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ORIGINAL PAPER

Docking and molecular dynamics simulation of quinone compounds with trypanocidal activity

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Abstract In this work, two different docking programs were used, AutoDock and FlexX, which use different types of scoring functions and searching methods. The docking poses of all quinone compounds studied stayed in the same region in the trypanothione reductase. This region is a hydrophobic pocket near to Phe396, Pro398 and Leu399 amino acid residues. The compounds studied displays a higher affinity in trypanothione reductase (TR) than glutathione reductase (GR), since only two out of 28 quinone compounds presented more favorable docking energy in the site of human enzyme. The interaction of quinone compounds with the TR enzyme is in agreement with other studies, which showed different binding sites from the ones formed by cysteines 52 and 58. To verify the results obtained by docking, we carried out a molecular dynamics simulation with the compounds that presented the highest and lowest docking energies. The results showed that the *root mean square deviation* (RMSD) between the initial and final pose were very small. In addition, the hydrogen bond pattern was conserved along the simulation. In the parasite enzyme, the amino acid residues Leu399, Met400 and Lys402 are replaced in the human enzyme by Met406, Tyr407 and Ala409, respectively. In view of the fact that Leu399 is an amino acid of the Z site, this difference could be explored to design selective inhibitors of TR.

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Keywords Chagas disease · Docking · Molecular dynamics · Quinone · Trypanothione reductase

Introduction

American trypanosomiasis, also known as Chagas' disease, is caused by hemoflagellate *Trypanosoma cruzi*. It is the most lethal parasitic disease in the tropics and subtropics of North and South America and causes over one million cases of infection annually and more than 50 000 deaths per year. Currently, there are between 16 and 18 million chronically infected individuals of Chagas' disease, and more than 100 million people at risk [1, 2]. Blood transfusion and congenital transmission are currently the major causes of the spread of the disease [3].

The chemotherapy of Chagas' disease is unsatisfactory and limited to only two drugs, nifurtimox and benznidazole. In addition, these drugs suffer from some toxicity and serious side effects [4]. Also, they are active only in the acute phase of the disease [5]. Therefore, there is an urgent need for new safe and effective drugs against Chagas' disease.

The maintenance of an intracellular reducing environment is essential in protecting the cell from the highly reactive oxygen species that arise as a result of aerobic respiration and host immune response to infection. In most organisms, the abundant thiol glutathione (GSH) is utilized for this purpose and its level is maintained by the action of glutathione reductase (GR), which regenerates GSH from glutathione disulfide ($[GS]_2$). The GR/GSH system is replaced in trypanosomes and leishmania by an analogous system based on trypanothione (N^1,N^8-bis(glutathionyl)-spermidine, $T[SH]_2$) and Trypanothione Reductase (TR), which regenerate $T[SH]_2$ from trypanothione disulfide ($T[S]_2$) [6].

Both mammalian GR and protozoal TR are homodimers with a molecular mass of 100–110 kDa. These enzymes share about 40% sequence identity including the residues necessary for enzyme catalysis. In spite of the close similarity between the two enzymes, each enzyme is specific for the cognate substrate [7]. Despite the structural similarities of trypanothione and glutathione, TR and GR demonstrate mutually exclusive substrate specificity, and consequently TR represents a promising target for the development of a range of anti-trypanosomal drugs [8].

Quinones, and particularly 1,4-naphthoquinones (1,4-NQs), are widespread among the secondary metabolites of plants and microorganisms. They can also be prepared synthetically and are widely produced by the chemical industry as organic dyes. The interest of 1,4-NQ is not restricted to the chemistry of dyes; a wide spectrum of biological activities is described for them, including antitumor, wound healing, anti-inflammatory, antiparasitic and cytotoxic activities, among others [9]. Some examples of naturally occurring 1,4-NQ are illustrated in Fig. 1.

Quinone and nitrofuran substrates are reduced by TR, and subsequently redox cycle *via* reaction of the reduced quinones and nitrofurans with molecular oxygen to form reactive oxygen species [10]. Besides, many naphthoquinone drugs such as menadione, plumbagin and lapachol display notable trypanocidal activities upon different trypanosomes and *Leishmania spp.* responsible for several human diseases including African sleeping sickness (*Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*), Chagas' disease (*Trypanosoma cruzi*) and Kala-azar (*Leishmania donovani*) [11].

Enzymatic and antitrypanosomal studies revealed that several naphthoquinone derivatives with strong trypanocidal activity were among the most effective TR inhibitors [11]. Considering that the TR-mepacrine structure is the only crystal structure of trypanothione reductase in complex with an inhibitor [12], molecular docking studies and molecular dynamics (MD) simulations were carried out to gain a better insight into the binding interaction between TR/GR and a series of quinone compounds. Thus, the development of new drugs to treat this disease would be aided by the discovery of compounds that interfere with processes that are essential to the parasites but absent in human hosts.

Methods

The quinone compounds studied here were reported in the literature as powerful and selective trypanocidal agents [13]. The central structure, numbering and the chemical structure of the 28 quinone compounds studied in this work are presented in Fig. 1. Some of these quinone compounds were tested against the enzyme trypanothione reductase from *T. cruzi* [14, 15].

