Can Tho University Journal of Science
website: ctujs.ctu.edu.vn

DOI: 10.22144/ctu.jen.2022.021

Docking-Based Virtual Screening for the Discovery of 1,3,4-Oxadiazoles as Aminoacyl-tRNA Synthetase Inhibitors

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Article info.

Received 20 Apr 2022
Revised 27 Apr 2022
Accepted 27 Apr 2022

Keywords

Aminoacyl-tRNA synthetase, inhibitor, antibiotics, docking, virtual screening, structure-based drug design

ABSTRACT

Aminoacyl-tRNA synthetases (aaRSs) are one of the leading targets for the development of antibiotic agents. In this paper, we reported the discovery of aaRS inhibitors using a structure-based virtual screening method. The interactions of 52 designed structures with the methionyl-tRNA synthetase (MetRS) target were performed by docking the ligands into the active zone of the MetRS using Autodock Vina. The data revealed 14 compounds displaying interactions with key amino acids (Asp287, Tyr250, Val473, Trp474, Phe522, Ile519, Ala477, Leu478, and His523) at the binding pocket of the enzyme, indicating their potential as MetRS inhibitors. These results could be served as the references for further synthetic work and bioassays experiments for discovering MetRS inhibitors and other pharmaceutical agents that may assist in the generation of new antibiotics.

1. INTRODUCTION

Aminoacyl-tRNA synthetase enzymes (aaRS) are important for protein synthesis in all organisms by catalyzing the production of methionyl-tRNA, a key molecule that binds AUG codons at the ribosome during translation, allowing the incorporation of methionine into nascent proteins (Duckworth et al., 2012). A total of 23 aaRSs have been described with methionyl-tRNA synthetase (MetRS) being one of the most studied enzymes since it recognizes an initiator tRNA as well as the tRNA delivering methionine for elongation of the protein chain. Due to the pivotal role that MetRS plays in protein synthesis, this enzyme has been described as a validated drug target in many disease-relevant organisms (Bouz & Zitko, 2021; Buckner et al., 2019; Torrie et al., 2017; Ojo et al., 2016; Hussain et al., 2015). In particular, MetRS has been successfully exploited as a target in Gram-positive bacteria with MetRS inhibitor CRS3123 having recently completed two-phase I clinical trials for the treatment of *Clostridium difficile* infections (Lomeli et al., 2019; Nayak et al., 2017). In addition to Gram-positive bacteria, MetRS has also been validated as a drug target in kinetoplastid parasites (Devine et al., 2017; Ojo et al., 2016; Zhang et al., 2016; Shibata et al., 2012). Several compounds targeting MetRS enzymes are in preclinical development for the treatment of infections caused by *C. difficile* and *Staphylococcus aureus* (Critchley et al., 2009, 2005).

1,3,4-Oxadiazole heterocycles have been known to possess a wide range of biological activities, including antiviral, anti-inflammatory, antitubercular, hypoglycemic, antineoplastic, fungicidal, and antibacterial properties (Desai et al., 2013; Barbuceanu et al., 2010; Kumar et al., 2010). Moreover, 1,3,4-oxadiazole heterocycles are excellent bioisosteres of amides and esters and can contribute to enhancing biological activity by

participating in hydrogen-bonding interactions (Guimaraes et al., 2005). Accordingly, compounds containing the 1,3,4-oxadiazole nucleus have demonstrated extensive antimicrobial activity (Teresa and Piotr, 2021; Zhaojun et al., 2018). On the other hand, benzimidazole, the isostere of a purine-based nucleic acid with a desirable electron-rich characteristic that is beneficial for binding with a variety of therapeutic targets, is one of the most prominent heterocycles with antimicrobial properties (Maria, 2021; Tahlan et al., 2019). There have been many reports in the literature related to the synthesis and antimicrobial activity evaluation of 1,3,4-oxadiazole based heterocycle hybrids, but very rare examples focus on the hybrid 1,3,4-oxadiazole/benzimidazole derivatives (Teresa and Piotr, 2021).

