Chapter

# Development of New Drugs to Treat *Taenia solium* Cysticercosis: Targeting 26 kDa Glutathione Transferase

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## Abstract

*Taenia solium* causes neurocysticercosis, a parasitic infection of the central nervous system in humans. The costs of management, treatment, and diagnosis of patients with neurocysticercosis are high, and some patients do not respond to the currently available treatments. Helminth cytosolic glutathione transferases (GSTs) are essential enzymes involved in the regulation of immune responses, transport, and detoxification. In *T. solium*, three cytosolic GSTs with molecular masses of 26.5 (Ts26GST), 25.5 (Ts25GST), and 24.3 kDa (TsMσGST), classified as mu-alpha, mu and sigma GST-classes, respectively, constitute the main detoxification system, and they may be immune targets for the development of vaccines and new anthelmintics. We performed a successful virtual screen, and identified I7, a novel selective inhibitor of Ts26GST that showed a non-competitive inhibition mechanism towards substrate glutathione with a Ki of 55.7 mM and mixed inhibition towards the electrophilic substrate 1-chloro-2,4-dinitrobenzene with a Ki of 8.64 mM. Docking simulation studies showed that I7 can bind to a site that is adjacent to the electrophilic site and the furthest from the glutathione site. This new inhibitor of Ts26GST will be used as a lead molecule to develop new effective and safe drugs against diseases caused by *T. solium*.

**Keywords:** Glutathione transferase, Inhibitor, *Taenia solium*, Neurocysticercosis

## 1. Introduction

### 1.1 Neurocysticercosis

*Taenia solium* is a cestode parasite in humans. Adult parasites cause taeniasis, and the larvae cause cysticercosis. Larvae located in the central nervous system cause neurocysticercosis (NCC), with a wide spectrum of clinical manifestations that depend on factors such as the location, number of larvae, and the intensity of host immune response [1, 2]. The disease may be asymptomatic or present with nonspecific symptoms, such as epilepsy, cognitive impairment, migraine-type headache, intracranial hypertension, and neurological deficits, among other symptoms [3, 4].

### 1.2 Clinical spectrum

According to the location of the larvae, NCC is classified into parenchymal NCC and extraparenchymal NCC. In the parenchymal NCC, the most frequent symptoms are seizures, which can occur at any stage of the cysticercus (viable or calcified) [5], and neurological signs, such as sensory deficits, language, and gait disturbances, as well as involuntary movements. Such manifestations have been reported in up to 15% of patients [3]. In the extraparenchymal NCC, cysticerci are usually found in the subarachnoid and ventricular locations. Hydrocephalus is observed in a significant number of cases of subarachnoid NCC, and neurological alterations associated with the obstruction of the cerebrospinal fluid flow have been observed in patients with ventricular NCC; the blockage of the cerebral aqueduct due to the presence of cysticerci in the fourth ventricle may result in the loss of consciousness or even death [3, 6, 7].

### 1.3 Treatment

NCC is a disease transmitted by food, which causes many disability-adjusted life years. In Mexico, the cost of management, treatment, and diagnosis of patients with NCC was approximately U.S. $52 million in 2015 [8]. In addition to these costs, a study in Peru estimated that two-thirds of patients who develop symptoms lose their jobs, and the sequelae make it impossible for 60% of them to return to work [9]. Treatment should be individualized according to the characteristics of the disease and location of cysticerci, but in general, it consists of a mixture of surgical intervention (recommended for cases of intraventricular or spinal NCC), antiparasitic and anti-inflammatory drugs, and drugs for the management of symptoms [10]. The antiparasitic treatment for NCC includes praziquantel or albendazole. Praziquantel is a pyrazino-isoquinoline derivative that affects calcium channels on the parasite's surface and causes muscle contractions, paralysis, and tegument damage [11]. Maximum serum levels of praziquantel are obtained in 1.5–2 h after administration [12]. Praziquantel is metabolized in the liver, and its mild side effects include gastric disturbances, dizziness, drowsiness, fever, headache, increased sweating, and sometimes allergic reactions; however, these reactions disappear when the drug is withdrawn [13]. Albendazole is a benzimidazole compound that leads to the selective degeneration of cytoplasmic microtubules, affecting the formation of ATP, and glucose intake, which depletes parasite of the energy source [14]. Maximum serum levels of albendazole are achieved in 2 to 3 h after ingestion. This drug penetrates the cerebrospinal fluid better than praziquantel [15]. Side effects in humans are mainly related to liver toxicity (increased liver enzymes), hematological effects, hair loss, and general symptoms that dissipate when treatment is withdrawn [14]. The use of antiparasitic drugs can cause adverse effects arising from the inflammatory reaction induced when cysticerci are damaged; therefore, the use of corticosteroids in addition to treatment is recommended. However, prolonged use of corticosteroids increases the risk of opportunistic infections, skin disorders, depression, osteopenia, among others [13]. Several drugs, including benzimidazole, praziquantel and nitazoxanide, have been evaluated for their ability to control swine cysticercosis in animals intended for consumption. Of these, oxfendazole has been shown to have close to 100% efficacy after a single dose in intramuscular cysticercosis, but the efficacy was lower in swine neurocysticercosis [16].

