

Triosephosphate Isomerase from *Mycobacterium tuberculosis* as Potential Target to Develop a New Anti-TB Drug

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Abstract: Tuberculosis (TB) is possibly the most prevalent infectious disease in the world, reports from the World Health Organization (WHO) indicate that TB is one of the top 10 causes of death and an estimated 10 million people worldwide, in addition, there are increasing the TB resistant to conventional antibiotics, multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). Lastly, TB has become more important and requires more attention since it has been proposed as a risk factor for the severity of COVID-19. Therefore, the need to develop new anti-TB drugs. In this study, we propose to use the glycolytic enzyme triosephosphate isomerase from *Mycobacterium tuberculosis* (MtTIM) as a therapeutic target against TB. The triosephosphate isomerase (TIM) is a target used in different proposals to develop new drugs against different organisms. The MtTIM is an extremely attractive drug target due to the characteristics of its amino acids sequence. In addition, it has been determined that this enzyme (MtTIM) is necessary for the viability of *in vitro* and *in vivo* cultures of *Mycobacterium tuberculosis*. In this way, using the MtTIM as a therapeutic target, we propose potential compounds against MtTIM by molecular docking.

Keywords: MtTIM inhibitors; triosephosphate isomerase; docking; *Mycobacterium tuberculosis*.

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1. Introduction

Tuberculosis (TB), caused by the bacillus *Mycobacterium tuberculosis*, is possibly the most prevalent infectious disease globally. The World Health Organization (WHO) indicates that TB is in the top ten causes of death and an estimated ten million cases worldwide per year, which shows a challenge for public health services. In addition, other increasing factors that favor TB, such as the development of *M. tuberculosis* resistant to conventional antibiotics, multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) [1-4], for which there are several anti-tuberculosis drugs [5, 6].

Lately, TB has become more important since pulmonary TB as a risk factor for the severity of COVID-19 has been identified, in addition to the fact that additivity or synergy can occur between the two diseases in their pathological processes [7-13].

Therefore, it demonstrates the need to improve the therapeutic options and develop more efficient drugs against *M. tuberculosis*. Currently, there are different proposals for the development of drugs using new therapeutic targets against this disease [6, 14, 15]. An example is the development of enzyme inhibitors; against cytochrome bd oxidase [16], probable cation-

transporting ATPase F (CtpF) [17], zinc metalloprotease 1 (Zmp1) [18], α -subunit of tryptophan synthase (α -TRPS) [19], decaprenylphosphoryl-beta-D-ribose oxidase (DprE1) [20], cytochrome bc1 complex cytochrome b subunit (QcrB) [21], and in this study is proposed the glycolytic enzyme, triosephosphate isomerase (TIM).

TIM is an enzyme that participates in glycolysis and gluconeogenesis, carrying out the interconversion between glyceraldehyde-3-phosphate and dihydroxyacetone phosphate [22], different proposals focus on TIM from different organisms to develop new drugs [23-29]. Hence, TIM from *Mycobacterium tuberculosis* (MtTIM), is one of the key enzymes of the glycolytic pathway, making it an attractive drug target [30, 31]. It has been shown that the functions of this enzyme influence the process of glycolysis and gluconeogenesis and that this enzyme (MtTIM) is necessary for the viability of *in vitro* and *in vivo* cultures [32].

In this study, MtTIM is proposed as a therapeutic target to develop anti-TB drugs, in which molecular docking is carried out using a library of 1772 bioactive agents that contain compounds with interaction in different proteins. In addition, is evaluated the interaction with a compound reported with inhibitory effect on TIM from *Entamoeba histolytica* (EhTIM) [29]; in this way, the MtTIM is evaluated for that it can be a new therapeutic target, as well as compounds with inhibitory potential are proposed.

2. Materials and Methods

2.1. Preparation of receptor protein and selection of binding sites.

Atomic coordinates were obtained from the Protein Data Bank [33], triosephosphate isomerase of *Mycobacterium tuberculosis* (MtTIM) and Homo sapiens (HsTIM) structures (PDB codes 3TA6 and 4POC, respectively), were used for molecular docking using Molecular Operating Environment (MOE) following procedures previously reported [34-36]. The potential binding sites were determined using a “site finder” in MOE, which identifies regions of high probability to interact and selects preferentially hydrophobic sites [37-39].

2.2. Compound library used for molecular docking.

The Bioactives Collection Stock screening library (Chembridge Corp.- Hit2Lead.com [40, 41]) was used for molecular docking. This collection of bioactive molecules contains 1772 compounds to evaluate the interaction with MtTIM, and we used the D4 compound with effect on triosephosphate isomerase of *Entamoeba histolytica* [29].

2.3. Molecular docking.

For molecular docking, 18 potential binding sites were used for MtTIM and were generated up to 100 conformers from each compound to interact (compound library against MtTIM), following procedures previously reported [34, 36]. The high-throughput molecular docking was carried out by the software MOE, and the analysis of ligand interaction per amino acid at MOE and Protein-Ligand Interaction Profiler [39, 41-43].

2.4. Selection of the best five compounds.

To select the best five compounds, the results of up to 30 conformers from each compound were used to select them. It was determining the binding free energy ($\Delta G_{\text{binding}}$) of each complex (Ligand-Protein), as previously reported [34, 36] using MOE [44, 45]. With

these results, the best averages of ΔG binding were determined between MtTIM and each compound and the standard deviation for each one using the Excel software (Microsoft-365). The better compounds selected were evaluated in HsTIM to discuss the selectivity only for MtTIM. In addition, it shows the description of chemical properties by PhysChem - ACD/Labs [46] and the theoretical toxicity (carcinogenicity and mutagenicity) [47-49].

3. Results and Discussion

3.1. Selection of compounds by Molecular Docking.

In this study were determined the potential interaction of the Bioactives Collection Stock screening library from Chembridge Corp. (1772 compounds) [40, 41] and D4 compound in MtTIM for this were generated up to 100 conformers of each compound to interact in the 18 potential binding sites (Figure 1) by molecular docking [34, 36]. The selection criteria for the best five compounds was based on the calculation of the average of $\Delta G_{\text{binding}}$ of each compound (Table 1). Using the best five values of their conformers of each compound, were determined the average of $\Delta G_{\text{binding}}$ between -9.83 to -18.70 kcal mol⁻¹ for MtTIM and -6.31 to -7.90 kcal mol⁻¹ for HsTIM (Table 2, and details on the supplementary material Table S1 and S2). The analysis of the interaction of the best five conformers from the five compounds selected (depicted here as T1 - T5) and D4 compound with MtTIM and HsTIM were carried out with the interaction report (Table 3 and details in Table S3 – S14). All averages of $\Delta G_{\text{binding}}$ calculated are related to the number of interactions generated by the conformers analyzed from the molecular docking results (mainly hydrogen bonding, Table S3 – S14).

In addition, the tables of the description of the theoretical toxicity (Table S15), ADME characteristics (Table S16), and chemical properties of each compound (T1 – T5 and D4, Table S17), are in the supplemental material.

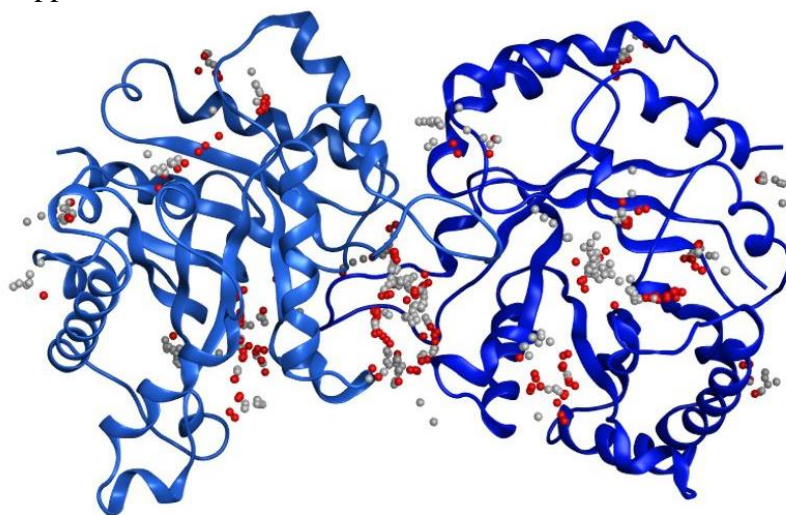
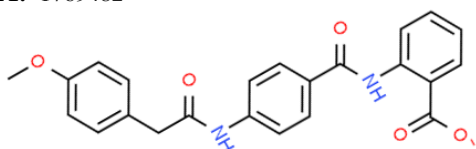


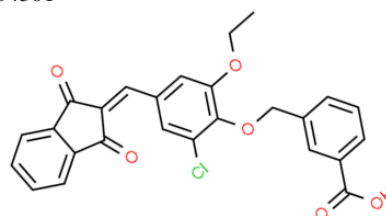
Figure 1. MtTIM (Blue) shows the 18 potential binding sites in balls red and white, determined by “site finder” in MOE for molecular docking.

Table 1. PubChem CID and Structure of the best compounds, T1 - T5 and D4 compounds.

T1.- 1769482



T2.- 1334501



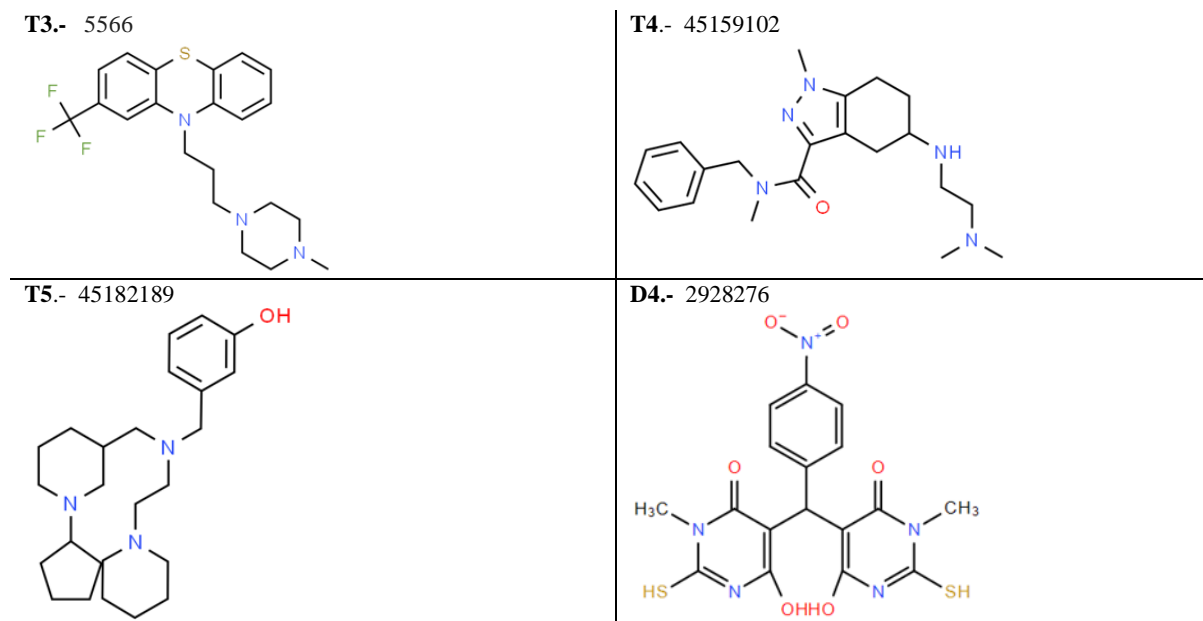


Table 2. Average of $\Delta G_{\text{binding}}$ (kcal mol⁻¹) and SD of T1 – T5 and D4 compounds in MtTIM and HsTIM.

