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Molecular Docking and DFT Study of Synthesized Oxazine Derivatives

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Abstract

Molecular docking and DFT calculation have been investigated for the synthesized oxazine derivatives. Theoretical calculations including DFT study has investigated and proved the reactivity of compound **4** is the greatest among others as follow **4**>**2**>**5**>**3**>**1**. On the other hand, the stability of compounds has been calculated to prove that compound **1** has the highest stability among others as follow **1**>**3**>**5**>**2**>**4**. Antibacterial activity test was confirmed potent activity for all the synthesized derivatives against *E. coli* and *S. aureus* bacterial *strains.* The synthesized compounds including **2**, **4** and **5** showed potent inhibitory activity against both bacterial strains compared to positive reference of tetracycline. Docking study revealed that oxazine derivatives with 4-chlorobenzamide group at position number 2 showed higher potency than tetracycline as antibacterial by inhibiting TetR.

Keywords: Molecular Docking; DFT; heterocycles; oxazine; antibacterial activity

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1. Introduction

Heterocycles are one of important tools used in development of pharmaceuticals, chemical industries and used for the synthesis of natural products as a building unit [1-5]. The oxaizne moiety is a six membered heterocyclic molecule containing one oxygen and one nitrogen that is of interest in pharmaceutical research [6]. Simple examples of dioxazines include naturally occurring cinnabarine and cinnabaric acid, which are derived from tryptophan biodegradation [7]. Oxazines play an important role as antihyperglycemic [8], antileishmanial [9], antitubercular [10, 11], antiulcer [12], anticancer [13, 14] and antibacterial [15]. Patil and his coworkers were synthesized some derivatives of oxazines and some compounds showed moderate potent antibacterial activity while some others showed potent activity towards *E.coli*, *S. aureus*, *Micrococcus* and *B. subtilis* bacterial strains [16]. Kategaonkar and co-workers were successfully designed and prepared novel derivatives of oxazine bearing naphthalene moiety and tested against two bacterial spices including G+ve *B. subtilis* and G-ve *E. coli* and showed strong inhibitory activity [17]. Oxazine derivatives were prepared by Chernov and co-workers displayed potent inhibitory activity against bacterial strain of *S. aureus* while it showed moderate activity against *E. coli* [18]. In 1944, Cope and Holly were first synthesized the oxaiznes using Mannich reaction. For the synthesis of oxazine derivatives, a variety of

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different methodologies were applied including green synthesis [19], eco-friendly synthesis [20] and some methods were published [21-23]. Herein molecular modeling properties and DFT calculations have studied for the synthesized oxazine derivatives (scheme 1). In addition, the similarity of the ligand-receptor interaction that provides an overview of the probability of whether the interaction of the test ligand will resemble the reference ligand or not has been investigated.

2. Experimental

2.1. Synthesis of Oxazine Derivatives

The oxazine derivatives have synthesized according to previous procedures [4b]. The derivatives including 4,6-Diphenyl-4H-1,3-oxazin-2-amine (1), 4-Chloro-N-(4,6-diphenyl-4H-1,3-oxazin-2-yl)benzamide (2), 4-(2-Bromopyridin-4-yl)-6-phenyl-4H-1,3-oxazin-2-amine (3), N-[4-(2-Bromopyridin-4-yl)-6-phenyl-4H-1,3-oxazin-2-yl]-4-chlorobenzamide (4) and 4-{2-[(4-Morpholinophenyl)amino]pyridin-4-yl}-6-phenyl-4H-1,3-oxazin-2-amine (5)

2.2. Antibacterial Activity

Materials

Staphylococcus aureus (*S. aureus,* G+ve) and *Escherichia coli* (*E. coli*, G-ve) were obtained from Applied Science Department at University of Technology. Nutrient broth was used to prepare fresh inoculants were prepared 37 °C for 24 h.

Test Method

Disk diffusion method on an agar plate was utilized to investigate the antibacterial activity. A cultured agar (10 mL) containing mixed samples (1 cm) was inoculated onto 10 µl of *microbe* culture and incubated for 24 hours at 37 °C. The samples were screened for zones of inhibition after the incubation period (in mm) [24].

