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.

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 (ΔGbinding) of each complex (Ligand-Protein), as previously reported [34, 36] using MOE [44, 45]. With
these results, the best averages of \( \Delta G \) 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 \( \Delta G \)binding of each compound (Table 1). Using the best five values of their conformers of each compound, were determined the average of \( \Delta G \)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 \( \Delta G \)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.](image)

![Table 1. PubChem CID and Structure of the best compounds, T1 - T5 and D4 compounds.](image)

T1.- 1769482

T2.- 1334501
Table 2. Average of $\Delta G_{binding}$ (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 LD$_{50}$ [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 | COC1=CC=C(C=C1)CC(=O)NC2=CC=C(C=C2)C(=O)NC3=CC=CC=C3C(=O)[O-] | His74, Asp82, Arg103, Thr105, Lys117, Leu136, His147, Ile177, Gly178 | Lys5, Lys58, Trp90, Arg98, Arg99, Asp106, Ser158 | Mutagen |
| T2.- 1334501 | CCOCl=C(C(=CC(=C1)C=C2C=C3=CC(=O)C)CC=C=C=C4C(=O)C)C | Lys12, Thr105, Leu136, Trp175, Ile177, Gly180 | Asn65, Tyr67, Glu77, Arg98 | Mutagen |
| T3.- 5566   | C1CCCN(C)CCCN2C3=CC=C=C3SC4=C2C(=C(C=C4)C(=O)F)F | Asp33, Tyr106, His107, Asp231 | Asp36, Glu107, Val142, Glu145, Glu186, Lys193, Gln223, Asp225 | Mutagen |
| T4.- 45159102 | CN1C2=C(CC(CC2)NCCN(C)C(=N1)C=O)N(C)CC3=CC=CC=C3 | Asp33, Arg63 | Arg98, Val101, Phe102, Gly103 | Mutagen |
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 ΔGbinding 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 ΔGbinding 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 ΔGbinding 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.

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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. Black: identical; gray: similar; yellow: amino acids important for interaction of T1 compound with MtTIM.

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

Figure S9. Alignment of HsTIM and MtTIM. Black: identical; gray: similar; yellow: amino acids important for interaction of T3 compound with MtTIM.
Figure S10. Alignment of HsTIM and MtTIM. Black: identical; gray: similar; yellow: amino acids important for interaction of T4 compound with MtTIM.

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

Figure S12. Alignment of HsTIM and MtTIM. Black: identical; gray: similar; yellow: amino acids important for interaction of D4 compound with MtTIM.
Table S1. $\Delta G_{\text{binding}}$ of 21 to 30 conformers from each compound, average $\Delta G_{\text{binding}}$ and SD for MtTIM.

| Compound | Conformer | $\Delta G_{\text{binding}}$ (kcal mol$^{-1}$) |
|----------|-----------|-------------------------------------------|
|          |           |                                           |
| T1       | 1         | -19.34875                                |
| T1       | 2         | -11.57174                                 |
| T1       | 3         | -9.763415                                 |
| T1       | 4         | -9.736414                                 |
| T1       | 5         | -9.705184                                 |
| T1       | 6         | -8.781687                                 |
| T1       | 7         | -8.660049                                 |
| T1       | 8         | -8.476028                                 |
| T1       | 9         | -8.418235                                 |
| T1       | 10        | -7.947825                                 |
| T1       | 11        | -7.707098                                 |
| T1       | 12        | -7.514373                                 |
| T1       | 13        | -7.375604                                 |
| T1       | 14        | -7.356618                                 |
| T1       | 15        | -7.229507                                 |
| T1       | 16        | -7.180483                                 |
| T1       | 17        | -7.116158                                 |
| T1       | 18        | -6.819993                                 |
| T1       | 19        | -6.426841                                 |
| T1       | 20        | -6.322961                                 |
| T1       | 21        | -6.201733                                 |
| T1       | 22        | -6.107904                                 |

