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In-Silico Evaluation of *Helix aspersa* Müller Slime-derived Biomolecules Targeting Acetyltransferase in Pathogenic Bacteria

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Abstract: Ensuring the safety and efficacy of drug candidates is a critical step in the drug development process. Protein acetylation, mediated by acetyltransferases, plays a pivotal role in cellular processes such as protein interactions, transcriptional regulation, enzymatic activity, and protein stability. Targeting these enzymes offers a promising strategy for combating pathogenic bacteria, especially in the face of increasing antibiotic resistance. This study aimed to identify bioactive compounds derived from *Helix aspersa* Müller snail slime that could effectively inhibit acetyltransferase proteins in pathogenic bacteria, using *in silico* methods. Sixteen bioactive compounds from *H. aspersa* Müller slime were evaluated for their drug-like properties based on the Lipinski rule of five, followed by molecular docking to assess their binding affinity to acetyltransferase proteins. Several compounds, including 2-ethylacridine, auramine, and 6-chloro-4-phenyl-2-propylquinoline, exhibited favorable drug-like properties, strong predicted binding affinities ≤ –7 kcal/mol, and interactions with key catalytic residues. While these results identify plausible acetyltransferase inhibitors, experimental validation is required to confirm their biological activity and therapeutic potential.

Keywords: *H. aspersa* Müller; protein transferase; drug development.

1. Introduction

The increase in antibiotic resistance represents one of the most urgent global health challenges of the 21st century, threatening to undermine decades of medical progress. According to the World Health Organization, antibiotic resistance is reaching dangerously high levels worldwide, making many standard treatments ineffective and increasing the risk of prolonged illness, disability, and death [1]. The overuse and misuse of antibiotics in human medicine, agriculture, and livestock have accelerated the emergence of multidrug-resistant pathogens, leaving clinicians with limited therapeutic options [2]. Without urgent action, we

risk entering a post-antibiotic era where common infections and minor injuries could become fatal again [3]. This crisis underscores the urgent need for innovative strategies to discover and develop new classes of antibiotics that can overcome resistance mechanisms.

In the midst of this urgent need, bacterial acetyltransferases have emerged as a promising yet underexplored target for the development of antibacterial drugs. Acetyltransferases are enzymes that catalyze the transfer of an acetyl group from acetyl-CoA to acceptor molecules, including proteins, thereby regulating critical cellular processes such as metabolism, gene expression, and protein interactions [4]. Although protein acetylation is well studied in eukaryotes, its role in bacterial physiology has only recently begun to be elucidated [5]. New evidence suggests that acetyltransferases play a central role in bacterial adaptation to environmental stress and pathogenesis, making them attractive targets for disrupting bacterial survival mechanisms [6,7].

Despite growing understanding of the biological significance of acetyltransferases, an important gap in research remains in developing effective inhibitors targeting these enzymes. Current antibiotics primarily target well-established pathways, such as cell wall synthesis and protein translation, but their widespread use has led to rapid resistance evolution [2]. The lack of clinically available inhibitors for bacterial acetyltransferases underscores a critical unmet need in antibiotic discovery. Filling this gap could provide a new therapeutic strategy to combat resistant bacterial infections and expand the available treatment arsenal.

Natural products have long been a rich source of bioactive compounds with various pharmacological properties, including antibacterial activity. Historically, many antibiotics, such as penicillin and streptomycin, were derived from natural sources, highlighting their potential in drug discovery [8]. Among unconventional sources, Helix aspersa Müller's slime has shown promise as a reservoir of new bioactive molecules [9]. Preliminary studies suggest that snail slime contains compounds with unique mechanisms of action, which makes it an intriguing candidate for the discovery of acetyltransferase inhibitors [10]. By taking advantage of the chemical diversity of natural products, researchers can explore new ways to target bacterial acetyltransferases and overcome resistance.

This study aims to advance the development of an acetyltransferase inhibitor by exploring the potential of natural compounds derived from the snail *Helix aspersa* Müller. Using advanced computational methods, we conducted an in silico docking study to evaluate the binding affinities of 16 bioactive snail slime compounds to the modeled acetyltransferase proteins. These compounds were compared with known antibiotics, such as ampicillin and tetracycline, to evaluate their potential as new inhibitors. By identifying natural compounds with a strong binding affinity with acetyltransferases, this research not only advances our understanding of the role of acetylation in bacterial physiology but also provides a basis for the development of new-generation antibiotics. The results have the potential to lead to new therapeutic avenues, offering hope in the fight against antibiotic-resistant infections.

2. Materials and Methods

2.1. Selection and preparation of ligands.

In total, 16 bioactive compounds present in the snail slime Helix aspersa Müller, previously reported in our study [11], were selected as ligands, and their structures were downloaded from the PubChem-NCBI database in SDF format (Figure 1). Using the PyMOL software, these ligands have been modified in PDB format, which makes them suitable for

docking analysis. The above step was followed by the definition of the torsion requirements for a suitable binding using the Autodock 4.2.6 parameters. Antibacterial agents, namely ampicillin and tetracycline, were used as positive controls and treated in the same manner.