### 1.4 New drugs

Patients who do not respond to therapy with the currently available drugs have been reported. Several factors have been proposed that may be involved in this

lack of sensitivity to the treatment: differential response according to the state of development of cysticerci, low penetration of the drug into the subarachnoid space, variability of albendazole sulfoxide levels in plasma in individual patients, or interference of corticosteroids with the activity of anti-helmintics [17, 18]. This has led to the search for new drugs that could improve the effectiveness of the anti-helminthic therapy. Therefore, cytosolic glutathione transferases (cGSTs) have been selected as targets for the development of vaccines and drugs against this parasite [19–22].

## 2. Overview of glutathione transferases

### 2.1 The catalytic reaction

GSTs (EC 2.5.1.18) are a multiprotein family highly expressed in all cells [23, 24]. They are part of phase II detoxification process and catalyze the conjugation of glutathione to a variety of endo- and exo-electrophilic substrates [25]. This conjugation produces soluble compounds and substrates for cellular export proteins, such as P-glycoprotein and multidrug resistance-related protein 1 [26]. The general reactions (GSH + RX → GSR + HX) comprise a nucleophilic attack, aromatic substitution, epoxide ring opening, reversible Michael addition, isomerization or peroxidation. Although nucleophilic attack can also be directed to nitrogen atoms in nitrate esters, sulfurs in organic thiocyanates or disulfites, and oxygen in organic hydroperoxides [25, 27–29].

### 2.2 Cellular distribution and GST classes

GSTs can be grouped into three subfamilies according to their cellular location: mitochondrial GSTs, microsomal GSTs or MAPEGs (membrane-associated proteins in eicosanoid and GSH metabolism), and cytosolic or canonical GSTs. In humans, genes encoding all expressed GSTs from a given subfamily are clustered on the same chromosome [30]. The mitochondrial GST subfamily includes a unique kappa (K) class. This class has very high peroxidase activity, and its location suggests an important role in β-oxidation of fatty acids and in lipid peroxidation. Moreover, it is also a key regulator of adiponectin biosynthesis and may function as a chaperone [31–34]. Microsomal GSTs are divided into four groups (I–IV). They share less than 20% sequence identity and are involved in eicosanoid metabolism, such as the synthesis of prostaglandins, thromboxanes, leukotrienes (inhibitors of inflammation), glutathione metabolism, and activation of some lipoxygenases [33, 35–37]. In the subfamily of cGSTs, members of the same class have more than 40% amino acid sequence identity, whereas sequence identity between classes is below 25%. cGSTs are divided into: (1) organism-specific GST classes, which include several GSTs, such as lambda (L), phi (F), and tau (U) in plants; delta (D), epsilon (E) in insects; beta (B) in prokaryotes; and 2) ubiquitous classes in any organism, including mu (M), alpha (A), pi (P), theta (T), sigma (S), zeta (Z), and omega (O) classes. Each of them displays distinct catalytic and non-catalytic binding properties, and their functions are very versatile and involve detoxification, signal modulation, catabolism of aromatic amino acids, ion channel modulation, chemotherapy resistance, prostaglandin and steroid hormone synthesis, and transport of molecules such as bilirubin, heme, steroids, hormones, and bile salts [25, 27, 29, 31, 33, 38–43].

### 2.3 Structural characteristics

All cGSTs are dimers with 24–27 kDa monomeric subunits containing ~250 amino acid residues on average. They share the same tertiary and quaternary

structures, and each subunit has two distinct functional domains. The first domain is the G site, which is located at the N-terminal region and is responsible for GSH binding. This domain is highly conserved in all classes and has a thioredoxin-like fold constructed by three helices and four sheets (βαβαββα). Activation of GSH occurs at the G-site by different amino acids, depending on the class, and is either a tyrosine (Y) found in M, P, A, and S-classes, a serine (S) found in T, Z, F, U, and D-classes, or a cysteine (C) to O, and B-classes. The activation allows a nucleophilic attack on the electrophilic compounds, allowing conjugation or thiol transfer. The first two amino acids, tyrosine and serine, promote the formation and stabilization of the thiolate anion of GSH, lowering its pKa to 6.2. This is achieved through hydrogen bond donation of the hydroxyl group, which makes GSH ready for conjugation. The C residue is used for thiol transfer, and it forms mixed disulfides with GSH. The N-terminal domain consensus sequence SNAIL/TRAIL is localized in the region between residues 68 and 77, and appears in all mammalian cGSTs [25, 29, 31, 44, 45]. The second domain is the H site, which is localized in the C-terminal region. This domain binds the electrophilic substrate, and it is constituted exclusively by α-helices. The number of helices varies from four to seven, depending on the class. This variation has been used to explain the wide range of electrophilic substrates for detoxification and specificity among classes. For example, the M-class has very efficient catalysis with molecules containing oxiranes and unsaturated carbonyl groups, whereas A-class acts on 4-hydroxyalkenals and peroxides [20, 25, 31, 33, 45]. Although GSTs do not present specificity for their hydrophobic substrates, they seem very specific for the γ-glutamyl portion of GSH, and there is evidence that a peptide portion in the conjugate binds to ATP pumps or the multidrug resistance-associated proteins to be exported [46, 47]. Furthermore, in these domains, there are also conserved motifs that identify GST classes. For example, the primary and secondary structures that form the mu-loop or α9-helix are characteristic of M, and A-GST classes [20, 45, 48].