Figure S13. Alignment of TIMs (HsTIM, MtTIM, BtTIM, CpTIM, NpTIM, ScTIM, DrTIM and TtTIM) [29]. The red box indicates the position of Phe46 and Lys230 and that HsTIM not have them in its sequence.
| Compound | Conformer | ΔGbinding (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        | -7.482631            |
| 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        | -6.325875            |
| 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        | -6.064059            |
| 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 | ΔGbinding (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                    |
| T5       | 1         | -17.860346                    |
| T5       | 2         | -15.663527                    |
| T5       | 3         | -12.467755                    |
| T5       | 4         | -9.4178664                    |
| 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        | -4.217681                     |
| T5       | 18        | -3.9669673                    |
| T5       | 19        | -3.5289178                    |
| T5       | 20        | -3.3479142                    |
| T5       | 21        | -3.1974645                    |
| T5       | 22        | -3.058063                     |
| D4       | 1         | -12.990515                    |
| D4       | 2         | -12.301793                    |
| D4       | 3         | -12.146478                    |
| D4       | 4         | -10.714886                    |
| D4       | 5         | -10.038652                    |
| 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        | -6.0711179                    |
| D4       | 17        | -6.0020676                    |
| D4       | 18        | -5.822444                     |
| D4       | 19        | -5.2859912                    |
| D4       | 20        | -4.7035975                    |
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$^{-1}$) |
|----------|-----------|---------------------------------------------|
| D4       | 21        | -4.4125037                                  |
| T1       | 1         | -7.7168059                                  |
| T1       | 2         | -7.360672                                   |
| T1       | 3         | -7.265059                                   |
| 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 | ΔGbinding (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         | -5.6154823            |
| 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            |
| 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        | -6.3233377            |
| 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        | -5.5676293            |
| T4       | 27        | -4.4412456            |
| T4       | 28        | -4.4044247            |
| T4       | 29        | -3.9639657            |
| T4       | 30        | -3.6974566            |
| 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 | ΔGbinding (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        | -5.495123             |
| T5       | 18        | -5.4517694            |
| T5       | 19        | -5.3147964            |
| T5       | 20        | -5.2868881            |
| T5       | 21        | -5.2818365            |
| T5       | 22        | -5.237505             |
| T5       | 23        | -5.151322             |
| 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         | -6.1111888            |
| 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     |
| 2         | 6-ring | ILE 177           | pi-H        | 4.49     |
| 3         | 6-ring | THR 105           | pi-H        | 3.67     |
| 4         | O      | LEU 136           | H-acceptor  | 3.43     |
| 5         | 6-ring | HIS 147           | H-acceptor  | 3.22     |
| 5         | N      | ASP 82            | H-donor     | 2.78     |
| 6-ring    | O      | HIS 74            | H-acceptor  | 3.07     |
| 6-ring    | 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        |
| 3         | C      | 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|                  |             | 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        |
| 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 | 6-ring 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 209| ionic             | 2.81        |
| 3         | N      | ASP 209           | ionic       | 3.98     |
| 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               | H-donor     | 2.95     |
| 2         | O      | LYS               | H-acceptor  | 2.94     |
| 6-ring    | GLY    | 240               | pi-H        | 3.55     |
| 3         | S      | GLU               | H-donor     | 3.46     |
| 4         | S      | GLY               | H-donor     | 3.2      |
| 5         | O      | GLY               | H-acceptor  | 2.96     |
| 6         | LYS    | 12                | ionic       | 2.89     |

### 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               | H-donor     | 3.06     |
| 2         | O      | TRP               | H-acceptor  | 2.96     |
| 3         | O      | LYS               | H-acceptor  | 3.19     |
| 4         | N      | SER               | H-donor     | 3.28     |
| 5         | O      | LYS               | H-acceptor  | 2.9     |
| 6         | LYS    | 5                 | ionic       | 2.98     |