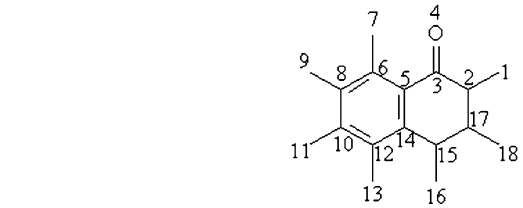
The docking problem is the search for the most energetically favorable binding pose of a ligand to its receptor. Docking is applied at different stages of drug discovery process to predict the docked structure of a ligand-receptor complex, as well as calculate a binding energy that can be used to rank different molecules according to their affinity for a receptor [16]. In this work a docking study was performed with the objective of elucidating the interaction mechanism of the quinones with the enzymes TR and GR. Along with, molecular dynamics simulations were carried out in order to check the robustness of the docking results. For the sake of comparison, AutoDock [17] and FlexX [18] methods were chosen. AutoDock [17] and FlexX [18] use different methods to dock the compound to the binding site and are therefore appropriate for comparison purposes. In addition, they contain a minimization stage for optimizing the proposed binding mode. AutoDock [17] uses a genetic algorithm for optimizing the translation, rotation, and conformation of the ligands. The FlexX [18] program uses a rapid incremental construction algorithm to assemble the ligand in the binding pocket [19].

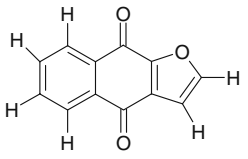
The binding affinity of the receptor-ligand complex is estimated by using a scoring function. The FlexX scoring function is based on an empirical function first reported by Böhm, which estimates the free binding energy (ΔG) of the protein-ligand complex [16, 20].

$$\begin{aligned}\Delta G = \; & \Delta G_0 + \Delta G_{rot}.N_{rot} \\ & + \Delta G_{hb} \sum_{neutral\ H\ bond} f(\Delta R, \Delta\alpha) \\ & + \Delta G_{io} \sum_{ionic\ int.} f(\Delta R, \Delta\alpha) + \Delta G_{aro} \sum_{aro\ int.} f(\Delta R, \Delta\alpha) \\ & + \Delta G_{lipo} \sum_{lipo\ int.} f^{*}(\Delta R, \Delta\alpha)\end{aligned} \tag{1}$$

In the Eq. (1) the term f(ΔR, $\Delta\alpha$) is a scaling function penalizing deviations from the ideal geometry and N_{rot} is the number of free rotatable bonds that are immobilized in the complex. The terms ΔG_{hb}, ΔG_{io}, ΔG_{rot}, and ΔG_0 are adjustable parameters. These values and the function *f* are taken as developed by Böhm. In addition, the term ΔG_{aro} is used for the interaction of aromatic groups and it was determined as −0.7 kJ mol^{-1}. The last term (ΔG_{lipo}) is a modification of Böhm's lipophilic contact energy. The function $f^{*}(\Delta R)$ in Eq. (1) accounts for contacts with a more or less ideal distance and penalize forbiddingly close contacts [20]. The FlexX score contains a hydrogen bonding term, a penalty for protein-ligand overlap, a pairwise hydrophobic potential, and additional terms for specific hydrophobic contacts [16].

Fig. 1 The central chemical structure, numbering used and chemical structure of the 28 quinone compounds studied





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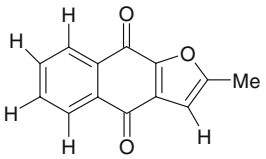

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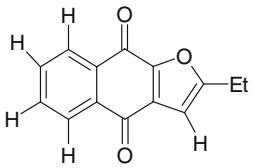

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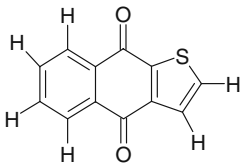

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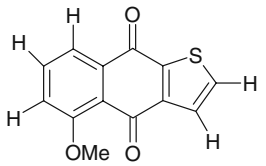

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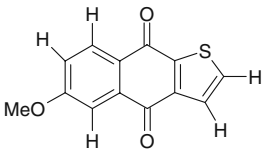

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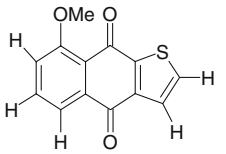

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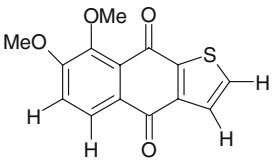

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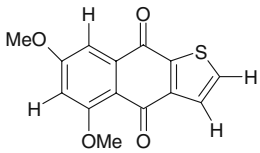

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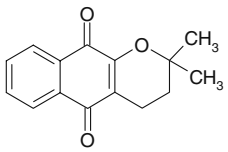

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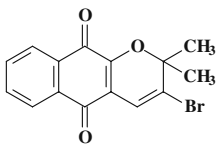

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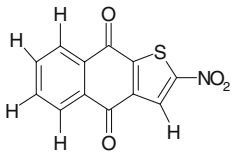

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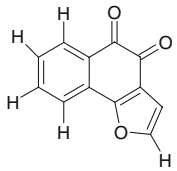

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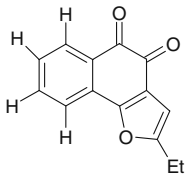

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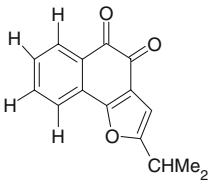

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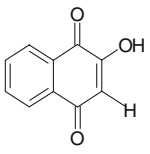

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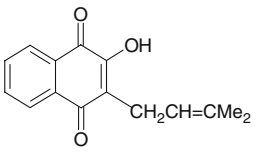