2.3. Docking Study

Materials

The Toshiba Portege Z30-C series Ultrabook was used, which had an IntelTM Core i7-6600U@2.6 GHz processor and Windows 10 Pro. Chem3D 20.0.0.41 for energy minimization, OpenBabel 3.1.1 for ligand and receptor format conversion, AutoDockTools 1.5.6 for docking protocol configuration, AutoDock Vina 1.1.2 for docking process, PyMOL 2.4.1 for docking protocol validation, UCSF Chimera 1.15 for docking result preparation, and Discovery Studio Visualizer 20.1.0.19295 for visualization and observation of docking results were used.

Ligands preparation

All test ligands were sketched using Chem3D 20.0.0.41 with energy minimization using the MMFF94 force field. Using Open Babel 3.1.1, the optimized structure was then converted from .hin to .pdb format. Using AutoDockTools 1.5.6, all test ligands were then charged and torqued by default.

Receptors Preparation

In accordance with *in vitro* tests conducted using tetracycline as a reference drug, the receptor used was those that had tetracycline as co-crystal ligands. The docking process used the tetracycline repressor (TetR) with PDB ID 2VKE [25] from the Protein Data Bank website (https://www.rcsb.org/). With the co-crystal tetracycline, the receptor is made up of only one chain (A). Receptor was downloaded in the format pdb. Using AutoDockTools 1.5.6, the unused part of the receptor, including the water molecules, was removed, polar hydrogen was added, the receptor was given a charge, and the size and coordinate grid were adjusted. The grid box's center coordinates were automatically changed to match the ligand co-crystal position of the receptor by making the ligand position as grid box's center. The orienting process obtained the smallest grid box that could be fitted with both co-crystal and test ligands.

Docking Protocol Validation

The redocking method described in our earlier report was used to validate the docking protocol. [4]. The investigated parameter was the root-mean-square deviation (RMSD), with a limit of less than 2 Å to infer that the technique was valid and could be utilized for docking. The free energy of binding (ΔG; kcal/mol) and amino acid interactions were obtained as a result of the docking process and were utilized as comparisons for the docking results of the test ligands.

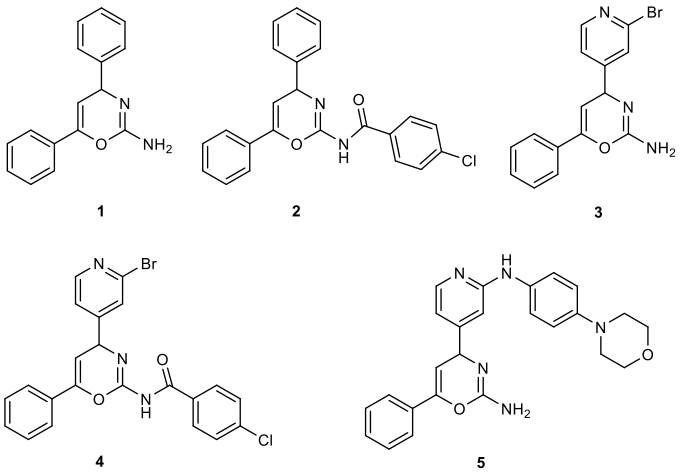
Molecular Docking

All test ligands were docked using the similar sizes and positions of the grid box as the validation process. The results obtained were grouped into two parameters: ΔG and ligand-receptor interactions. As reported in our previous study, the similarity of ligand-receptor interaction was computed as a percentage by comparing two factors: amino acid type similarity and kind of interaction [26]. The two parameters of each test ligand were then compared with tetracycline.

3. Results and Discussion

3.1. Theoretical Studies

Theoretical studies are essential to understand the molecular structure of the compounds (figure 1). All compounds were optimized with the help of Becke's 3-parameters, B3LYP functional level under 6-311G basis set.



Scheme 1. Oxazine derivatives

The Gaussian 09 program, which included the Gauss View 5.0.9 program, was used to calculate and analyze chemical reactivity, as well as to select the active site of the synthesized compounds. The energy gap between HOMO and LUMO orbitals was used to investigate electron transfer interaction utilizing frontier molecular orbitals [27]. Parameters are called

chemical reactivity values such as global hardness (η), electronegativity (χ), chemical potential (μ)**,** global softness (S) and global electrophilicity index (ω) [28].