Virtual screening has recently emerged as an important tool to access novel drug-like compounds as it is fast and cost-efficient (Reddy et al., 2007). This computational technique is applied to identify lead molecules using a huge and diverse collection of chemical compounds libraries. Structure-based virtual screening involves automated and fast docking of a large number of chemical compounds against a protein-binding or active site, provided that a three-dimensional structure of the target is known (Bender & Glen, 2005; Bissantz et al., 2000; Willett et al., 1998). Virtual screening methods undoubtedly play a major role in pharmacogenomics by finding the very first leads of new targets, especially in cases of orphan receptors for which no information on potential ligands is known (Bissantz et al., 2000). Based on crystal structures of *Trypanosoma brucei* methionyl-tRNA synthetase (*Tb*MetRS) bound to inhibitors, Zhang et al. (2016) reported the design and synthesis of compound **A** (Figure 1) as a novel *Tb*MetRS inhibitor (IC_{50} < 40 nM) which contains a linker of 5-membered 1,3-dihydro-imidazol-2-one ring connecting to the lefthand side phenyl ring and the benzimidazole moiety on the righthand side (Zhang et al., 2020).

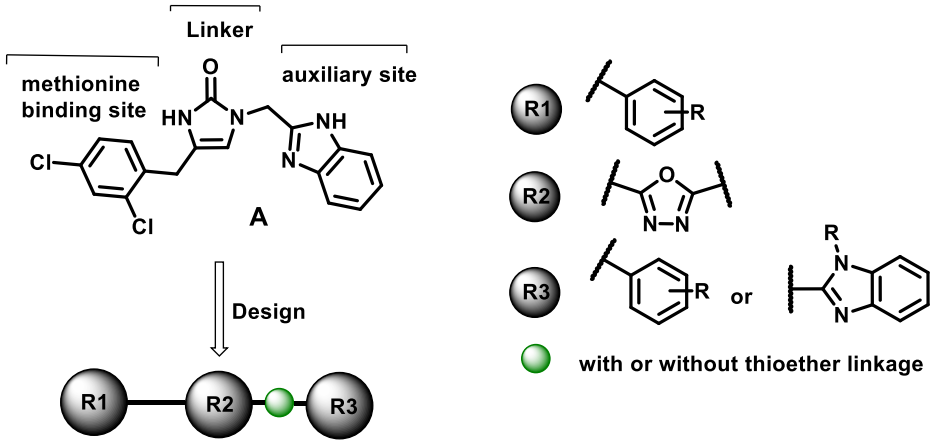


Figure 1. General structures of the designed compounds for virtual screening

Based on these findings and also in the continuation of our studies on the synthesis and bioactivity evaluation of hybrid heterocycles, especially benzimidazole derivatives (Bui et al., 2022, 2021, 2019; Hue et al., 2020), in the current study, the structure-based virtual screening of a small library of fifty-two 1,3,4-oxadiazole based benzimidazole hybrid derivatives against the methionyl-tRNA synthetase (MetRS) was performed. These preliminary results could serve as references for *in vitro* screening investigation of new and potent MetRS inhibitors and also aaRS inhibitors in general.

2. MATERIALS AND METHODS

2.1. Method for preparation of protein and chemical structures

The crystal structure of methionyl-tRNA synthetase from *T. brucei* in complex with inhibitor (ID: 6MES) was retrieved from the PDB (Zhang et al., 2020). The protein was pre-processed and the water molecules were eliminated. In addition, heteroatoms that showed no influence on the protein structure, function, or conformation were removed from the protein. The 3D structures of the designed compounds were created by Gaussview and the energy minimization was carried out with the

B3LYP/6-31g(d,p) in Gaussian 09 (Frisch et al., 2009).

2.2. Molecular docking simulation

AutoDock Vina was used to set up and perform docking calculations using the PyRx program (Dallakyan & Olson, 2015). After docking was finished, the ligands were ranked based on their minimum binding energy; the more negative the binding energy, the better the binding affinity of the compound toward the targeted receptor. The docking study was performed by assuming a rigid structure of the protein and considering the conformational space of the ligands to analyze the inductive effect of the hybrid compounds. The binding site was enclosed in a box with the number of grid points in x × y × z dimensions (46.7514 Å × 48.2834 Å × 45.5232 Å), and the center of the grid box was placed at x = -39.1800, y = 9.0610, z = 9.1222. The output of the AutoDock Vina studies was analyzed using Discovery Studio Visualizer. Ligand efficiency value (LE) is calculated by using equation (1), which is an important informative parameter when selecting a lead compound.

$$LE = \frac{-BE}{HA} \quad (1)$$

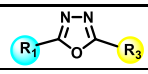
where BE is binding energy and HA is the number of heavy (non-hydrogen) atoms of the ligand.