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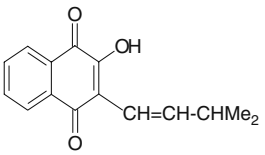

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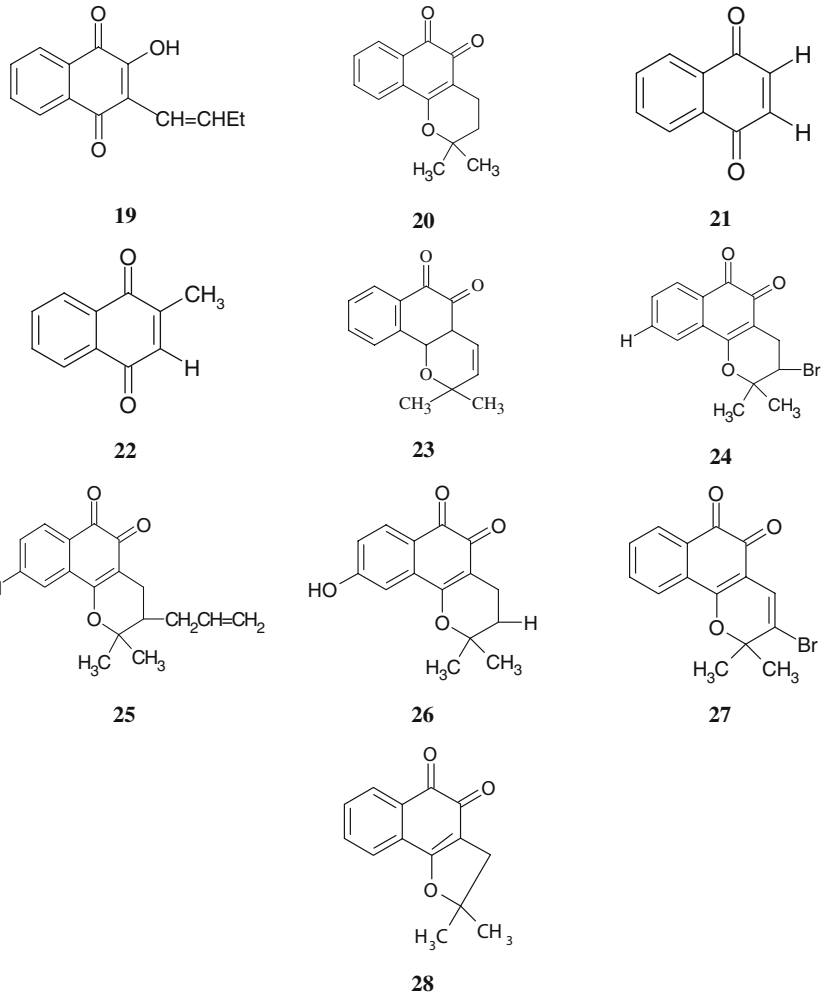
Fig. 1 (continued)



The scoring function used by AutoDock, which is a function that combines both force-field-like and empirically based attributes has the form

$$\Delta G = \Delta G_{VDW} \sum_{ij} \left(\frac{A_{ij}}{R_{ij}^{12}} - \frac{B_{ij}}{R_{ij}^{6}} \right) + \Delta G_{hb} \sum_{ij} E(t) \left(\frac{C_{ij}}{R_{ij}^{12}} - \frac{D_{ij}}{R_{ij}^{6}} \right) + \Delta G_{elec} \sum_{ij} \frac{QiQi}{\varepsilon(R_{ij}) R_{ij}} + \Delta G_{TOR} N_{TOR} + \Delta G_{SOL} \sum_{ij} \left(S_i V_j + S_j V_j \right) e^{\frac{-R^2}{2\sigma}} \quad (2)$$

.

The first three terms of this equation are similar to ones found in molecular mechanics force field for VDW, H-bonds, and electrostatics, but in this instance, they are weighted by empirical weighting factors (the ΔGs). The last term is the protein-ligand desolvation penalty where the terms S_i and S_j are the atomic solvation parameters for ligand atom i and protein atom j, respectively. The term V_j is the volume of protein atom j in the vicinity of ligand atom i, and V_i is the volume of ligand atom i in the vicinity of protein. The whole term is scaled by an exponential function of the distance between the atom pair. The empirical free energy function was calibrated using a set of 30 known protein-ligand complexes with known binding constants [16, 17].

The docking calculations were carried out with the crystal structures of human glutathione reductase and trypanothione reductase from *T. cruzi*. The protein structures used in the docking study were obtained from the Protein

Data Bank (1BZL and 2GH5). The structures of 1BZL [21] and 2GH5 [22] have 2.4 Å and 1.70 Å of resolution and R-factor of 0.209 and 0.224, respectively. The two structures were selected because they present a reasonable resolution and are complexed with non-covalent ligands.