In order to better understand the chemical reactivity of a compound, it is essential to compare orbital energies. The electron donor orbital (HOMO) has more energy than the electron accepter orbital (LUMO). Negative values of the compounds clearly indicate that the prepared compounds are thermodynamically stable. The calculated energy gap (ΔE) of the compound 4 is less than all other compound, which clearly indicates that the reactivity of compound 4 is greater as 4>2>5>3>1. Compound with lower energy gap (Soft compound) is reactive because it easily offers electrons to an acceptor.

$$\boldsymbol{\chi = -\frac{(E_{LUMO} + E_{HOMO})}{2}}$$

$$\boldsymbol{\mu = -\chi = \frac{(E_{LUMO} + E_{HOMO})}{2}}$$

$$\boldsymbol{\eta = \frac{(E_{LUMO} - E_{HOMO})}{2}}$$

$$\boldsymbol{S = \frac{1}{2\eta}}$$

$$\boldsymbol{\omega = \frac{\mu^2}{2\eta}}$$

$$\boldsymbol{\sigma = \frac{1}{\eta}}$$

The best electron donor is compound 4, which has the highest HOMO energy (E_{HOMO} = -0.14793 eV) and the lowest ionization value (I = 0.14793 eV), while the best electron acceptor is compound 3, which has the lowest LUMO energy (E_{LUMO} = -0.16299 eV), the highest electron affinity (A= 0.16299 eV), and the highest ionization value (I = 0.34581 eV) (Table 1). Chemical hardness revealed that compound 4 (η= 0.024885 eV, S= 20.09242 eV) is the least (greatest) among all of the studied products. Compound 3 (ω= 0.35400590 eV) has among other compounds the best electrophilic properties, based on the electrophilicity index. Furthermore, compound 4 showed the smallest orbital energy gap (ΔE= 0.04977 eV) among the investigated products is the consequence of the highest chemical reactivity, least kinetically stable "soft molecule", and the most polarizable form (figure 2). The reactivity of this compound is the greatest based on energy gap (ΔE) parameters in which confirm that compound 1 is the most stable as compared to others as follow the order 1>3>5>2>4 (figure 2). The other important properties to index the stability of the complex is Global Hardness (η), global softness (S) and the chemical potential (μ) are also important parameters to measure the stability of the compound.

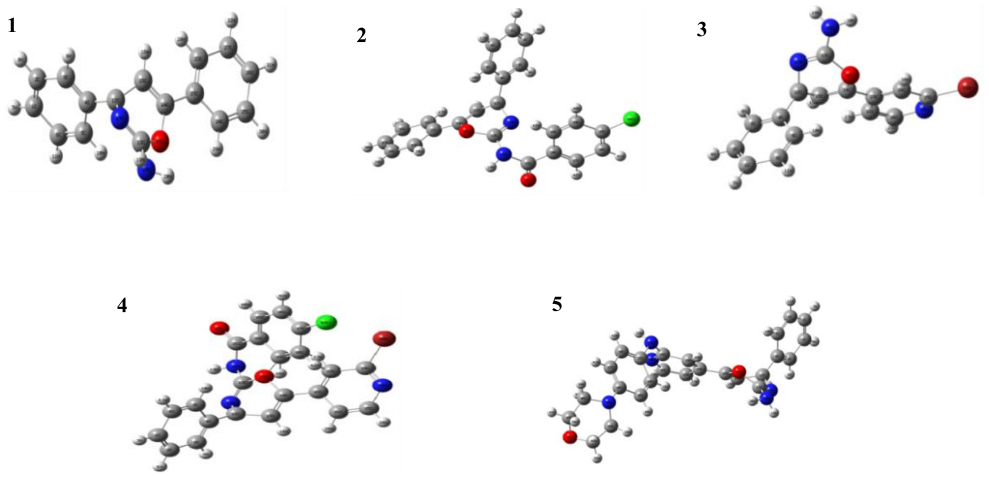


Figure 1. Molecular structure of compounds **1-5**

Table 1. Calculated quantum chemical parameters for all compounds

Parameters	1	2	3	4	5
E_{HOMO} (eV)	-0.18769	-0.17802	-0.34581	-0.14793	-0.19972
E_{LUMO} (eV)	-0.00198	-0.08384	-0.16299	-0.09816	-0.06391
ΔE_{gap} (eV)	0.18571	0.09418	0.18282	0.04977	0.13581
IE	0.18769	0.17802	0.34581	0.14793	0.19972
A	0.00198	0.08384	0.16299	0.09816	0.06391
η	0.092855	0.04709	0.09141	0.024885	0.067905
ω	0.04842861	0.18202022	0.35400590	0.30420076	0.12792781
χ	0.094835	0.13093	0.25440	0.123045	0.13181
μ	-0.094835	-0.13093	-0.25440	-0.123045	-0.13181
S	5.38474	10.61797	5.469861	20.09242	7.363228
σ	10.76948	21.23593	10.93972	40.18485	14.72646