3. RESULTS AND DISCUSSION

3.1. Design of hybrid 1,3,4-oxadiazole structures

Based on the structure of compound **A** (Figure 1), a known MetRS inhibitor (Zhang et al., 2020), we designed three series of hybrid 1,3,4-oxadiazoles by replacing the linker 5-membered 1,3-dihydro-imidazol-2-one ring of **A** with 1,3,4-oxadiazole ring. The substituted phenyl (Ph) moieties were installed on the left-hand side of the oxadiazole heterocycle and three different pharmacophores were introduced on the right-hand side including (1) substituted phenyl groups (series A), (2) benzimidazole moieties (series B), and (3) methylthioether linked benzimidazoles (series C). Substituents with different characteristics such as alkyl ($CH_2CH_2OCH_3$, CH_2CH_2OH), cyclohexyl, or phenyl groups; electron-releasing (OMe) or electron-withdrawing (F, Cl, pyridin-3-yl-methyl, pyridin-4-yl-methyl) groups were introduced on both sides to search for the structure-activity relationship. The structures of the fifty-two designed compounds are presented in Table 1-3.

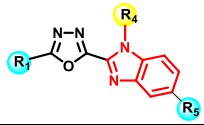
Table 1. Designed structures of series A



Code	R_1	R_3
1	3,4,5-$(OMe)_3$-Ph	Ph
2	3,4,5-$(OMe)_3$-Ph	3,4,5-$(OMe)_3$-Ph
3	Ph	2,5-$(OMe)_2$-Ph
4	4-OMe-Ph	3-Cl-Ph
5	3,4,5-$(OMe)_3$-Ph	2,4-$(OMe)_2$-Ph

Ph: phenyl; Me: methyl.

Table 2. Designed structures of series B



Code	R_1	R_4	R_5
6	3,4,5-$(OMe)_3$-Ph	H	H
7	3,4,5-$(OMe)_3$-Ph	Ph-CH_2	H
8	3,4,5-$(OMe)_3$-Ph	3-F-Bn	H
9	3,4,5-$(OMe)_3$-Ph	2,4-F_2-Bn	H
10	3,4,5-$(OMe)_3$-Ph	3-F-Bn	CF_3
11	3,4,5-$(OMe)_3$-Ph	pyridin-4-yl-methyl	H
12	3,4,5-$(OMe)_3$-Ph	$CH_2CH_2OCH_3$	H
13	3,4,5-$(OMe)_3$-Ph	4-F-Bn	H
14	3,4,5-$(OMe)_3$-Ph	2-Cl-Bn	OMe
15	3,4,5-$(OMe)_3$-Ph	2-Cl-Bn	H
16	3,4,5-$(OMe)_3$-Ph	Bn	CF_3
17	3,4,5-$(OMe)_3$-Ph	2-Cl-Bn	CF_3
18	3,4,5-$(OMe)_3$-Ph	4-Me-Bn	H
19	3,4-$(OMe)_2$-Ph	H	H
20	3,4-$(OMe)_2$-Ph	2-F-Bn	H
21	3,4-$(OMe)_2$-Ph	Bn	CF_3
22	3,4-$(OMe)_2$-Ph	2-Cl-Bn	CF_3
23	3,4-$(OMe)_2$-Ph	Bn	H
24	3,4-$(OMe)_2$-Ph	3-F-Bn	H
25	3,4-$(OMe)_2$-Ph	2,4-F_2-Bn	H
26	3,4-$(OMe)_2$-Ph	pyridin-3-yl-methyl	H
27	3,4-$(OMe)_2$-Ph	$CH_2CH_2OCH_3$	H
28	3,4-$(OMe)_2$-Ph	4-F-Bn	H
29	3,4-$(OMe)_2$-Ph	4-Me-Bn	H

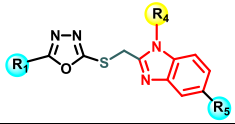
Ph: phenyl; Me: methyl.

3.2. The validation of the docking protocol

Among available molecular docking software including MOE (Kitchen et al., 2004), GOLD (Jones et al., 1997), AutoDock4 (Morris et al., 1998), AutoDock Vina (Trott & Olson, 2010), GLIDE (Friesner et al., 2004), and several docking servers such as SwissDock (Grosdidier et al., 2011) or PatchDock (Schneidman-Duhovny et al., 2005),

AutoDock Vina is a non-commercial package that is widely used as a useful tool to rapidly predict the binding affinity of ligands towards a specific protein/enzyme target (Nguyen et al., 2020). Before the virtual screening of compounds targeting MetRS, re-docking was performed with the co-crystallized ligand. The results showed that the ligand had a low free binding energy (-9.8 kcal/mol) and a root-mean-square deviation (RMSD) value of 0.8208 Å (RMSD calculated using Maestro 11 software) (Figure 2). A docking result is considered good if the RMSD value is less than 2 Å. This result shows that the selected coordinate parameters are suitable for screening targets into *Tb*MetRS.