The binding site for calculations was defined as all atoms of 1BZL and 2GH5 within 15 Å of His461 (His467, in 2GH5). Ligand and all water molecules were removed from the protein structure. For the ligands, Gasteiger charges [23] were calculated using AutoDock Tools [24]. The receptor model was prepared with AutoDock Tools adding polar hydrogens and loading Kollman United Atom charges [25]. With the use of AutoGrid (part of the AutoDock package), affinity grids centered on and encompassing the active site were calculated for the relative ligand atom types with a 0.375 Å spaced box (70 Å×66 Å×64 Å). The torsion and rotatable bonds in the ligands were defined by AutoTors, an auxiliary program of AutoDock 3.0.5 [26] which also united the nonpolar hydrogens and partial atomic charges to the bonded carbon atoms. A flexible docking was carried out with AutoDock 3.0.5 to evaluate ligand binding energies over the conformational search space using the Lamarckian genetic algorithm. Default docking parameters were used with the following exceptions: ga_pop_size, 300; ga_num_evals, 500000; ga_num_generations, 27000; and ga_run, 20. The docking conformations of each ligand were clustered on the basis of root-mean-square deviation (RMSD) and ranked on the basis of free energy of binding. The top-ranked conformations were visually analyzed.

The molecular docking program FlexX integrated in Sybyl 7.3 was used in default setting, and the binding site was the same as in AutoDock. For each ligand, the docked conformation with the best score was saved in Sybyl database format.

The molecular dynamics study was done with both the best and worst ranked ligands (compound **25** and **21**, respectively) in the original crystal structure of trypanothione reductase from *T. cruzi*, according to the program AutoDock3.0.5 [26]. The complex was minimized using Amber force field in the presence of GB/SA continuum water model before performing molecular dynamics simulations. The complex was minimized for 3000 steps of Steepest Descent plus 1000 steps of Polak-Ribiere conjugate gradient using the Macromodel 9.1 program [27]. All hydrogen atoms were constrained using the SHAKE algorithm. Nonbonded energy terms were included up to 15 Å for van der Waals interactions and 20 Å for charge-charge electrostatics. All atomic charges were derived from AMBER force field.

For simulation of the receptor-ligand complex, the residues further away than 15 Å from the best pose of compounds **21** and **25** were constrained by harmonic constraints. Only residues inside the 15 Å sphere from the His461 were allowed to move freely. An initial random velocity to all atoms corresponding to 300 K was applied. At this point, the rigid part of the system was kept frozen and the thermalization of the mobile part was started by running four molecular dynamics (MD) simulations: initial temperature of 500 K; cooling down of 100 K at 10 ps with 1.5 fs time step up to 200 K. The molecular dynamics was carried out at constant temperature (298 K) for 2 ns and 1.5 fs time step. The saved conformations (40) from molecular dynamics runs were fully minimized for 500 steps of conjugate gradient. All molecular dynamics were carried out by using Macromodel 9.1 program [27].

Results and discussion

The ESPript 2.2 program [28] was used to align the amino acid sequence of the structure 1BZL and 2GH5. The analysis from Fig. 2 showed that two structures have meaningful differences in the amino acids sequence. The percent of identity and similarity between the two structures, calculated by Align program [29], was 35.7% and 52%, respectively. Moreover, 9.3% of the alignment present gaps (regions where one or more amino acids are absent).

The amino acids of the active site with a distance ≤ 5 Å from the ligand in the TR aligned with GR showed several distinct amino acids between two enzymes. The amino acids Glu19, Trp22, Ala53, Ile107, Ser110, Met114, Gly459, Ala465 and Ser470 are present in TR but are replaced in human enzyme by Ala34, Arg37, Tyr106, Leu110, Ile113, Asn117, Ala465, Ser471 e Thr476, respectively. On the other hand, the amino acids of Z site and some neighbors (formed by Lys62, Thr66, Phe396, Pro398, Leu399, His461, Pro462, Ser464, Glu466 and Glu467) exhibit more correspondence with GR amino acids. There is only one mutation in the Z-site: Leu399 are replaced by the Met406 in the human enzyme. The existence of gaps and regions where there is no identity between both enzymes can be explored for the development of selective drugs against the parasite enzyme.

Docking

It was observed that the interaction of quinones with trypanothione reductase occurred in a distinct site, different from the active site formed by Cys52 and Cys58. Modeling studies on TR have suggested a new site, which is a hydrophobic pocket in the outer region of the active site formed by Phe396, Pro398 and Leu399 [12], called Z site.

Molecular docking studies reported with chlorpromazine and phenothiazine derivatives showed that these com-

pounds interact with amino acids of Z site in TR enzyme [30–32]. However, the use of docking on 1,4-NQ is the first work that discloses the feasibility of these compounds to bind the hydrophobic Z site. As an attempt to explain the binding site and the interaction mechanism between the quinone compounds with the trypanothione and glutathione reductase, docking studies were carried out with these enzymes through AutoDock [17] and FlexX [18] programs.

FlexX

Table 1 shows the results obtained with FlexX [18] program for the two enzymes, TR and GR, respectively. The docking energy of the quinone compounds with TR range from −6.32 to −12.15 kJ mol^{-1}, and for GR enzyme the docking energies span of −14.09 to −2.86 kJ mol^{-1}. Almost all quinone compounds docked with FlexX stayed