Compound	MtTIM	HsTIM
T1	-12.02 ± 4.17	-7.30 ± 0.21
T2	-12.21 ± 2.34	-7.69 ± 0.20
T3	-9.83 ± 3.93	-6.31 ± 0.16
T4	-18.70 ± 5.03	-7.90 ± 0.34
T5	-11.84 ± 5.14	-6.71 ± 0.15
D4	-11.64 ± 1.20	-6.83 ± 0.71

Table 3. PubChem CID, Canonical SMILES, Interaction with amino acids in MtTIM and HsTIM, Ames test and strain used (positive or negative) and LD₅₀ [47,49].

PubChem CID	Canonical SMILES	Interaction with amino acids in AXL (Table S2 – S11), in bold are greater interaction.	Interaction with amino acids in AXL (Table S2 – S11), in bold are greater interaction.	PreADMET Ames test and LD ₅₀
T1.- 1769482	<chem>COC1=CC=C(C=C1)CC(=O)NC2=CC=C(C=C2)C(=O)NC3=CC=CC=C3C(=O)[O-]</chem>	His74, Asp82, Arg103, Thr105, Lys117, Leu136, His147, Ile177, Gly178	Lys5 , Lys58, Trp90, Arg98 , Arg99, Asp106, Ser158	Mutagen -TA100_10RL -TA100_NA -TA1535_10R -TA1535_NA Predicted LD ₅₀ mg/kg
T2.- 1334501	<chem>CCOC1=C(C(=CC(=C1)C=C2C(=O)C3=CC=CC=C3C2=O)Cl)OCC4=CC(=CC=C4)C(=O)O</chem>	Lys12, Thr105, Leu136, Trp175, Ile177, Gly180	Asn65, Tyr67, Glu77, Arg98	Mutagen -Negative -Negative -Negative -Negative 1600 mg/kg
T3.- 5566	<chem>CN1CCN(CC1)CCCN2C3=CC=C(C=C3)SC4=C2C=C(C=C4)C(F)(F)F</chem>	Asp33 , Tyr106, His107, Asp231	Asp36 , Glu107, Val142, Glu145 , Glu186, Lys193, Gln223, Asp225	Mutagen -Negative -Negative -Negative -Negative 424 mg/kg
T4.- 45159102	<chem>CN1C2=C(CC(CC2)NCCN(C)C)C(=N1)C(=O)N(C)CC3=CC=CC=C3</chem>	Asp33 , Arg63	Arg98 , Val101 , Phe102, Gly103	Mutagen -Negative -Negative -Negative

PubChem CID	Canonical SMILES	Interaction with amino acids in AXL (Table S2 – S11), in bold are greater interaction.	Interaction with amino acids in AXL (Table S2 – S11), in bold are greater interaction.	PreADMET Ames test and LD ₅₀
				-TA100_10RL -TA100_NA -TA1535_10R -TA1535_NA Predicted LD₅₀ mg/kg
				-Negative 300 mg/kg
T5.- 45182189	<chem>C1CCN(CC1)CCN(CC2CCCN(C2)C3CCCC3)CC4=CC(=CC=C4)O</chem>	Asp33 , Arg104, Glu109, Asp110, Asp209	Glu104, Asp106 , Glu133 , Glu145	Mutagen -Negative -Negative -Positive -Negative 410 mg/kg
D4.- 2928276	<chem>CN1C(=C(C(=O)NC1=S)C(C2=CC=C(C=C2)[N+](=O)[O-])C3=C(N(C(=S)NC3=O)C)O)O</chem>	Lys12 , Asn14, Glu102, Glu172, Ile177, Gly216, Gly240	Lys13 , Asn15, Lys68, Ala73, Lys112, Trp191, Gly210, Val212, Gly232	Mutagen -Positive -Negative -Negative -Negative 800 mg/kg

3.2. Interaction of T1 – T5 and D4 compounds with MtTIM and HsTIM.

To describe the probable interaction between each compound (T1-T5 and D4) with MtTIM and HsTIM, they analyzed the best five conformers from each compound interacting on each TIM (Figure 2, Figure S1 – S6).

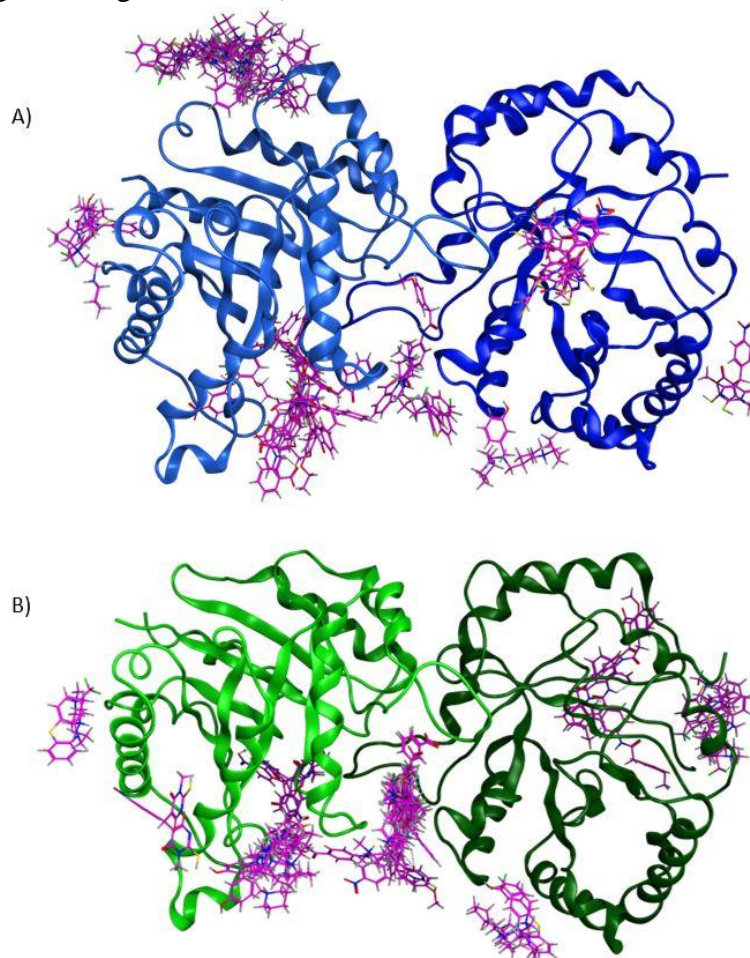


Figure 2. The best five conformers of T1 – T5 and D4 compounds (Pink); in total 36 conformers are indicated in their interaction site from docking results. A) MtTIM (Blue) and B) HsTIM (Green).

From molecular docking results (Table S3 – S14), were determined the main amino acids for MtTIM to interact with T1 – T5 and D4 compounds in Lys12, Asn14, Asp33, Arg63, His74, Asp82, Glu102, Arg103, Arg104, Thr105, Tyr106, Glu109, Asp110, Lys117, Leu136, His147, Glu172, Trp175, Ile177, Gly178, Gly180, Gly216, Asp231 and Gly240 amino acids, and Lys5, Lys13, Asn15, Asp36, Lys58, Asn65, Tyr67, Lys68, Ala73, Glu77, Trp90, Arg98, Arg99, Val101, Phe102, Gly103, Glu104, Asp106, Glu107, Lys112, Glu133, Val142, Glu145, Ser158, Glu186, Trp191, Lys193, Gly210, Val212, Gln223, Asp225 and Gly232 amino acids for HsTIM; both TIMs have conserved Lys13, Asn15, Glu97, Arg98, Arg99, Glu104, Lys112, Leu132, Glu166, Trp169, Ile171, Gly172, Gly174, Gly210 and Gly234 amino acids (Figure 3); despite it, these compounds have a better average of $\Delta G_{binding}$ of interaction in their interaction site (Table 2, Table S1 and S2), particularly a greater interaction with Asp and His amino acids in MtTIM (Figure 3). The details of the interaction for each TIM with conformers of each compound are shown in the supplementary material (Figure S1 – S6).



Figure 3. Alignment of HsTIM and MtTIM, with an identity of 36.12 % between both. Black: identical; gray: similar; green: amino acids important for T1-T5 and D4 compounds interacting in MtTIM; yellow: amino acids conserved in HsTIM.

3.3. Discussion.

Global efforts continue to develop new drugs for anti-TB due to the increasing factors that favor this disease, such as multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) [1-4], recently, TB has become more important, since TB as a risk factor for COVID-19 [7, 9-13, 50].

As already mentioned, it is still necessary to develop new drugs anti-TB, here we propose the MtTIM as a therapeutic target, and TIMs from other organisms are being used to develop new drugs, for example, TIM from *Trichomonas vaginalis* (TvTIM), TIM from *Burkholderia thailandensis* (BtTIM), TIM from *Clostridium perfringens* (CpTIM), TIM from *Nostoc punctiforme* (NpTIM), TIM from *Thermus thermophiles* (TtTIM), TIM from *Streptomyces coelicolor* (ScTIM) and TIM from *Deinococcus radiodurans* (DrTIM) [27, 28]. The MtTIM has characteristics that might help develop a specific TIM inhibitor because there are studies that propose amino acids that are important to generate interactions and selectivity between compounds and TIMs, without affecting the HsTIM [26-29].

Moreover, this study proposes compounds that might interact in MtTIM, the molecular docking using against 18 potential sites (Figure 1), and the results indicate the main amino acids in MtTIM for better interaction and selectivity with the compounds selected. Due to more Asp and His in MtTIM, we propose that the interaction with the compounds is better (Figure 3). Therefore the averages of $\Delta G_{\text{binding}}$ are higher in MtTIM (Table 2). For the compounds (T1 - T5 and D4), the main amino acids to interact in MtTIM and HsTIM are indicated in Table 3 and Tables S3 - S14. Despite some amino acids conserved between MtTIM and HsTIM, it is highly probable that the selectivity is only for MtTIM because previously, some of them were tested in HsTIM without effects on its glycolytic activity [26-29]. The analysis of the interactions of conformers of each compound shows the different interactions in the interaction sites between MtTIM and HsTIM (Figure 2, 3, and Table 3).

From docking results, we determine the T1 - T5 and D4 compounds with a better $\Delta G_{\text{binding}}$ in MtTIM. These compounds interact more Asp, Arg, Lys, and His amino acids in MtTIM than HsTIM (Figure 3, Figure S7 – S12, Table 3 and S3 - S14). It is important to note that MtTIM conserves amino acids that are important in other TIMs (TvTIM, CpTIM, BtTIM, NpTIM, TtTIM, ScTIM, DrTIM, and MtTIM), that are inhibited by compounds interacting with more Asp, Arg, Lys, and His. MtTIM has Asp33, Arg63, His74, Asp82, Thr105, Tyr106, His107, Asp110, His147, Asp209, Asp231 amino acids (Figure 3, Figure S7 – S12), these amino acids with characteristics (positives and negative charges) that increase the interaction with molecules [51], which are important for generating the capacity to interact with the T1 – T5 and D4 compounds. Probably these amino acids could help to other compounds to interact with MtTIM. Also, it is important to consider other consequences that some compound/molecule interact with this kind of amino acids, in particular Asp, Arg, and Lys, since these amino acids could influence the conformational stability of the MtTIM [20].

