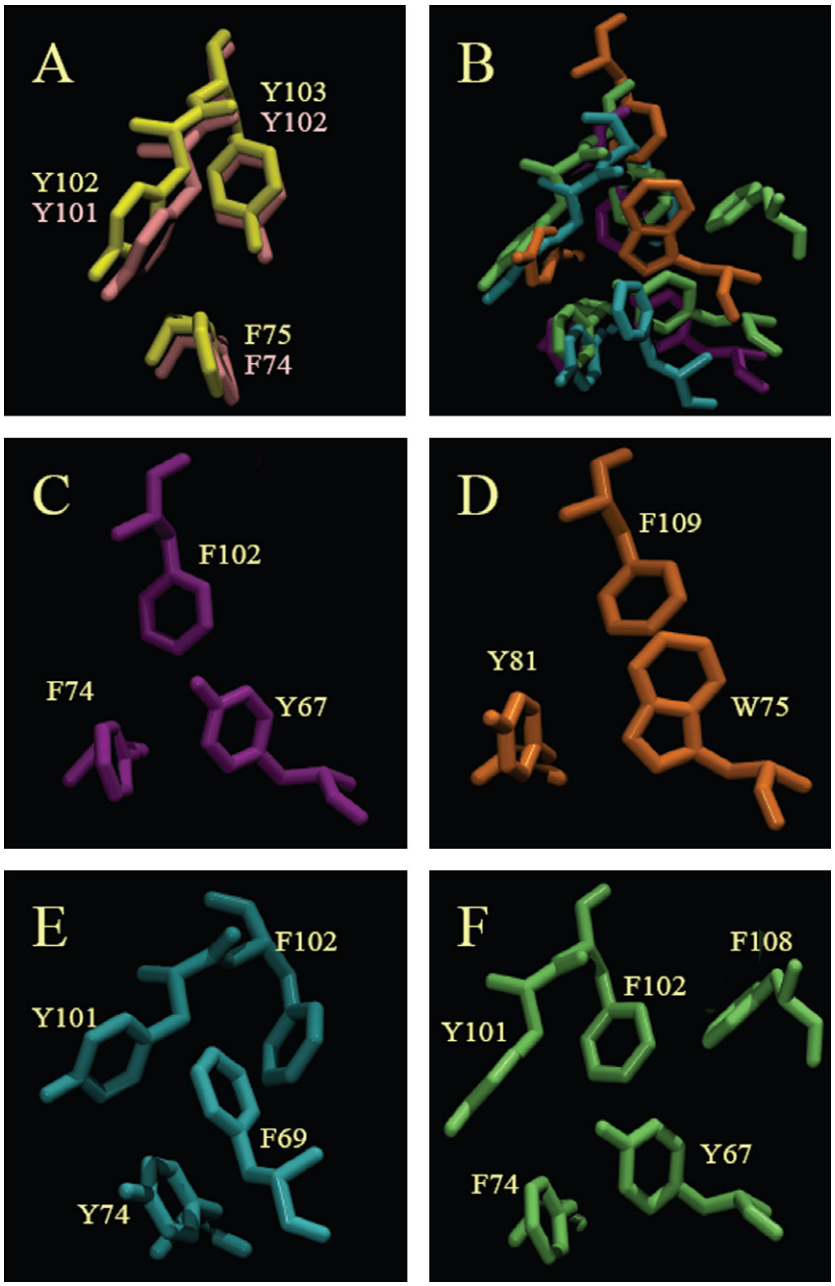

Figure 3. Structure and composition of the aromatic clusters of the six TIMs. (A) Comparison between TIM from *Trypanosoma cruzi* (yellow) and *Trypanosoma brucei* (pink). (B) Superimposition of the aromatic clusters of TIM from human (purple), *Entamoeba histolytica* (orange), *Plasmodium falciparum* (cyan), and yeast (lime). The composition of such aromatic clusters is depicted in: (C) for human, (D) for *Entamoeba histolytica*, (E) for *Plasmodium falciparum*, and (F) for yeast.

similar to that observed for compounds that are homologous to **V7**. Moreover, it was observed that the binding modes of **V7** over the TIMs from *T. cruzi* and *T. brucei* were very similar. The calculated RMSD from both binding modes was <0.5 Å. This observation could explain, in part, why this compound is highly selective for these two strains. In contrast, neither such well-defined binding site nor similar binding modes were observed for the TIMs from *P. falciparum*, *E. histolytica*, human, and yeast.

Considering the structural data obtained in this study, we hypothesize that the accessibility to the aromatic sites located at the interface of the TIM could potentially play an important role in the selective inhibition of trypanosomal TIMs (Fig. 3). The regions located at the interface containing clusters of aromatic residues such as Phe and Tyr were found to be involved important for the stabilization of the quaternary structure of the TIM's dimer from *P. falciparum*.[14] Moreover, such aromatic clusters are highly conserved, although the number, composition, and distribution of the aromatic amino acids vary along the phylogenetic tree. Such structural changes on individual aromatic clusters at the TIM's interface, together with the presence of bulky residues surrounding them, alter the accessibility to such

sites, impeding the optimal binding of chemical agents that can disturb the stabilizing interactions of the dimer. Although this study clarifies some of the questions previously raised on the selective effect of benzothiazoles over parasite's TIMs, more work on this area needs to be performed. Thus, further studies addressing the dynamics of the complex between multi-trypanosomatid agents and TIMs from different species, together with the calculation of the absolute free energies of binding, would provide a more complete view on how the parasite's TIM could be effectively inhibited, leading to a fully rational design of highly efficient multi-trypanosomatid inhibitors.

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