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In silico Evaluation of Antimicrobial Activity of Some Thiadiazoles Using Molecular Docking Approach †

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Abstract: Molecular docking studies have been performed to assess antimicrobial potential of three 1,3,4-Thiadiazole derivatives containing azulene rings. The simulations were conducted on *Mycobacterium tuberculosis* DNA gyrase, *Staphylococcus aureus* DNA gyrase and *Escherichia coli* DNA adenine methylase. The relationships between the structures of compounds and their potential antimicrobial activity were investigated. Interactions with amino acids residues form the active binding site were elucidated and the results of docking are reported in terms of docking score. Better docking scores are obtained for the investigated compounds than for the natural ligand, (4S)-2-methyl-2,4-pentanediol, in the case of the *Mycobacterium tuberculosis*. Two of the studied ligands present better binding affinities against *Escherichia coli* than the co-crystallized. Regarding S. aureus gyrase, the thiadiazole derivatives exhibit lower docking scores and less interactions than the aminobenzimidazole urea inhibitor. Our study can be useful to screen and design similar hybrid active compounds.

Keywords: molecular docking; antimicrobial virtual screening; thiadiazoles; azulenes

1. Introduction

Heterocyclic compounds, such as thiadizoles, play important role among organic compounds possessing pharmacological activity, with potential applications in medicinal chemistry. In literature, some hybrid thiadiazoles based structures (e.g., 2-phenylamino-5-(4-fluorophenyl)-1,3,4-thiadiazole) are reported as pharmacophore system, with antituberculosis activity against *Mycobacterium tuberculosis* [1]. 2-Amino-1,3,4-thiadiazole is reported as promising scaffold to designs antimicrobial agents [2].

Starting from such premises, the goal of this study was to examine some hybrid structures containing azulene and thiadiazoles, by computational means as molecular docking approach to realize a virtual screening for the assessment of their potential biological activity. We chose three different protein targets to evaluate their ability to interact and interfere in the replication process of important and opportunistic pathogens such as *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Escherichia coli*. A series of 1,3,4-thiadiazoles, unsubstituted or substituted either at azulen-1-yl moiety or at 5-position of thiadiazole ring were previously synthesized and characterized [3]. By our investigation, we intent to evaluate their possible applications in the field of medicinal chemistry.

2. Computational Methodology. Docking Protocol

The docking simulations were carried out using CLC Drug Discovery Workbench (Qiagen). The protein fragments were imported from Protein Data Bank: 3M4I: Crystal structure of the second part of the *Mycobacterium tuberculosis* DNA gyrase reaction core: the TOPRIM domain at 1.95 Å resolution, containing the co-crystalized): (4S)-2-methyl-2,4-pentanediol (three-letter code: MPD) [4]; 4P8O: *Staphylococcus aureus* gyrase bound to an aminobenzimidazole urea inhibitor (1-ethyl-3-[5-(5-fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1H-benzimidazol-2-yl]ure a (three-letter code: 883)) [5] and 4RTO: Complex of *Escherichia coli* DNA Adenine Methyltransferase (DAM) with Sinefungin and with DNA containing proximal Pap Regulon Sequence [6]. Investigated ligands structures, **T**1-**T**3 (see Figure 1a–c) were generated with Spartan 16 Software, Wavefunction Inc, Irvine, USA [7,8], and optimized by energy minimization to prepare *.sdf files used as input in the docking program. The co-crystalized ligand's pose was validated by redocking and the binding active site was set up. The water molecules and co-factors were removed. Ligands' properties were calculated and their accordance with the Lipinski's rule of five [9]. The results are given as docking score function and Root Mean Square Deviation (RMSD). Interactions of ligands by Hydrogen-bonding with amino acids form the interacting amino acids group of protein fragment's active binding site, are depicted and their length were measured.