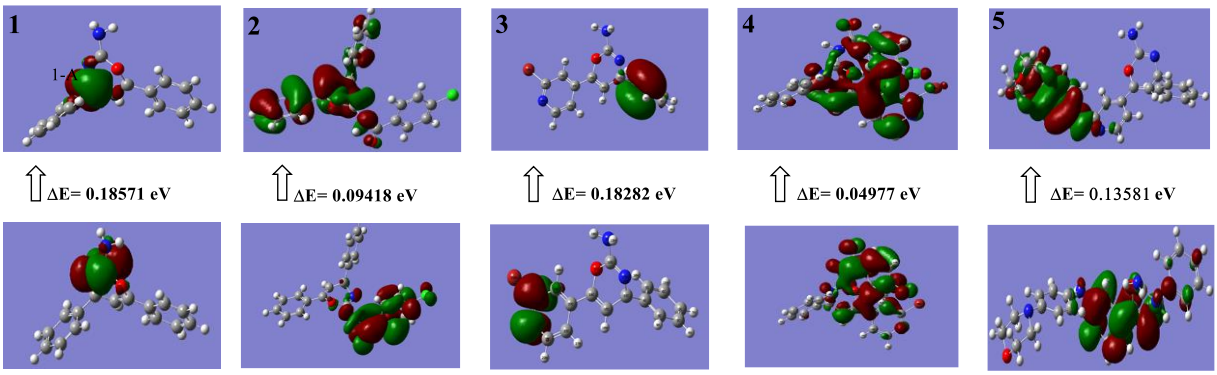


Figure 2. HOMO-LUMO energy gap of compounds **1-5**

3.2. Antibacterial Activity Study

The synthesized oxazine derivatives were evaluated for their antibacterial activities against bacterial strains and using tetracycline as positive reference. The targets showed considerable activity against the bacterial strains (Table 2). The Compounds 2, 4 and 5 showed an excellent activity against both *S. aureus and E. coli* while the compounds 1 and 3 showed less activity against both strains compared to tetracycline. The activity in compounds 2 and 4 may belongs to the presence of amide group, while the activity of compound 5 due to the morpholine moiety.

Table 2. Antibacterial data for the synthesized derivatives

	Inhibition Zone (mm)	
Compound	***S. aureus***	***E. coli***
1	20	14
2	33	31.5
3	18	13.5
4	34.5	35
5	30.5	29.5
Tetracycline	26	24

3.3. Docking Protocol Validation

The redocking process yielded an RMSD value of 0.896 Å for the 2VKE receptor. These findings suggest that the docking procedure adopted satisfies the docking process's validity requirements. Ligand overlay visualization of redocking with crystallographic reference ligands is presented in Figure 3. Apart from a minor shift in position, the redocking ligands have the same orientation as the

crystallographic ligands. Table 3 presents the validation results as well as the docking protocol employed. Of the 15 interactions that occurred, five of them were hydrogen bonds. These findings explain why although the number of interactions was relatively short, the ΔG of tetracycline was comparatively low, considering that hydrogen bonding is a strong ligand-receptor interaction [29].

The two ligands with the highest ΔG values (ligands number 1 and 3) had the lowest inhibition zone diameters. Meanwhile, ligand number 4, which had the largest inhibition zone diameter in both test microbes, was one of the two ligands with the lowest ΔG value. These findings show a consistent correlation between *in vitro* assay results with *in silico* results, with a tendency for ligands with low ΔG also to have promising *in vitro* antibacterial activity, consistent with that reported by Nybond *et al* [30].