As already mentioned, there are other TIMs with reports of compounds with some inhibitory effect [28]. It is possible to propose to use the compounds against other TIMs, because the MtTIM has a higher percentage of identity with the TIMs tested, the MtTIM has an identity of 42.58 % with BtTIM, 40.23 % with CpTIM, 38.68 % with NpTIM, 64.50 % with ScTIM, 45.59 % with DrRTIM and 49.42 % with TmTIM, and less identity with HsTIM of 36.12%. On the other hand, the Phe46 and Lys230 amino acids are important to decrease the glycolytic activity in the TIMs tested [28], MtTIM has these amino acids, but in HsTIM are not conserved both amino acids (Figure S13). Therefore, the reported compounds could be tested against other TIMs, maintaining the safety of these compounds in the HsTIM, since it has less identity with the TIMs tested.

Currently, there are many developments of anti-tuberculosis drugs that use other therapeutics targets [16-21]. As already mentioned, TIM is growing as a therapeutic target in different organisms, and there are advances *in vivo* assays, such as D4-compound against *E. histolytica* [29]. Therefore, MtTIM could be another alternative to affect the viability of *M. tuberculosis*, and to be able to contribute to efforts to develop better anti-TB drugs.

4. Conclusions

In this study, we propose another therapeutic target against *M. tuberculosis*, the triosephosphate isomerase (MtTIM), because this enzyme has amino acids similar to other TIMs that reduce the enzymatic activity with specific compounds [28, 29] to develop a new drug against *M. tuberculosis*.

As well, we propose six compounds with high probability to be selective against MtTIM because the main amino acids that are important for interacting in MtTIM are not conserved in HsTIM, these amino acids could generate the selectivity for MtTIM, it was demonstrated in other TIMs by specific compounds [28], these compounds might be tested on MtTIM too. Therefore, future research, such as *in vitro* and *in vivo* will be necessary to evaluate this therapeutic target against TB.

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Supplementary Data

Supporting information includes figures and tables of interactions for compounds with MtTIM, as well as details of the interaction of each compound with MtTIM per amino acid, theoretical toxicity results, ADME characteristics, and physical chemistry, which support the information given in the results and discussion.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Sanou, A.; Bañuls, A-L.; Van Anh, N.T.; Godreuil, S. Mycobacterium tuberculosis: ecology and evolution of a human bacterium. *J. Med. Microbiol.* **2015**, *64*, 1261–1269, <https://doi.org/10.1099/jmm.0.000171>.
2. Koch, A.; Mizrahi, V. Mycobacterium tuberculosis. *Trends in Microbiology* **2018**, *26*, 555–556, <https://doi.org/10.1016/j.tim.2018.02.012>.
3. Araujo, Z.; Macias-Segura, N.; Lopez-Ramos, J.E.; De Waard, J.H.; Vanegas, M.; Patarroyo, M.A.; Salgado, A.; Enciso-Moreno, J.A. Diagnostic accuracy of combinations of serological biomarkers for identifying clinical tuberculosis. *J. Infect. Dev. Ctries.* **2018**, *12*, 429–441, <https://doi.org/10.3855/jidc.9554>.
4. Senghore, M.; Diarra, B. *et al.* Evolution of Mycobacterium tuberculosis complex lineages and their role in an emerging threat of multidrug resistant tuberculosis in Bamako, Mali. *Sci. Rep.* **2020**, *10*, 327, <https://doi.org/10.1038/s41598-019-56001-0>.
5. CDC. Tratamiento para la enfermedad de tuberculosis Available online: <https://www.cdc.gov/tb/esp/topic/treatment/tbdisease.htm>.
6. Chetty, S.; Ramesh, M.; Singh-Pillay, A.; Soliman, M.E.S. Recent advancements in the development of anti-tuberculosis drugs. *Bioorg. Med. Chem. Lett.* **2017**, *27*, 370–386, <https://doi.org/10.1016/j.bmcl.2016.11.084>.
7. Chen, Y.; Wang, Y. *et al.* Active or latent tuberculosis increases susceptibility to COVID-19 and disease severity. *medRxiv The preprint server for health sciences* **2020**, <https://doi.org/10.1101/2020.03.10.20033795>.
8. Aguilar-León, P.; Cotrina-Castañeda, J.; Zavala-Flores, E. SARS-CoV-2 infection and pulmonary tuberculosis: an analysis of the situation in Peru. *Cad. Saude Publica* **2020**, *36*, e00094520, <https://doi.org/10.1590/0102-311x00094520>.
9. Motta, I.; Centis, R. *et al.* Tuberculosis, COVID-19 and migrants: Preliminary analysis of deaths occurring in 69 patients from two cohorts. *Pulmonology* **2020**, *26*, 233–240, <https://doi.org/10.1016/j.pulmoe.2020.05.002>.
10. Tadolini, M.; Codecasa, L.R. *et al.* Active tuberculosis, sequelae and COVID-19 co-infection: first cohort of 49 cases. *Eur. Respir. J.* **2020**, *56*, 2001398, <https://doi.org/10.1183/13993003.01398-2020>.
11. McQuaid, C.F.; McCreesh, N.; Read, J.M.; Sumner, T.; Houben, R.M.G.J.; White, R.G.; Harris, R.C. The potential impact of COVID-19-related disruption on tuberculosis burden. *Eur. Respir. J.* **2020**, *56*, 2001718, <https://doi.org/10.1183/13993003.01718-2020>.

12. Tapela, K.; Ochieng' Olwal, C.; Quaye, O. Parallels in the pathogenesis of SARS-CoV-2 and M. tuberculosis: a synergistic or antagonistic alliance? *Future Microbiol.* **2020**, *15*, 1691–1695, <https://doi.org/10.2217/fmb-2020-0179>.
13. Chopra, K.K.; Arora, V.K.; Singh, S. COVID 19 and tuberculosis. *Indian J. Tuberc.* **2020**, *67*, 149–151, <https://doi.org/10.1016/j.ijtb.2020.06.001>.
14. Sawicki, R.; Ginalska, G. *Mycobacterium tuberculosis* topoisomerases and EthR as the targets for new anti-TB drugs development. *Future Med. Chem.* **2019**, *11*, 2193–2203, <https://doi.org/10.4155/fmc-2018-0232>.
15. Torfs, E.; Piller, T.; Cos, P.; Cappoen, D. Opportunities for Overcoming *Mycobacterium tuberculosis* Drug Resistance: Emerging Mycobacterial Targets and Host-Directed Therapy. *Int. J. Mol. Sci.* **2019**, *20*, 2868, <https://doi.org/10.3390/ijms20122868>.
16. Harikishore, A.; Chong, S.S.M.; Ragunathan, P.; Bates, R.W.; Grüber, G. Targeting the menaquinol binding loop of mycobacterial cytochrome bd oxidase. *Mol. Divers.* **2020**, *25*, 517–524, <https://doi.org/10.1007/s11030-020-10034-0>.
17. Santos, P.; Lopez-Vallejo, F.; Ramírez, D.; Caballero, J.; Mata Espinosa, D.; Hernández-Pando, R.; Soto, C.Y. Identification of *Mycobacterium tuberculosis* CtpF as a target for designing new antituberculous compounds. *Bioorg. Med. Chem.* **2020**, *28*, 115256, <https://doi.org/10.1016/j.bmc.2019.115256>.
18. Šlachťová, V.; Šebela, M.; Torfs, E.; Oorts, L.; Cappoen, D.; Berka, K.; Bazgier, V.; Brulíková, L. Novel thiazolidinedione-hydroxamates as inhibitors of *Mycobacterium tuberculosis* virulence factor Zmp1. *Eur. J. Med. Chem.* **2020**, *185*, 111812, <https://doi.org/10.1016/j.ejmech.2019.111812>.
19. Naz, S.; Farooq, U.; Ali, S.; Sarwar, R.; Khan, S.; Abagyan, R. Identification of new benzamide inhibitor against α -subunit of tryptophan synthase from *Mycobacterium tuberculosis* through structure-based virtual screening, anti-tuberculosis activity and molecular dynamics simulations. *J. Biomol. Struct. Dyn.* **2019**, *37*, 1043–1053, <https://doi.org/10.1080/07391102.2018.1448303>.
20. Meuzelaar, H.; Vreede, J.; Woutersen, S. Influence of Glu/Arg, Asp/Arg, and Glu/Lys Salt Bridges on α -Helical Stability and Folding Kinetics. *Biophys. J.* **2016**, *110*, 2328–2341, <https://doi.org/10.1016/j.bpj.2016.04.015>.
21. Karale, U.B.; Shinde, A.U.; Babar, D.A.; Sangu, K.G.; Vagolu, S.K.; Eruva, V.K.; Jadav, S.S.; Misra, S.; Dharmarajan, S.; Rode, H.B. 3-Aryl-substituted imidazo[1,2- α]pyridines as antituberculosis agents. *Arch. Pharm. (Weinheim)* **2021**, <https://doi.org/10.1002/ardp.202000419>.
22. Jimenez-Sandoval, P.; Vique-Sanchez, J.L. *et al.* A competent catalytic active site is necessary for substrate induced dimer assembly in triosephosphate isomerase. *Biochim. Biophys. Acta - Proteins Proteomics* **2017**, *1865*, 1423–1432 <https://doi.org/10.1016/j.bbapap.2017.07.014>.
23. Alvarez, G.; Martínez, J.; Aguirre-López, B.; Cabrera, N.; Pérez-Díaz, L.; Gómez-Puyou, M.T. de; Gómez-Puyou, A.; Pérez-Montfort, R.; Garat, B.; Merlino, A.; Gonzales, M.; Cerecetto, H. New chemotypes as *Trypanosoma cruzi* triosephosphate isomerase inhibitors: a deeper insight into the mechanism of inhibition. *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 198–204, <http://dx.doi.org/10.3109/14756366.2013.765415>.
24. Velanker, S.S.; Ray, S.S.; Gokhale, R.S.; Suma, S.; Balaram, H.; Balaram, P.; Murthy, M.R. Triosephosphate isomerase from *Plasmodium falciparum*: the crystal structure provides insights into antimalarial drug design. *Structure* **1997**, *5*, 751–61, [https://doi.org/10.1016/s0969-2126\(97\)00230-x](https://doi.org/10.1016/s0969-2126(97)00230-x).
25. Minini, L.; Álvarez, G.; González, M.; Cerecetto, H.; Merlino, A. Molecular docking and molecular dynamics simulation studies of *Trypanosoma cruzi* triosephosphate isomerase inhibitors. Insights into the inhibition mechanism and selectivity. *J. Mol. Graph. Model.* **2015**, *58*, 40–9, <https://doi.org/10.1016/j.jmgm.2015.02.002>.
26. Vique-Sánchez, J.L.; Caro-Gómez, L.A.; Brieba, L.G.; Benítez-Cardoza, C.G. Developing a new drug against trichomoniasis, new inhibitory compounds of the protein triosephosphate isomerase. *Parasitol. Int.* **2020**, *76*, 102086, <https://doi.org/10.1016/j.parint.2020.102086>.
27. Benítez-Cardoza, C.G.; Fernández-Velasco, D.A.; Vique-Sánchez, J.L. Triosephosphate Isomerase Inhibitors as Potential Drugs against *Clostridium perfringens*. *ChemistrySelect* **2020**, *5*, 2365–2370, <https://doi.org/10.1002/slct.201904632>.
28. Benítez-Cardoza, C.G.; Jiménez-Pineda, A.; Angles-Falconi, S.I.; Fernández-Velasco, D.A.; Vique-Sánchez, J.L. Potential Site to Direct Selective Compounds in the Triosephosphate Isomerase for the Development of New Drugs. *ChemistrySelect* **2020**, *5*, 4866–4874, <https://doi.org/10.1002/slct.202000820>.
29. Vique-Sánchez, J.L.; Jiménez-Pineda, A.; Benítez-Cardoza, C.G. Amoebicidal effect of 5,5'-[(4-nitrophenyl)methylene]bis-6-hydroxy-2-mercapto-3-methyl-4(3H)-pyrimidinone), a new drug against *Entamoeba histolytica*. *Arch. Pharm. (Weinheim)*. **2020**, <http://dx.doi.org/10.1002/ardp.202000263>.
30. Mathur, D.; Malik, G.; Garg, L.C. Biochemical and functional characterization of triosephosphate isomerase from *Mycobacterium tuberculosis* H37Rv. *FEMS Microbiol. Lett.* **2006**, *263*, 229–235, <https://doi.org/10.1111/j.1574-6968.2006.00420.x>.
31. Connor, S.E.; Capodagli, G.C.; Deaton, M.K.; Pegan, S.D. Structural and functional characterization of *Mycobacterium tuberculosis* triosephosphate isomerase. *Acta Crystallogr. Sect. D Biol. Crystallogr.* **2011**, *67*, 1017–1022, <https://doi.org/10.1107/s0907444911042971>.
32. Trujillo, C.; Blumenthal, A.; Marrero, J.; Rhee, K.Y.; Schnappinger, D.; Ehrt, S. Triosephosphate Isomerase Is Dispensable *In vitro* yet Essential for *Mycobacterium tuberculosis* To Establish Infection. *mBio* **2014**, *5*,

