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Exploring the possible binding sites at the interface of triosephosphate isomerase dimer as a potential target for anti-tripanosomal drug design

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Abstract—To explore the possible binding sites at the interface of tripanosomal triosephosphate isomerase, fully flexible benzothiazoles were docked onto the dimer interface. Docking studies revealed that the most favorable interactions occur in the aromatic clusters of the dimeric form. Hence is purposed that the dimer disruption is not via Cys 15, as presented in last studies, but it could be carried out through the unstabilization of π–π interactions of two aromatic clusters present in the interface. These studies enable a novel alternative for rational structure-based anti-tripanosomal drug design.

Triosephosphate isomerase (TIM) plays a crucial role in catalyzing the interconversion between the trioses glyceraldehyde 3-phosphate and dihydroxyacetone 3-phosphate in the glycolytic pathway, and except for *Thermotoga maritima* and *Pyrococcus woesei*, it is found as a homodimeric protein.[1,2] Many studies have established that the 'natural' TIM is active only as a dimer and the active site along the philogenetic tree is highly conserved.[3,4] Since this enzyme has been studied as an important target model to develop anti-parasitic drugs, new strategies, principally focused on the selective perturbation of the dimeric form of the parasite enzyme, has received considerable attention. Although a vast body literature about the structure, biochemistry and stability of the TIM interface is available,[5] there is no certain information about where and how the dimeric form may be targeted and disrupted into the monomeric form, and the main problem for the design of strong and selective TIM interface inhibitors is the lack of information on the interactions between the potential binding sites and the inhibitors. Thus, the information concerning the atomic structure of the complex of the TIM with its interface inhibitors is essential to better understand the mechanisms involved in the dimer stabilization and for enabling the structure-based drug design efforts to proceed. Here we present computational flexible docking studies to explore the possible binding sites where the interface of *Tripanosoma cruzi* TIM (TcTIM) may be disrupted.

For docking studies, the latest version of AutoDock (3.0.5) was chosen because it allows full flexibility in small ligands in its algorithm. It has been shown that it successfully reproduces many crystal structure complexes and includes an empirical binding free energy evaluation.[6] Recently, 10 benzothiazoles were assayed as TcTIM interface inhibitors, those being the first non-peptidic agents that reached a considerable inhibition potency via interface perturbation.[7] Nine of those ten compounds were used here to search the possible binding sites at the TcTIM interface, to be exploited as drug-design targets. These nine structures are presented in Scheme 1.

Docking to TcTIM was carried out using the hybrid Lamarckian Genetic Algorithm, with an initial population of 100 randomly placed individuals, a maximum number of 1.0×10^7 energy evaluations and a maximum number of iterations in the pseudo-Solis-and-Wets-minimization/local search of 300. Fifty independent docking runs were carried out for each ligand. Resulting docked orientations within 1.0 Å rmsd were clustered together. The largest cluster returned by AutoDock for each compound was used for further analyses. All other

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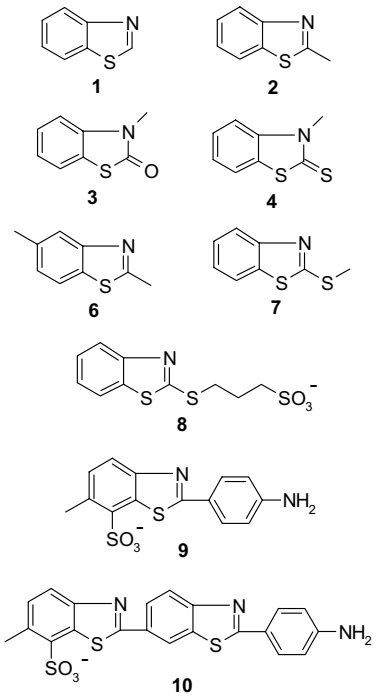

Scheme 1. Nine benzothiazoles employed here for docking studies.

parameters were maintained at their default settings. Ligands were generated with SYBYL[8] and the atomic charges added using the Gasteiger–Marsili formalism.[9] The TcTIM dimer crystal structure used in the present analysis was obtained from the Protein Data Bank under 1TCD entry. Before docking studies, crystallographic waters were removed. Polar hydrogens and atom charges were added to the protein, followed by a 500-step Steepest Descent minimization employing the CHARM22 force field implemented in the NAMD 2.5 software.[10]

Of the nine structures studied here, only six correctly docked in the TcTIM interface. In the case of structures **8**–**10**, output files were not useful for analysis. Out of the 50 of these runs, 40–45 different orientations were generated and many of them presented steric clashes with the residues of the dimer (e.g., distances less than 0.5 Å between atoms). Those three benzothiazoles were discarded for further analysis.