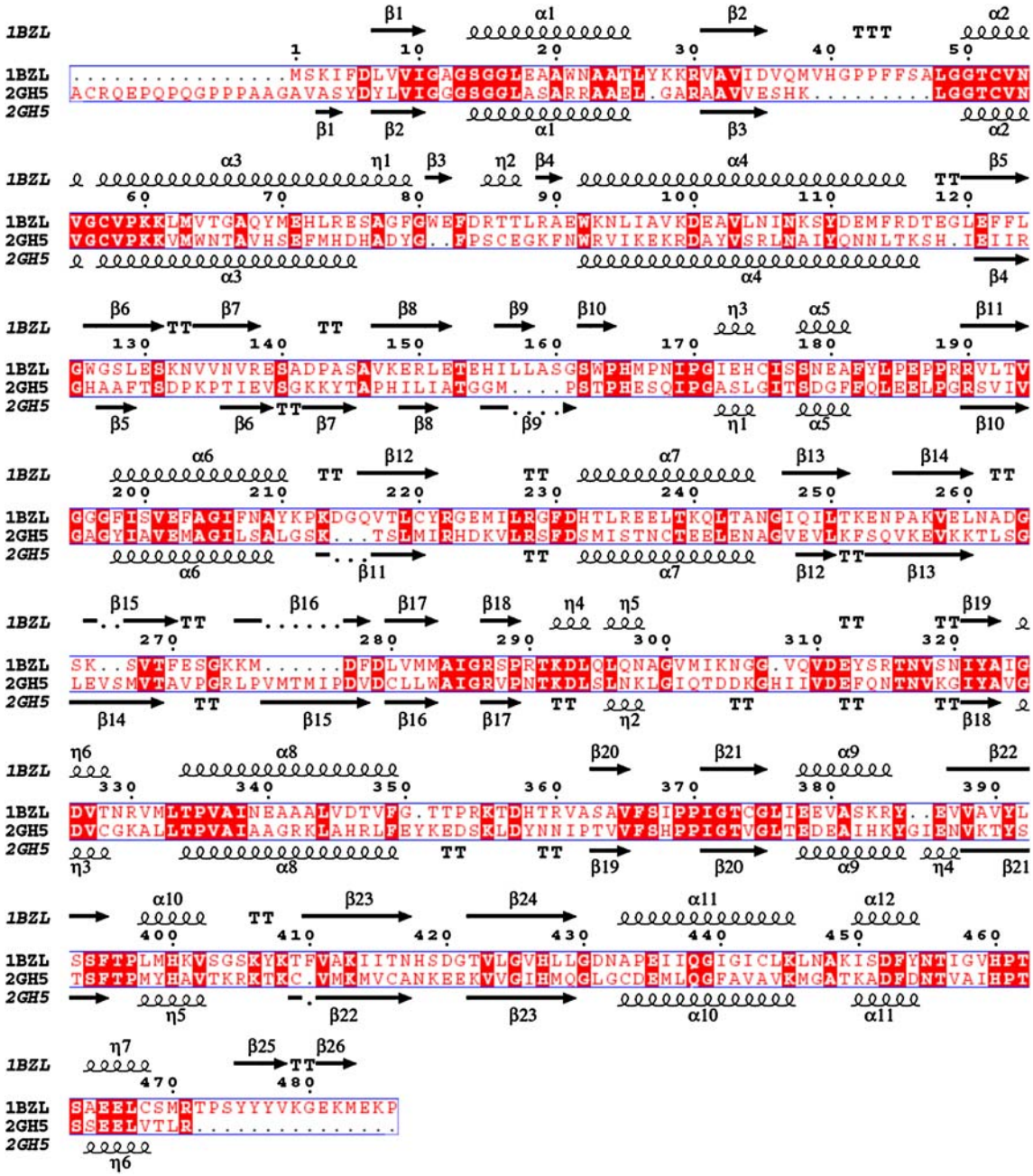


Fig. 2 Structural alignment of primary structure of 1BZL and 2GH5 enzymes. The first line showns the amino acids sequence of 1BZL and the second line is the sequence for 2GH5. The amino acids that are presented in both enzymes are enclosed in red-filled boxes. The gaps are represented by (.) where there are absences of amino acid residues

Table 1 Docking energies (kJ mol−1) of the quinone compounds obtained with FlexX and AutoDock programs

Compound	FlexX (kJ mol^{-1})		AutoDock (kJ mol^{-1})	
	TR	*GR*	*TR*	*GR*
1	−8.40	−2.86	−28.45	−25.68
2	−7.90	−3.96	−29.67	−26.40
3	−7.31	−6.90	−31.22	−28.20
4	−8.76	−4.67	−28.87	−26.31
5	−9.88	−5.40	−30.80	−26.98
6	−11.82	−9.06	−29.67	−26.36
7	−9.92	−3.56	−31.72	−27.32
8	−11.06	−6.33	−29.54	−26.69
9	−10.93	−8.19	−33.10	−28.49
10	−9.32	−4.32	−34.86	−29.87
11	−7.26	−3.42	−35.66	−32.64
12	−9.18	−14.09	−29.08	−26.23
13	−8.71	−5.33	−30.55	−25.31
14	−8.14	−6.37	−31.89	−29.46
15	−7.59	−4.56	−32.26	−30.75
16	−10.79	−9.64	−26.19	−22.33
17	−10.46	−9.38	−35.45	−32.98
18	−12.15	−10.34	−33.86	−31.89
19	−11.78	−12.39	−34.65	−29.75
20	−6.60	−5.07	−29.87	−31.38
21	−8.74	−2.87	−24.97	−21.37
22	−7.87	−3.82	−26.48	−23.46
23	−8.22	−5.46	−29.46	−30.67
24	−9.79	−5.48	−33.81	−32.72
25	−6.32	−4.44	−36.24	−35.53
26	−10.58	−10.00	−30.59	−29.71
27	−10.08	−5.49	−33.77	−30.71
28	−7.84	−6.13	−31.17	−28.62

at the hydrophobic Z site formed by Phe396, Pro398 and Leu399 amino acid residues.