- e00085-14, <https://doi.org/10.1128/mBio.00085-14>.
33. RCSB, Protein Data Bank. Available online: <https://www.rcsb.org>.
 34. Benítez-Cardoza, C.G.; Vique-Sánchez, J.L. Potential inhibitors of the interaction between ACE2 and SARS-CoV-2 (RBD), to develop a drug. *Life Sci.* **2020**, *256*, 117970, <https://doi.org/10.1016/j.lfs.2020.117970>.
 35. Halgren, T.A. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.* **1996**, *17*, 490–519, [https://doi.org/10.1002/\(SICI\)1096-987X\(199604\)17:5/6%3C490::AID-JCC1%3E3.0.CO;2-P](https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6%3C490::AID-JCC1%3E3.0.CO;2-P).
 36. Vique-Sánchez, J.L. Potential inhibitors interacting in Neuropilin-1 to develop an adjuvant drug against COVID-19, by molecular docking. *Bioorg. Med. Chem.* **2021**, *33*, 116040, <https://doi.org/10.1016/j.bmc.2021.116040>.
 37. Del Carpio, C.A.; Takahashi, Y.; Sasaki, S. A new approach to the automatic identification of candidates for ligand receptor sites in proteins: (I) Search for pocket regions. *J. Mol. Graph.* **1993**, *11*, 23–29, [https://doi.org/10.1016/0263-7855\(93\)85003-9](https://doi.org/10.1016/0263-7855(93)85003-9).
 38. Miranker, A.; Karplus, M. Functionality maps of binding sites: A multiple copy simultaneous search method. *Proteins Struct. Funct. Bioinform.* **1991**, *11*, 29–34, <https://doi.org/10.1002/prot.340110104>.
 39. Soga, S.; Shirai, H.; Kobori, M.; Hirayama, N. Use of Amino Acid Composition to Predict Ligand-Binding Sites. *J. Chem. Inf. Model.* **2007**, *47*, 400–406, <https://doi.org/10.1021/ci6002202>.
 40. Corporation, Hit2Lead.com. Available online: <https://www.hit2lead.com/bioactives.asp>.
 41. Corporation, ChemBridge. Available online: http://www.chembridge.com/screening_libraries.
 42. Protein-Ligand Interaction Profiler. Available online: <https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>.
 43. Salentin, S.; Schreiber, S.; Haupt, V.J.; Adasme, M.F.; Schroeder, M. PLIP: fully automated protein–ligand interaction profiler. *Nucleic Acids Res.* **2015**, *43*, W443–W447, <https://doi.org/10.1093/nar/gkv315>.
 44. Labute, P. The generalized Born/volume integral implicit solvent model: estimation of the free energy of hydration using London dispersion instead of atomic surface area. *J. Comput. Chem.* **2008**, *29*, 1693–1698, <https://doi.org/10.1002/jcc.20933>.
 45. Wadood, A.; Ghufuran, M.; Hassan, S.F.; Khan, H.; Azam, S.S.; Rashid, U. *In silico* identification of promiscuous scaffolds as potential inhibitors of 1-deoxy- d -xylulose 5-phosphate reductoisomerase for treatment of Falciparum malaria. *Pharm. Biol.* **2017**, *55*, 19–32, <http://dx.doi.org/10.1080/13880209.2016.1225778>.
 46. PhysChem, ADME & Toxicity, version 2021.1.1, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2021, www.acdlabs.com.
 47. ADMETlab. Available online: http://admet.scbdd.com/calcpred/index_sys.
 48. PreADMET. Available online: <https://preadmet.bmdrc.kr/toxicity>.
 49. ProTox-II - Prediction of Toxicity of Chemicals. Available online: http://tox.charite.de/protox_II/index.php?site=compound_input.
 50. Aguilar-León, P.; Cotrina-Castañeda, J.; Zavala-Flores, E. SARS-CoV-2 infection and pulmonary tuberculosis: an analysis of the situation in Peru. *Cad. Saude Publica* **2020**, *36*, e00094520, <https://doi.org/10.1590/0102-311x00094520>.
 51. Ellington, A.; Cherry, J.M. Characteristics of Amino Acids. *Curr. Protoc. Mol. Biol.* **1996**, Appendix 1:Appendix 1C, <https://doi.org/10.1002/0471142727.mba01cs33>.

Supplementary materials

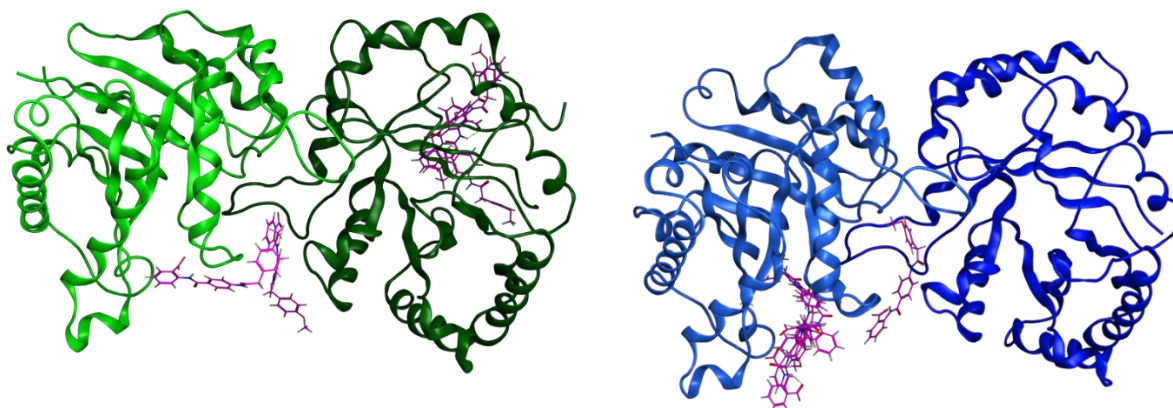


Figure S1. From docking results, the best five conformers of T1 compound (Pink) interacting in MtTIM (Blue) and HsTIM (Green).

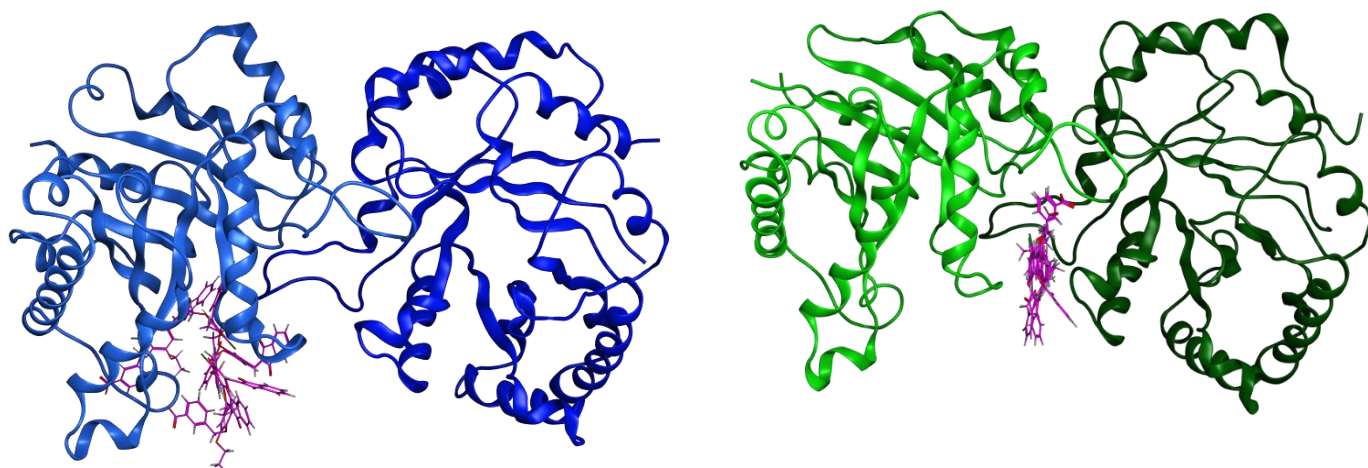


Figure S2. From docking results, the best five conformers of T2 compound (Pink) interacting in MtTIM (Blue) and HsTIM (Green).