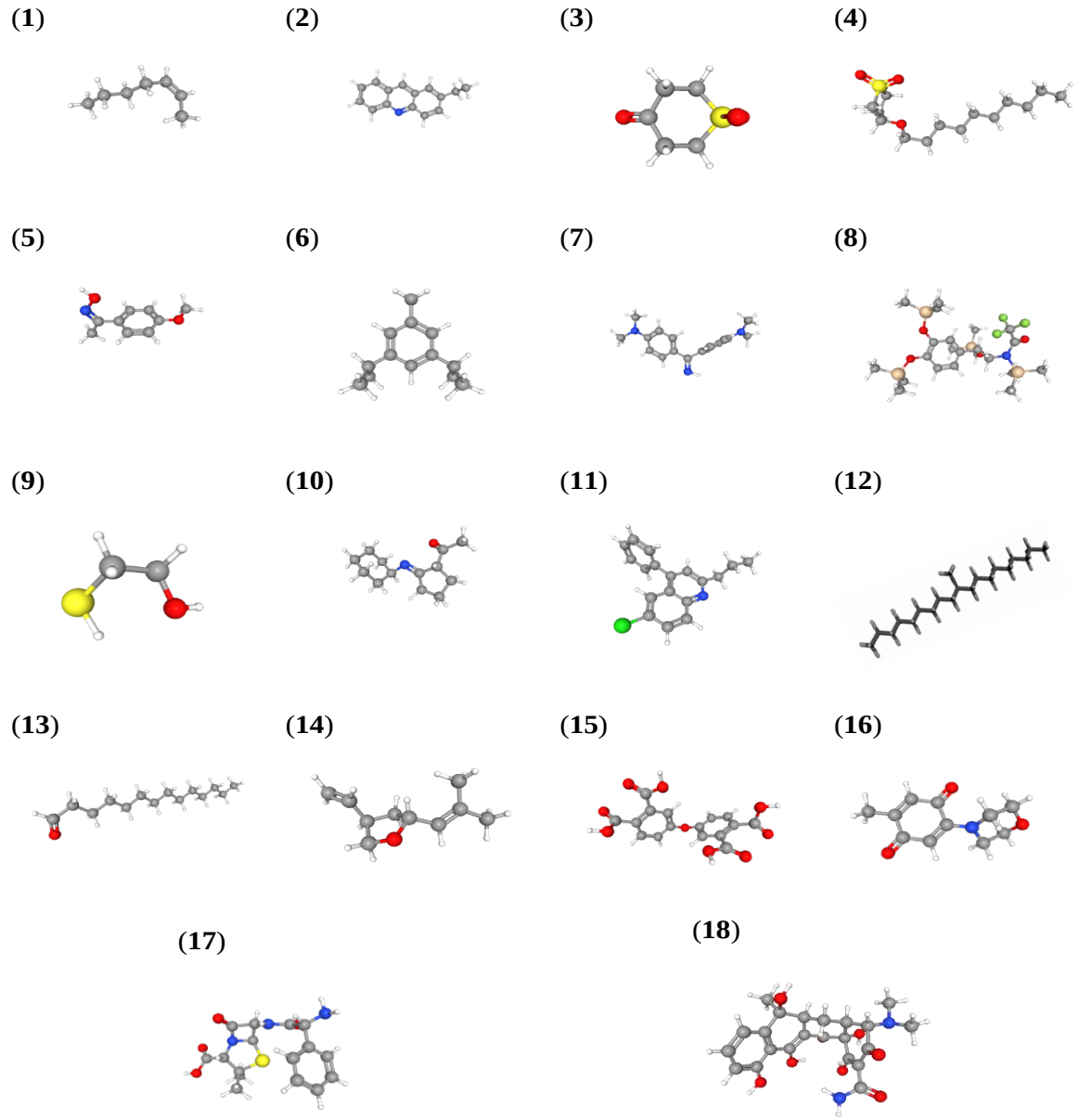


Figure 1. 3D structures of the biocompounds derived from *H. aspersa* Müller.
(1) (Z)-2-heptene; **(2)** 2-ethylacridine; **(3)** 4H-Thiopyran-4-one, tetrahydro, 1,1-dioxyde; **(4)** Thiophene, 3-(decyloxy)tetrahydro, 1,1-dioxyde; **(5)** Methoxyphenyl-Oxime; **(6)** 1-Butyl-2,4,6-trimethyl benzene; **(7)** Auramine; **(8)** N-(trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine; **(9)** Mercaptoethanol; **(10)** N-(2-acetylcyclopentylidene)cyclohexylamine; **(11)** 6-chloro-4-phenyl-2-propylquinoline; **(12)** 10-methylnonadecane; **(13)** Tetradecanal; **(14)** Furan, 2 isobutenyl-4-vinyl; **(15)** 1,2-Benzene dicarboxilic acid; **(16)** Cyclohexa-2,5-diene-1,4-diene, 2-methyl-5-(4-morpholinyl); **(17)** Ampicillin; **(18)** Tetracycline.

2.2. Preparation of proteins.

The 3D structures of the acetyltransferase proteins were downloaded from the RCSB protein database with the PDB IDs 2J8N and 4QVT (Figure 2). These proteins were captured in PDBQT format after being stripped of water molecules and assigned polar hydrogen atoms and Kollman charges using AutoDock 4.2.6.

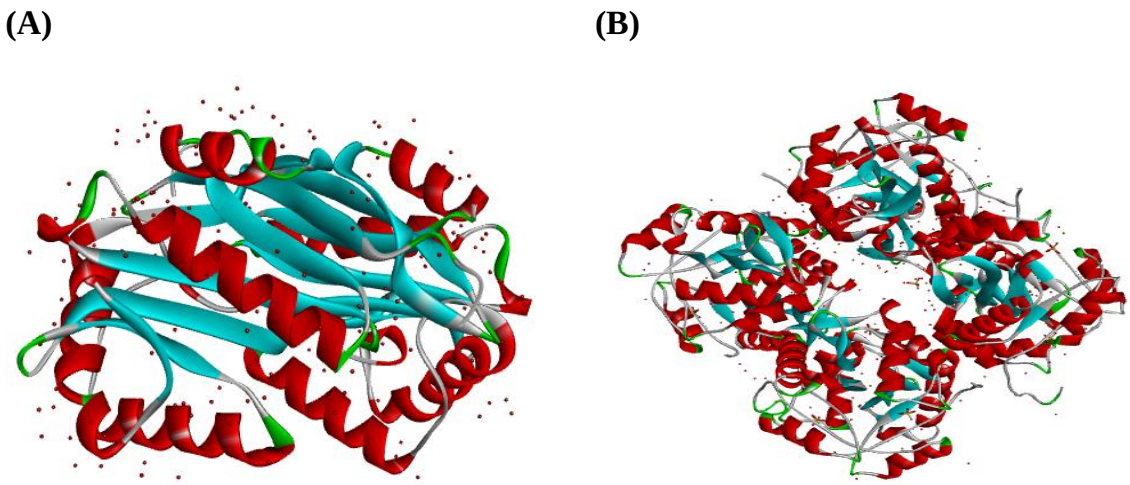


Figure 2. 3D structure of acetyltransferase **(A)** *Pseudomonas aeruginosa* (2J8N); **(B)** *Escherichia coli* (4QVT).

2.3. ADME analysis and other pharmacokinetic properties.

The analysis of drug similarity was carried out on the basis of Lipinski's rule of five [12]. The absorption, distribution, metabolism, and excretion of a ligand throughout the human body are the primary pharmacokinetic features known as ADMEs. SwissADME was used to construct the primary parameter, Lipinski's rule of five [13], to evaluate the pharmacological significance. For a compound to be qualified as a ligand, it must have a molecular weight < 500 Da, a high lipophilicity, that is to say, a Log P value < 5, hydrogen bond acceptors of less than 10, and H bond donors < 5. Any compound with 2 or more violations was excluded from further study [12].

2.4. Bioactivity score and bioavailability radar.

The online Molinspiration program was used to calculate the ligands' bioactivity score. Using the canonical SMILES of the ligands that were acquired from PubChem, this was accomplished. G protein-coupled receptors (GPCR), kinase inhibitors (KI), nuclear receptor ligands (NRL), enzyme inhibitors (EI), and ion channel modulators (ICM) are among the characteristics examined. The probability of a candidate drug with a binding energy lower than that of the control was exhaustively analyzed by taking into account 6 physicochemical properties and by forming a bioavailability radar using the SwissADME tools. Six parameters have been taken into account: solubility, size, polarity, lipophilicity, flexibility, and saturation. The ideal values of the six parameters are defined by the pink-highlighted zone; a significant departure from these values indicates that the ligand is not orally accessible [13].

2.5. Docking analysis.

After the preparation of the proteins and ligands, the molecular docking was executed using Autodock 4.2.6. For the connection to take place, the x, y, and z dimensions were defined at 60 × 60 × 60 with a resolution of 0.500 Å, and the (x, y, z) coordinates centered on the grid to obtain favorable docking conformations. The box of acetyltransferase from *Pseudomonas aeruginosa* (2J8N) (6.723, 1.573, 51.621), and from acetyltransferase *Escherichia coli* (4QVT) (–28.805, –31.551, 100.859). The grid file has been saved as a file (.gpf) and executed in autogrid mode. Next, the Lamarckian evolutionary method was used to calculate the tie-down, with ten executions set as the default value. The docking file was saved as a DPF file, and after

running Autodock, the final docking results were obtained in a file (.dlg), which presented information such as the binding residues, the binding energy (kcal/mol), and the inhibition constant. The structures showing the interaction between ligands and proteins were visualized using Discovery Studio (V 24) [14].

3. Results and Discussion

3.1. ADME analysis and pharmacokinetic analysis.

None of these compounds violated more than one parameter, so the 16 substrates satisfied the five Lipinski criteria and were subjected to linkage studies (Table 1).

Table 1. Dependent variables of chemical compounds.

Sub. No	PubChem ID	LogP	MW	nOH	nOHNH	Nb	Nb viol	Mol ref
(1)	643836	2.75	98.19	0	0	3	0	34.34
(2)	610161	3.95	207.27	1	0	1	0	68.63
(3)	262230	0.84	148.18	3	0	0	0	32.94
(4)	86787	4.41	276.44	3	0	10	0	75.66
(5)	5355943	1.79	165.19	3	1	2	0	47.14
(6)	137809	4.24	176.30	0	0	2	0	59.36
(7)	10298	3.24	267.38	3	1	4	0	87.47
(8)	553890	3.73	553.92	5	0	11	1	149.84
(9)	1567	–0.09	78.14	1	1	1	0	20.94
(10)	610112	3.15	207.32	2	0	2	0	62.33
(11)	620147	5.51	281.79	1	0	3	1	86.18
(12)	530070	6.38	282.56	0	0	16	1	111.40
(13)	31291	4.88	212.38	1	0	12	0	67.14
(14)	550408	2.54	152.24	1	0	2	0	47.48
(15)	82136	2.27	346.25	9	4	6	0	80.80
(16)	610024	0.30	207.23	4	0	1	0	54.25
(17)	6249	0.32	349.40	7	4	5	0	89.03
(18)	54675776	–0.60	444.43	10	7	2	1	109.18

LogP: lipophilicity; Mol Wt: molecular weight; nOH: no. of H bond acceptors; nOHNH: no. of H bond donors; Nb: no. of rotatable bonds; Nb viol: no of violations; Mol ref: molecular refractivity.