As observed in Figure 1, six benzothiazoles employed for the study presented here, did dock in the TcTIM interface. Must important contacts of these structures with the protein were observed with two aromatic clusters (in blue, Fig. 1) present in the dimer interface. Both aromatic clusters are formed by residue Phe75 from one monomer and residues Tyr102 and Tyr103 from the other monomer. Aromatic residues have been found to be essential in binding several ligands to receptors via π–π interactions.[11] In our study, we found that the distance between the centroids of the aromatic residues from the enzyme and the aromatic rings present in the molecules studied are between 3 and 4 Å, which corresponds to distances found for this kind of interactions. We also observed that AutoDock software

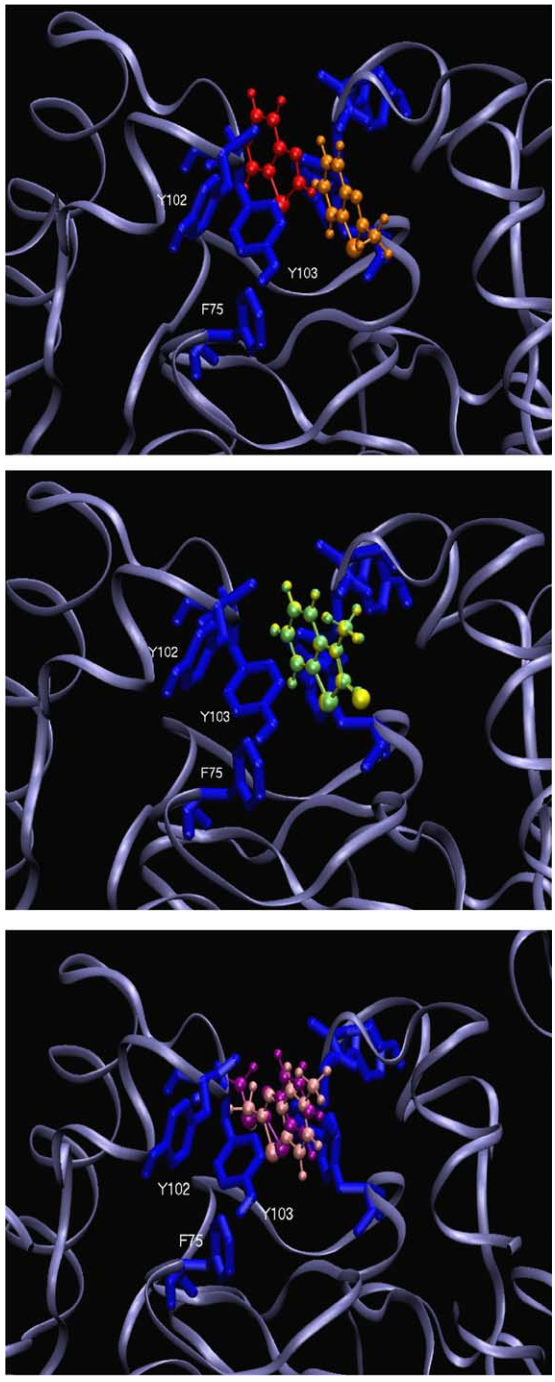

Figure 1. Graphical representation of the top-ranked binding orientations obtained for six benzothiazoles at the TcTIM interface. Both aromatic clusters (F75-Y102-Y103) are shown in blue. Top: **1**, red; **2**, orange. Center: **3**, green; **4**, yellow. Bottom: **6**, pink; **7**, purple. In the case of compounds **3** and **4** it is observed that they bind identically, suggesting that the change of oxygen by sulfur does not produce significant changes in the mode of recognition at the dimer interface.

successfully simulates these π–π interactions between benzothiazoles and the aromatic residues of the enzyme: the aromatic rings of the ligands are turned in the direction of the aromatic sidechains of the enzyme, so that both stacking and T-shaped forms are reached, giving the complex important stability. Thus, we infer that the aromatic rings present in the interface of the TcTIM dimer could play the principal role in binding

Table 1. Binding free-energies calculated for benzothiazoles here presented

Compound	ΔG_{bind} (kcal/mol)	N_{tot}	f_{occ}
1	−12.90	3	36
2	−13.60	4	24
3	−12.98	6	19
4	−12.40	5	22
6	−13.40	6	20
7	−15.80	8	18

N_{tot} is the number of clusters generated by AutoDock; f_{occ} is the number of results in the top clusters.

and accommodating the ligands to the interface of the protein; the rest of the non-aromatic residues involved in the molecular recognition could contribute to the van der Waals and electrostatic forces acting in the binding mechanism.