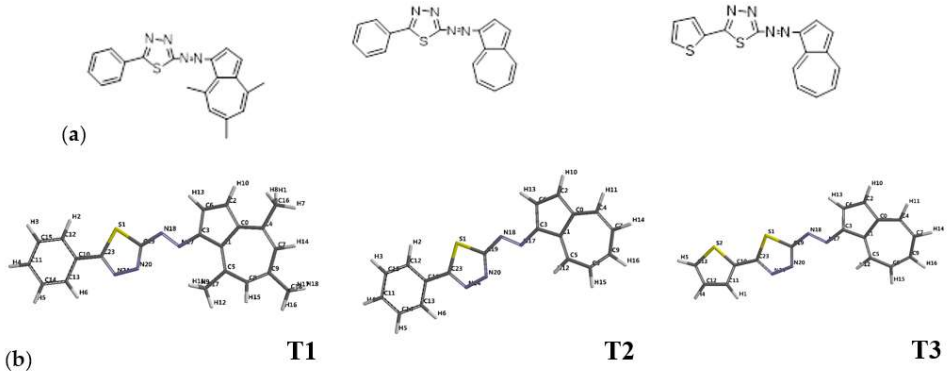


Figure 1. 2D structure of **T1–T3** thiadiazole derivatives (**a**) and their 3D optimized structures with atomic numbering labels (**b**).

3. Results and Discussion

Figure 1 illustrates the structure of 1,3,4—thiadiazoles (**T1–T3**) under investigation, as 2D (a) and optimized 3D structures with atomic labels (b) arbitrary chosen by Spartan Software.

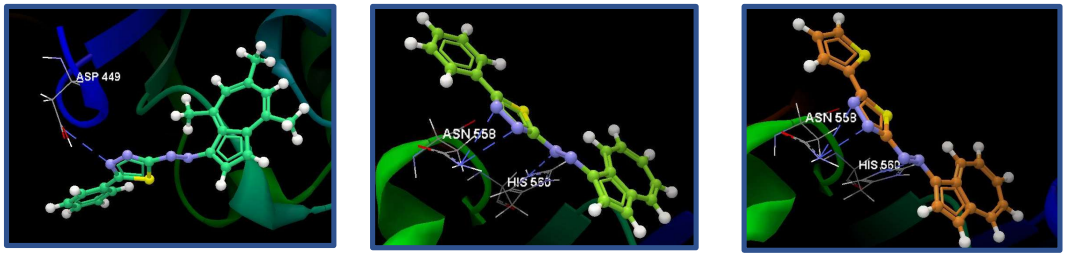
In Table 1 are listed important molecular descriptors and properties to assess the oral bioavailability according Lipinski's rule [9], where: MW is the molecular weight, that should be less than 500 Daltons, HBD—the number of hydrogen bond donors, recommended to be lower than 5, HBD—the number of hydrogen bond acceptors with acceptable values less than 10 and the water—octanol partition coefficient (logP) that should be less than 5. The investigated **T1–T3** structures reveal one Lipinski's violation, given by the logP > 5, thus suggesting their hydrophobic character. These calculations are useful to predict the drug-likeness for drug candidates in virtual screening methodologies. The calculated values of LogP parameter suggest that all investigated 1,3,4—thiadiazoles are highly lipophilic, with poor aqueous solubility. Generally, values of LogP over 5 suggest poor absorption or permeation. Further optimization of such ligands containing together azulene and thiadiazole moieties, is required in order to increase the hydrophilicity and to favor hydrophilic interactions by means of NH/OH/N/O groups. Thus, the probability to interact with proteins and the ability to become biologically active, can be successfully achieved.