Figure 3 displays that practically all compounds have a higher docking energy in TR than GR. The exceptions were compounds **12** and **19** that exhibit opposite behavior (high docking energy in GR). The compound **12** was localized in the entrance of the Z site. This compound has hydrophobic interactions with the amino acids Phe396, Pro398 and Leu399. In the GR, the compound **12** was not localized in the Z site and this compound formed three hydrogen bonds with the amino acids Arg37. This interaction could be responsible by the high docking energy for this compound in the human enzyme.

Compound **19** stayed in the Z site in the trypanothione reductase and was stabilized by two hydrogen bonds with Glu466 and Leu399 amino acids, respectively. In the human

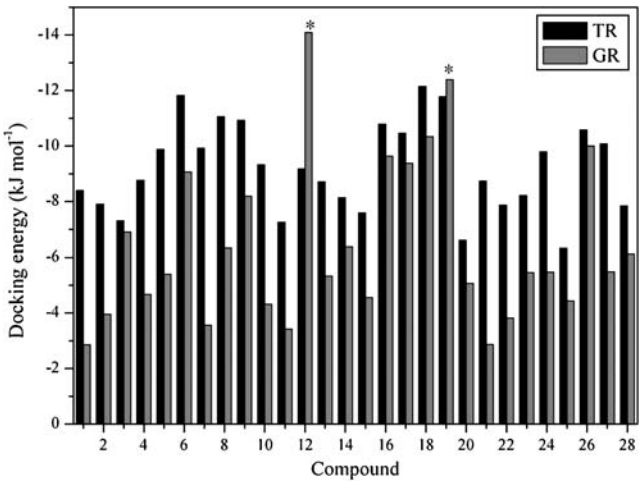


Fig. 3 Histogram with docking energies using FlexX program

enzyme, the compound **19** was also localized in the same way that observed with TR. Furthermore, this compound forms three hydrogen bonds with Glu472, Thr469 and Met406 amino acids. The number and likeness of hydrogen bonds that were observed in this case could be related to the slight energy difference between the docking energies in TR (−11.78 kJ mol^{-1}) and GR (−12.39 kJ mol^{-1}).

Autodock

According to Table 1, the docking energy of the quinones with an AutoDock ranges from −36.24 to −24.97 kJ mol^{-1} in TR and from −35.53 to −21.37 kJ mol^{-1} in GR. As showed in FlexX program, in the AutoDock program all of the quinone compounds stayed also in the same region near to the Z site formed by Phe396, Pro398 and Leu399 amino acid residues. This could be an indication that these quinone compounds have a good interaction with the amino acids.

Likewise in the energies obtained from FlexX, almost all compounds present a docking energy higher in TR than GR (Table 1 and Fig. 4). In the AutoDock, the exceptions were the compounds **20** and **23** that exhibited more favorable docking energies in the site of human enzyme, but the differences are very small. The compound **20** form one hydrogen bond with Thr66 in TR and none with amino acids of the Z site. In the human enzyme, this compound was also localized in the Z site in the same way that observed with TR, i.e., it forms one hydrogen bond with the Met406 amino acid. For the compound **23** either did not form any hydrogen bond with GR or TR.

From Fig. 3 and Table 1 we can see that compound **18** was the best docked according to FlexX program. Also, there are four amino acids surrounding this compound (Pro398, Leu399, His402 and Thr463, respectively) with distances lower than 3.5 Å and whose interactions are weak hydrogen

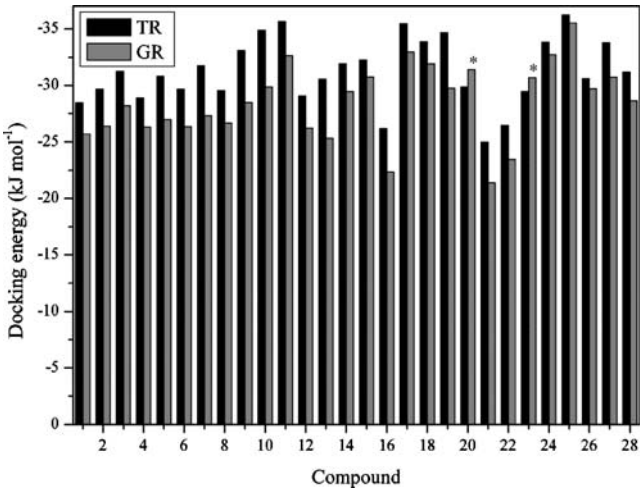


Fig. 4 Histogram with docking energies using AutoDock program

bonds of the kind CH···O and CH···N [33, 34]. By using the AutoDock program, we can see that compound **25** has the best docking energy (Fig. 4 and Table 1), and there are six amino acids (Lys62, Leu399, Met400, Ser464, Thr463, Glu466 and Glu467, respectively) surrounding this compound (distance lower than 3.5 Å), which can be stabilized by weak hydrogen bonds as compound **18** [33, 34].