The binding free energy (ΔG_{bind}) values obtained for the best docked orientation of six benzothiazoles are presented in Table 1. The largest cluster returned by AutoDock for each compound was used for analysis, as mentioned above. In the case of all compounds studied here, the best occurred clusters were generally the top-placed in the histogram, as shown in Table 1. Generally, the top clusters (associated with the most favorable ΔG_{bind}) are also associated with the highest frequency of occurrence, which suggests a good convergence behavior of the algorithm. In this case, the best results in terms of free energy are those located in a quite similar position in the aromatic clusters of the TcTIM interface. Obtained energies serve as a guide for supporting that the inhibitors will bind in the site we are studying. Telléz-Valencia et al. have inferred that the sulfonate group present in the three of the molecules they studied should be necessary for molecular recognition. We found that benzothiazole moiety is essential for molecular recognition, and not the sulfonate group, judged by the energies obtained for all compounds and the orientation of the structural moieties in the three-dimensional representation. Despite of the lack of the sulfonate group, has been demonstrated that some compounds at a concentration of 250 M presented inhibitory activity on TcTIM, as reported by Telléz-Valencia et al. Thus, the main function of the sulfonate group must be only to increase the capacity of molecules to be dissolved, and not in molecular recognition by the enzyme. In addition, all these energies seem to be overestimated. This could be explained due to the dimer hast two possible binding sites (two aromatic clusters), and the program evaluates the energy only in one of two possible aromatic clusters. Experimentally, it has been inferred by means of kinetic studies, that two molecules of the benzothiazoles here presented are needed to reach an efficient inhibition, evaluated by the measure of co-operativity, which is near 2.[7] According with experimental data, we found that, in all cases, molecules do bind to one of the aromatic clusters, because in our study we only consider a one-to-one system, and the optimal ratio to reach an efficient inhibition should be 2:1.

With the values of binding free-energy we obtained in our docking experiments, we do not claim that the inhibition potency of the compounds depends on binding energy, for example, if we obtain a very good binding free energy does not mean that the inhibitory potency will be optimal. In this sense, detailed studies on the nature of interactions along the dimer interface of different TIM sources should be started to better clarify the interactions leading to the successful disruption of the dimeric form of the enzyme.

Essentially, here we have focused our attention on the residue Phe75 and it relationship with residues Tyr102 and Tyr103. This side chain is important because it makes several contacts at the dimer interface, particularly with residues Cys15 and Tyr102 from the other subunit, and is also part of an aromatic cluster involving residues Tyr102 and Tyr103 from the other subunit. Aromatic clusters have frequently been implicated in the stabilization of folded protein structures through π–π interactions. So, π–π interactions involving proximal aromatic rings are suggested to be important contributors to protein stability.[12]

In this manner, Phe75 could stabilize an important part of the enzyme–inhibitor complex at the aromatic cluster. On the basis of the information we obtained in our docking study, the inhibition mechanism at the interface of TcTIM could be rationalized as follows: first, all the aromatic rings of the inhibitors bind at the interface via π–π interactions, and the rest of the non-aromatic regions of the inhibitor, if present, may contribute many of the van der Waals and electrostatic forces; the inhibitor molecules are accommodated in the most favorable orientation, binding principally with each aromatic cluster. This favorable orientation may create an interference of the 'natural' π–π interactions in the aromatic cluster (Phe75-Tyr102-Tyr103), and as consequence of these events, diverse conformational changes inside aromatic clusters and the regions around them will be started. Part of these conformational changes could be favored by the van der Waals and electrostatic forces applied to many residues of the loop 3, for example, Ile69, Thr70 and Arg71. The sum of these forces will be big enough to produce a chain reaction, which ruptures all interactions along the interface, and as a consequence the dimer disruption will occur. A positively charged residue (Arg71) occurs close to the aromatic cluster. Therefore, it is possible that the binding of inhibitors is also favored by π-cation interactions.

In this sense, Phe75 should play a crucial role. It could be considered as a key piece in the aromatic cluster support, and in the dimer stability. Interestingly, along the philogenetic tree, the presence of aromatic clusters inside the dimer is highly conserved. On the other hand, it has been affirmed that the selectivity of inhibitors on different TIM sources and vulnerability of dimeric form depends of the presence of the inter-subunit residue Cysteine. In this study, we did not observe any relationships between inhibitors and Cys15, characteristic in TcTIM. Instead of that hypothesis, we propose that the binding and potency of inhibitors are independent of the structure, and should be studied separately and in more detail.

With the information presented above, the consideration of the docking results has provided better indications of which regions should be exploited as drug-design target and how the inhibitors could act on these binding sites. In this study, we tried to answer these main questions. With all findings in our study, we purpose that the aromatic clusters located inside the TcTIM interface are the starting point of the dimer disruption instead the residue Cys15, which has received important attention as the principal residue involved in the dimer separation,[7] and these aromatic clusters may be found crucial in the dimer stabilization. Also we demonstrated that benzothiazole moiety and not sulfonate group is essential for molecular recognition, and as consequence, for the dimer disruption. Molecular dynamics simulations have been started to further clarify the binding modes of this benzothiazoles, taking into account solvation and flexibility of the dimer. These simulations together with docking studies should help to obtain a consensus view of inhibitor interactions at the interface of the TcTIM dimer and will provide important support to the structure-based design efforts.

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