Table 3. Designed structures of series C



Code	R_1	R_4	R_5
30	3,4,5-$(OMe)_3$-Ph	H	H
31	3,4,5-$(OMe)_3$-Ph	Bn	H
32	3,4,5-$(OMe)_3$-Ph	H	H
33	3,4,5-$(OMe)_3$-Ph	4-F-Bn	H
34	3,4,5-$(OMe)_3$-Ph	Bn	OMe
35	3,4,5-$(OMe)_3$-Ph	$CH_2CH_2OCH_3$	H
36	3,4,5-$(OMe)_3$-Ph	CH_2CH_2OH	H
37	3,4,5-$(OMe)_3$-Ph	Cyclohexyl	H
38	3,4,5-$(OMe)_3$-Ph	3-F-Bn	H
39	3,4,5-$(OMe)_3$-Ph	4-Me-Bn	H
40	3,4-$(OMe)_2$-Ph	H	H
41	3,4-$(OMe)_2$-Ph	2-Me-Bn	CF_3
42	3,4-$(OMe)_2$-Ph	2-Cl-Bn	H
43	3,4,5-$(OMe)_3$-Ph	H	Cl
44	3,4-$(OMe)_2$-Ph	Bn	Cl
45	3,4,5-$(OMe)_3$-Ph	H	CF_3
46	4-OH-Ph	H	Cl
47	4-OH-Ph	H	Me
48	4-OH-Ph	H	H
49	3,4,5-$(OMe)_3$-Ph	H	NO_2
50	3,4-$(OMe)_2$-Ph	Bn	Me
51	3,4-$(OMe)_2$-Ph	Bn	H
52	3,4,5-$(OMe)_3$-Ph	3,4-F_2-Bn	H

Ph: phenyl; Me: methyl.

3.3. Docking-based virtual screening

Docking-based virtual screening of 52 designed compounds (Tables 1-3) targeting *Tb*MetRS was then performed. The results showed that 29 compounds (**7**-**10**, **13**-**18**, **20**-**26**, **28**, **29**, **31**, **33**, **38**-**39**, **41**-**42**, **44**, and **50**-**52**) had lower free binding energies of -9.9 kcal/mol and three compounds (**34**, **46**, and **47**) gave the same binding energy compared to the control (compound **A,** -9.8 kcal/mol) (Figure 3). Fourteen compounds including 12 compounds of series B and two compounds of series C, which showed the lowest free binding energies (<10 kcal/mol), were then selected and analyzed for their interactions at the *Tb*MetRS binding site. Table 4 presents dock results of selected compounds in combination with the ligand LE. A ligand would be considered as a potential lead when it has an LE value greater than 0.3 (Schultes et al., 2010). As compared with series A (having the binding energies ranging from -9.0 to -8.2 kcal/mol), the substitutions on the benzimidazole rings in series B and the sulfur moieties as the linkers in series C resulted in higher stability of the compounds in the active site and would be the reason for the observed low binding energies, (Figure 3).

Unlike the compounds of series B and C, the orientations of the compounds of series A were not foolproof for both vesicles. This may be due to the lesser free rotation around a bond in the absence of a linker. Within the series B and C, visual inspection of the pose in the inhibitor binding cavity revealed that these compounds were inserted into the methionine and auxiliary pocket framed with amino acids in the same way as the reference ligand. However, the designed compounds did not show profound interactions into the auxiliary pocket (AP). Only bonds with the amino acids near the mouth of the pocket were formed, probably because of the short length of the linker. The 1,3,4-oxadiazole rings were found to interact with the two amino acids (Tyr250 and Val473) and formed critical hydrogen bonds with Asp287. All structures were sandwiched between the side chains of Tyr250-Val473 and Ile519-Tyr250 around the mouth of the pocket. The compounds of series B were found to fill two binding pockets, where, compounds **21** and **22** were considered as the potential candidates with the lowest binding energy of -11.0 and -11.1 kcal/mol, respectively (Figures 3 and 4). The substituted (R_4) on the benzimidazole rings were inserted into the enlarged methionyl (Met) pocket (MP), while the substituted phenyl moieties on the left-hand side of the oxadiazole ring were filled into the auxiliary pocket. The molecular mechanisms underlying the induction of *Tb*MetRS inhibition of inhibitors at auxiliary pocket rim (APR) and methionine pocket rim (MPR) have not been elucidated to date. However, targeting MP and AP is considered the most favorable for protein-ligand interaction, which will be a necessary condition for receptor inhibition (Zhang et al., 2020).