### 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             | H-donor     | 3.66     |
| 2         | O      | ARG             | H-acceptor  | 3.27     |
| 3         | O      | ARG             | H-acceptor  | 3.07     |
| 4         | O      | ARG             | ionic       | 3.27     |
| 5         | O      | ASP             | H-acceptor  | 3.52     |
| 6         | ASP    | 99              | ionic       | 2.87     |

### 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               | H-donor     | 3.33     |
| 2         | S      | ASP               | H-donor     | 3.18     |
| 6-ring    | ASP    | 36                | pi-H        | 4.73     |
| 3         | C      | ASP               | H-donor     | 3.49     |
| 4         | C      | ASP               | H-donor     | 3.45     |
| 5         | N      | ASP               | H-donor     | 3.49     |
| 6         | ASP    | 225               | ionic       | 3.79     |
| 7         | LYS    | 193               | pi-cation   | 3.96     |
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     |
|           | ARG    | 98                | pi-cation   | 4.05     |
| 2         | N      | GLY 103           | H-donor     | 3.18     |
|           | N      | VAL 101           | H-donor     | 3.16     |
| 5-ring    | ARG    | 98                | pi-cation   | 3.21     |
|           | 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     |
| 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      |
|           | ASP    | 106               | H-donor     | 3.49     |
|           | ASP    | 106               | ionic       | 3.94     |
|           | GLU    | 133               | ionic       | 2.83     |
| 2         | C      | ASP 106           | H-donor     | 3.32     |
|           | GLU    | 104               | H-donor     | 3.02     |
|           | ASP    | 106               | ionic       | 3.96     |
|           | GLU    | 145               | ionic       | 3.9      |
| 3         | N      | GLU 133           | H-donor     | 2.84     |
|           | ASP    | 106               | ionic       | 3.75     |
|           | GLU    | 145               | ionic       | 3.86     |
|           | GLU    | 133               | ionic       | 2.84     |
|           | 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     |
| 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     |
| 0-ring    | GLY    | 232               | pi-H        | 4.16     |
| 0-ring    | ALA    | 73                | pi-H        | 4.67     |
| 3         | N      | ASN 15            | H-donor     | 3.55     |
|           | O      | LYS 13            | H-acceptor  | 2.95     |
## Table S15. Toxicity – PreADMET | Prediction of ADME/Tox of compounds T1–T5 and D4.

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

### T1.
- **algae** at 0.0451961
  - Ames test mutagen: negative
  - [Carcino_Mouse](#): negative
  - [Carcino_Rat](#): positive
  - [daphnia](#) at 0.127897: high risk
  - [hERG](#) inhibition: medium risk
  - [medaka](#) at 0.0335621
  - [minnow](#) at 0.00280155

- **TA100** 10RLI: negative
- **TA100** NA: negative
- **TA1535** 10RLI: negative
- **TA1535** NA: positive

### T2.
- **algae** at 0.00554922
  - Ames test mutagen: negative
  - [Carcino_Mouse](#): negative
  - [Carcino_Rat](#): negative
  - [daphnia](#) at 0.00650143: medium risk
  - [hERG](#) inhibition: medium risk
  - [medaka](#) at 0.000116801
  - [minnow](#) at 0.000129914

- **TA100** 10RLI: negative
- **TA100** NA: negative
- **TA1535** 10RLI: negative
- **TA1535** NA: negative

### T3.
- **algae** at 0.00859781
  - Ames test mutagen: negative
  - [Carcino_Mouse](#): negative
  - [Carcino_Rat](#): positive
  - [daphnia](#) at 0.0154404: high risk
  - [hERG](#) inhibition: medium risk
  - [medaka](#) at 0.0000516688
  - [minnow](#) at 0.000664166

- **TA100** 10RLI: negative
- **TA100** NA: negative
- **TA1535** 10RLI: negative
- **TA1535** NA: negative

