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IN SILICO STUDY OF VANILLIN-DERIVTAIVES AS A GLUTAMATE RACEMASE (MurI) INHIBITORS

Adnan Shahzad[*1], Naheed Fazal[2], Zakia Ahmad[3], Shahida Bibi[4], Nasib Zaman[5], Fazal Dayan[6], Bilal Khan[7], Mian Sayed Jamil[8], Inam Uddin[9], Mansoor Ali Khan[10]

[*1,8,9]*Institute of Chemical Sciences, University of Swat, Swat, Pakistan*
[2,3,4]*Center for Plant Science and Biodiversity, University of Swat, Swat,*
[5]*Center for Microbiology and Biotechnology, University of Swat, Swat,*
[6]*Department of Chemistry Quaid-i-Azam University, Islamabad, Pakistan*
[7,10]*Department of Chemistry Abdul Wali Khan University, Mardan, Pakistan*

[*1]adnanshahzad@uswat.edu.pk

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Corresponding Author: *
Adnan Shahzad

Abstract

Vanillin and its structural analogues have drawn interest among natural and semi-synthetic small molecules because of their wide range of biological activities, low toxicity, and ease of chemical modification. As a result, we chose different Vanillin derivatives (V1-V4) based on their ability to bind to the glutamate racemase (MurI) target enzyme. Molecular docking software AutoDock Vina were used to investigate the inhibitory potential of vanillin-derivatives (V1-V4). According to the investigation of the molecular docking research, the compounds (V1-V4) show encouraging binding interactions with glutamate racemase (MurI) target enzyme, with favourable binding energies ranging from -8.68 kcal/mol to -9.94 kcal/mol. These inhibitor compounds are excellent prospects for the development of new treatments due to their large binding energies, which show that they can successfully change the activity of the Mycobacterium enzyme. Potent Mycobacterium inhibitors with improved efficacy and fewer side effects may be created with additional research and testing of these drugs. The results show that substances based on vanillin have better inhibitory activity against particular enzymes glutamate racemase (MurI)

INTRODUCTION

A crucial, cofactor-independent enzyme called glutamate racemase (MurI) catalyses the interconversion of L-glutamate to D-glutamate, which is a necessary component of bacterial peptidoglycan biosynthesis [1-3]. Glutamate racemase is an appealing and highly selective target for antibacterial drugs because [4], in contrast to many metabolic enzymes, it is universally conserved among pathogenic Gram-positive and Gram-negative bacteria but lacks a human homolog [5]. When MurI is inhibited, D-glutamate production is disrupted, cell wall assembly is hampered, structural integrity is weakened, and eventually bacterial cell death results [3, 6]. Finding strong MurI inhibitors has become a viable approach for next-generation antimicrobial treatments due to the growing incidence of antimicrobial resistance (AMR) and the narrow pipeline for new antibiotic classes [7, 8]. Vanillin and its structural analogues have drawn interest among natural and semi-synthetic small molecules because of their wide range of biological activities, low toxicity, and ease of chemical modification [9-11]. Vanillin's phenolic hydroxyl, aldehyde, and methoxy functional groups offer a flexible scaffold that can be logically optimized for improved enzyme binding and antibacterial efficacy [12-15].

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Vanillin derivatives may interact favorably with important catalytic residues of MurI, potentially preventing the racemization process through competitive or allosteric inhibition, according to a number of in-silico and in-vitro studies [16]. Nevertheless, systematic molecular testing of vanillin-based inhibitors against glutamate racemase is still scarce, despite their encouraging pharmaco-chemical characteristics.

Molecular docking, binding energy prediction are examples of advances in computational drug design that provide effective tools to find, screen, and optimize inhibitors derived from natural products prior to experimental validation [17-21]. By using these methods on vanillin derivatives, new antibacterial leads can be found more quickly by clarifying their binding behavior, stability, and inhibitory potential against MurI. Limited docking studies incorporate induced-fit, ensemble docking approaches, meaning conformational changes in MurI that influence binding of vanillin derivatives remain unexplored. So in this study vanillin derivative will be investigated for MurI inhibition. This crisis has resulted in a call for the discovery of drugs that have a novel mode of action

1. Methodology

Molecular docking studies were used to assess the binding interaction mechanism of the vanillin-derivatives for competitive and noncompetitive inhibition of the mark enzymes of Mycobacterium tuberculosis glutamate racemase (MurI) [22]. The crystal structures of the relevant enzymes were obtained from Protein Data Bank. Theoretically, protein was constructed using AutoDock MGL tools (1.5.6 version), and to make calculations easier, all water molecules, heteroatoms, and cofactors were eliminated. For additional research, the optimal docking conformer with the lowest binding energy was chosen. Highly stable confirmer was used to analyse the protein-ligand interaction data, and PyMol (3.1), AutoDock Vina (1.5.6 version), BIOvia Discovery Studio visualisation (V 4), and LigPlot (2.2) were used to visualise the docking poses of selective vanillin derivatives against target enzymes [23].