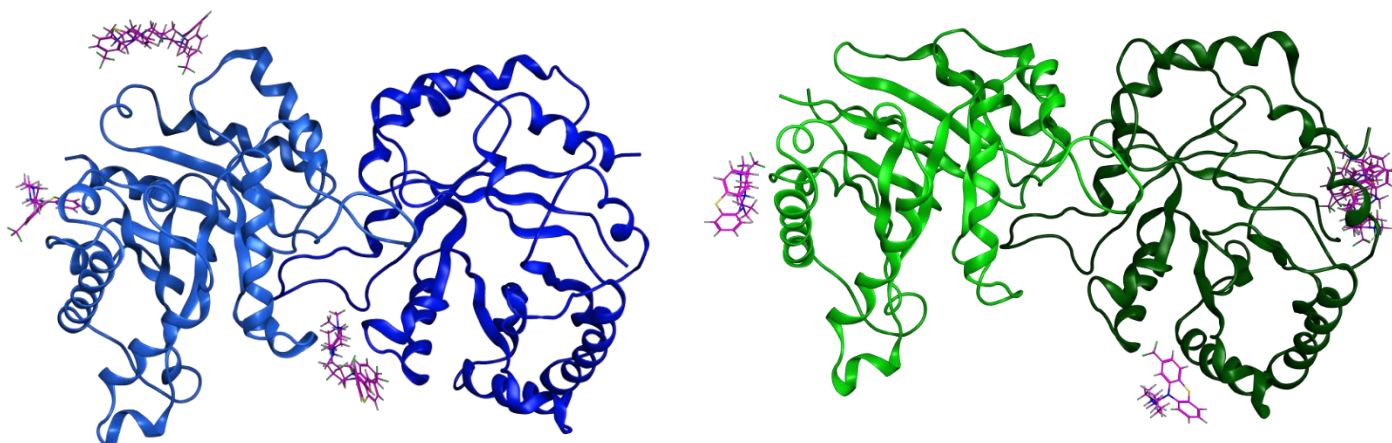


Figure S3. From docking results, the best five conformers of T3 compound (Pink) interacting in MtTIM (Blue) and HsTIM (Green).

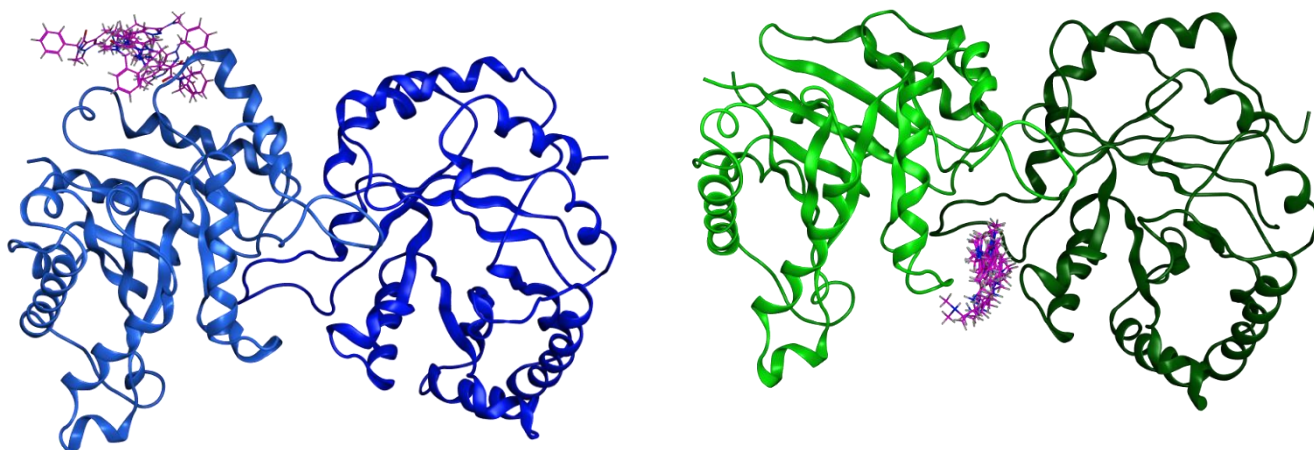


Figure S4. From docking results, the best five conformers of T4 compound (Pink) interacting in MtTIM (Blue) and HsTIM (Green).

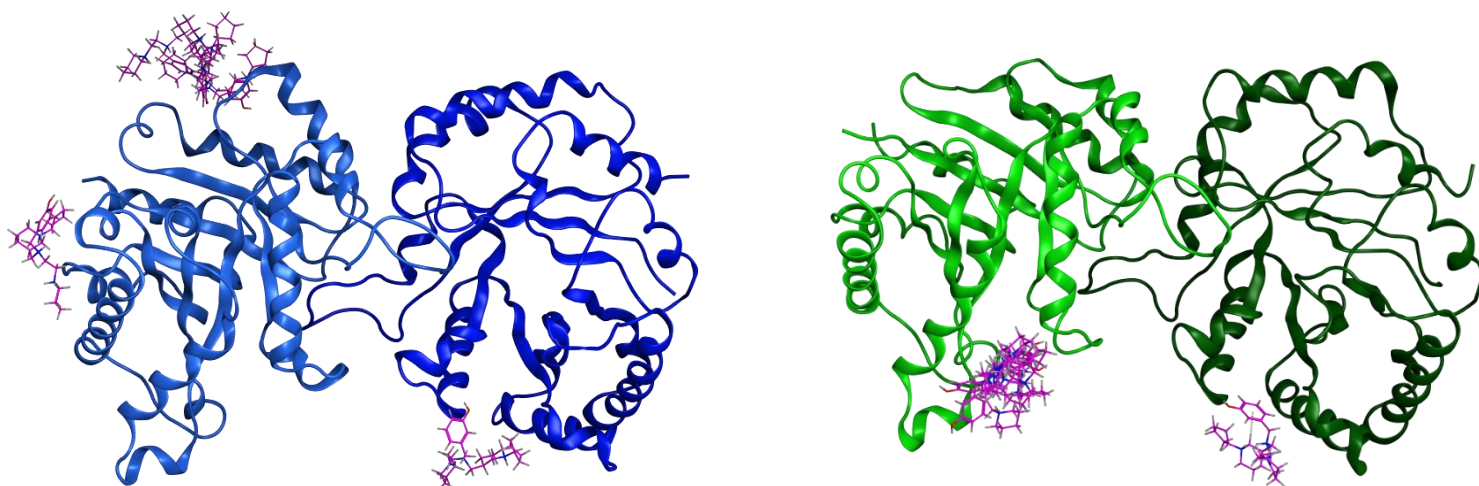


Figure S5. From docking results, the best five conformers of T5 compound (Pink) interacting in MtTIM (Blue) and HsTIM (Green).

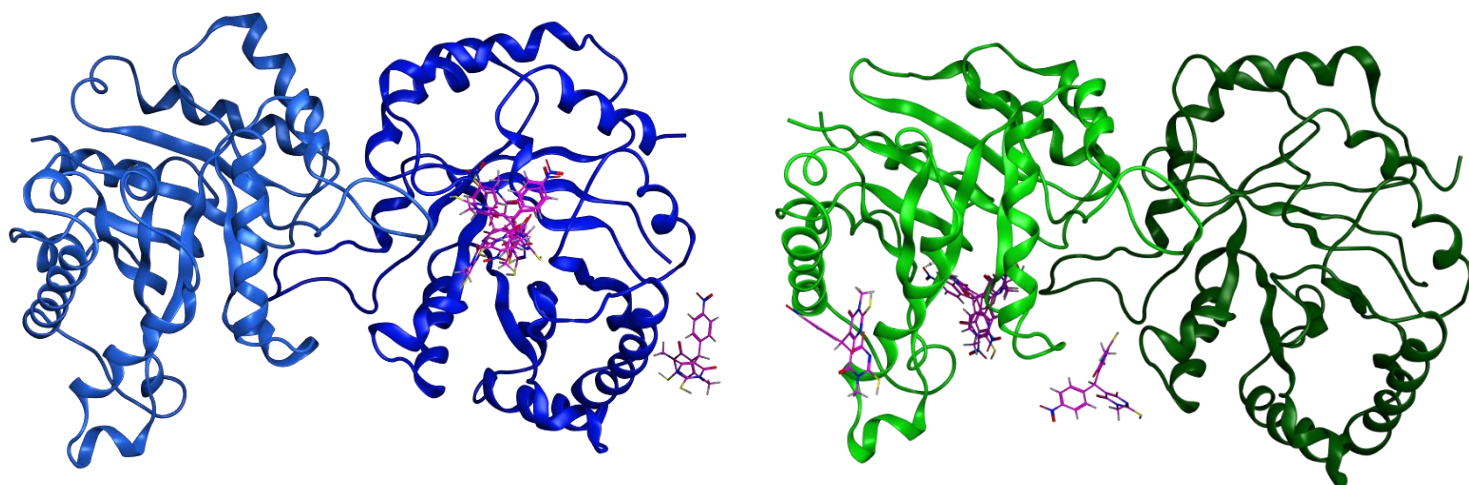


Figure S6. From docking results, the best five conformers of D4 compound (Pink) interacting in MtTIM (Blue) and HsTIM (Green).



Figure S7. Alignment of HsTIM and MtTIM. Blck: identical; gray: similar; yellow: amino acids important for interaction of T1 compound with MtTIM.



Figure S8. Alignment of HsTIM and MtTIM. Blck: identical; gray: similar; yellow: amino acids important for interaction of T2 compound with MtTIM.



Figure S9. Alignment of HsTIM and MtTIM. Blck: identical; gray: similar; yellow: amino acids important for interaction of T3 compound with MtTIM.

```

HsTIM      1  MAPSRKFFVGGNWKMNGRKQSLGELIGTINAA---KVPADTEVVCAPPTAYDFARQK--
MtTIM      1  --MSRKPLIAGNWKMNLNHYEAIALVQKIAFSLPDKYYDRVEVAVIPPFTDRSVQTLVD

HsTIM      56  -LDPKIAVAAAQNCYKVTNGAFTGELSPGMKDCGATWVVLGHSERRHVFGESDELIGQKV
MtTIM      59  GDKLRLTYGAQDLSPHDSGAYTGDVSGAFLAKLGCSYVVVGHSERRTYHNEEDDALVAAKA

HsTIM      115  AHALAEGLGVIACIGEKLDEREAGITEKVVFEQTKV--IADNVKDWSKVVLAYEPVWAIG
MtTIM      119  ATALKHGLTPIVCIGEHLDVREAGNHVAHNIEQLRGSLAGLLAEQIGSVVLAYEPVWAIG

HsTIM      173  TGRVASAAADAQEVHEKIRGNLKSNVSDAVAQSTRIIYGGSVTGATCKELASCEDVDGFLV
MtTIM      179  TGRVASAAADAQEVCAAIRKELASLASPRIADIVRVLYGGSVNAKNVGDIVAQDDDVDGGLV

HsTIM      233  GGASLKPEFVDIINAKQ-----
MtTIM      239  GGASLDGEHFATIAAIAAGGPLE
    
```

Figure S10. Alignment of HsTIM and MtTIM. Bck: identical; gray: similar; yellow: amino acids important for interaction of T4 compound with MtTIM.

```

HsTIM      1  MAPSRKFFVGGNWKMNGRKQSLGELIGTINAA---KVPADTEVVCAPPTAYDFARQK--
MtTIM      1  --MSRKPLIAGNWKMNLNHYEAIALVQKIAFSLPDKYYDRVEVAVIPPFTDRSVQTLVD

HsTIM      56  -LDPKIAVAAAQNCYKVTNGAFTGELSPGMKDCGATWVVLGHSERRHVFGESDELIGQKV
MtTIM      59  GDKLRLTYGAQDLSPHDSGAYTGDVSGAFLAKLGCSYVVVGHSERRTYHNEEDDALVAAKA

HsTIM      115  AHALAEGLGVIACIGEKLDEREAGITEKVVFEQTKV--IADNVKDWSKVVLAYEPVWAIG
MtTIM      119  ATALKHGLTPIVCIGEHLDVREAGNHVAHNIEQLRGSLAGLLAEQIGSVVLAYEPVWAIG

HsTIM      173  TGRVASAAADAQEVHEKIRGNLKSNVSDAVAQSTRIIYGGSVTGATCKELASCEDVDGFLV
MtTIM      179  TGRVASAAADAQEVCAAIRKELASLASPRIADIVRVLYGGSVNAKNVGDIVAQDDDVDGGLV

HsTIM      233  GGASLKPEFVDIINAKQ-----
MtTIM      239  GGASLDGEHFATIAAIAAGGPLE
    
```