### 2.4 Alternative functions of GSTs

Besides their catalytic role, ligandin activity has been identified in GSTs because they bind toxic non-substrate ligands, such as hemin, bilirubin, bile salts, steroids, thyroid hormones, fatty acids, drugs (albendazole and praziquantel), and members of the MAPK protein kinase family, which are involved in processes such as the production, storage, and rapid transport of prostaglandins out of cells, intrinsic and acquired drug resistance, cell survival and apoptosis, contributing to passive detoxification or intracellular transport in cells. The ligandin site is different from the G and H sites, and the above-mentioned toxic non-substrates are able to inhibit the catalytic activity of GSTs [49–52]. Another striking property of the GST enzyme is its translocation from the outside to the inside of various cells. This internalization occurs through endocytosis mediated by receptors or by the GST-fold structure, and it is independent of GST function as an enzyme [53, 54].

### 2.5 GSTs in platyhelminthes

In these parasites, GSTs also act as xenobiotic detoxifying enzymes, catalyzing conjugation of GSH (active detoxification) or, in the case of ligandin, transporting toxic substrates (passive detoxification) and acting as protective antigens to the host [23, 55]. Finally, many reports on vaccination experiments have described reductions in parasite burden, fecal egg counts, tissue egg densities, and female fecundity in experimental cysticercosis, schistosomiasis, and fascioliasis [23, 56–60]. The World Health Organization has recommended the use of *Schistosoma japonicum*

GST (SjGST) as a vaccine antigen in the form of a DNA vaccine (pcDNA/sjGST) in nanoparticles combined with pIL-12 [61, 62].

### 2.6 GSTs in *T. solium*

In the cestode *T. solium*, GST activity has been identified in the microsomal fraction, and it was noncompetitively inhibited by triphenyltin chloride and bromosulfophthalein [63]. Moreover, three cGSTs classes have been identified according to the classification of mammalian GSTs [20]: (i) a moderately abundant S-class GST denoted as TsMσGST, (ii) the least abundant M-class GST named Ts25GST (previously referred to as SGSTM1), which has a high capacity to conjugate reactive carbonyls, the secondary products of lipid peroxidation, and (iii) the most abundant M and A-class GST named Ts26GST (previously referred to as SGSTM2). The characteristics and properties of these enzymes are listed in **Table 1**.

The specific antibodies produced against each TsGST (TsMσGST, Ts25GST, and Ts26GST) showed that they are not antigenically related to each other, nor to trematode, cestode, or mammalian GSTs [19, 20, 64]. Interestingly, these specific antibodies recognized the homologous GST class in *T. saginata*, *T. taeniaeformis*, and *T. crassiceps*. On the other hand, immunizations of a murine model of cysticercosis with the SGSTF fraction purified from cysticerci (comprising both Ts25GST and Ts26GST) or with recombinant Ts26GST alone were highly effective in reducing cysticerci load by 90% and 74%, respectively, whereas the use of the native and recombinant Ts25GST as immunogens afforded lower protection rates, 46% and 25%, respectively [19].

The aforementioned result as well as the known lack of catalase and low activities of CYP450 and glutathione peroxidase have led us to postulate that GSTs are the major detoxification system for this parasite. In addition, the properties of cGSTs as immunogens and vaccination candidates make them attractive targets for the development of new drugs against this parasite [19, 20, 22, 64].

Anti-helminthic compounds such as mebendazole and praziquantel inhibited Ts26GST and TsMσGST *in vitro*, but they did not reach plasma concentrations *in vivo* that would allow effective inhibition of enzyme activity [20, 64, 65, 66]. To date, a

| | **TsMσGST** | **Ts25GST** | **Ts26GST** |
|---|---|---|---|
| Number of amino acids | 212 | 219 | 221 |
| Molecular mass (Da) | 24,290 | 25,496 | 25,936 |
| Number of isoforms | 4 | 2 | 4 |
| Isoelectric point | 8.2–8.7 | 5.7–6.3 | 7.2–8.5 |
| $V_{max}$(CDNB) (μmol $min^{-1}$ $mg^{-1}$) | 1.08 | 12.0 | 51.5 |
| $K_M$(CDNB) (mM) | 0.16 | 1.38 | 1.06 |
| $V_{max}$(GSH) (μmol $min^{-1}$ $mg^{-1}$) | 0.78 | 10.2 | 39.9 |
| $K_M$(GSH) (mM) | 0.17 | 0.905 | 0.20 |
| Classes of effective inhibitors | A, M | A, M | A, M |
| Optimal conditions | pH 8.0, 40 °C | pH 8.0, 37–40°C | pH 7–5, 37–40°C |
| Main location | Scolex | Tegument, and parenchyma | Tegument, and parenchyma |