The in-silico pharmacokinetic profile of the ligands, as illustrated in Table 2, indicates that the drug similarity and the pharmacokinetic properties of certain compounds are analogous to those of the established pharmaceutical products.

Table 2. In-silico pharmacokinetics of ligands using SwissADME.

Sub. No	ESOL (Log S)	GIads	BBB	P-gp	CYP3A4	CYP1A2	(iLOGP)	Bio score
(1)	−2.25 (S)	Low	Yes	No	No	No	2.51	0.55
(2)	−4.23 (MS)	High	Yes	No	No	Yes	2.73	0.55
(3)	−0.17 (VS)	High	Yes	No	No	No	0.52	0.55
(4)	−3.51 (S)	High	Yes	No	No	Yes	3.13	0.55
(5)	−2.36 (S)	High	Yes	No	No	No	1.98	0.55
(6)	−4.00 (MS)	Low	Yes	No	No	No	3.20	0.55
(7)	−4.17 (MS)	High	Yes	No	Yes	Yes	2.73	0.55
(8)	−7.56 (PS)	Low	No	Yes	Yes	No	5.61	0.55
(9)	−0.15 (VS)	High	No	No	No	No	1.09	0.55
(10)	−2.40 (S)	High	Yes	No	No	No	2.85	0.55
(11)	−5.53 (MS)	High	No	No	Yes	No	3.45	0.55
(12)	−7.31 (PS)	Low	No	No	No	Yes	5.52	0.55
(13)	−4.13 (MS)	High	Yes	No	No	Yes	3.63	0.55
(14)	−2.32 (S)	High	Yes	No	No	No	2.74	0.55
(15)	−2.93 (S)	Low	No	No	No	No	0.19	0.11
(16)	−1.25 (VS)	High	No	No	No	No	1.90	0.55
(17)	−1.15 (VS)	Low	No	No	No	No	1.15	0.55
(18)	−2.77 (S)	Low	No	Yes	No	No	1.19	0.11

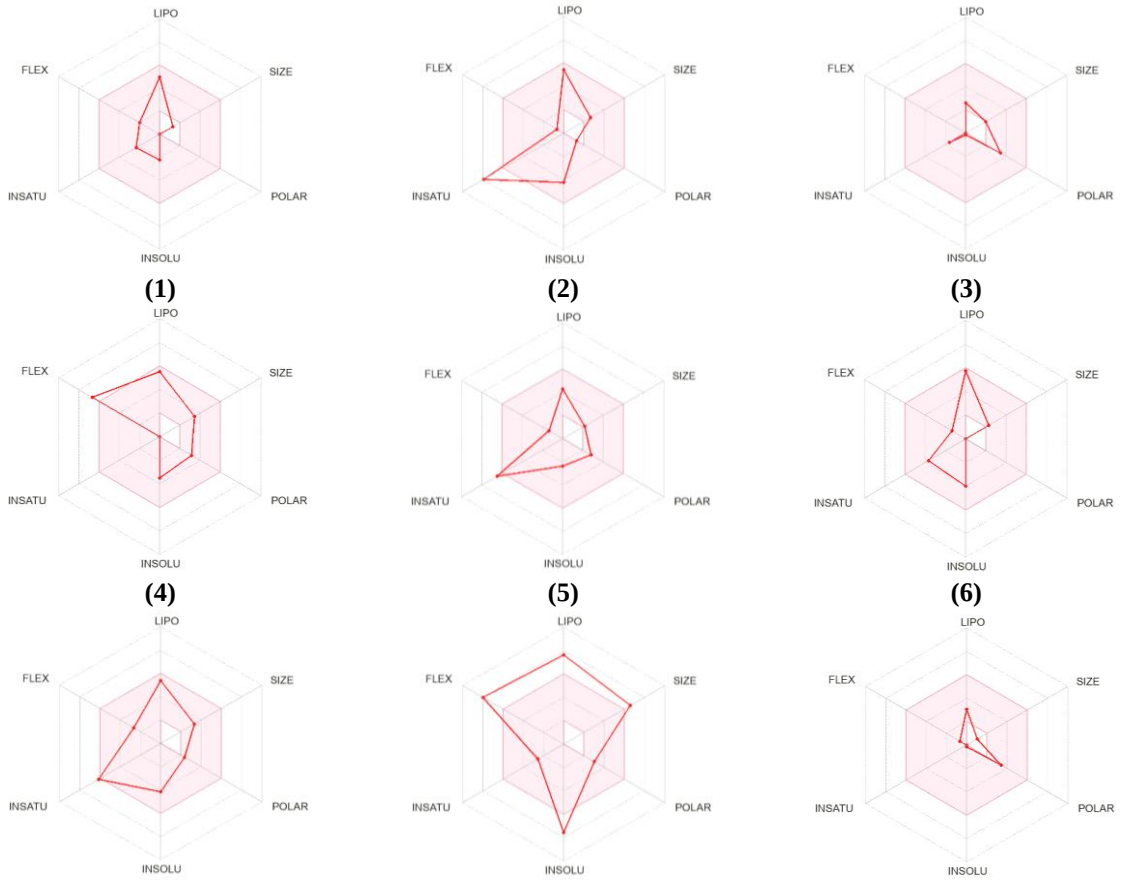
BBB: blood–brain barrier.

On the basis of the evaluation of the solubility (ESOL), it has been demonstrated that the chemical compounds 4H-Thiopyran-4-one, tetrahydro (3), and Mercaptoethanol (9) have a higher solubility than that of the reference compounds ampicillin (17) and tetracycline (18), possessing a lipophilicity similar to that of ampicillin (17) but exceeding that of tetracycline (18). The GIads were particularly high for all ligands, with the exception of (Z)-2-heptene (1), 1-Butyl-2,4,6-trimethyl benzene (6), N-(trifluoroacetyl)-N,O,O',O"-tetrakis(trimethylsilyl)norepinephrine (8), 10-methylnonadecane (12), and 1,2-Benzenedicarboxylic acid (15).

As Table 2 shows, the majority of phytochemicals do not serve as inhibitors of CYP3A4 and CYP1A2, which are part of the drug-metabolizing enzymes known as cytochrome P450, an essential enzyme involved in the metabolism of pharmaceutical products. The interaction of cytochrome P450 isoenzymes with drugs can lead either to an accelerated metabolism when the drug acts as a substrate for a CYP, resulting in induction, or to an accumulation of the drug when the drug acts as an inhibitor, resulting in inhibition, both factors generally considered to be unfavorable results [15]. Consequently, in the context of drug development, in silico approaches for forecasting the interactions of substances or medications with CYP isoenzymes are essential.

3.2. Bioavailability radar.

The bioavailability radar enables rapid evaluation of a compound's similarity to an established pharmacological agent. As shown in Figure 3, the magenta region delimits the ideal spectrum for each parameter. When evaluating the characteristics of a compound, the radar representation of this compound must be located in the magenta zone to be considered as a drug; therefore, the ligands should present an oral bioavailability or an absence of bioavailability based on the radar representation.