### T4.
- **algae** at 0.0318207
  - Ames test mutagen: negative
  - [Carcino_Mouse](#): negative
  - [Carcino_Rat](#): negative
  - [daphnia](#) at 0.0179538: medium risk
  - [hERG](#) inhibition: medium risk
  - [medaka](#) at 0.000116801
  - [minnow](#) at 0.000129914

- **TA100** 10RLI: negative
- **TA100** NA: negative
- **TA1535** 10RLI: negative
- **TA1535** NA: negative

### T5.
- **algae** at 0.00466884
  - Ames test mutagen: negative
  - [Carcino_Mouse](#): negative
  - [Carcino_Rat](#): positive
  - [daphnia](#) at 0.0219124: high risk
  - [hERG](#) inhibition: medium risk
  - [medaka](#) at 0.000884984
  - [minnow](#) at 0.00125159

- **TA100** 10RLI: negative
- **TA100** NA: negative
- **TA1535** 10RLI: negative
- **TA1535** NA: negative

### T6.
- **algae** at 0.0162351
  - Ames test mutagen: negative
  - [Carcino_Mouse](#): negative
  - [Carcino_Rat](#): negative
  - [daphnia](#) at 0.0261622: medium risk
  - [hERG](#) inhibition: ambiguous
  - [medaka](#) at 0.00190628
  - [minnow](#) at 0.00152966

- **TA100** 10RLI: negative
- **TA100** NA: negative
- **TA1535** 10RLI: negative
- **TA1535** NA: negative

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

| Conformer | BBB | Buffer_solvability_mg_L | Caco2 | CYP_2C9 inhibition | CYP_2D6 inhibition | CYP_3A4 inhibition | HIA | MDCK | Pgp_inhibition | Plasma_Protein_Binding | Pure_water_solvability_mg_L | Skin_Permeability | SKlogD_value | SKlogP_value | SKlogS_buffer | SKlogS_pure | T1. |
|-----------|-----|-------------------------|-------|-------------------|-------------------|-------------------|-----|------|----------------|------------------------|--------------------------|---------------------|-------------|-------------|-------------|-------------|------|
| T1.       |     | 0.0319453               | 874.844** | 21.0136          | Non               | Non               | 95.12408 | 9.16919 | Non            | 92.872463              | 7.12722                  | -2.95411            | 2.2739      | 2.2739      | -2.663820** | -4.75283   |      |
| T2.       |     | 0.0215146               |       | 21.4132          | Inhibitor        | Non               |       |       | Inhibitor     | 94.13655                | 94.13655                 | -2.34608            | 4.41304     | 5.66104     | -6.97946   | -7.93446    |      |
| T3.       | 0.384245 | Buffer_solvability_mg_L | 221.745 | 25.9121          |                  |                  |       |       |                |                        |                          |                    |             |             |             |             |      |
| T4.       | 0.026848 | Buffer_solvability_mg_L | 68065.1 | 54.4744          |                  |                  |       |       |                |                        |                          |                    |             |             |             |             |      |
Table S17. Properties predicted by PhysChem - ACD/Labs of compounds T1–T5 and D4.

**T1.**
- Density: 1.4±0.1 g/cm³
- Boiling Point: 615.5±55.0 °C at 760 mmHg
- Vapour Pressure: 0.0±1.9 mmHg at 25°C
- Enthalpy of Vaporization: 96.0±3.0 kJ/mol
- Flash Point: 326.0±31.5 °C
- Index of Refraction: 1.00
- Molar Refractivity: 302.0±30.1 °C
- Molar Volume: 108.2±0.3 cm³
- Polarizability: 49.0±0.5 10⁻24cm³
- Surface Tension: 63.7±3.0 dyn/cm
- Molar Volume: 329.0±3.0 cm³