However, comparing the ΔG values from the docking results alone is not enough to obtain comprehensive results. It can be seen that ligand number 5 also has the same ΔG value as ligand number 4, so it will be challenging to determine the ranking of docking results if only the ΔG value is considered. The similarity of ΔG values is often difficult in determining the ranking of docking results, especially with software that only provides a one-digit decimal value, such as AutoDock Vina [31]. Therefore, it is important to determine the similarity of the ligand-receptor interaction, which is used to determine ranking and provides an overview of the probability of whether the interaction of the test ligand will resemble the reference ligand or not [32, 33].

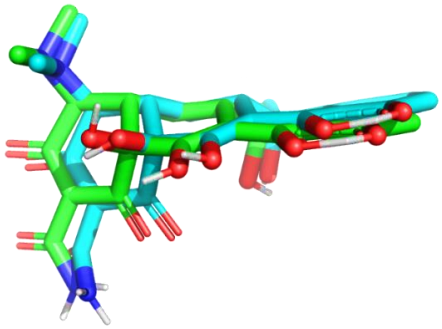


Figure 3. At receptors 2VKE with RMSD 0.896 Å, overlays of redocking ligands (blue) with reference ligands from crystallographic data (green).

Table 3. The validation process results

Parameters	Value
PDB ID	2VKE
Reference ligand	Tetracycline
Grid box size (Å)	20 x 30 x 20
Grid box position	x: 19.115
	y: 35.724
	z: 35.074
RMSD (Å)	0.896
ΔG (kcal/mol)	-8.5
Amino acid residues	60-Leu[a]
	64-His[b]
	82-Asn[b]
	86-Phe[a]
	100-His[b]
	103-Thr[a]
	104-Arg[a]
	105-Pro[c]
	109-Gln[a]
	112-Thr[a]
	113-Val[a]
	116-Gln[b]
	117-Leu[a]
	131-Leu[c]
	134-Ile[a]
	138-Ser[b]

The similarity of ligand-receptor interactions between ligands number 4 and 5 showed a significant difference (68.75 vs. 50%). The similarity of ligand number 5 was even smaller than that of ligand number 2 (62.5%), which incidentally had a higher ΔG value. The sequence was found to be in harmony with the results of *in vitro* tests, with the diameter of the inhibition zone on both microbes from ligand number 2 being larger than number 5. These results further confirm the correlation between the *in vitro* test and molecular docking [34], with a note also taking into account the similarity of ligand-receptor interactions. In addition, these results also imply a potential mechanism of action of the number 4 (and possibly number 2) ligand, which resembles tetracycline in inhibiting TetR. This finding is very interesting, considering the two structures are very different, but the interaction similarity is more than ⅔. Such a high similarity of ligand-receptor interactions is usually indicated if the test and reference ligands are from the same group of compounds, mainly due to the similarity of the pharmacophores [35]. However, ligand number 4 and tetracycline showed different pharmacophore profiles, so the similarity of the interactions was unique and exciting. The overall docking results could be seen in Table 4.

The substituent at position number 2 of oxazines has a considerable influence on the ligand-receptor interaction, according to the structure of each test ligand. The two test ligands with the highest interaction similarity had the same substituent at that position: 4-chlorobenzamide. The group interacts with 60-leucine and 61-alanine by forming alkyl/Pi-alkyl interactions and forming a hydrogen bond with 67-serine. The amino acid at this position is an important binding site on TetR [36], which interacts with the carboxamide group, one of the main pharmacophores of the tetracyclines [37]. On the other hand, the presence of a phenyl group (ligand number 2) or 2-bromopyridine (ligand number 4) at position number 4 of oxazines turned out to have a significant impact on ΔG value (0.6 kcal/mol difference), so the choice of 2-bromopyridine substituent was more reasonable at the position.

A different situation is indicated by ligand number 5, which also has a low ΔG value. A much smaller amine group occupies the substituent at position number 2 of oxazines. Instead, ligand number 5 has a 4-morpholinoaniline group on the number 2 substituent of the pyridine group, which is the major pharmacophore of the ligand. The pharmacophore interacts at the amino acid positions 60-67, which is dominated by the van der Waals interaction though weaker than the interaction of the 4-chlorobenzamide group [38]. The difference in the position of these substituents causes the difference in the orientation of the ligand number 5 compared to other ligands, as shown in Figure 4. Thus, selecting a 4-chlorobenzamide substituent is more beneficial than

4-morpholinoaniline for developing antibacterial compounds from oxazines derivatives. Derivatives with a 4-chlorobenzamide substituent are also known to exhibit increased antibacterial activity, as reported by Desai *et al*. [39] and Zhou *et al* [40].