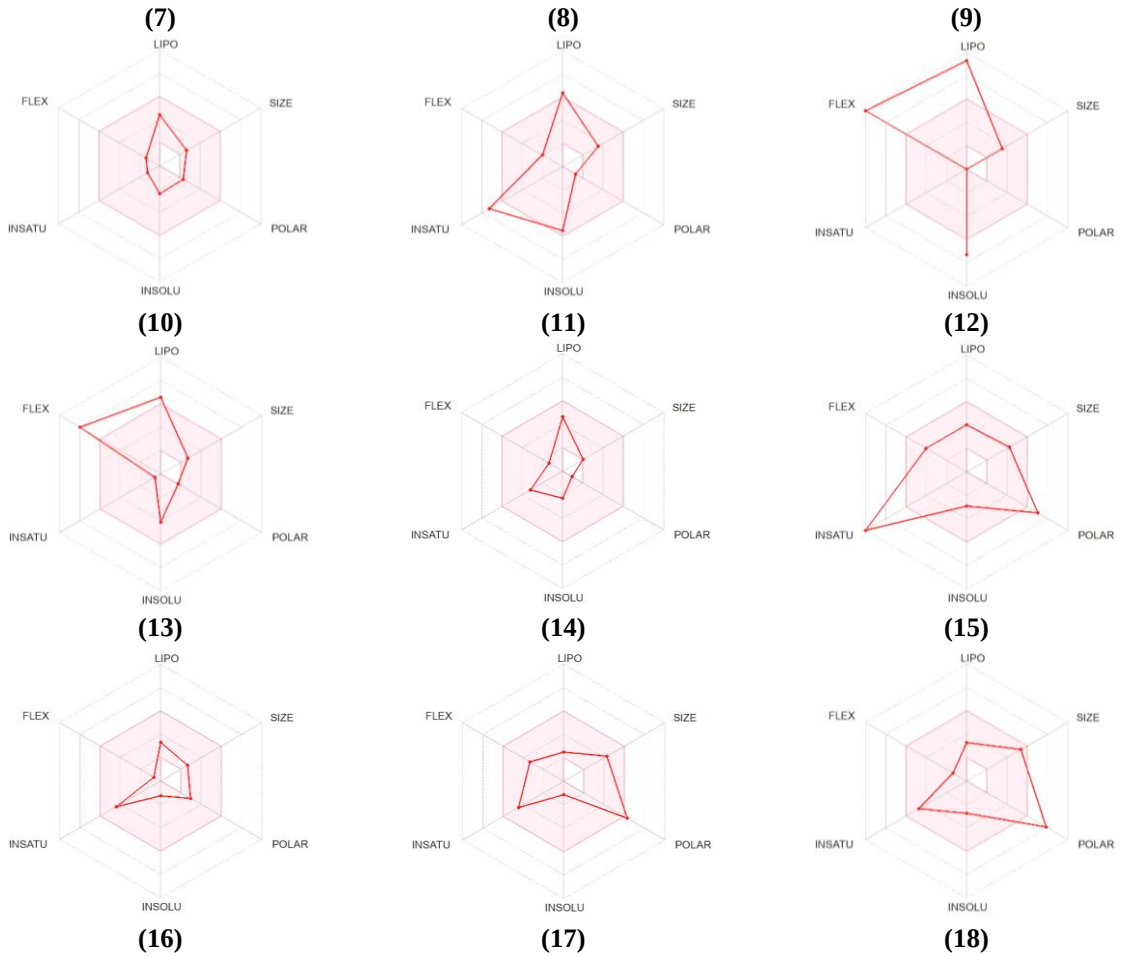


Figure 3. Radar graphs of the ligands.

Nine of the compounds studied ((Z)-2-heptene (1), 4H-Thiopyran-4-one, tetrahydro-, 1,1-dioxide (3), 1-Butyl-2,4,6-trimethyl benzene (6), Cyclohexa-2,5-diene-1,4-diene, 2-methyl-5-(4-morpholinyl) (16), Furan, 2 isobutenyl-4-vinyl (14), N- (2-acetylcyclopentylidene) cyclohexylamine (10), Mercaptoethanol (9)) satisfy the criteria of the radar plot and can therefore be suggested as being bioavailable orally (Figure 3).

3.3. Bioactivity score.

For EI, GPCR, and PI, N-(trifluoroacetyl)-N,O,O',O'-tetrakis(trimethylsilyl)norepinephrine (8) showed the highest scores of 0.51, 0.32, and 0.38, respectively. 1,2-Benzenedicarboxylic acid (15) showed the highest score of 0.23 for NRL, 6-chloro-4-phenyl-2- propylquinoline (11) had the highest score of 0.19 for KI, and Tetradecanal (13) showed a high score of 0.24 for ICM. Comparing this to standard antibiotics, (17) has good scores for GPCR, PI, and EI, while (18) showed inactivity in some of the properties, as shown in Table 3. The potential of these bioactive compounds as powerful therapeutic agents has been highlighted by good bioactivity scores; the higher the scores, the higher the activity.

Table 3. Bioactivity score of the compounds

Sub. No	GPCR	ICM	KI	NRL	PI	EI
(1)	−3.37	−3.17	−3.75	−3.22	−3.52	−3.01
(2)	−0.30	0.01	−0.38	−0.57	−0.52	−0.01
(3)	−2.47	−2.26	−2.92	−2.58	−2.06	−1.76
(4)	−0.64	−0.89	−1.09	−0.92	−0.14	−0.54
(5)	−0.98	−0.92	−0.98	−0.57	−0.97	−0.41
(6)	−0.73	−0.33	−0.89	−0.72	−0.93	−0.45

Sub. No	GPCR	ICM	KI	NRL	PI	EI
(7)	0.07	0.13	−0.10	−0.04	−0.03	0.07
(8)	0.32	0.10	−0.01	0.10	0.38	0.51
(9)	−3.76	−3.76	−3.74	−3.78	−3.12	−3.02
(10)	−0.59	−0.20	−1.26	−0.72	−0.77	−0.02
(11)	0.21	0.14	0.19	0.17	−0.15	0.27
(12)	−0.04	−0.02	−0.22	−0.04	−0.05	0.02
(13)	−0.24	0.24	−0.56	−0.34	−0.15	0.12
(14)	−0.98	−0.40	−1.48	−0.93	−1.01	0.01
(15)	0.04	−0.02	0.02	0.23	0.07	0.08
(16)	−0.76	−0.54	−0.35	−1.16	−0.97	−0.07
(17)	0.04	−0.47	−0.71	−0.61	0.87	0.25
(18)	−0.38	−0.34	−0.67	−0.30	−0.17	0.60