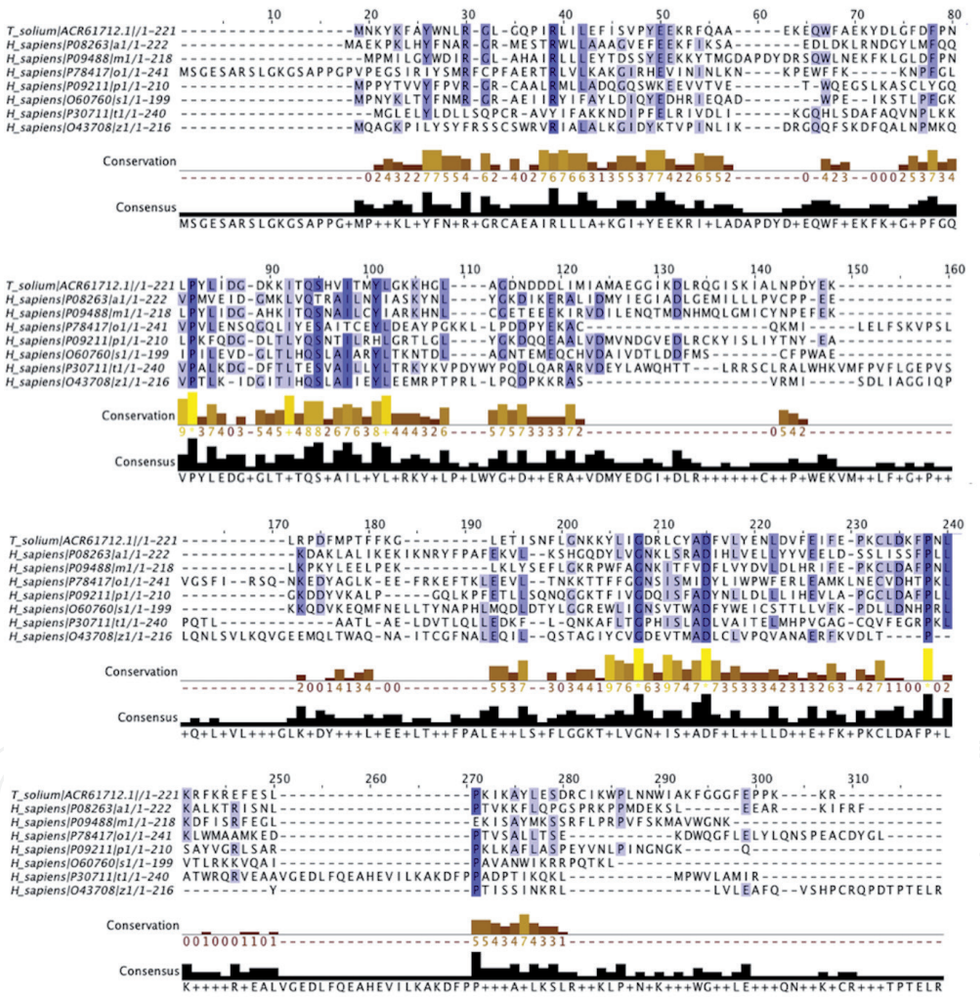
**Table 1.**
*Cytosolic glutathione transferases from* Taenia solium.

non-toxic inhibitor for GST has not been developed, but ethacrynic acid, haloenol lactone, disulfiram, and curcumin are potent inhibitors of human GST-P1 [67, 68]. A new generation of drugs, such as modified ethacrynic acid, γ-glutamyl-S-(benzyl) cysteinyl-R(−)-phenyl glycine diethyl ester (TER 199), and prodrug (TER 286), provide a starting point for development of novel powerful and specific inhibitors against human GST-P1. However, the clinical side effects have limited their application [24].

## 3. Kinetic and structural properties of Ts26GST

### 3.1 Kinetic mechanism of Ts26GST in the CDNB conjugation reaction

Ts26GST is a bisustrate enzyme that exhibits a higher affinity for glutathione (GSH) than for 1-chloro-2,4-dinitrobenzene (CDNB), unlike other two cGSTs of