Figure 2 reveals the interactions by Hydrogen-bonding of **T1–T3**, with the crystal structure of the second part of the *Mycobacterium tuberculosis* DNA gyrase reaction core: the TOPRIM domain at 1.95 Å resolution. **T2** and **T3** reveal similar scores (43.19 and 40.95, respectively), by forming 3

Hydrogen bonds with the same amino acids residues, with N (sp^2) HIS560 and N (sp^2) ASN558, respectively, at the two nitrogen atoms of the thiadiazole aromatic ring, that is known as structural motif common in pharmacology [10] and one interaction by the diazo bond that link the thiazole with the azulene. The planar five-member thiadiazole ring acts as an acceptor in the H-bond formation, in the biological media. Some of thiadiazole based structures possess antimicrobial activities, e.g., oxazolidinone analogues possessing 1,3,4—thiadiazole C-ring, designed as hybrids of linezolid [11,12]. Against *Mycobacterium tuberculosis* DNA gyrase, **T1** compound reveals lower score than its analogues, **T2** and **T3**, respectively. **T1** forms a single H-bonding with—O (sp^3) ASP449, as depicted in Table 2.

Table 1. Ligands' calculated properties.

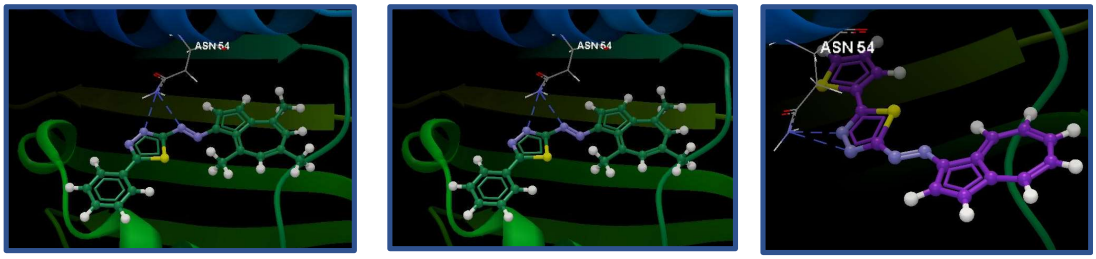
Ligand /Protein Fragment Source	MW ($g \cdot mol^{-1}$)	HBD	HBA	LogP	Flexible Bonds	Lipinski's Violations
co-crystalized MPDA-1 /3M4I (M. tuberculosis)	118.17	2	2	0.27	2	0
co-crystalized 883B 301 /4P8O (S. aureus)	376.37	2	8	1.61	4	0
co-crystalized SFG /4RTO (E. coli)	382.39	10	12	−3.22	7	2
T1	358.46	0	4	**5.24**	3	1
T2	326.46	0	4	**5.49**	3	1
T3	322.41	0	4	**5.21**	3	1



(**a**) **T1** H-bonding with ASP449 (**b**) **T2** H-bonding with ASN558 and HIS560 (**c**) **T3** H-bonding with ASN558 and HIS560

Figure 2. T1–T3 Hydrogen-bonding interactions with amino acid residues form the active binding site of 3M4I (*Mycobacterium tuberculosis* DNA gyrase).

Figure 3 reveals the interactions by Hydrogen-bonding of **T1–T3**, with 4P8O protein fragment from *S. aureus* gyrase.



(**a**) **T1** H-bonding with ASN54 (**b**) **T2** H-bonding with ASN54 (**c**) **T3** H-bonding with ASN54

Figure 3. T1–T3 Hydrogen-bonding interactions with amino acid residues form the active binding site of 4P8O (*S. aureus* gyrase).

Table 2. Docking results for 3M4I (*Mycobacterium tuberculosis* DNA gyrase).