3.4. Docking analysis.

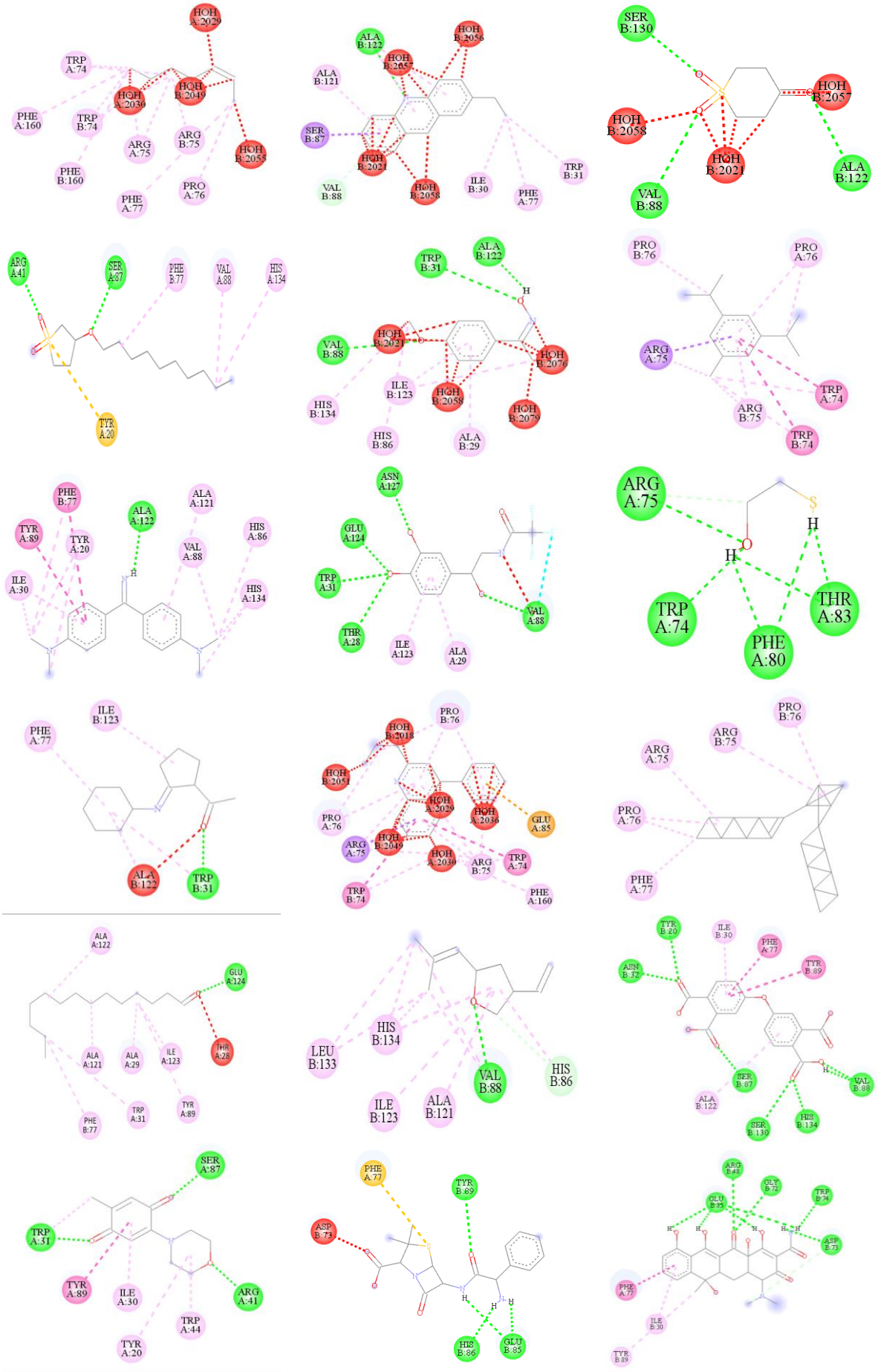
To achieve this objective, 16 bioactive compounds were tested against protein transferases. The conventional antibiotics (ampicillin and tetracycline) were bound to the proteins, and their binding energies were compared with the binding energies of the selected proteins. The binding energy (kcal/mol), the number of hydrogen bonds, the inhibitory constant (µM/mM), and the amino acids involved in the hydrogen bond were recorded after docking.

Among the bioactive compounds, six have a binding energy comparable to that of the standard antibiotics for 2J8N (Table 4 and Figure 4, 5). Ampicillin and tetracycline have binding energies of –7.4 kcal/mol and –8.7 kcal/mol, respectively, with an amino acid involved in the hydrogen bond, such as His B:86, Glu B:85, and Tyr B:89 for ampicillin and Glu B:85, Arg B:48, Gly B:72, Trp B:74, and Asp B:73 for tetracycline. The best inhibitory profiles are carried by the compound (11) 6-chloro-4-phenyl-2-propylquinoline (Be = –7.61 kcal/mol; Ic = 2.64 µM) and the compound (12) 10-methylnonadecane (Be = –7.42 kcal/mol; Ic = 3.95 µM), which display comparable inhibition constants and sufficiently low to consider them as priority candidates. The compound (2) (2-ethylacridine) (Be = –7.16 kcal/mol; Ic = 5.65 µM) and (7) Auramine (Be = –7.14 kcal/mol; Ic = 8.50 µM) follow in potential, while (10) (Be = –6.97; Ic = 7.81 µM) also exhibits moderate activity. Conversely, 1,2-benzene dicarboxylic acid (15) displays the most favorable Be (–8.00 kcal/mol) and a very high Ic (388.59 µM).

Table 4. Interaction scores between bioactive compounds and 2J8N transferase proteins.

Sub. No	Be (Kcal/mol)	Ic (µM)	Amino acids involved in hydrogen bonding
(1)	−3.77	1.73 mM	—
(2)	−7.16	5.65	Ala B:122
(3)	−5.34	122.41	Ser B:130, Val B:88, Ala B:122
(4)	−6.04	37.45	Ser A:87, Arg A:41
(5)	−6.06	36.30	Ala B:122, Trp B:31, Val B:88
(6)	−6.23	27.28	—
(7)	−7.14	8.50	Ala A:122
(8)	−6.54	16.10	Thr A:25, Trp A:31: Glu A:124, Asn A:127, Val A:88
(9)	−2.72	10.13 mM	Arg A:75, Trp A:74, Phe A:80, Thr A:83
(10)	−6.97	7.81	Trp B:31
(11)	−7.61	2.64	—
(12)	−7.42	3.95	—
(13)	−4.75	332.34	Glu A:124
(14)	−5.17	161.94	Val B:188
(15)	−8.00	388.59	Tyr B:20, Asn B:32, Ser B:97, Ser B:130, His B:134, Val B:88
(16)	−6.50	29.48	Ser A:87, Trp A:31, Arg A:41
(17)	−6.85	9.47	His B:86, Glu B:85, Tyr B:89
(18)	−8.00	1.37	Glu B:85, Arg B:48, Gly B:72, Trp B:74, Asp B:73

Be: Binding energy; Ic: Inhibition constant.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TRP A:74
HOH A:2030
HOH B:2049
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TRP B:74
ARG A:75
ARG B:75
HOH B:2055
PHE B:160
PRO A:76
PHE A:77
ALA B:122
HOH B:2056
HOH B:2054
ALA B:121
SER B:87
HOH B:2021
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ILE B:30
TYR B:89

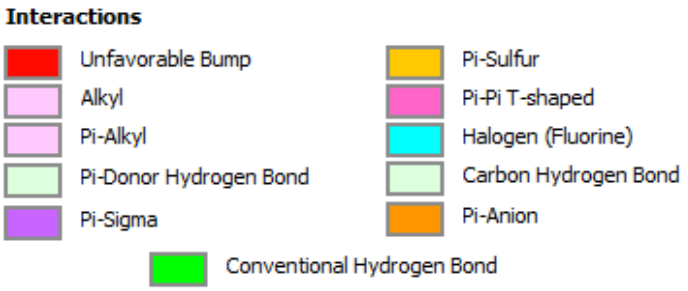


Figure 4. 2D interaction of ligands with 2J8N.