In previous works [35, 36], we showed that four descriptors were important to discriminate these quinone compounds. The descriptors were: T_5 (torsion angle), QTS1 (sum of absolute values of the atomic charges), VOLS2 (volume of the substituent at region B) and HOMO-1 (energy of the molecular orbital below HOMO). These descriptors give information on the kind of interaction that occurs between the compounds and the biological receptor and are in agreement with the docking results obtained here. From Fig. 5, we can explain the importance of the molecular orbital HOMO-1 in our docking results, since this orbital should be a possible electron donor of quinone compounds. To demonstrate this, Fig. (5a) shows the orbital

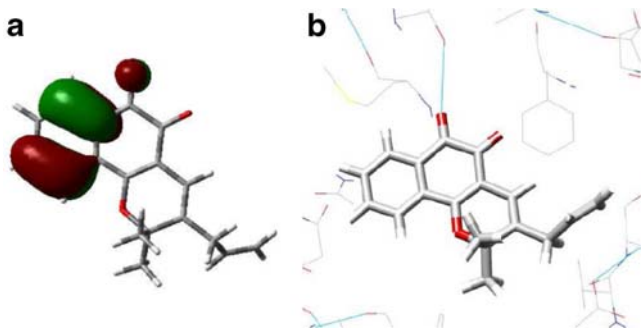


Fig. 5 **a**) Molecular orbital HOMO-1 for the compound 25 and **b**) Docking pose for compound 25

HOMO-1 for the compound 25 and Fig. (5b) the docking pose for the same compound. Thus, this result using chemometrics methods corroborates very well with our docking results.

It is worth saying that the most striking results of our docking studies was that despite the use of two different docking programs, AutoDock and FlexX, which use different scoring functions and search algorithms, practically all quinone compounds studied here were docked in the same region with both programs and the most important residues involved in the TR-quinones interactions include Lys62, Pro398, Leu399, Met400, Lys402, Thr463, Ser464, Glu466, and Glu467. These results are in agreement with previous work [11, 37] where inhibitor molecules bind in another cavity that is in the dimer interface, which is distinct from the active site, FAD (Flavin Adenine Dinucleotide) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate) binding sites.

In addition, the residues Thr66, Phe396, Pro398, Leu399 and Thr463 are conserved in different TRs. Moreover, the amino acids Leu399, Met400 and Lys402 are substituted in human enzyme by Met406, Tyr407 and Ala409, and could be explored in the design of new molecules in a more effective and selective way.

Molecular dynamics

In order to verify if the results obtained here by docking were a robust or a fortuitous result, we carried out a 2 ns molecular dynamics simulation with the compounds that presented the highest and lowest docking energy (compound **25** and **21**, respectively) with TR and GR according to AutoDock.

Analyses by Ligplot program [38] on the structure of TR enzyme with both compounds are presented in Fig. 6a and b, respectively. The result shows that compounds 21 and 25 make hydrogen bonding with Lys62. Therefore, there are four residues that interact with compound 21 by hydrophobic interactions (Pro398, Met400, Pro462 and Thr463). The compound 25 has nine hydrophobic interactions with the amino acids Val59, Ile107, Phe396, Pro398, Leu399, Met400, Ser464, Thr463 and Glu466. Thus, these interactions can be responsible for the high docking energy for compound 25 in TR enzyme.

The inspection of molecular dynamics (MD) shows that the binding mode of compound **21** is very similar before (Fig. 7a, blue) and after (Fig. 7b, gold) the simulation. The amino acids that are localized at a distance ≤ 5.0 Å showed little difference from their original positions. Only the amino acids Phe396, Pro398 and Leu399 displayed a more remarkable variation in their positions. It could be seen that the position adopted by this compound is very stable, since the hydrogen bonding formed between compound 21 and