Target/Ligand	Interacting Group	Hydrogen Bonds/Length(Å)	Docking Score/RMSD
3M4I/co-crystalized MPDA-1	ARG451, HIS525, PRO450, TYR524, HIS560, GLY520, ILE519, LEU522, ARG523	O4(sp^3)—O(sp^2) LEU522/3.302	−25.91/0.86
3M4I/T1	ASP449, ARG451, PRO450, TYR524, ARG523, LEU522, LYS521, GLY520, ALA508, LEU509, GLY510, THR507	N24(sp^2)—O(sp^3) ASP449/3.247	−38.19/0.06
3M4I/T2	ASN558, HIS560, ILE519, HIS525, ARG451, PRO450, GLY520, TYR524, LEU522, ARG523, LYS521	N18(sp^2)—N(sp^2) HIS560/3.057 N20(sp^2)—N(sp^2) ASN558/3.126 N24(sp^2)—N(sp^2) ASN558/3.103	−43.19/0.69
3M4I/T3	GLU557, ASN558, HIS560, ILE519, HIS525, ARG451, LYS452, ASP449, PRO450, TYR524, GLY520, LEU522, ARG523, LYS521	N17 (sp^2)—N (sp^2) HIS560/3.187 N20 (sp^2)—N(sp^2) ASN558/2.914 N24 (sp^2)—N (sp^2) ASN558/3.135	−40.95/0.72

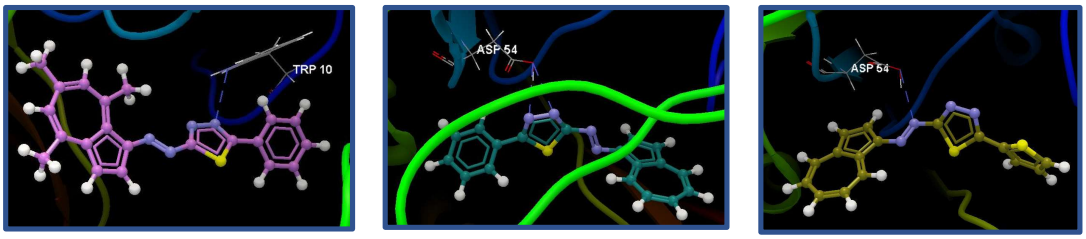
Table 3. Docking results for 4P8O (*Staphylococcus aureus* gyrase).

Target/Ligand	Interacting Group	Hydrogen Bonds/Length (Å)	Docking Score/RMSD
4P8O/ co-crystalized	ASN54, VAL52, ILE51, ILE102, VAL79, ILE175, VAL174, THR80, THR173, PRO87, GLY85, ASP81, ARG144, ARG84, GLY83, GLU58, SER55, ILE86	N25(sp^2)—N(sp^2) ARG144/2.769 N6(sp^2)—O(sp^2) ASP81/2.797 N3(sp^2)—O(sp^2) ASP81/2.914 N3(sp^2)—O(sp^3) SER55/3.081	−70.22/0.08
4P8O/T1	SER55, ASN54, GLU58, ASP81, GLY83, GLY172, ARG84, GLY85, ILE86, PRO87, ARG144, ILE102, SER128, THR173	N20(sp^2)—N(sp^2) ASN54/3.062 N18(sp^2)—N(sp^2) ASN54/3.135	−58.08/0.10
4P8O/T2	VAL52, VAL79, ASN54, ILE51, GLU50, SER55, THR80, ASP81, GLU88, GLY83, ARG84, THR173, VAL174, ILE175, GLY85, ARG144, ILE86, PRO87, ILE102	N24(sp^2)—N(sp^2) ASN54/2.790 N20(sp^2)—N(sp^2) ASN54/2.944	−56.49/0.18
4P8O/T3	ASP81, GLU58, GLY83, THR80, SER55, VAL79, ASN54, ILE51, ILE175, VAL174, THR173, ARG84, GLY85, ARG144, ILE86, PRO87, ILE102	N24(sp^2)—N(sp^2) ASN54/2.954 N20(sp^2)—N(sp^2) ASN54/2.903	−53.61/0.19

Regarding docking against *S. aureus* 4P8O fragment, all 1,3,4-thiadiazoles exhibit lower docking score than the natural ligand. ASN54 amino acid residue is involved by its Nsp^2 in two H-bond forming with T1-T3 ligands. Although present in the interacting surrounding group of co-crystalized ligand and thiadizoles ligands, ASN54 don't interact by hydrogen bonding with the natural ligand. This compound reveals more interactions (4 H-bonding and greater docking score). So, lower, maybe inefficient activity of investigated thiadiazoles against *S. aureus* gyrase is expected.