Percent Identity Matrix (created by Clustal 2.1

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1: T_solium\|ACR61712.1\| | 100.00 | 26.79 | 41.78 | 17.22 | 26.47 | 24.34 | 16.84 | 16.36 |
| 2: H_sapiens\|P08263\|a1 | 26.79 | 100.00 | 23.76 | 22.40 | 28.99 | 25.26 | 16.32 | 21.76 |
| 3: H_sapiens\|P09488\|m1 | 41.78 | 23.76 | 100.00 | 19.08 | 28.71 | 25.93 | 19.25 | 14.56 |
| 4: H_sapiens\|P78417\|o1 | 17.22 | 22.40 | 19.08 | 100.00 | 20.59 | 21.05 | 18.65 | 25.87 |
| 5: H_sapiens\|P09211\|p1 | 26.47 | 28.99 | 28.71 | 20.59 | 100.00 | 24.48 | 19.57 | 17.72 |
| 6: H_sapiens\|O60760\|s1 | 24.34 | 25.26 | 25.93 | 21.05 | 24.48 | 100.00 | 19.10 | 22.78 |
| 7: H_sapiens\|P30711\|t1 | 16.84 | 16.32 | 19.25 | 18.65 | 19.57 | 19.10 | 100.00 | 25.28 |
| 8: H_sapiens\|O43708\|z1 | 16.36 | 21.76 | 14.56 | 25.87 | 17.72 | 22.78 | 25.28 | 100.00 |

**Figure 1.**
*Alignment of the amino acid sequences of Ts26GST with representatives of different human GST classes. The percent identity matrix shows that Ts26GST is most related to human M-class GST (m1).*

*T. solium* (see **Table 1**). Furthermore, the kinetic curves for both substrates showed positive cooperativity, indicating that the binding of the first substrate stabilizes the right conformation of Ts26GST to bind the second substrate [21]. This positive cooperativity, previously described for the GSTs of *P. falciparum* and in classes P1 and Z1 of mammals, allows the parasite to adapt to changes in the amounts of toxic molecules secreted by the host's immune cells or induced by oral drugs, and to inactivate them through efficient processing of these substrates [69].

Kinetic analyses performed at different concentrations of GSH and CDNB produced intersecting double-reciprocal plots that provided evidence of ternary complex formation during enzymatic conjugation [70]. Furthermore, because the intersection occurred on the abscissa, the mechanism proceeds through the random sequential binding of co-substrates [71].

To determine the GST class to which Ts26GST belongs, various class marker substrates and inhibitors were tested. Ts26GST conjugates the A-class markers cumene hydroperoxide and ethacrynic acid better than the M-class marker 1,2-dichloro-4-nitrobenzene [20]. However, Ts26GST is more sensitive to the M-class inhibitors cibacron blue and triphenyltin chloride than to bromosulfophtalein, an A-class inhibitor. This enzyme is also sensitive to the anthelminthic mebendazole, displaying a non-competitive inhibition pattern, which suggests that at least two molecules bind to Ts26GST [21].

### 3.2 Structural similarity of Ts26GST to human cGSTs

Multiple amino acid sequence alignments of Ts26GST with all classes of human cGSTs are shown in **Figure 1**. It can be seen from the percent identity matrix that the primary structure of Ts26GST is closely related to M-class (42% sequence identity) and A-class (27% sequence identity) but is more distant from other human GST classes. The G-site of Ts26GST belongs to class Y, with Y8 being the catalytic residue that activates GSH. This site also has the essential conserved residues for γ-glutamyl binding: P(57), Q(68), and S(69). The last two residues are part of the (Q)SHVIT sequence, which in mammalian GSTs constitutes the consensus motif (Q)SNAIL /(Q)TRAIL. Notably, amino acid variation in this consensus motif is one of the markers for distinguishing between mammalian and

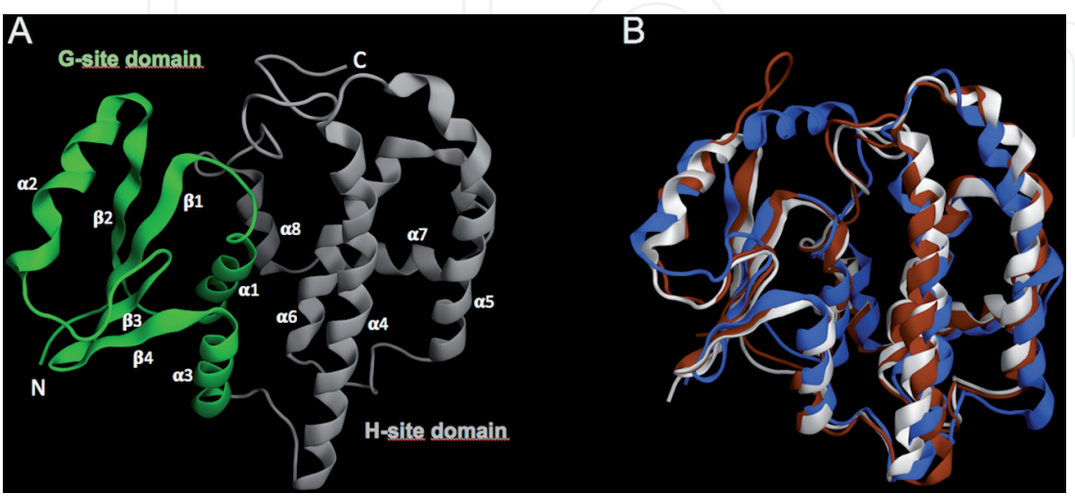



**Figure 2.**
*Modeled structure of Ts26GST. (A) The domain with the site where glutathione binds, is highlighted in green, and the domain with the hydrophobic site, to which electrophilic substrates bind, is highlighted in gray. (B) The structure of Ts26GST in white is compared to human A-class GST structure (blue) and M-class structure (brown).*

parasite cGSTs [20]. Ts26GST has ligandin activity and is internalized by macrophages, suggesting an important role in transport and the parasite–host relationship [72, 73].