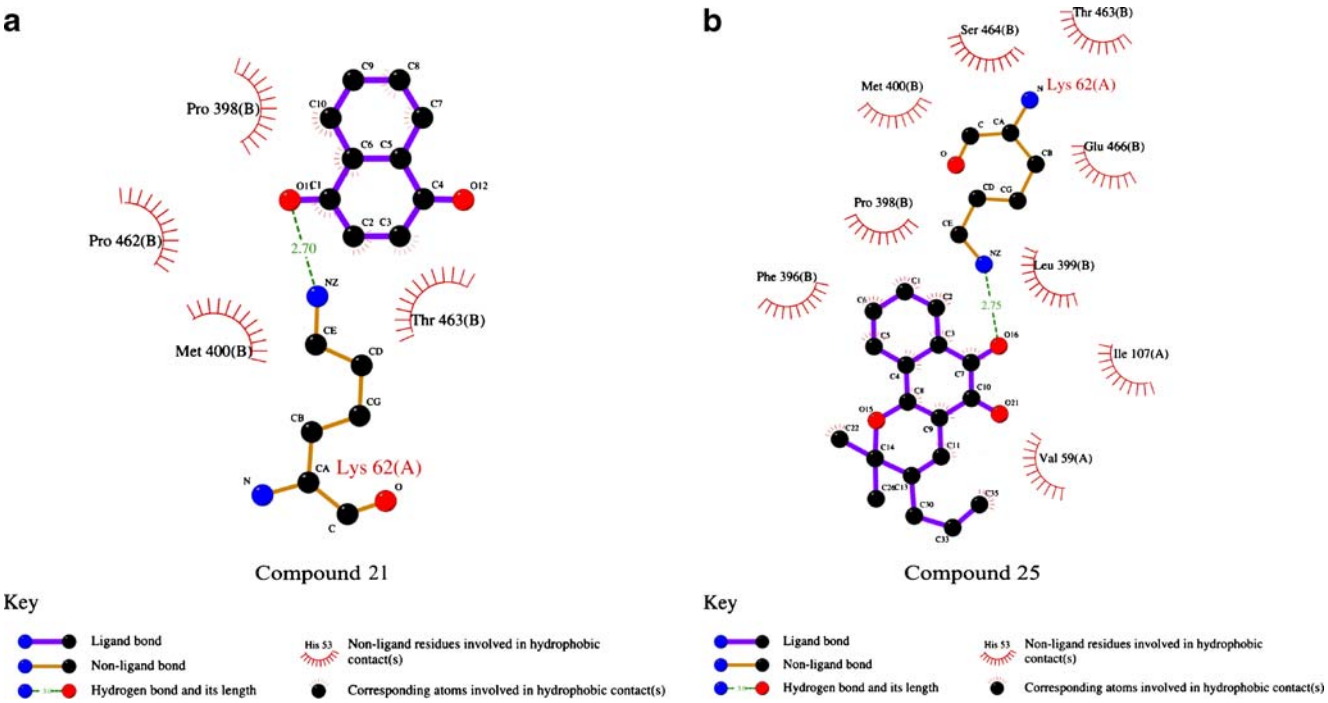


Fig. 6 Structure of compounds 21(6**a**) and 25(6**b**), respectively, using the Ligplot program to identify some specific contacts between atoms of these quinone compounds with the trypanotione reductase enzyme

the Lys62 is conserved along the whole simulation. Also, the RMSD (only for the heavy atoms) between the initial and final pose of compound **21** was very small (1.344 Å), confirming that the interactions presented by this compound with the Z-site are very strong.

Compound **25** adopted a similar arrangement in the first and in the last step of the MD simulation. In this case, the differences were more subtle since the main alteration occurs at the side chain of some amino acids. Also, compound **25** makes hydrogen bond with Lys62, which is preserved during the simulation. In addition, the final pose of compound **25** formed another hydrogen bond with Leu399. The latter observation, and the fact that the RMSD between the initial and final pose of compound **25** was slightly lower (1.259 Å), gives support to affirm that the pose observed in all quinone compounds studied here can be exploited carefully in the design of new inhibitors of trypanothione reductase.

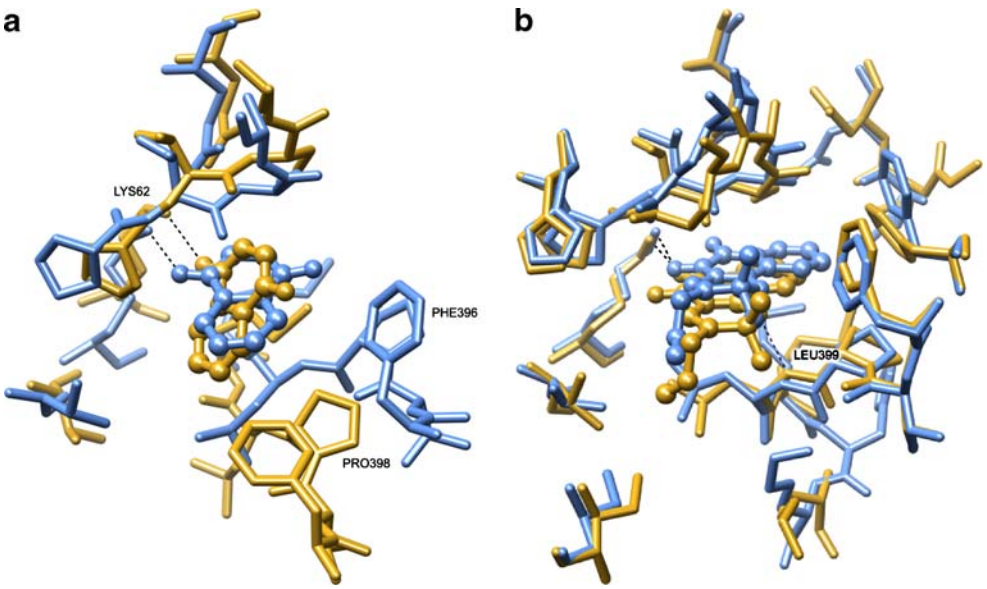


Fig. 7 Representation of the main interaction between compounds 21(7**a**) and 25(7**b**), respectively. The blue and gold models represent the initial and final pose, respectively. Hydrogen-bonded contacts are shown by dotted lines. For the sake of clarity, hydrogen atoms are not displayed

Conclusions

The docking studies carried out with two different programs (AutoDock and FlexX) showed that all quinone compounds studied in this work stayed in the same region in the trypanothione reductase. This region is a hydrophobic pocket near to the Z site formed by Phe396, Pro398 and Leu399 amino acid residues. Also, the docking energies in TR were higher than GR for practically all compounds.

The results described above are amazing, since crystallographic studies have been demonstrated that TR has a large binding site, which allows the ligands to adopt many different poses in the site of this enzyme. In spite of this, we were able to demonstrate that quinone, even being small compounds, binds in a hydrophobic pocket on the enzyme that is not utilized in the binding of natural substrate.

The molecular dynamics simulation with the quinone compounds that presented the highest and lowest docking energies showed that the *root mean square deviation* (RMSD) between the initial and final pose were very small. Furthermore, the hydrogen bond pattern was conserved along the simulation. It seems that the quinone compounds studied here present a strong interaction with the amino acids in the Z site and could be used as a starting point in the design of new trypanothione reductase inhibitors.

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