**T2.**
- Density: 1.4±0.1 g/cm³
- Boiling Point: 706.8±60.0 °C at 760 mmHg
- Vapour Pressure: 0.0±2.4 mmHg at 25°C
- Enthalpy of Vaporization: 108.6±3.0 kJ/mol
- Flash Point: 381.2±32.9 °C
- Index of Refraction: 1.675
- Molar Refractivity: 123.6±0.3 cm³
- #H bond acceptors: 6
- #H bond donors: 1
- #Freely Rotating Bonds: 7
- #Rule of 5 Violations: 1
- ACD/LogP: 4.16
- ACD/LogD (pH 5.5): 2.24
- ACD/BCF (pH 5.5): 9.04
- ACD/KOC (pH 5.5): 41.47
- ACD/LogD (pH 7.4): 1.27
- ACD/BCF (pH 7.4): 1.00
- ACD/KOC (pH 7.4): 4.53

**T3.**
- Density: 1.2±0.1 g/cm³
- Boiling Point: 506.0±50.0 °C at 760 mmHg
- Vapour Pressure: 0.0±1.3 mmHg at 25°C
- Enthalpy of Vaporization: 77.6±3.0 kJ/mol
- Flash Point: 259.8±30.1 °C
- Index of Refraction: 1.572
- Molar Refractivity: 108.2±0.3 cm³
- #H bond acceptors: 3
- #H bond donors: 0
- #Freely Rotating Bonds: 5
- #Rule of 5 Violations: 1
- ACD/LogP: 5.11
- ACD/LogD (pH 5.5): 2.63
- ACD/BCF (pH 5.5): 16.20
- ACD/KOC (pH 5.5): 56.14
- ACD/LogD (pH 7.4): 4.34
- ACD/BCF (pH 7.4): 835.29

**T4.**
- Density: 1.2±0.1 g/cm³
- Boiling Point: 575.7±50.0 °C at 760 mmHg
- Vapour Pressure: 0.0±1.6 mmHg at 25°C
- Enthalpy of Vaporization: 86.2±3.0 kJ/mol
- Flash Point: 302.0±30.1 °C
- Index of Refraction: 1.601
- Molar Refractivity: 109.8±0.5 cm³
- #H bond acceptors: 6
- #H bond donors: 1
- #Freely Rotating Bonds: 7
- #Rule of 5 Violations: 0
- ACD/LogP: 0.17
- ACD/LogD (pH 5.5): 2.11
- ACD/BCF (pH 5.5): 1.00
- ACD/KOC (pH 5.5): 1.00
- ACD/LogD (pH 7.4): -0.62
- ACD/BCF (pH 7.4): 1.00

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| Property | Value |
|----------|-------|
| ACD/KOC (pH 7.4) | 2894.38 |
| Polar Surface Area: | 35 Å² |
| Polarizability: | 42.9±0.5 10⁻²⁴cm³ |
| Surface Tension: | 40.4±3.0 dyne/cm |
| Molar Volume: | 328.8±3.0 cm³ |

| Property | Value |
|----------|-------|
| ACD/KOC (pH 7.4) | 1.00 |
| Polar Surface Area: | 33 Å² |
| Polarizability: | 43.5±0.5 10⁻²⁴cm³ |
| Surface Tension: | 42.4±7.0 dyne/cm |
| Molar Volume: | 320.6±7.0 cm³ |

**T5**

| Density | 1.1±0.1 g/cm³ |
| 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 cm³ |
| #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 Å² |
| Polarizability: | 48.0±0.5 10⁻²⁴cm³ |
| Surface Tension: | 46.4±3.0 dyne/cm |
| Molar Volume: | 370.4±3.0 cm³ |

**D4**

| Density | 1.7±0.1 g/cm³ |
| 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 cm³ |
| #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 Å² |
| Polarizability: | 43.9±0.5 10⁻²⁴cm³ |
| Surface Tension: | 73.0±7.0 dyne/cm |
| Molar Volume: | 261.7±7.0 cm³ |