A homology model for Ts26GST was built from the structure of *Fasciola hepatica* M-class GST (PDB ID 2FHE), whose sequence has 47% identity, with 96% query coverage [70]. The analysis of this model with PROCHECK showed that 91.5% of residues are in favored regions in the Ramachandran plot, with no residues in the disallowed region. In addition, verification with ERRAT yielded an Overall Quality Factor of 93.55 and the Verify3D score was 95.18. A comparison of the Ts26GST model with M and A-class human GST structures is shown in **Figure 2**. It is clear that Ts26GST does not have the classical mammalian mu-loop or the canonical α9-helix observed in A-class GSTs.

## 4. Structure-based discovery of Ts26GST selective inhibitors

### 4.1 Search and selection of cavities with non-conserved residues as potential targets

Knowing the structure of the target whose activity we wish to inhibit is an essential step for the discovery and optimization of specific inhibitors. Furthermore, if the target is a parasitic enzyme, and the host has orthologs, knowing and comparing their structures allows us to take advantage of their differences and design more specific inhibitors [74]. Different strategies have been used to find appropriate inhibitors, and we decided to look for a non-competitive inhibitor that cannot be displaced by excess substrate, i.e., the one that would not bind to either the G-site or the H-site. Thus, we focused our search on the area of the dimer interface, trying to find a site whose occupation would alter the architecture of at least one of the substrate sites and prevent catalysis. Furthermore, the binding of a molecule in this interfacial region could destabilize the quaternary structure of this enzyme, which is only active as a dimer. Likewise, we assumed that the site has a predominantly hydrophobic surface and contains a considerable fraction of non-conserved residues with respect to its human orthologs. Using the MOE's Site Finder tool [75], we found only one site that met all these requirements; its location is shown in **Figure 3**.

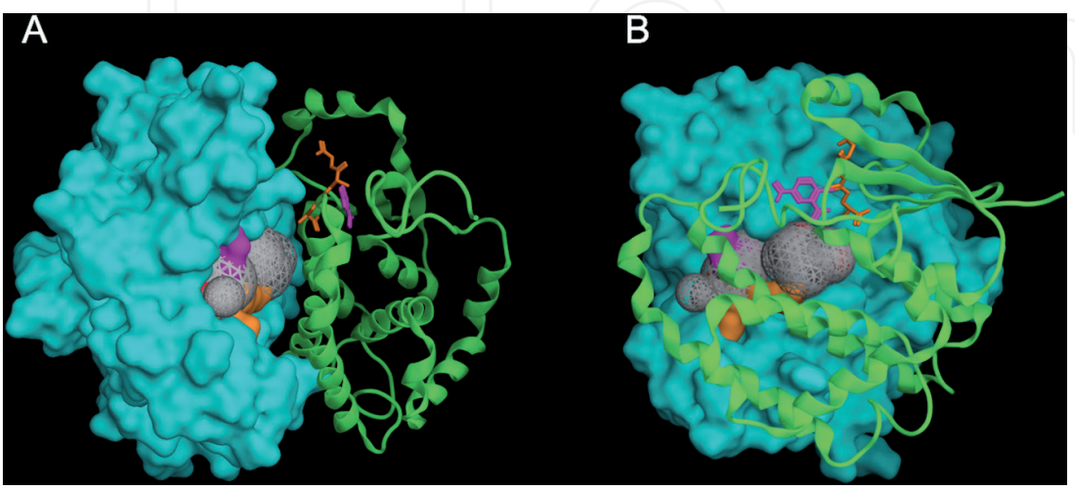



**Figure 3.**
*Putative binding site for TS26GST inhibitors whose occupancy could produce non-competitive inhibition. Just one subunit is represented with van der Waals surface for clarity. Bound GSH and CDNB molecules are shown in orange and magenta, respectively, whereas the spheres represent the space that the ligand could occupy.*

### 4.2 Virtual screening with a commercial diverse library set

Once a potential inhibitor binding site has been located, we must find molecules that conform to its surface and interact favorably to form stable complexes. To explore how to cover this site in the chemical space, we used Enamine's library of non-redundant organic compounds called the Discovery Diversity Set, which consists of 50,240 drug-like compounds, and performed virtual screening using AutoDock Vina [76]. The scores of the best candidates were verified using MOE's Dock Tool [75].

### 4.3 Assortment of candidates

The best putative binders for Ts26GST were selected using the conventional criteria: the highest docking scores, the highest number of hydrogen bonds, and Lipinski's rule of five [77], but in addition, those ligands were prioritized that established the lowest number of contacts with conserved residues in relation to human GSTs. The best 23 candidates are shown in **Figure 4** and their docking scores obtained using AutoDock Vina and MOE are given in **Table 2**.