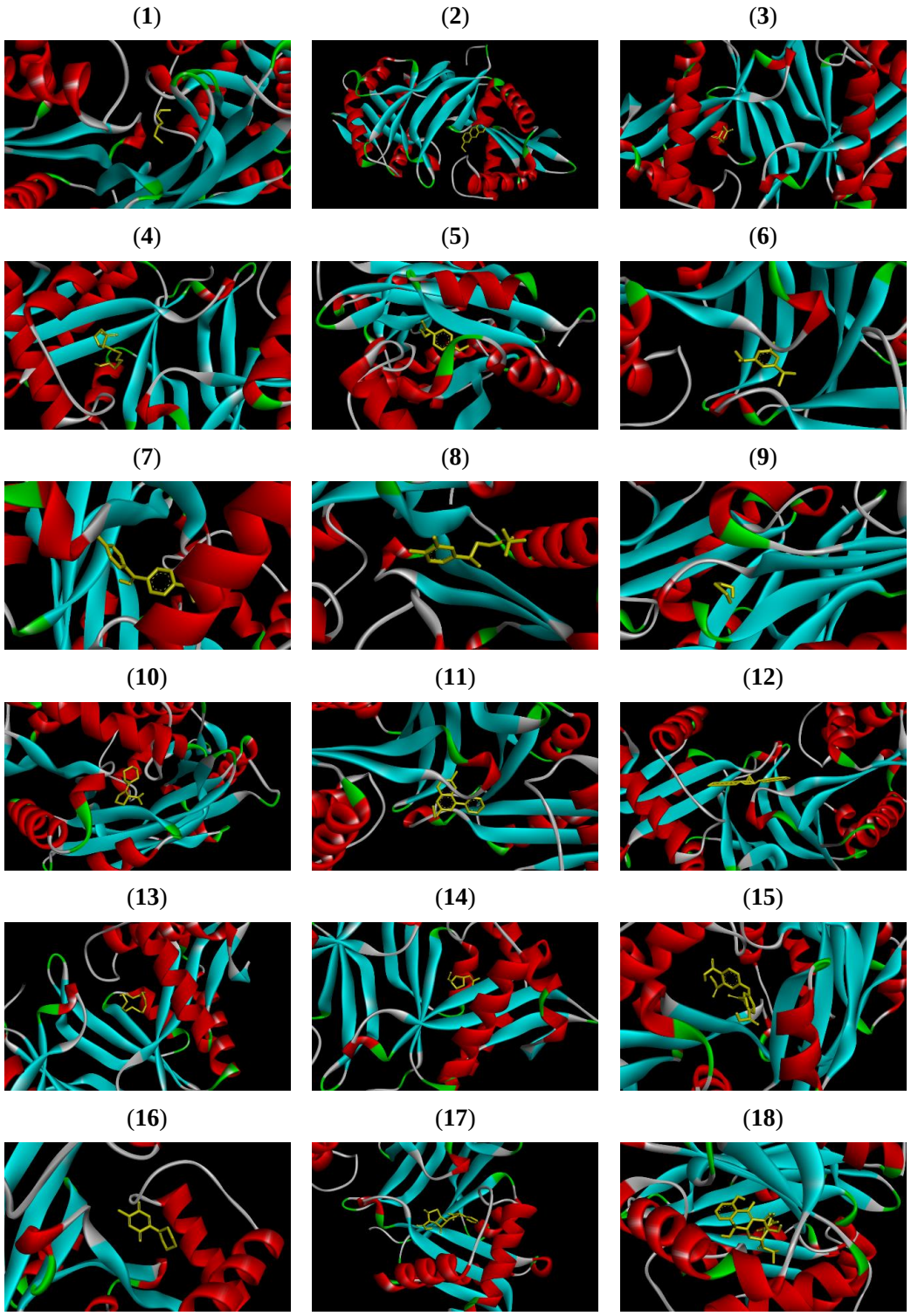


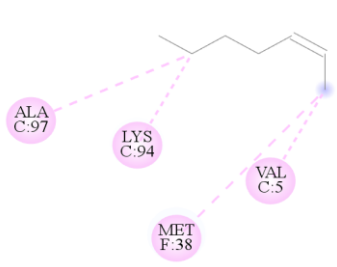
Figure 5. 3D interaction of ligands with 2J8N.

According to Table 5, five bioactive compounds have a binding energy similar to that of the standard antibiotics for 4QVT. The binding energies of ampicillin and tetracycline are respectively –7.58 kcal/mol and –7.60 kcal/mol, with amino acids that participate in the hydrogen bond, such as Lys D:94, SO A:4202 for ampicillin and Arg E:98, His E:40, Asp E:41, Asn D:39 for tetracycline (Figures 6 and 7). 10-methylnonadecane (12) stands out with a Be of –7.88 kcal/mol and an Ic of 41.28 µM, followed by 6-chloro-4-phenyl-2-propylquinoline (11) (Be = –7.81 kcal/mol, Ic = 23.75 µM), 2-ethylacridine (2) (Be = –7.79 kcal/mol, Ic = 28.83 µM), N-(2-acetylcyclopentylidene)cyclohexylamine (10) (Be = –7.79 kcal/mol, Ic = 68.04 µM) and 1,2-Benzene dicarboxilic acid (15) (Be = –7.73 kcal/mol, Ic = 27.57 µM). In contrast, (Z)-2-heptene (1) (Be = –3.81 kcal/mol, Ic = 3.15 mM) and -Butyl-2,4,6-trimethylbenzene (6) (Be = –4.87, Ic = 5.66 mM) display too high inhibition values.

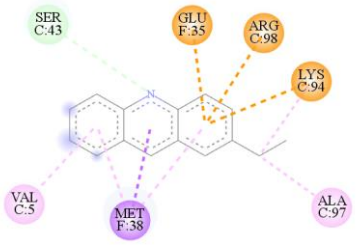
Table 5. Interaction scores between the bioactive compounds and the 4QVT transferase proteins.

Sub. No	Be (Kcal/mol)	Ic (µM)	Amino acids involved in hydrogen bonding
(1)	−3.81	3.15 mM	—
(2)	−7.79	28.83	—
(3)	−5.30	205.98	Arg C:98, Lys C:94
(4)	−5.44	782.59	Asn A:39
(5)	−5.67	380.12	Agr C:98, Asn D:39, His D:40
(6)	−6.42	347.23	—
(7)	−6.81	91.86	Arg C:98
(8)	−7.47	191.97	Ser B:43, Agr B:98, Lys B:94
(9)	−6.75	4.17 mM	His A:40, Lys B:37, Asp B:41
(10)	−7.79	68.04	Arg C:98, Ser C:43
(11)	−7.81	23.75	Arg C:98
(12)	−7.88	41.28	—
(13)	−4.87	5.66 mM	—
(14)	−5.45	389.43	Asn D:39
(15)	−7.73	27.57	Asn C:39, Glu C:35, Arg B:98, Gln C:8
(16)	−6.22	346.20	—
(17)	–7.58	2.78	Lys D:94, So A:4202
(18)	–7.60	2.70	Arg E:98, His E:40, Asp E:41, Asn D:39

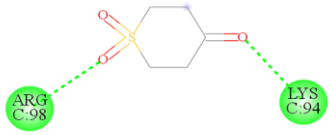
Be: Binding energy; Ic: Inhibition constant.



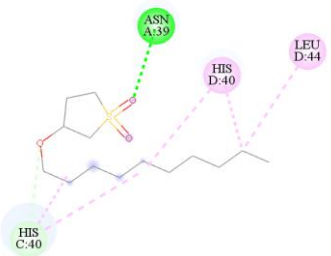
(1)



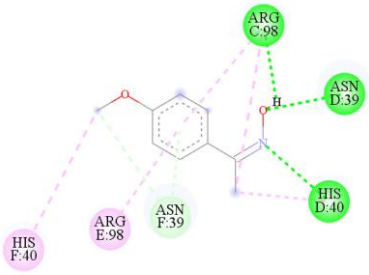
(2)



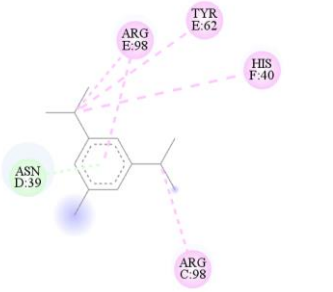
(3)



(4)



(5)



(6)