In Figure 4 are depicted the intramolecular interactions of T1-T3 with 4RTO (*Escherichia coli* DNA Adenine Methyltransferase). Concerning T1 and T2, the thiadiazole ring is involved in H-bonding with different amino acid residues (TRP10 and ASP54, respectively). T3 acts differently, by a nitrogen

of the azo bond, that forms Hydrogen bond with ASP54. **T1** and **T2** reveals greater docking scores than the natural ligand. The obtained score for **T3** is lower, as seen in Table 4. The co-crystallized ligand presents interactions within the active binding site, whiles our investigated thiadiazoles are poorly interacting.



(**a**) **T1** H-bonding with TRP10 (**b**) **T2** H-bonding with ASP54 (**c**) **T3** H-bonding with ASP54

Figure 4. T1–T3 Hydrogen-bonding interactions with amino acid residues form the active binding site of 4RTO (*Escherichia coli* DNA Adenine Methyltransferase).

Table 4. Docking results for 4RTO (*Escherichia coli* DNA Adenine Methyltransferase)**.**

Target/Ligand	Interacting Group	Hydrogen Bonds/Length (Å)	Docking Score/RMSD
4RTO/co-crystalized SFG	ASN56, ILE55, PHE201, GLU163, SER164, GLN205, TYR165, SER168, LEU59, ASP54, PRO183, PHE35, ALA53, PRO182, PRO34, ASP181, GLU33, TYR179, VAL36, TYR184, VAL41, LYS14, SER40, GLY39, GLY13, GLY12, GLY37, ALA38, ALA11, TRP10	N1 (sp^2)—N (sp^2) TYR165/3.129 O2′ (sp^3)—O (sp^2) ASP54/2.654 O3′ (sp^3)—O (sp^3) ASP54/2.567 O3′ (sp^3)—N(sp^2) TRP10/3.128 O (sp^2)—N (sp^2) ALA38/2.834 OXT (sp^2)—O (sp^3) SER40/2.980 N (sp^3)—O s(sp^3) ASP181/2.426	−67.74/0.79
4RTO/T1	ALA53, VAL36, GLU163, PRO34, ASP54, PHE35, ILE55, SER164, TYR165, ALA166, GLN205, PHE201, SER200, PRO183, ASN120, LEU122, CYS123, ALA11, TRP10, LYS59, ASN115, GLY121	N24 (sp^2)—N(sp^2) TRP10/3.101	−72.21/0.07
4RTO/ T2	ALA53, ASP54, GLU163, PRO34, PHE35, ILE55, SER164, TYR165, GLN205, PHE201, TYR184, PRO183, PRO182, ASP181, ALA11, GLY12, TRP10, GLY13	N24 (sp^2)—O (sp^3) ASP54/3.000 N20 (sp^2)—O (sp^3) ASP54/2.982	−71.15/0.07
4RTO/T3	TRP10, ALA11, GLY12, GLY37, VAL36, ASP54, ALA53, ILE55, GLU163, SER164, PHE35, PRO34, TYR165, ASP181, PRO183, PRO182, SER200, TYR184, PHE201, GLN205	N18 (sp^2)—O (sp^3) ASP54/3.304	−66.42/0.23

4. Conclusions

This study opened new opportunities to consider the synthesis and development of new structures derived from thiadiazoles coupled with azulene moieties, as possible antimicrobial agents. Further analyses are required in order to establish certainly a possible inhibitory action against pathogens and hybrid structures containing skeletons similar to those used in the present study, must be optimized to acquire high inhibitory activity against pathogenic microorganisms.

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