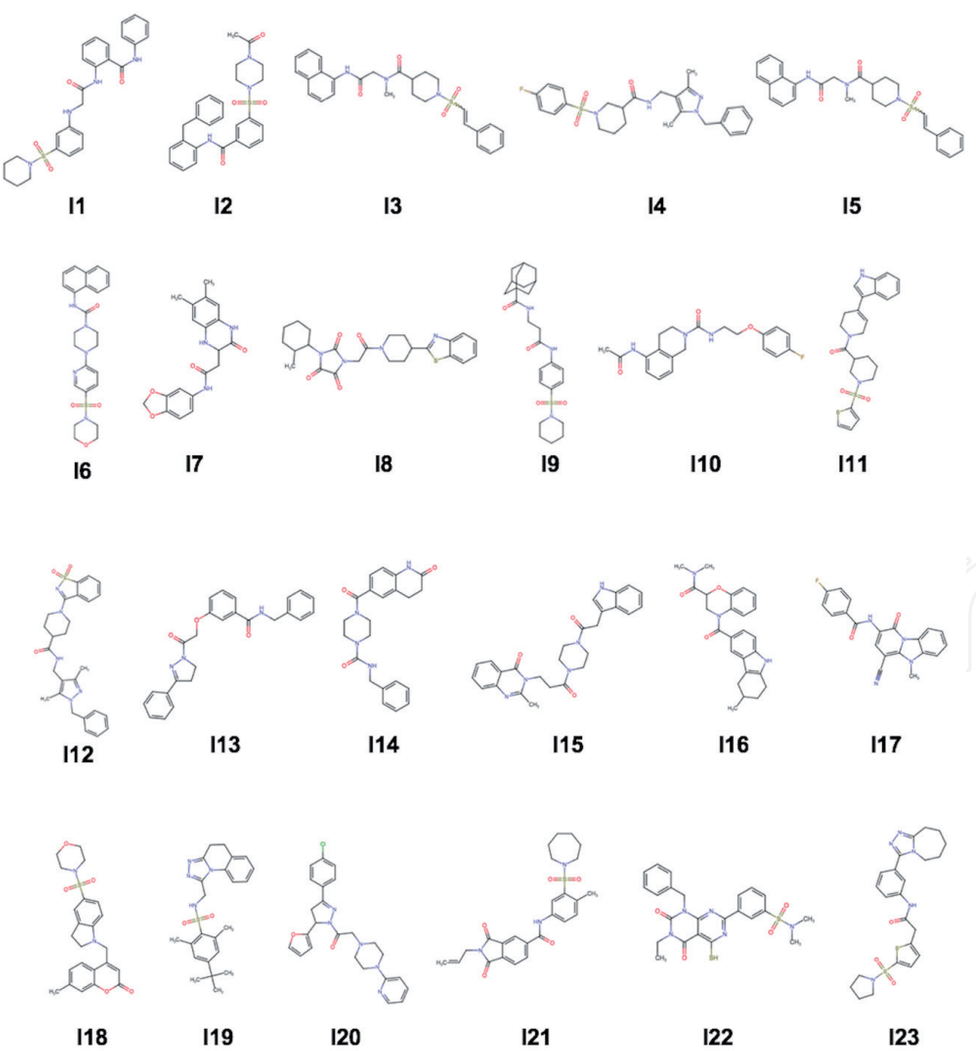



**Figure 4.**
*Best candidate inhibitors found by virtual screening.*

| Compound | Enamine ID | Chemical formula | Docking score VINA/MOE | *Residual activity (%) |
|---|---|---|---|---|
| I1 | Z30971507 | $C_{26}H_{28}N_4O_4S$ | −9.6/−11.1 | 92 |
| I2 | Z26762460 | $C_{26}H_{27}N_3O_4S$ | −10.0/−10.4 | 129 |
| I3 | Z27434387 | $C_{22}H_{21}FN_4O_3S_2$ | −9.4/−10.1 | 98 |
| I4 | Z109818646 | $C_{25}H_{29}FN_4O_3S$ | −9.4/−11.0 | 120 |
| I5 | Z27205337 | $C_{27}H_{29}N_3O_4S$ | −9.5/−10.3 | 82 |
| I6 | Z235256042 | $C_{24}H_{27}N_5O_4S$ | −10.0/−10.3 | 74 |
| I7 | Z56803795 | $C_{19}H_{19}N_3O_4$ | −9.9/−9.7 | 30 |
| I8 | Z98069587 | $C_{24}H_{28}N_4O_4S$ | −9.9/−9.7 | 107 |
| I9 | Z51980171 | $C_{25}H_{35}N_3O_4S$ | −9.5/−10.8 | 76 |
| I10 | Z744434314 | $C_{20}H_{22}FN_3O_3$ | −9.4/−10.2 | 135 |
| I11 | Z231257554 | $C_{23}H_{25}N_3O_3S_2$ | −9.5/−10.0 | 83 |
| I12 | Z109816768 | $C_{26}H_{29}N_5O_3S$ | −9.9/−9.8 | 117 |
| I13 | Z225448008 | $C_{25}H_{23}N_3O_3$ | −9.4/−10.1 | 55 |
| I14 | Z283658802 | $C_{22}H_{24}N_4O_3$ | −9.7/−8.8 | 115 |
| I15 | Z512929356 | $C_{26}H_{27}N_5O_3$ | −9.8/−9.3 | 114 |
| I16 | Z131580092 | $C_{25}H_{27}N_3O_3$ | −9.7/−9.9 | 108 |
| I17 | Z90661741 | $C_{20}H_{13}FN_4O_2$ | −9.8/−8.5 | 116 |
| I18 | Z134793448 | $C_{23}H_{24}N_2O_5S$ | −9.9/−10.3 | 66 |
| I19 | Z317185036 | $C_{23}H_{28}N_4O_2S$ | −9.9/−10.0 | 113 |
| I20 | Z30996502 | $C_{24}H_{24}ClN_5O_2$ | −9.4/−10.6 | 72 |
| I21 | Z26496603 | $C_{25}H_{27}N_3O_5S$ | −9.9/−11.5 | 78 |
| I22 | Z55180729 | $C_{23}H_{23}N_5O_4S_2$ | −9.6/−9.9 | 88 |
| I23 | Z30414302 | $C_{23}H_{27}N_5O_3S_2$ | −9.7/−10.0 | 82 |