The Met pocket of the *Tb*MetRS enzyme is hydrophobic, which is surrounded by several side chains including Tyr250, Val473, Trp474, Phe522, Ile519, Ala477, Leu478, and His523 (Koh et al., 2012; Shibata et al., 2011). For compounds of series B and C, the benzimidazole rings and substituents (R_5) interacted with some amino acids (histidine, tyrosine, aspartate, isoleucine) around the mouth of the Met pocket. The binding was further stabilized by multiple interactions of R_5 substituents such as OCH_3, CF_3 with His259, Asp518, and Tyr249 in the Met pocket rim. The 1,3,4-oxadiazole rings were oriented between the two binding pockets with two bonds being formed including a pi-pi bond with Tyr250 and pi-alkyl interaction with Val473 (Figure 4). All these characteristics were observed in all top compounds of series B and C (Table 4). These details would contribute to the observed higher negative binding energies compared to others.

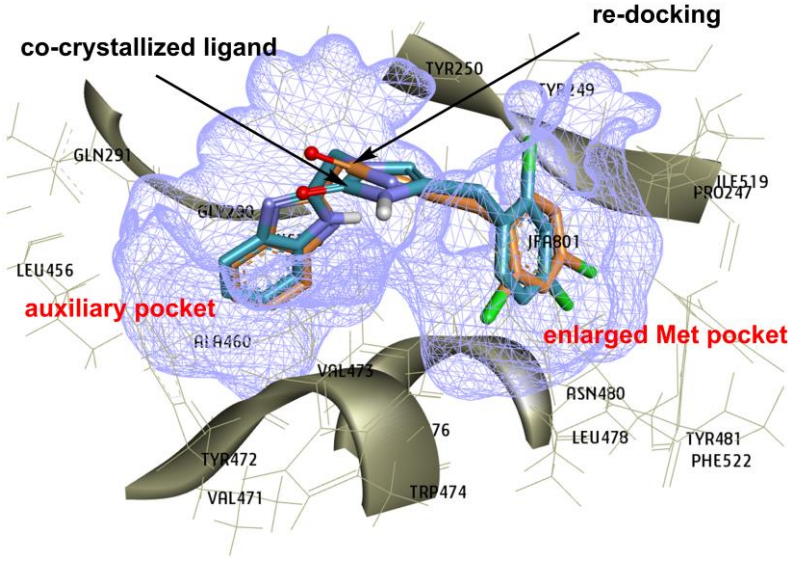


Figure 2. Re-docking results

re-docking ligand-orange, co-crystallized ligand-medium azure

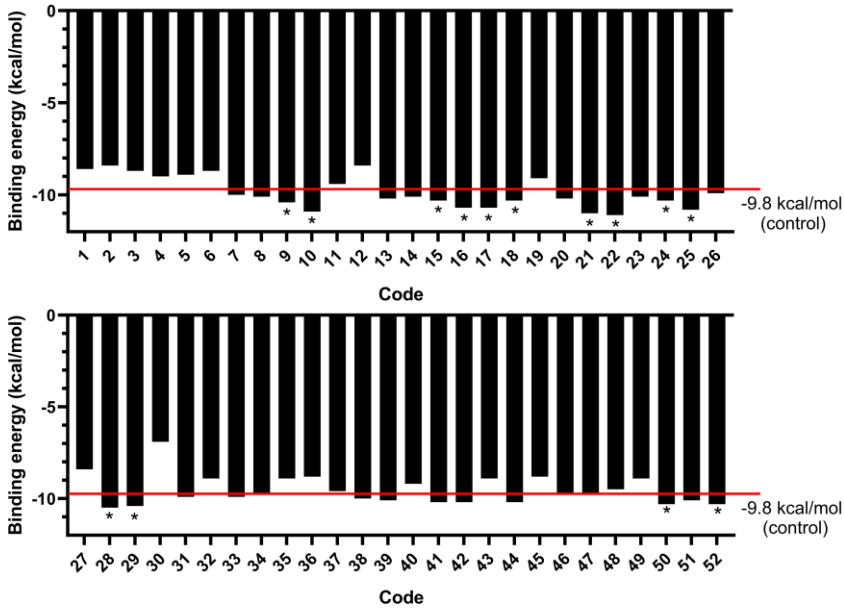


Figure 3. Docking-based virtual screening of *Tb*MetRS inhibitors

Table 4. Analysis of the interaction of the hit compounds with *Tb*MetRS

Rank[a]	Code	Energy of binding (kcal/mol)	Interaction regions[d]	Hydrogen bonding with key residues	LE[b]
1	22	-11.1	AP, MPR	Pro247, Ile248, Tyr249, Tyr250, Asp287, Tyr472, Val473, Ala477, Ile519, Asp518	0.30
2	21	-11.0	APR, MPR	Pro247, Ile248, Tyr249, Tyr250, His259, Asp287, Glu368, Tyr472, Val473, Ala477, Asp518, Ile519	0.31