2. Results and discussion

2.1. Molecular docking of vanillin-derivatives (V1-V4)

Receptor proteins, Glutamate racemase (MurI) with distinct 3D structures and functions were taken into consideration in order to evaluate the multi-domain anti-mycobacterial activity of ligands; in particular, they all had to have some crucial functions necessary for the survival of the target bacterial cell and its infection. Glutamate racemase (MurI), the chosen receptor protein (PDB ID: 5hj7), plays crucial roles in executing vital cellular functions. The enzyme glutamate racemase (MurI) is necessary for the mycobacteria to catalyses the interconversion of L-glutamate to D-glutamate, which is a necessary component of bacterial peptidoglycan biosynthesis. The vanillin derivatives (V1-V4) was chosen for inhibitory potential against glutamate racemase (MurI) and one standard Macozinone in order to impede the action of specific enzymes in our current investigation (as illustrated in figure 1). For comparative research, test against this enzyme as well.

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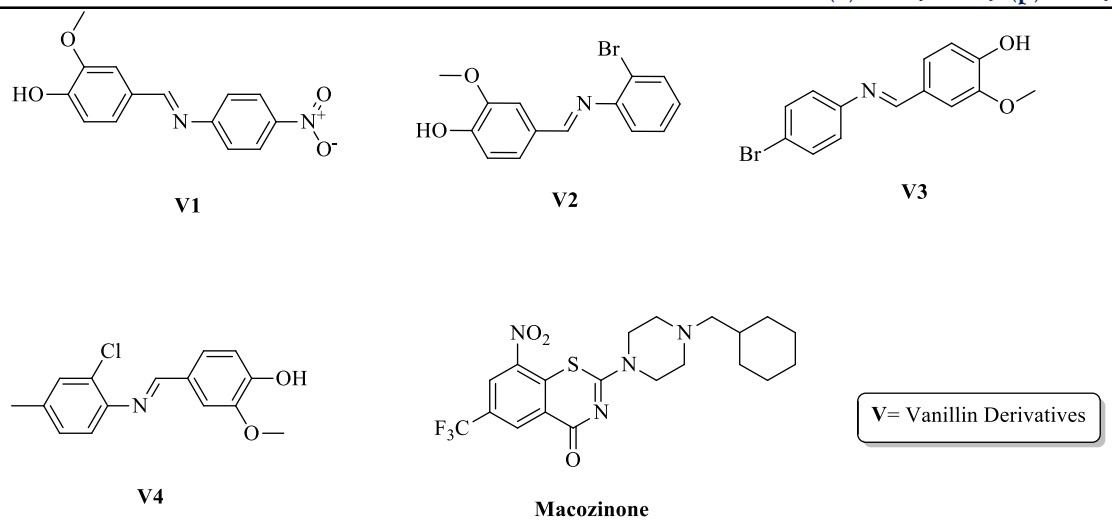

Figure 1. Structural formulae of vanillin-derivatives (V1-V4) and Macozinone

Interaction between V1 and glutamate racemase (MurI): Compound **(V1)** exhibits great inhibitory activity against the MurI enzyme with a docking score of -9.94 kcal/mol (shown in **table 1)** and an inhibition constant of 51.53 nM. The compound **(V1)** interact with residue of MurI enzyme is SER-13, THR-186, ASN-76, SER-77, THR-122. The above selected compound **(V1)** is fitted with active pocket of MurI enzyme which allow N-O group to form hydrogen-bond interaction with ASN-76 (N-H...O=N), THR-186 (N-H...O=N), SER-77 (N-H...O=N), THR-122 (O-H...O=N) with the residue of MurI enzyme the bond length is 1.8Å, 2.0Å, 2.4Å, 2.5Å [24] (shown in **table 2)** respectively. Beside these Hydrogen-bonds in active site, **V1** also exhibit hydrophobic, weak Vander Wall interaction and π-π interaction with SER-13(H-O...N) amino acid of MurI represent in **Figure 2.**