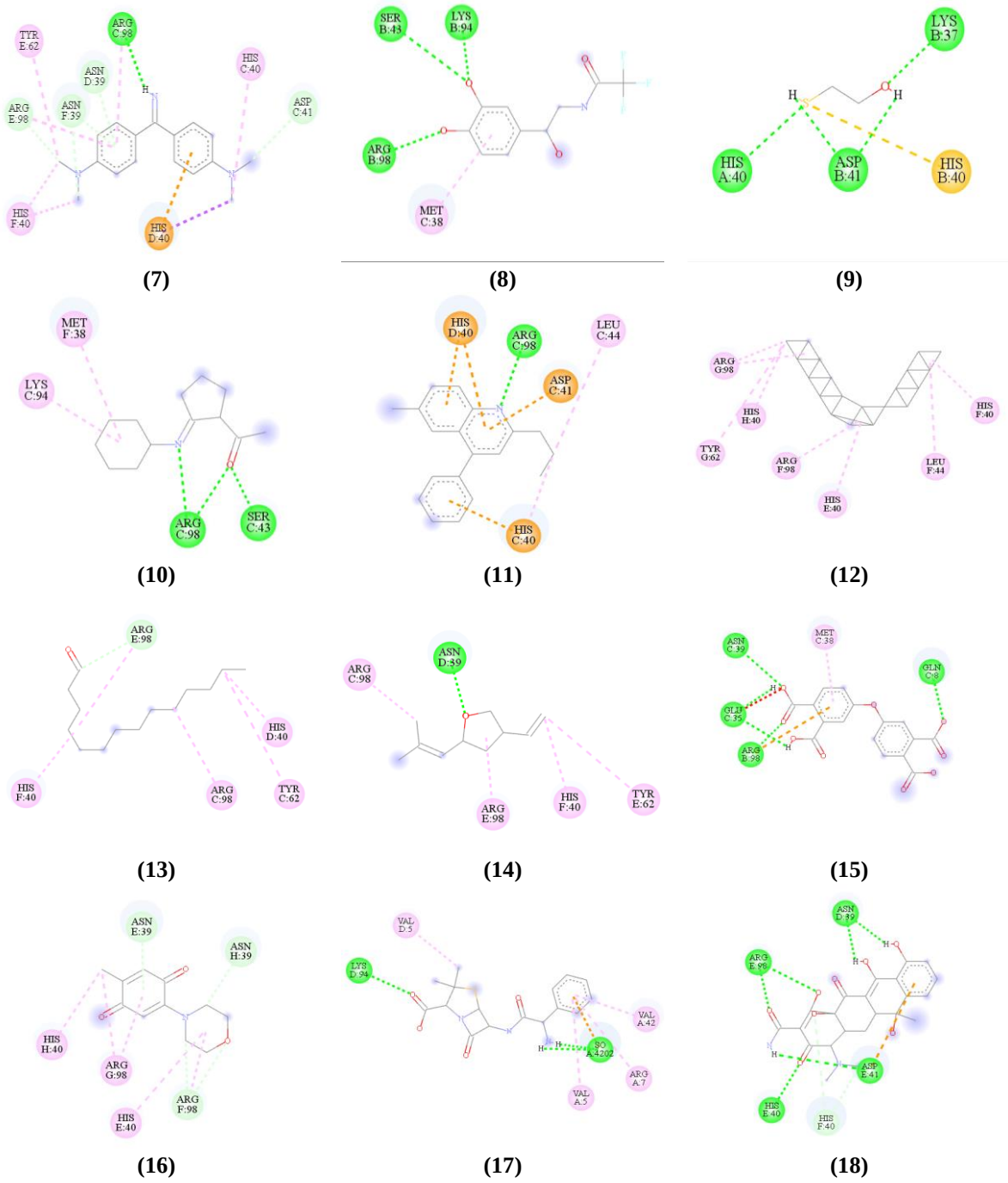


Figure 6. 2D ligand interaction with 4QVT.

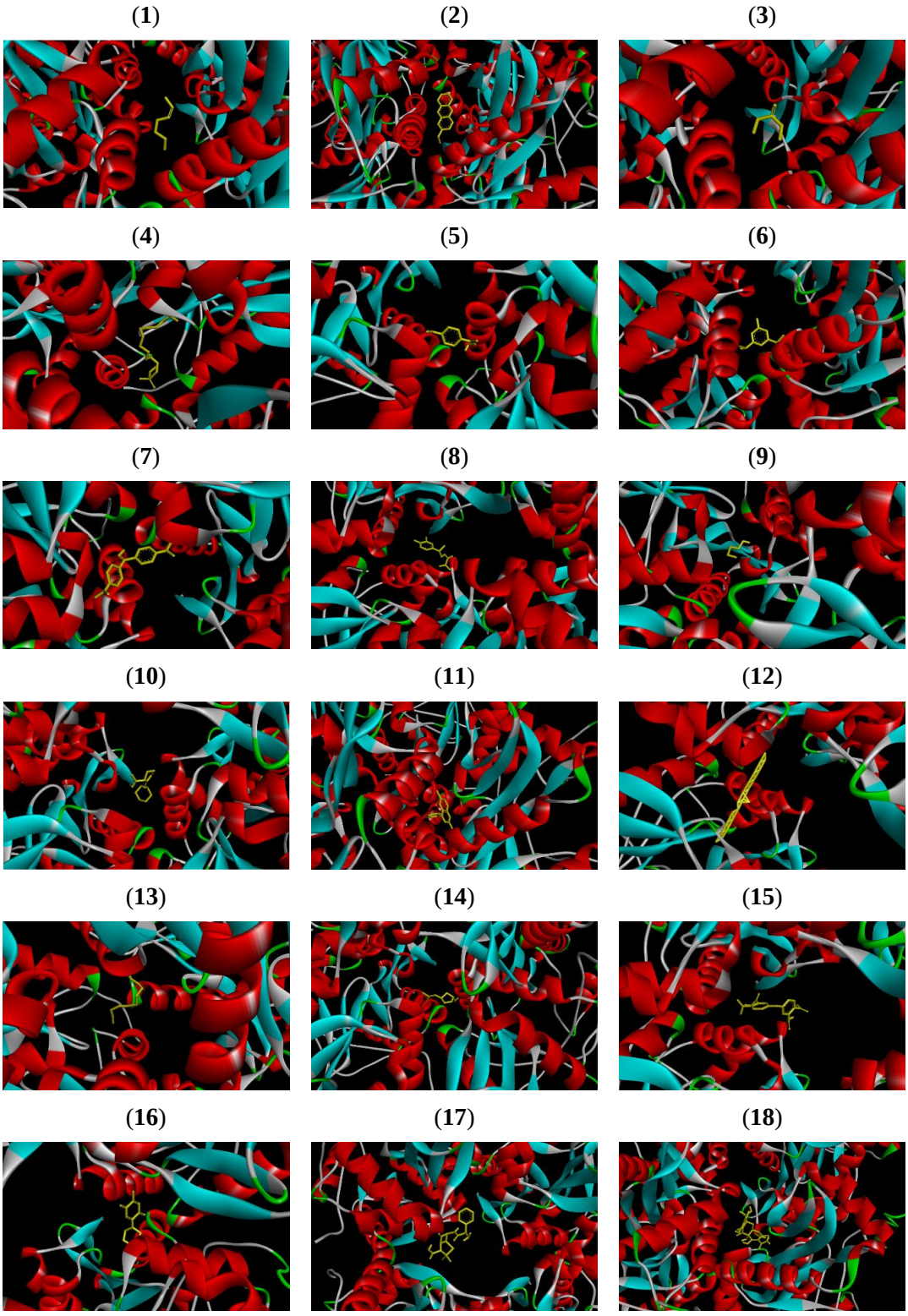


Figure 7. 3D interaction of ligands with 4QVT.

3.5. Discussion.

Given the role of acetyltransferases in bacterial adaptation to different environments, it is necessary to develop suitable drug candidates that can effectively inhibit receptor sites with minimal or no effects. In general, bioactive compounds are known to have one or more medicinal properties. For proper integration during the in silico drug design protocol, bioactive compounds must be qualified for the drug-likeness test; that is, they must fall within the ranges

defined by Lipinski's rule for various descriptors. The drug probability of the chosen candidates was estimated using the Lipinski rule of five. By using a comparison approach, we are able to rule out some substances based on their physicochemical characteristics. Compounds that violated two or more parameters were eliminated, while the remaining compounds were considered as ligands for the linkage study.