3	10	-10.9	AP, MPR	Pro247, Ile248, Tyr249, Tyr250, His259, Ser262, Lys292, Asp287, Tyr472, Val473, Ala477, Asn480, Tyr481, Gly516, Asp518, Ile519, His523	0.29
4	25	-10.8	AP, MPR	Pro247, Ile248, Tyr250, Lys292, Asp287, Glu368, Val473, Ala477, Ile519, His523	0.33
5	16	-10.7	APR, MPR	Pro247, Tyr249, Tyr250, His256, Asp287, Asp367, Glu368, Val473, Ile519, His523	0.29
6	17	-10.7	AP, MP	Pro247, Tyr249, Tyr250, His259, His289, Val471, Tyr472, Val473, Asp476, Ile519, His523	0.28
7	28	-10.5	MP, MPR	Tyr250, Asn252, Ala253, Asp287, Val473, Ile519	0.32
8	9	-10.4	MP, MPR	Tyr250, Asn252, Ala253, His256, His259, Asp287, Leu478, Ile519, Phe522	0.30
9	29	-10.4	AP, MP	Ile248, Tyr250, Asp287, Val473, Trp474, Ala477, Leu478, Tyr481, Ile519, Phe522, His523	0.32
10	15	-10.3	APR, MPR	Pro247, Ile248, Tyr250, His259, Asp287, Tyr472, Val473, Ile519, His523	0.30
11	18	-10.3	MPR, APR	Pro247, Tyr250, His259, Asp287, Tyr472, Val473, Trp474, Ile519, Phe522, His523	0.30
12	24	-10.3	AP, MP	Tyr250, Asp287, Glu368, Leu456, Ala460, Tyr472, Val473, Ala477, Leu478, Ile519	0.30
13	50	-10.3	AP, MP	Pro247, Ile248, Tyr249, Tyr250, Tyr472, Val473, Ala477, Tyr481, Ile519, Phe522, His523	0.30
14	52	-10.3	AP, MPR	Tyr250, His259, His289, Lys292, Tyr472, Val473, Asp518	0.28
Control[c]		-9.8	AP, MP	Pro247, Ile248, Tyr250, Asp287, Tyr472, Val473, Trp474, Ala477, Leu478, Tyr481, Ile519, His523, Phe522	0.39

[a]Ranking by docking score. [b]LE: ligand efficiency (compounds with LE > 0.3 are considered as potential lead compounds). [c]Control: co-crystallized ligand in TbMetRS (ID:6MES). [d]AP: auxiliary pocket; MP: methionine pocket; APR: auxiliary pocket rim; MPR: methionine pocket rim.