Interaction between V2 and glutamate racemase (MurI): Compound **(V2)** show inhibition opposed to MurI enzyme with a docking score of -8.68 kcal/mol (shown in **table 1)** and inhibition constant is 430.51 nM. Interaction between, compound **(V2)** with residues of MurI enzyme is ASP-12 respectively. Among this compound **(V2)** form a hydrogen-bond interaction with ASP-12 (OH...O=N) residue of MurI enzyme with a bond distance is 2.7Å (shown in **table 2)**.The Binding poses of compound **(V2)** with MurI enzyme 3D structures are illustrated in **Figure 3.**

Interaction between V3 and glutamate racemase (MurI): Compound **(V3)** select against the MurI enzyme with a binding energy is -9.11kcal/mol (shown in **table 1)** and inhibition constant is 209.01nM. Interaction between compound **(V3)** and the residue of MurI enzyme is SER-77, ASN-76. Compound **(V3)** form a hydrogen bond with the active amino site of MurI enzyme is SER-77 (-C-O...H-N), ASN-76 (H-O...H-N) with a bond distance is 2.1Å and 2.5Å (shown in **table 2)**. The Binding poses of compound **(V3)** with MurI enzyme 3D structures are illustrated in **Figure 4.**

Interaction between V4 and glutamate racemase (MurI): Compound **V4** was selected for inhibition potential opposed to MurI enzyme with docking score -8.69kcal/mol (shown in **table 1)** and inhibition constant is 430.07nM. Interaction between the V4 and active site of MurI enzyme is THR-186. Among these interaction hydrogen bond form with THR-186 (-C-O...H-N) and THR-186 (H-O...H-O) with a bond distance is 2.8Å and 2.9Å (shown in **table 2)**. The Binding poses of compound **(V4)** with MurI enzyme 3D structures are illustrated in **Figure 5.**

Interaction between Macozinone and glutamate racemase (MurI): Compound macozinone select against the MurI enzyme with a binding energy is -9.88 kcal/mol (shown in **table 1)** and inhibition constant is 57.41 nM. Interaction between compound macozinone and the residue of MurI enzyme is ARG-120.

Frontier in Medical & Health Research

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Compound macozinone form a hydrogen bond with the active amino site of MurI enzyme is ARG-120 (N-O...H-N) and ARG-120 (N-O...H-N) with a bond distance is 2.1Å and 2.4Å.

Table 1. Binding energy in (kcal/mol) of compound (V1-V4) and macozinone with MurI

Compounds	MurI	Inhibition constant (nM)
V1	-9.94	51.53
V2	-8.68	430.51
V3	-9.11	204.01
V4	-8.69	430.07
Macozinone	-9.88	57.41

Table 2. Residues involved in interactions and their distances of selected compounds with MurI

Compounds	Interacting amino acids	Interactions	Distances(Å)
V1	ASN-76, THR-186, SER-77, THR-122	N-H...O-N, N-H...O-N N-H...O-N, O-H...O-N	1.8, 2.0, 2.4, 2.5
V2	ASP-12	O-H...ON	2.7Å
V3	SER-77, ASN-76	C-O...H-N, H-O...H-N	2.1, 2.5
V4	THR-186, THR-186	C-O...H-N, H-O...H-O	2.8, 2.9
Macozinone	ARG-120	N-O...H-N	2.1, 2.4