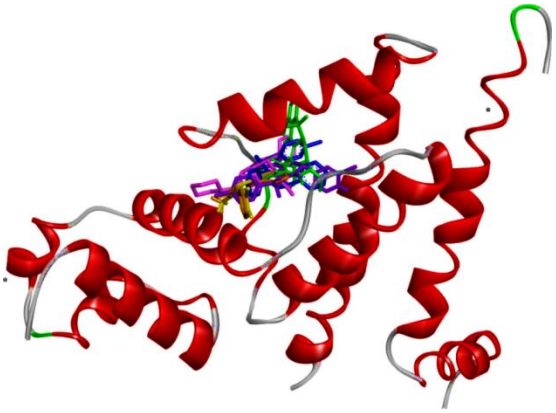


Figure 4. Overlays of tetracycline (green) with test ligands of 1 (orange), 2 (blue), 3 (yellow), 4 (magenta), and 5 (pink) at receptors 2VKE

4. Conclusions

The synthesized derivatives of oxazine were subjected to antibacterial inhibitory activity test and most of them showed significant antibacterial inhibitory activity against bacterial strains especially compounds 2, 4, and 5 while other compounds showed moderate activity compared to reference of tetracycline. Oxazine derivatives with 4-chlorobenzamide group at position number 2 showed higher potency than tetracycline as antibacterial by inhibiting TetR. stability of compounds has been calculated to prove that compound **1** has the highest stability among others as follow **1**>**3**>**5**>**2**>**4**.

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Table 4. Docking results for test ligands at the 2VKE receptor's binding site

Parameters	Value				
Ligands	1	2	3	4	5
ΔG (kcal/mol)	-7.7	-9.1	-8	-9.7	-9.7
Amino acid residues	-	-	-	-	56-Ala[c]
	57-Val[a]	-	57-Val[a]	-	57-Val[c]
	60-Leu[b]	60-Leu[b]	60-Leu[b]	60-Leu[b]	60-Leu[b]
	61-Ala[c]	-	61-Ala[c]	61-Ala[b]	61-Ala[b]
	64-His[c]	64-His[c]	64-His[a]	64-His[c]	64-His[c]
	67-Ser[a]	67-Ser[c]	67-Ser[c]	67-Ser[a]	67-Ser[c]
	-	-	68-Leu[b]	-	-
	-	-	-	79-Leu[b]	-
	82-Asn[c]	82-Asn[c]	82-Asn[c]	82-Asn[c]	82-Asn[c]
	-	-	-	83-Ala[c]	-
	-	86-Phe[d]	-	86-Phe[d]	86-Phe[c]
	-	100-His[c]	-	100-His[d]	100-His[c]
	-	103-Thr[c]	-	103-Thr[c]	103-Thr[a]
	-	104-Arg[c]	-	104-Arg[c]	104-Arg[c]
	-	105-Pro[b]	-	105-Pro[b]	105-Pro[c]
	-	-	-	-	108-Lys[b]
	109-Gln[a]	109-Gln[a]	109-Gln[c]	109-Gln[c]	109-Gln[a]
	112-Thr[c]	112-Thr[c]	112-Thr[c]	112-Thr[c]	112-Thr[c]
	113-Val[b]	113-Val[c]	113-Val[b]	113-Val[c]	113-Val[c]
	116-Gln[c]	116-Gln[c]	116-Gln[c]	116-Gln[a]	116-Gln[e]
	-	134-Ile[b]	-	134-Ile[b]	-
	-	135-Ser[c]	-	-	-
	-	-	-	137-Val[b]	-
	-	138-Ser[a]	-	138-Ser[c]	-
	-	-	-	-	139-His[c]
	-	-	-	141-Thr[c]	-
Similarity of ligand-receptor interaction with tetracycline (%)	25	62.5	31.25	68.75	50

[a]Hydrogen bond; [b]Alkyl/Pi-alkyl interaction; [c]van der Waals interaction; [d]ePi-Pi T-shaped/Pi-Pi stacked/Amide-Pi stacked; [e]Unfavorable bump/Donor-donor

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