Figure S11. Alignment of HsTIM and MtTIM. Bck: identical; gray: similar; yellow: amino acids important for interaction of T5 compound with MtTIM.

```

HsTIM      1  MAPSRKFFVGGNWKMNGRKQSLGELIGTINAA---KVPADTEVVCAPPTAYDFARQK--
MtTIM      1  --MSRKPLIAGNWKMNLNHYEAIALVQKIAFSLPDKYYDRVEVAVIPPFTDRSVQTLVD

HsTIM      56  -LDPKIAVAAAQNCYKVTNGAFTGELSPGMKDCGATWVVLGHSERRHVFGESDELIGQKV
MtTIM      59  GDKLRLTYGAQDLSPHDSGAYTGDVSGAFLAKLGCSYVVVGHSERRTYHNEEDDALVAAKA

HsTIM      115  AHALAEGLGVIACIGEKLDEREAGITEKVVFEQTKV--IADNVKDWSKVVLAYEPVWAIG
MtTIM      119  ATALKHGLTPIVCIGEHLDVREAGNHVAHNIEQLRGSLAGLLAEQIGSVVLAYEPVWAIG

HsTIM      173  TGRVASAAADAQEVHEKIRGNLKSNVSDAVAQSTRIIYGGSVTGATCKELASCEDVDGFLV
MtTIM      179  TGRVASAAADAQEVCAAIRKELASLASPRIADIVRVLYGGSVNAKNVGDIVAQDDDVDGGLV

HsTIM      233  GGASLKPEFVDIINAKQ-----
MtTIM      239  GGASLDGEHFATIAAIAAGGPLE
    
```

Figure S12. Alignment of HsTIM and MtTIM. Bck: identical; gray: similar; yellow: amino acids important for interaction of D4 compound with MtTIM.

Compound	Conformer	$\Delta G_{\text{binding}}$ (kcal mol ⁻¹)
T1	27	-5.7765675
T1	28	-5.6680088
T1	29	-5.6583171
T1	30	-5.3566537
T2	1	-15.537114
T2	2	-13.553677
T2	3	-11.770159
T2	4	-10.279182
T2	5	-9.9367619
T2	6	-9.8412666
T2	7	-9.2330456
T2	8	-8.8120222
T2	9	-7.979362
T2	10	-7.7080684
T2	11	-7.6696777
T2	12	-7.6442399
T2	13	-7.5763412
T2	14	-7.4826231
T2	15	-7.2848735
T2	16	-7.0824809
T2	17	-6.7282276
T2	18	-6.7118812
T2	19	-6.6992998
T2	20	-6.6481018
T2	21	-6.5618019
T2	22	-6.4962931
T2	23	-6.4591956
T2	24	-6.3704786
T2	25	-6.3360152
T2	26	-6.3225875
T2	27	-6.3079095
T2	28	-6.1934919
T2	29	-6.1128836
T2	30	-5.3612046
T3	1	-15.116655
T3	2	-13.008216
T3	3	-7.0577545
T3	4	-7.0203171
T3	5	-6.9499674
T3	6	-6.8818908
T3	7	-6.8359346
T3	8	-6.7382536
T3	9	-6.3743525
T3	10	-6.0799451
T3	11	-6.0681424
T3	12	-6.0644059
T3	13	-5.9516473
T3	14	-5.8327732
T3	15	-5.5505624
T3	16	-5.4287672
T3	17	-5.4237223
T3	18	-5.4153743
T3	19	-5.2977958
T3	20	-5.0905232
T3	21	-4.6928897
T3	22	-4.2618294
T3	23	-4.1460981
T3	24	-4.1179452
T4	1	-24.330847
T4	2	-23.882708
T4	3	-16.255596
T4	4	-15.581671
T4	5	-13.486635

Compound	Conformer	$\Delta G_{\text{binding}}$ (kcal mol ⁻¹)
T4	6	-8.3607273
T4	7	-7.1885114
T4	8	-6.9169402
T4	9	-6.888463
T4	10	-6.8775702
T4	11	-6.7225504
T4	12	-6.651176
T4	13	-6.648479
T4	14	-6.5183239
T4	15	-6.1678424
T4	16	-5.8116722
T4	17	-5.6924958
T4	18	-5.0737009
T4	19	-4.9741526
T4	20	-4.9722543
T4	21	-4.9010634
T4	22	-4.8302636
T4	23	-4.7352643
T4	24	-4.6384964
T4	25	-3.9685273
T4	26	-3.8633578
T4	27	-3.4467297
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T5	1	-17.860346
T5	2	-15.663527
T5	3	-12.467755
T5	4	-6.9478664
T5	5	-6.2957191
T5	6	-5.6617556
T5	7	-5.6608129
T5	8	-5.639112
T5	9	-5.4046063
T5	10	-5.0869069
T5	11	-5.0007954
T5	12	-4.8458681
T5	13	-4.5041757
T5	14	-4.4739423
T5	15	-4.4557891
T5	16	-4.3064985
T5	17	-4.2176781
T5	18	-3.9669673
T5	19	-3.5289178
T5	20	-3.3479142
T5	21	-3.1974645
T5	22	-3.058063
<hr/>		
D4	1	-12.990515
D4	2	-12.301793
D4	3	-12.146478
D4	4	-10.714886
D4	5	-10.083652
D4	6	-9.6891546
D4	7	-9.5278368
D4	8	-7.8773251
D4	9	-7.6345749
D4	10	-7.2739491
D4	11	-7.1685681
D4	12	-6.9002981
D4	13	-6.7073278
D4	14	-6.6391988
D4	15	-6.279294
D4	16	-6.0771179
D4	17	-6.0020676
D4	18	-5.822444
D4	19	-5.2859912
D4	20	-4.7035975

Compound	Conformer	$\Delta G_{\text{binding}}$ (kcal mol ⁻¹)
D4	21	-4.4125037

Table S2. $\Delta G_{\text{binding}}$ of 25 to 29 conformers from each compound, average $\Delta G_{\text{binding}}$ and SD for HsTIM.

Compound	Conformer	$\Delta G_{\text{binding}}$ (kcal mol ⁻¹)
T1	1	-7.7168059
T1	2	-7.360672
T1	3	-7.2650509
T1	4	-7.2606478
T1	5	-7.1813784
T1	6	-7.179544
T1	7	-7.0657287
T1	8	-6.9319258
T1	9	-6.76088
T1	10	-6.6002774
T1	11	-6.5789924
T1	12	-6.565639
T1	13	-6.5065255
T1	14	-6.5045161
T1	15	-6.4828606
T1	16	-6.445879
T1	17	-6.4457469
T1	18	-6.3822765
T1	19	-6.3690825
T1	20	-6.3519197
T1	21	-6.2274313
T1	22	-6.1355376
T1	23	-6.1147327
T1	24	-6.0977507
T1	25	-6.0827899
T1	26	-6.0152578
T1	27	-5.7975535
T1	28	-5.7748508
T1	29	-5.7234125
T2	1	-7.9971185
T2	2	-7.7562976
T2	3	-7.6788526
T2	4	-7.591114
T2	5	-7.4447231
T2	6	-7.3959584
T2	7	-7.3230925
T2	8	-7.304625
T2	9	-7.1931901
T2	10	-7.0935645
T2	11	-7.0325017
T2	12	-6.989707
T2	13	-6.9020367
T2	14	-6.8814683
T2	15	-6.8244319
T2	16	-6.7677431
T2	17	-6.7304602
T2	18	-6.5045209
T2	19	-6.2630115
T2	20	-6.0394382
T2	21	-5.9408007
T2	22	-5.8978119
T2	23	-5.887135
T2	24	-5.8579259
T2	25	-5.3863916
T2	26	-5.3284125
T2	27	-5.2395229
T2	28	-5.0759993
T2	29	-2.8577199
T3	1	-6.5749302

Compound	Conformer	$\Delta G_{\text{binding}}$ (kcal mol ⁻¹)
T3	2	-6.3824253
T3	3	-6.2241406
T3	4	-6.2208524
T3	5	-6.1575103
T3	6	-5.9685321
T3	7	-5.8002906
T3	8	-5.6772218
T3	9	-5.6754823
T3	10	-5.6222467
T3	11	-5.5276299
T3	12	-5.4636011
T3	13	-5.4501634
T3	14	-5.3239136
T3	15	-5.2912912
T3	16	-5.26686
T3	17	-5.2463126
T3	18	-5.2417669
T3	19	-5.226068
T3	20	-5.2058945
T3	21	-5.172924
T3	22	-5.0544639
T3	23	-4.6577363
T3	24	-4.6173983
T3	25	-4.4684453
T3	26	-4.3001547
T3	27	-4.2011528
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T4	1	-8.3689117
T4	2	-8.1757193
T4	3	-7.764658
T4	4	-7.6395993
T4	5	-7.582962
T4	6	-7.4218984
T4	7	-7.0887237
T4	8	-6.987906
T4	9	-6.8171511
T4	10	-6.7192264
T4	11	-6.6646791
T4	12	-6.561758
T4	13	-6.5300455
T4	14	-6.3378372
T4	15	-6.3233337
T4	16	-6.2640481
T4	17	-6.181746
T4	18	-6.1525826
T4	19	-6.0715342
T4	20	-5.7689247
T4	21	-5.7037458
T4	22	-5.5879264
T4	23	-5.5096035
T4	24	-5.4134665
T4	25	-5.3651433
T4	26	-4.5676293
T4	27	-4.4412456
T4	28	-4.4044247
T4	29	-3.9639657
T4	30	-3.6974566
<hr/>		
T5	1	-6.8720546
T5	2	-6.8253832
T5	3	-6.7739153
T5	4	-6.5734506
T5	5	-6.5388331
T5	6	-6.4891763
T5	7	-6.4298067
T5	8	-6.3848505

Compound	Conformer	$\Delta G_{\text{binding}}$ (kcal mol ⁻¹)
T5	9	-6.3394074
T5	10	-6.2334661
T5	11	-6.131846
T5	12	-5.9663172
T5	13	-5.9041052
T5	14	-5.8545771
T5	15	-5.8322792
T5	16	-5.5169511
T5	17	-5.4954123
T5	18	-5.4517694
T5	19	-5.3147964
T5	20	-5.2868881
T5	21	-5.2818365
T5	22	-5.237505
T5	23	-5.1515322
T5	24	-5.1090546
T5	25	-5.0306735
T5	26	-5.0156164
T5	27	-4.693058
T5	28	-4.6785226
T5	29	-4.4189272
D4	1	-7.9663849
D4	2	-7.0733962
D4	3	-6.6277122
D4	4	-6.2691174
D4	5	-6.2620864
D4	6	-6.2564354
D4	7	-6.1517792
D4	8	-6.1448488
D4	9	-6.1111188
D4	10	-6.0154591
D4	11	-5.7929511
D4	12	-5.6190715
D4	13	-5.570888
D4	14	-5.4607539
D4	15	-5.3625689
D4	16	-5.3184571
D4	17	-5.311152
D4	18	-5.297945
D4	19	-5.1712837
D4	20	-5.1551957
D4	21	-4.9263911
D4	22	-4.856832
D4	23	-4.7874279
D4	24	-4.7247987
D4	25	-4.7180524