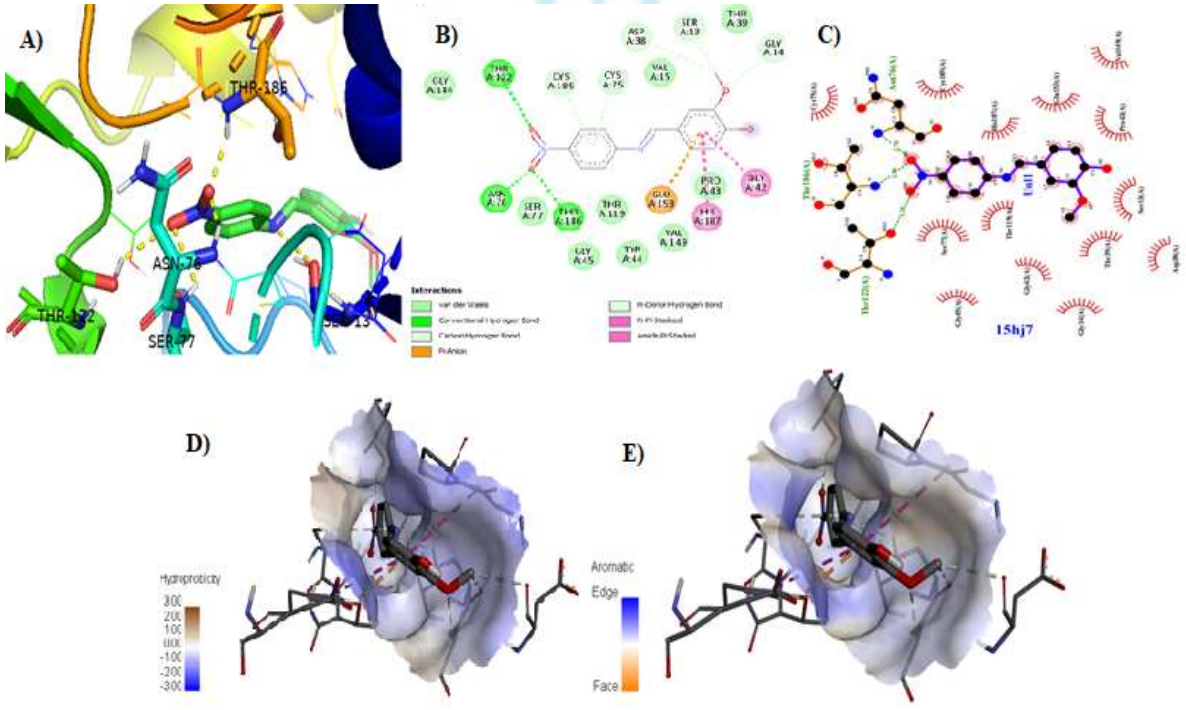

Figure 2. Binding poses of Compound (V1) with MurI enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

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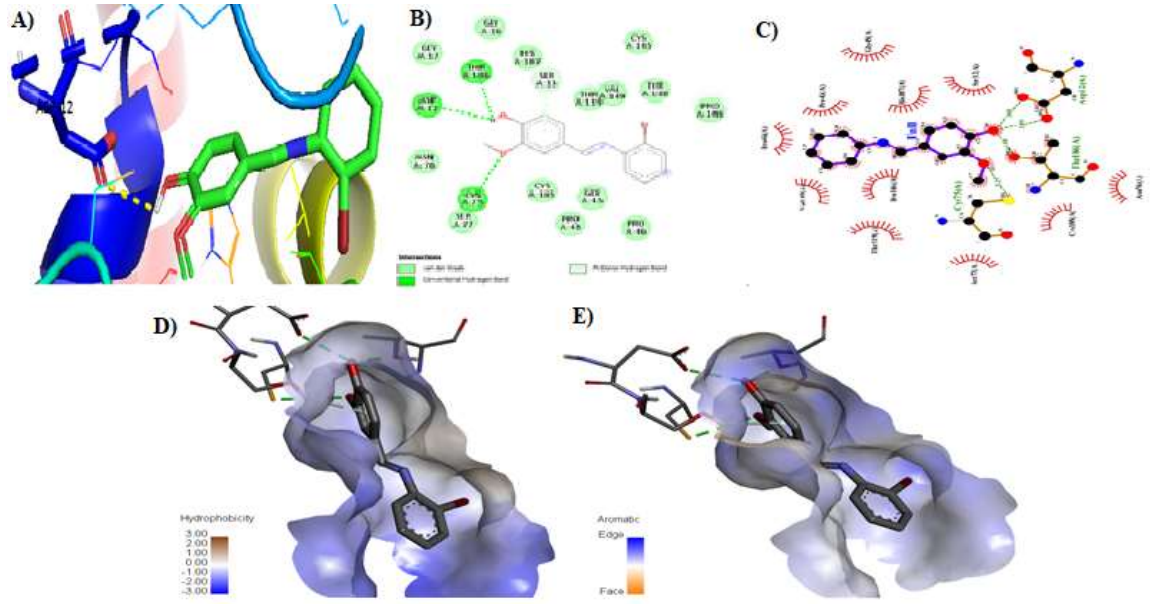

Figure 3. Binding poses of Compound (V2) with MurI enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