*Residual activity of 5 μg of recombinant Ts26GST in presence of 100 μM of potential inhibitors.

**Table 2.**
*Docking scores of the predicted potential inhibitors determined using Vina and MOE_Dock. The inhibitory capacity of the compounds was determined by measuring the enzymatic activity of T26GST in the presence of each compound at a concentration of 100 μM, with 5.0 mM GSH and 2.5 mM CDNB. The reaction rate was monitored by ultraviolet–visible absorption at 340 nm and compared with that obtained in the absence of the compound (100% activity).*

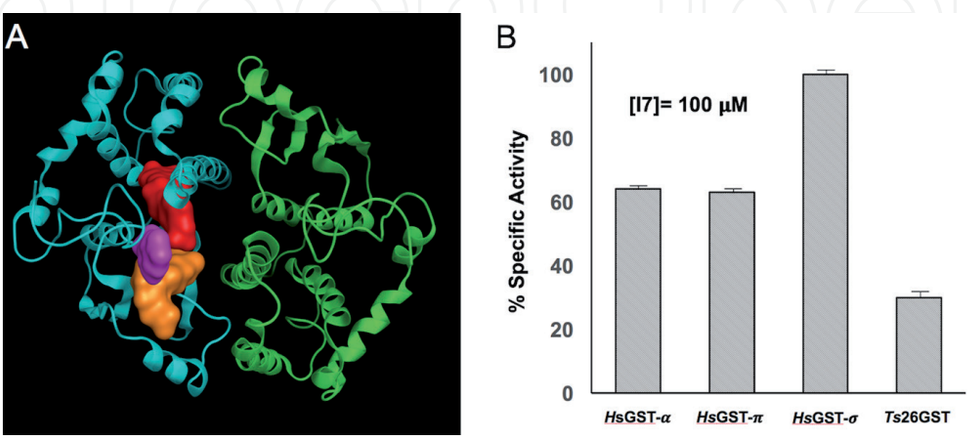



**Figure 5.**
*Relative position of the substrates GSH (orange) and CDNB (magenta), and the inhibitor I7 (red) in the structure of Ts26GST. (A) This figure was obtained by the superposition of the crystallographic structures of the complex M-class GS-DNB-HsGST (PDB ID: 1XWK) with the modeled complex of Ts26GST-I7, hiding the protein chain of the human GST. (B) Percent residual activity of Ts26GST and three human GSTs in the presence of 100 μM I7.*

### 4.4 *In vitro* assay of selected compounds with the best scores

The twenty-three compounds previously identified as potential ligands of Ts26GST were purchased and tested for their inhibitory activity using *in vitro* enzymatic assays. **Table 2** shows the residual activity obtained with 5.0 μg of recombinant Ts26GST in the presence of each compound at a concentration of 100 μM. I7 was the best Enamine compound that inhibited enzymatic activity of Ts26GST by 70%. **Figure 5A** shows the location of the I7 binding site, as derived from the docking protocol. We also tested the inhibitory effect of I7 on several human GSTs and observed that it had much smaller or no effect (**Figure 5B**).

## 5. Conclusions

Human NCC caused by *T. solium* larvae can be asymptomatic, disabling, and sometimes fatal. Currently, its diagnosis and treatment are expensive, and the approved drugs have associated unwanted effects. The search for the essential targets in *T. solium*, such as GST, and the methodology used to obtain the inhibitor I7 and its derivatives, shows that it is possible to develop safe, specific, and effective drugs that will contribute to eradicating this parasite. We are currently working on the crystallization of Ts26GST and site-directed mutagenesis to verify the location of the I7 binding site.

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## Conflict of interest

The authors declare that they have no known conflict of interest.

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