Table S3. Interaction report of each conformer of T1 compound. Number of conformer, Atom of compound, Amino acid in MtTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in MtTIM		Interaction	Distance
1	O	LEU	136	H-acceptor	2.98
	6-ring	ILE	177	pi-H	4.49
2	6-ring	THR	105	pi-H	3.67
3	O	LEU	136	H-acceptor	3.43
4	O	HIS	147	H-acceptor	3.22
	6-ring	GLY	178	pi-H	4.02
5	N	ASP	82	H-donor	2.78
	O	HIS	74	H-acceptor	3.07
	O	LYS	117	H-acceptor	2.77
	6-ring	ARG	103	pi-cation	4.66

Table S4. Interaction report of each conformer of T2 compound. Number of conformer, Atom of compound, Amino acid in MtTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in MtTIM		Interaction	Distance
1	O	LEU	136	H-acceptor	3.45
	O	LEU	136	H-acceptor	3.25
	O	TRP	175	H-acceptor	3.38
2	CL	THR	105	H-donor	3.12
3	O	LYS	12	H-acceptor	3.17
	6-ring	ILE	177	pi-H	4.11
	6-ring	GLY	180	pi-H	3.52

Table S5. Interaction report of each conformer of T3 compound. Number of conformer, Atom of compound, Amino acid in MtTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in MtTIM		Interaction	Distance
1	C	ASP	33	H-donor	3.64
2	N	ASP	33	H-donor	3
	C	ASP	33	H-donor	3.32
	N	ASP	33	ionic	3
	N	ASP	33	ionic	3.75
3	C	ASP	231	H-donor	3.37
4	C	TYR	106	H-pi	4.27
5	C	TYR	106	H-pi	4.9
	C	HIS	107	H-pi	4.64

Table S6. Interaction report of each conformer of T4 compound. Number of conformer, Atom of compound, Amino acid in MtTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in MtTIM		Interaction	Distance
1	N	ASP	33	H-donor	2.74
	N	ASP	33	H-donor	2.99
	N	ASP	33	ionic	2.74
	N	ASP	33	ionic	2.99
	N	ASP	33	ionic	2.99
2	N	ASP	33	H-donor	2.88
	N	ASP	33	H-donor	3.35
	N	ASP	33	ionic	2.88
	N	ASP	33	ionic	3.35
3	N	ASP	33	H-donor	2.7
	N	ASP	33	ionic	3
	N	ASP	33	ionic	2.7
4	N	ASP	33	ionic	2.87
	N	ASP	33	ionic	3.38
	6-ring	ARG	63	pi-cation	3.86
5	N	ASP	33	H-donor	2.74
	N	ASP	33	ionic	2.74
	N	ASP	33	ionic	3.82

Table S7. Interaction report of each conformer of T5 compound. Number of conformer, Atom of compound, Amino acid in MtTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in MtTIM		Interaction	Distance
1	N	ASP	33	ionic	3.63
2	N	ASP	33	H-donor	2.81
	C	ASP	33	H-donor	3.37
	N	ASP	33	ionic	2.81
3	N	ASP	209	H-donor	3.28
	N	ASP	209	ionic	3.98
	N	ASP	209	ionic	3.28
4	O	GLU	109	H-donor	3.24
	O	ARG	104	H-acceptor	3.24
	N	ASP	110	ionic	3.66

Table S8. Interaction report of each conformer of D4 compound. Number of conformer, Atom of compound, Amino acid in MtTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in MtTIM		Interaction	Distance
1	S	GLU	172	H-donor	2.95
	O	LYS	12	H-acceptor	2.94
	O	LYS	12	ionic	2.94
2	O	LYS	12	H-acceptor	2.91
	O	LYS	12	ionic	2.91
3	6-ring	GLY	240	pi-H	3.55
	S	GLU	172	H-donor	3.46
	S	GLY	216	H-donor	3.2
	S	ASN	14	H-donor	3.79
4	O	GLY	240	H-acceptor	2.96
	S	GLU	102	H-donor	3.91
	O	LYS	12	H-acceptor	2.89
	O	LYS	12	ionic	2.89
	6-ring	ILE	177	pi-H	4.46

Table S9. Interaction report of each conformer of T1 compound. Number of conformer, Atom of compound, Amino acid in HsTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in HsTIM		Interaction	Distance
1	N	LYS	58	H-donor	3.06
	O	TRP	90	H-acceptor	2.96
	O	LYS	5	H-acceptor	3.19
2	N	SER	158	H-donor	3.28
	O	LYS	5	H-acceptor	2.9
	O	LYS	5	ionic	2.9
3	O	LYS	5	ionic	2.98
	O	TRP	90	H-acceptor	3.03
4	O	ARG	98	H-acceptor	2.96
	O	ARG	98	H-acceptor	3.27
	O	ARG	98	H-acceptor	3.07
	O	ARG	98	ionic	2.96
	O	ARG	98	ionic	3.27
	O	ARG	98	ionic	3.07
	O	ARG	98	ionic	3.82
5	O	ARG	98	ionic	3.63
	O	ASP	106	H-acceptor	3.52
	O	ARG	99	ionic	2.87

Table S10. Interaction report of each conformer of T2 compound. Number of conformer, Atom of compound, Amino acid in HsTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in AXL		Interaction	Distance
1	CL	GLU	77	H-donor	3.66
	O	ARG	98	H-acceptor	2.88
	O	ARG	98	ionic	2.88
	O	ARG	98	ionic	3.99
2	O	ASN	65	H-acceptor	3.39
3	O	TYR	67	H-acceptor	3.3
4	CL	GLU	77	H-donor	3.05
	O	ASN	65	H-acceptor	3.4

Table S11. Interaction report of each conformer of T3 compound. Number of conformer, Atom of compound, Amino acid in HsTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in HsTIM		Interaction	Distance
1	C	GLN	223	H-donor	3.33
2	S	ASP	36	H-donor	3.18
	6-ring	ASP	36	pi-H	4.73
3	C	ASP	225	H-donor	3.49
	C	ASP	225	H-donor	3.45
	C	GLU	186	H-donor	3.49
	N	ASP	225	ionic	3.79
	N	ASP	225	ionic	3.67
	6-ring	LYS	193	pi-cation	3.96

Conformer	Ligand	Residues in HsTIM		Interaction	Distance
4	6-ring	LYS	193	pi-cation	4.35
	S	GLU	145	H-donor	3.47
	N	GLU	145	H-donor	2.8
	C	GLU	107	H-donor	3.35
	N	GLU	145	ionic	3.86
	N	GLU	107	ionic	3.99
	N	GLU	145	ionic	2.8
5	6-ring	VAL	142	pi-H	4.56
	N	ASP	36	H-donor	2.78
	N	ASP	36	ionic	3.99
	N	ASP	36	ionic	2.78

Table S12. Interaction report of each conformer of T4 compound. Number of conformer, Atom of compound, Amino acid in HsTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in HsTIM		Interaction	Distance
1	N	VAL	101	H-donor	3.02
	C	GLY	103	H-donor	3.46
	5-ring	ARG	98	pi-cation	4.01
	5-ring	ARG	98	pi-cation	4.05
2	N	GLY	103	H-donor	3.18
	N	VAL	101	H-donor	3.16
	N	ARG	98	H-acceptor	3.21
	5-ring	ARG	98	pi-cation	4.42
3	N	VAL	101	H-donor	2.93
	C	GLY	103	H-donor	3.46
	5-ring	ARG	98	pi-cation	4.15
4	C	VAL	101	H-donor	3.33
	N	ARG	98	H-acceptor	3.08
	5-ring	ARG	98	pi-cation	4.31

Table S13. Interaction report of each conformer of T5 compound. Number of conformer, Atom of compound, Amino acid in HsTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in HsTIM		Interaction	Distance
1	N	GLU	133	H-donor	2.83
	C	ASP	106	H-donor	3.5
	C	ASP	106	H-donor	3.49
	N	ASP	106	ionic	3.94
	N	GLU	133	ionic	2.83
2	C	ASP	106	H-donor	3.32
	O	GLU	104	H-donor	3.02
	N	ASP	106	ionic	3.96
3	N	GLU	145	ionic	3.9
4	N	ASP	106	ionic	3.3
5	N	GLU	133	H-donor	2.84
	N	ASP	106	ionic	3.75
	N	GLU	145	ionic	3.86
	N	GLU	133	ionic	2.84
	N	GLU	133	ionic	3.82

Table S14. Interaction report of each conformer of D4 compound. Number of conformer, Atom of compound, Amino acid in HsTIM, Type of interaction and Distance in angstroms.

Conformer	Ligand	Residues in HsTIM		Interaction	Distance
1	S	GLY	210	H-donor	2.94
	S	VAL	212	H-donor	3.68
	O	LYS	13	H-acceptor	2.86
	O	LYS	13	ionic	2.86
	O	LYS	13	ionic	2.86
2	S	GLY	210	H-donor	2.96
	S	VAL	212	H-donor	3.9
	O	LYS	13	H-acceptor	2.86
	O	LYS	13	ionic	2.86
3	6-ring	GLY	232	pi-H	4.16
	6-ring	ALA	73	pi-H	4.67
	S	ASN	15	H-donor	3.55
	O	LYS	13	H-acceptor	2.95

Conformer	Ligand	Residues in HsTIM	Interaction	Distance	
	O	LYS	13	ionic	2.97
	6-ring	ALA	73	pi-H	4.38
4	O	LYS	68	H-acceptor	3.28
	S	LYS	112	H-acceptor	3.51
	O	LYS	68	ionic	3.09
5	O	TRP	191	H-acceptor	3.21

Table S15. Toxicity – PreADMET | Prediction of ADME/Tox of compounds T1–T5 and D4.

<p>T1.- algae_at 0.0451961 Ames_test mutagen Carcino_Mouse negative Carcino_Rat positive daphnia_at0.127897 hERG_inhibition high_risk medaka_at0.0335621 minnow_at0.0280155 TA100_10RLI negative TA100_NA negative TA1535_10RLI negative TA1535_NA positive</p>	<p>T2.- algae_at 0.00554922 Ames_test mutagen Carcino_Mouse negative Carcino_Rat negative daphnia_at0.00650143 hERG_inhibition medium_risk medaka_at0.000116801 minnow_at0.000129914 TA100_10RLI negative TA100_NA negative TA1535_10RLI negative TA1535_NA negative</p>
<p>T3.- algae_at 0.00859781 Ames_test mutagen Carcino_Mouse negative Carcino_Rat negative daphnia_at0.0154404 hERG_inhibition medium_risk medaka_at0.000516688 minnow_at0.000664616 TA100_10RLI negative TA100_NA negative TA1535_10RLI negative TA1535_NA negative</p>	<p>T4.- algae_at 0.0318207 Ames_test mutagen Carcino_Mouse negative Carcino_Rat negative daphnia_at0.179538 hERG_inhibition medium_risk medaka_at0.05414 minnow_at0.0876528 TA100_10RLI negative TA100_NA negative TA1535_10RLI negative TA1535_NA negative</p>
<p>T5.- algae_at 0.00416684 Ames_test mutagen Carcino_Mouse negative Carcino_Rat positive daphnia_at0.0219124 hERG_inhibition medium_risk medaka_at0.000884984 minnow_at0.00121519 TA100_10RLI negative TA100_NA negative TA1535_10RLI positive TA1535_NA negative</p>	<p>D4.- algae_at 0.0162351 Ames_test mutagen Carcino_Mouse negative Carcino_Rat negative daphnia_at0.0261622 hERG_inhibition ambiguous medaka_at0.00190628 minnow_at0.00152966 TA100_10RLI positive TA100_NA negative TA1535_10RLI negative TA1535_NA negative</p>

Table S16. ADME - PreADMET | Prediction of ADME/Tox of compounds T1–T5 and D4.