The orientation of all top compounds in Table 4 at the active site showed similarities with the results of previous studies (Huang et al., 2016; Kovalenko et al., 2019). Specifically, based on the docking results, two new series of potent *Tb*MetRS inhibitors were obtained which exhibited high activity in a *T. brucei* growth inhibition assays with EC_{50} of 39 and 22 nM, respectively (Huang et al., 2016). In another study, screening data revealed 28 *N*-benzylidene-*N′*-thiazol-2-yl-hydrazine derivatives which showed activities against *Tb*MetRS with the IC_{50} values of 4.2 to 123 µM (Kovalenko et al., 2019). These compounds interacted with the auxiliary and methionine pocket of *Tb*MetRS through hydrophobic interactions with key residues (Asp287, Phe522, His289, Gly290, and Val473). Our docking studies also gave similar results where the selected compounds in Table 4 also occupied a favorable position and formed interactions with key amino acids in the binding pocket of the studied enzyme. In summary, the fourteen compounds in Table 4 displayed high affinity towards the *Tb*MetRS enzyme based on the predicted low binding energies as well as the interactions with keys amino acids in the active site of the enzyme. These findings could be served as the references for the design and synthesis of compounds for *in vitro* and *in vivo* screening studies to find leads for the development of antimicrobial agents in the future.

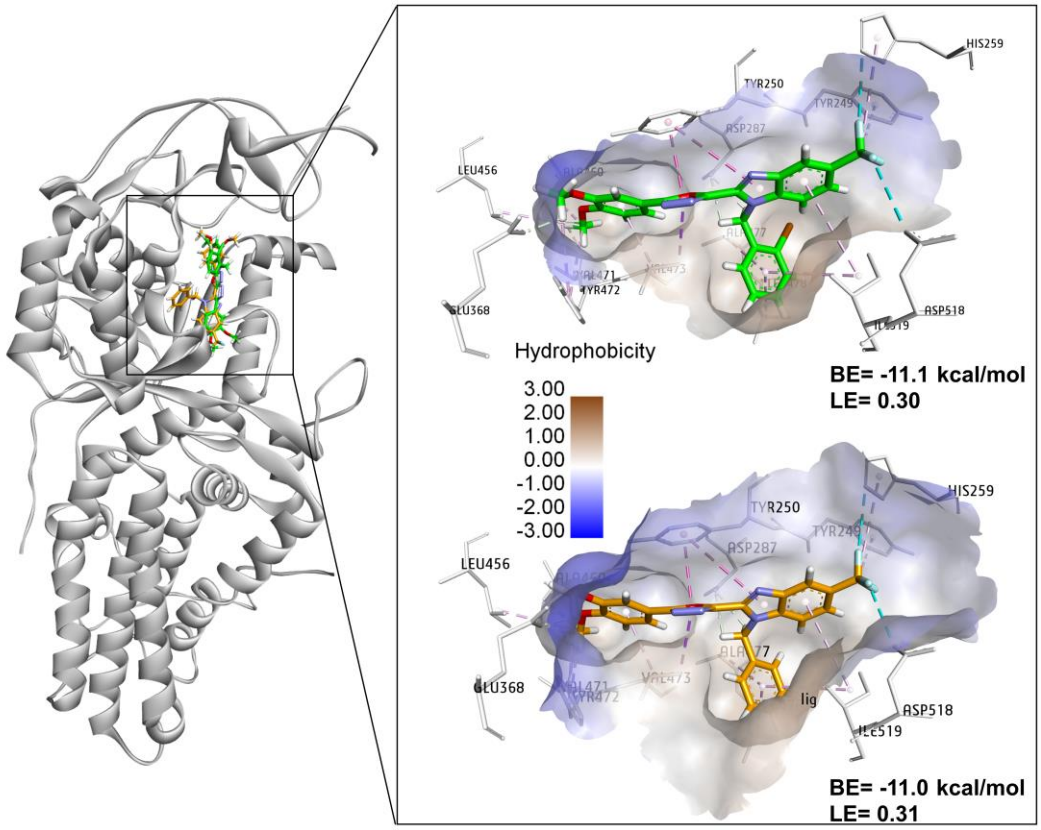


Figure 4. Three-dimensional interaction map of the top representative compounds to the binding site of *Tb*MetRS (22-green, 21- orange, BE: binding energy, LE: ligand efficiency)

4. CONCLUSION

In this study, fifty-two 1,3,4-oxadiazole based hybrids bearing benzimidazole heterocycles were designed based on a known *Tb*MetRS inhibitor. The docking protocols confirmed 29 compounds showing binding energies lower than that of the reference ligand. Notably, 14 compounds showed lower binding energy of -10.3 kcal/mol. Furthermore, these compounds showed binding with key amino acids at the active pocket of the *Tb*MetRS receptor. These findings provide references for the further studies in design, synthesis, and screening new aminoacyl-tRNA synthase inhibitors.

ACKNOWLEDGMENT

This work was supported by the Ministry of Education and Training of Vietnam under Grant number of B2020-TCT-12.

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