The biocompounds were analyzed for their ADME (Absorption, Distribution, Metabolism, and Excretion) characteristics as determined by SwissADME, which predicts and evaluates the pharmacokinetics and drug similarity of various molecules, using several computer models [13]. The lipophilicity indicated by ILogP influences the absorption of the drug, with a decreased ILogP value correlating with an increased absorption, and vice versa. The BOILED model, which evaluates the polarity and the lipophilicity of the compounds, predicts the gastrointestinal absorption (GIads) and the permeability of the blood-brain barrier (BBB) [16]. P-glycoprotein substrates are the primary therapeutic agents, and they typically have the capacity to decrease a drug's oral bioavailability, permeability, absorption, and retention duration [17,18]. However, it is important to note that in silico predictions have inherent limitations and must be validated experimentally.

The bioavailability of the compounds is influenced by two essential properties: flexibility (FLEX) and polarity (polar). According to Ji et al. [15], flexibility is influenced by rotary links. Compounds with rotary bonds greater than 10 are associated with low oral bioavailability. On the other hand, the polarity determined by the topological polar surface indicates that the compounds with a TPSA > 20 Å2 < 130 Å2 have a high oral bioavailability.

The pharmacovigilance characteristics of the ligands are evaluated using the bioactivity score. The online server Molinspiration was used to predict the performance of the ligands. According to Khan et al. [19], scores above 0.00 indicate high activity, while scores ranging from –0.5 to 0.00 indicate moderate activity, and scores below –0.5 suggest inactivity.

The ADME profiles of the compounds provide essential information on their potential as drug candidates. The compounds 4H-Thiopyran-4-one, tetrahydro- (3), and Mercaptoethanol (9) have a high solubility, which is favorable to oral bioavailability. The majority of the compounds have a high gastrointestinal absorption, with the exception of the compounds (Z)-2-heptene (1), 1-Butyl-2,4,6-trimethyl benzene (6), N-(trifluoroacetyl)-N,O,O',O'-tetrakis(trimethylsilyl)norepinephrine (8), 10-methylnonadecane (12), and 1,2-Benzene dicarboxylic acid (15), which may require other methods of administration. The permeability of the blood-brain barrier is favorable for most compounds, suggesting potential efficacy in the treatment of central nervous system infections. However, the compound N-(trifluoroacetyl)-N,O,O',O'-tetrakis(trimethylsilyl)norepinephrine (8) is a substrate of P-glycoprotein, which could limit its bioavailability due to efflux mechanisms.

A molecular docking analysis was carried out on all the ligands filtered from the ADME analysis. The computer tool of molecular docking plays a crucial role in the search for drugs. This is done to more accurately select potential compounds and examine bond formation at the binding site level in the protein-ligand complex. The binding energy influences the binding affinity between ligands and receptors; the lower the energy, the higher the binding affinity [20].

Molecular docking analysis revealed high binding affinities for several compounds, as evidenced by their negative *Be* and low *Ic*, suggesting their potential as acetyltransferase inhibitors. These compounds could play a crucial role in developing new antibacterial agents, especially in contexts where resistance to current antibiotics is becoming a major public health

problem. Nevertheless, these results must be interpreted with caution, since in silico docking does not fully account for the complexity of biological systems.

The compounds 2-ethylacridine (2), Auramine (7), N-(2-acetylcyclopentylidene)cyclohexylamine (10), 6-chloro-4-phenyl-2-propylquinoline (11), and 10-methylnonadecane (12) are distinguished by their particularly high binding energies (Be ranging from −7.14 to −7.61 Kcal/mol) and their low inhibition constants. For example, the 10-methylnonadecane compound (12) shows a Be of −7.61 Kcal/mol and an Ic of 2.64 µM, which makes it a very promising candidate for the inhibition of *P. aeruginosa*. These values suggest that these compounds could rival the effectiveness of known antibiotics such as ampicillin and tetracycline in terms of affinity and inhibitory potency.

The compounds 2-ethylacridine (2), Auramine (7), N-(trifluoroacetyl)-N,O,O',O'-tetrakis(trimethylsilyl)norepinephrine (8), N-(2-acetylcyclopentylidene)cyclohexylamine (10), 6-chloro-4-phenyl-2-propylquinoline (11), 10-methylnonadecane (12), and 1,2-Benzene dicarboxilic acid (15) are distinguished by their particularly high binding energies (*Be* ranging from -6.81 to -7.88 Kcal/mol) and their low inhibition constants. For example, the compound 6-chloro-4-phenyl-2-propylquinoline (11) shows a Be of −7.81 Kcal/mol and an Ic of 23.75 µM, which makes it a very promising candidate for the inhibition of *E. coli*. These values indicate that these compounds could equal, or even surpass, the effectiveness of certain antibiotics such as ampicillin and tetracycline in terms of affinity and inhibitory activity, highlighting their potential as effective inhibitors. Unlike conventional drugs that target well-established pathways, the compounds identified in this study inhibit acetyltransferases, a relatively unexplored target. This new mechanism of action could bypass existing resistance mechanisms, offering a significant advantage in the treatment of resistant infections.

Acetyltransferases have emerged as promising targets for the development of antibacterial drugs due to their roles in bacterial adaptation and pathogenesis. While the acetylation of proteins is well studied in eukaryotes, its role in bacteria is less well understood. Recent studies have highlighted the importance of lysine acetyltransferases in bacterial physiology [6,7,21]. Recent research on octyl gallate, an inhibitor of the enzyme sérine O-acétyltransferase, which is essential for the biosynthesis of L-cysteine, has shown that it can potentially increase the activity of carbapenems against *enterobacteria* that produce allo-β-lactamases by weakening bacterial antioxidant defenses [22]. Our results are in line with these studies, demonstrating that the natural compounds of *Helix aspersa* Müller can effectively inhibit acetyltransferases, filling an important gap in the current understanding of bacterial acetylation and its therapeutic potential.

Based on the docking results, the compounds of the snail slime *Helix aspersa* Müller inhibit acetyltransferases by binding to residues of key active sites, such as Ser B:130, Val B:88, Lys C:94, and Arg C:98, which are essential for enzymatic activity. For example, the compound 6-chloro-4-phenyl-2-propylquinoline (11) interacts with Arg C:98, a residue involved in substrate binding, potentially disrupting the function of the enzyme. This mechanism differs from that of conventional antibiotics, which usually target cell wall synthesis or protein translation, offering a new pathway for drug development. According to Kouzarides [23], the neutralization of lysine's positive charge can influence protein stability; thus, several studies highlight the physiological importance and regulation of lysine acetyltransferases in various bacteria [24–26].

Even if these results seem encouraging, experimental validation is still necessary. To validate the biological activity of these candidates, it is now essential to conduct enzymatic

tests, bacterial re-sensitization assays, and cellular toxicity analyses. If these steps prove successful, these natural compounds could serve as the basis for new anti-resistance agents, capable of revitalizing current antibiotics in the battle against infections resistant to several treatments.

4. Conclusions

This study identified several bioactive compounds from *Helix aspersa* Müller snail slime that meet standard drug-likeness criteria and show strong *in silico* binding affinity for bacterial acetyltransferases. Molecular docking predicted that compounds such as 2-ethylacridine, auramine, and 6-chloro-4-phenyl-2-propylquinoline interact with conserved active-site residues (Arg C:98, Lys C:94) and exhibit binding energies between –7.14 and –7.81 kcal/mol. Additionally, these compounds have positive bioavailability radar signatures and ADME profiles, indicating their feasibility for additional research. These results, however, are restricted to computational forecasts. To determine whether these substances indeed block acetyltransferase activity, experimental validation—using enzymatic inhibition assays, bacterial susceptibility testing, and toxicity studies—is crucial. Prior to confirming any therapeutic promise, further research should concentrate on such in vitro and in vivo characterization.

Author Contributions

Conceptualization, M.A. and M.Z.; methodology, M.A. and A.R.; software, M.A., M.Z. and G.K.; validation, M.A., M.Z. and G.K.; formal analysis, M.A.; investigation, M.A.; data curation, M.A.; writing—original draft preparation, M.A., M.Z. and Y.T.; writing—review and editing, M.A.; visualization, G.K., H.I. and A.R.; supervision, R.B. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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