<p>T1.- BBB 0.0139453 Buffer_solubility_mg_L 874.844** Caco2 21.0136 CYP_2C19_inhibition Non CYP_2C9_inhibition Non CYP_2D6_inhibition Non CYP_2D6_substrate Non CYP_3A4_inhibition Non CYP_3A4_substrate Non HIA 95.124084 MDCK 9.16919 Pgp_inhibition Non Plasma_Protein_Binding 92.872463 Pure_water_solubility_mg_L 7.12722 Skin_Permability -2.95411 SKlogD_value 2.2739 SKlogP_value 2.2739 SKlogS_buffer -2.663820** SKlogS_pure -4.75283</p>	<p>T2.- BBB 0.0215146 Buffer_solubility_mg_L 0.0485304 Caco2 21.4132 CYP_2C19_inhibition Inhibitor CYP_2C9_inhibition Inhibitor CYP_2D6_inhibition Non CYP_2D6_substrate Non CYP_3A4_inhibition Non CYP_3A4_substrate Weakly HIA 98.49618 MDCK 0.0589242 Pgp_inhibition Inhibitor Plasma_Protein_Binding 94.136555 Pure_water_solubility_mg_L 0.00538287 Skin_Permability -2.34608 SKlogD_value 4.41304 SKlogP_value 5.66104 SKlogS_buffer -6.97946 SKlogS_pure -7.93446</p>
<p>T3.- BBB 0.384245 Buffer_solubility_mg_L 221.745 Caco2 25.9121</p>	<p>T4.- BBB 0.0268428 Buffer_solubility_mg_L 68065.1 Caco2 54.4744</p>

CYP_2C19_inhibition	Non	CYP_2C19_inhibition	Inhibitor
CYP_2C9_inhibition	Non	CYP_2C9_inhibition	Non
CYP_2D6_inhibition	Inhibitor	CYP_2D6_inhibition	Non
CYP_2D6_substrate	Substrate	CYP_2D6_substrate	Substrate
CYP_3A4_inhibition	Non	CYP_3A4_inhibition	Non
CYP_3A4_substrate	Weakly	CYP_3A4_substrate	Substrate
HIA	98.766413	HIA	96.023819
MDCK	0.0499068	MDCK	57.2976
Pgp_inhibition	Inhibitor	Pgp_inhibition	Non
Plasma_Protein_Binding	83.498175	Plasma_Protein_Binding	49.658153
Pure_water_solubility_mg_L	3.17069	Pure_water_solubility_mg_L	11991.8
Skin_Permeability	-2.14204	Skin_Permeability	-3.72035
SKlogD_value	3.48853	SKlogD_value	0.682080
SKlogP_value	5.05299	SKlogP_value	2.02606
SKlogS_buffer	-3.26427	SKlogS_buffer	-0.7347
SKlogS_pure	-5.10897	SKlogS_pure	-1.48874
T5.-		D4.-	
BBB	9.45103	BBB	0.0349711
Buffer_solubility_mg_L	112.703	Buffer_solubility_mg_L	0.34811
Caco2	56.4024	Caco2	19.32
CYP_2C19_inhibition	Inhibitor	CYP_2C19_inhibition	Non
CYP_2C9_inhibition	Non	CYP_2C9_inhibition	Non
CYP_2D6_inhibition	Inhibitor	CYP_2D6_inhibition	Non
CYP_2D6_substrate	Substrate	CYP_2D6_substrate	Non
CYP_3A4_inhibition	Non	CYP_3A4_inhibition	Inhibitor
CYP_3A4_substrate	Weakly	CYP_3A4_substrate	Weakly
HIA	97.105389	HIA	40.443246
MDCK	0.872546	MDCK	1.68617
Pgp_inhibition	Inhibitor	Pgp_inhibition	Inhibitor
Plasma_Protein_Binding	64.300766	Plasma_Protein_Binding	94.330258
Pure_water_solubility_mg_L	383.156	Pure_water_solubility_mg_L	0.0804723
Skin_Permeability	-3.25132	Skin_Permeability	-3.79902
SKlogD_value	1.3476	SKlogD_value	1.85403
SKlogP_value	4.47652	SKlogP_value	1.85403
SKlogS_buffer	-3.54971	SKlogS_buffer	-6.11097
SKlogS_pure	-3.01827	SKlogS_pure	-6.74704

Table S17. Properties predicted by PhysChem - ACD/Labs of compounds T1–T5 and D4.

T1.-		T2.-	
Density:		Density:	1.4±0.1 g/cm3
Boiling Point:	615.5±55.0 °C at 760 mmHg	Boiling Point:	706.8±60.0 °C at 760 mmHg
Vapour Pressure:	0.0±1.9 mmHg at 25°C	Vapour Pressure:	0.0±2.4 mmHg at 25°C
Enthalpy of Vaporization:	96.0±3.0 kJ/mol	Enthalpy of Vaporization:	108.6±3.0 kJ/mol
Flash Point:	326.0±31.5 °C	Flash Point:	381.2±32.9 °C
Index of Refraction:		Index of Refraction:	1.675
Molar Refractivity:		Molar Refractivity:	123.6±0.3 cm3
#H bond acceptors:	7	#H bond acceptors:	6
#H bond donors:	3	#H bond donors:	1
#Freely Rotating Bonds:	7	#Freely Rotating Bonds:	7
#Rule of 5 Violations:	0	#Rule of 5 Violations:	1
ACD/LogP:	4.16	ACD/LogP:	6.42
ACD/LogD (pH 5.5):	2.24	ACD/LogD (pH 5.5):	4.16
ACD/BCF (pH 5.5):	9.04	ACD/BCF (pH 5.5):	399.19
ACD/KOC (pH 5.5):	41.47	ACD/KOC (pH 5.5):	1019.71
ACD/LogD (pH 7.4):	1.27	ACD/LogD (pH 7.4):	2.64
ACD/BCF (pH 7.4):	1.00	ACD/BCF (pH 7.4):	12.08
ACD/KOC (pH 7.4):	4.53	ACD/KOC (pH 7.4):	30.85
Polar Surface Area:	108 Å ²	Polar Surface Area:	90 Å ²
Polarizability:		Polarizability:	49.0±0.5 10 ⁻²⁴ cm ³
Surface Tension:		Surface Tension:	63.7±3.0 dyne/cm
Molar Volume:		Molar Volume:	329.0±3.0 cm ³
T3.-		T4.-	
Density:	1.2±0.1 g/cm3	Density:	1.2±0.1 g/cm3
Boiling Point:	506.0±50.0 °C at 760 mmHg	Boiling Point:	575.7±50.0 °C at 760 mmHg
Vapour Pressure:	0.0±1.3 mmHg at 25°C	Vapour Pressure:	0.0±1.6 mmHg at 25°C
Enthalpy of Vaporization:	77.6±3.0 kJ/mol	Enthalpy of Vaporization:	86.2±3.0 kJ/mol
Flash Point:	259.8±30.1 °C	Flash Point:	302.0±30.1 °C
Index of Refraction:	1.572	Index of Refraction:	1.601
Molar Refractivity:	108.2±0.3 cm3	Molar Refractivity:	109.8±0.5 cm3
#H bond acceptors:	3	#H bond acceptors:	6
#H bond donors:	0	#H bond donors:	1
#Freely Rotating Bonds:	5	#Freely Rotating Bonds:	7
#Rule of 5 Violations:	1	#Rule of 5 Violations:	0
ACD/LogP:	5.11	ACD/LogP:	0.17
ACD/LogD (pH 5.5):	2.63	ACD/LogD (pH 5.5):	-2.11
ACD/BCF (pH 5.5):	16.20	ACD/BCF (pH 5.5):	1.00
ACD/KOC (pH 5.5):	56.14	ACD/KOC (pH 5.5):	1.00
ACD/LogD (pH 7.4):	4.34	ACD/LogD (pH 7.4):	-0.62
ACD/BCF (pH 7.4):	835.29	ACD/BCF (pH 7.4):	1.00

ACD/KOC (pH 7.4): 2894.38 Polar Surface Area: 35 Å ² Polarizability: 42.9±0.5 10-24cm ³ Surface Tension: 40.4±3.0 dyne/cm Molar Volume: 328.8±3.0 cm ³	ACD/KOC (pH 7.4): 1.00 Polar Surface Area: 53 Å ² Polarizability: 43.5±0.5 10-24cm ³ Surface Tension: 42.4±7.0 dyne/cm Molar Volume: 320.6±7.0 cm ³
T5.- Density: 1.1±0.1 g/cm ³ Boiling Point: 530.8±35.0 °C at 760 mmHg Vapour Pressure: 0.0±1.5 mmHg at 25°C Enthalpy of Vaporization: 83.6±3.0 kJ/mol Flash Point: 246.3±24.6 °C Index of Refraction: 1.567 Molar Refractivity: 121.0±0.3 cm ³ #H bond acceptors: 4 #H bond donors: 1 #Freely Rotating Bonds: 8 #Rule of 5 Violations:0 ACD/LogP: 4.71 ACD/LogD (pH 5.5): -0.42 ACD/BCF (pH 5.5): 1.00 ACD/KOC (pH 5.5): 1.00 ACD/LogD (pH 7.4): 0.51 ACD/BCF (pH 7.4): 1.00 ACD/KOC (pH 7.4): 1.00 Polar Surface Area: 30 Å ² Polarizability: 48.0±0.5 10-24cm ³ Surface Tension: 46.4±3.0 dyne/cm Molar Volume: 370.4±3.0 cm ³	D4.- Density: 1.7±0.1 g/cm ³ Boiling Point: 648.7±65.0 °C at 760 mmHg Vapour Pressure: 0.0±2.0 mmHg at 25°C Enthalpy of Vaporization: 100.5±3.0 kJ/mol Flash Point: 346.1±34.3 °C Index of Refraction: 1.788 Molar Refractivity: 110.6±0.5 cm ³ #H bond acceptors: 11 #H bond donors: 2 #Freely Rotating Bonds: 4 #Rule of 5 Violations:1 ACD/LogP: 1.57 ACD/LogD (pH 5.5): -3.25 ACD/BCF (pH 5.5): 1.00 ACD/KOC (pH 5.5): 1.00 ACD/LogD (pH 7.4): -4.12 ACD/BCF (pH 7.4): 1.00 ACD/KOC (pH 7.4): 1.00 Polar Surface Area: 229 Å ² Polarizability: 43.9±0.5 10-24cm ³ Surface Tension: 73.0±7.0 dyne/cm Molar Volume: 261.7±7.0 cm ³