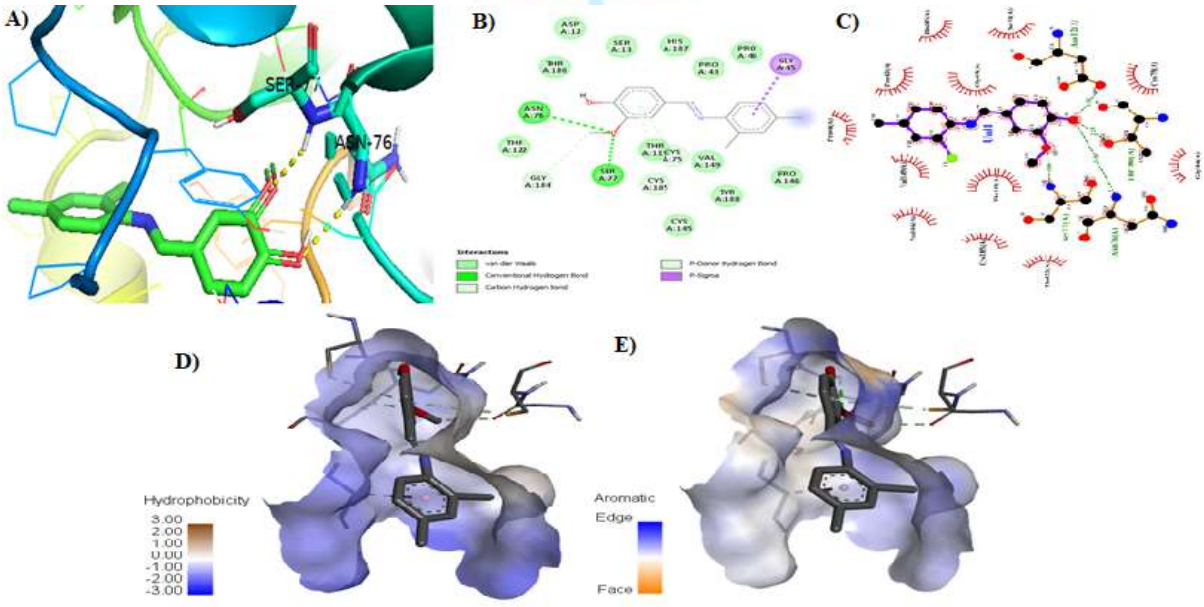

Figure 4. Binding poses of Compound (V3) with MurI enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

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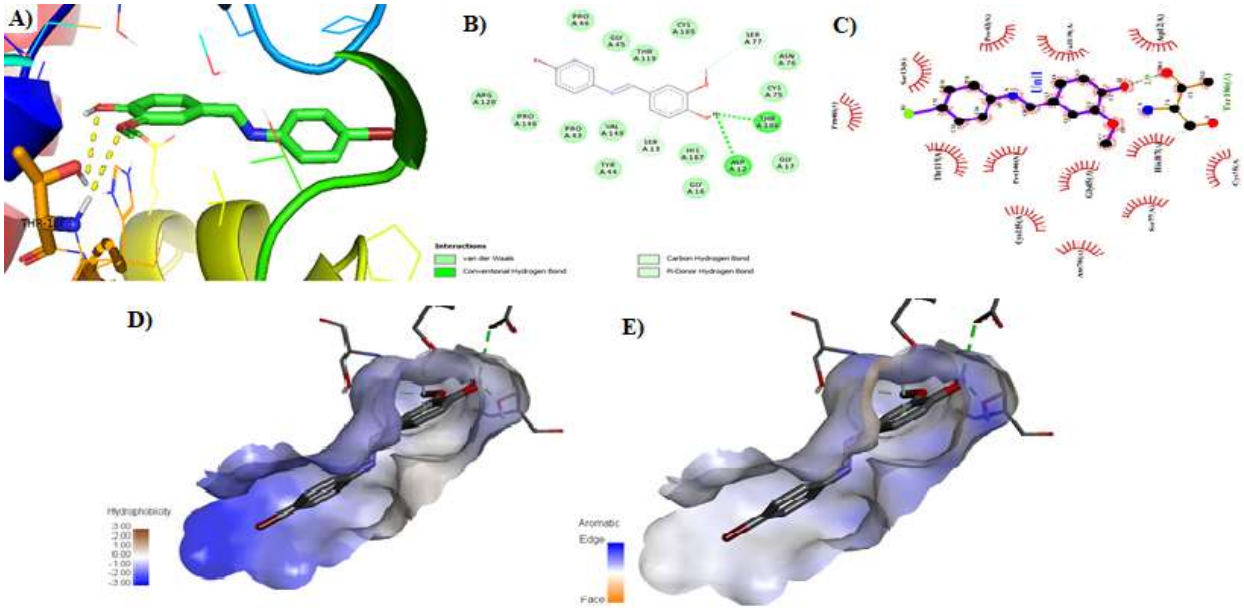

Figure 5. Binding poses of Compound (V4) with MurI enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

Conclusion

In this work, we used molecular docking techniques to examine the inhibitory potential of standard drug Macozinone and Vanillin derivatives V1-V4 against Glutamate racemase (MurI). The enzyme glutamate racemase (MurI) was used to treat with these ligands. According to our findings, compounds V1-V4 have stronger inhibitory effect against glutamate racemase (MurI) than previously reported. Additionally, we discovered that the binding energy of compounds V1was substantially higher to that of the Macozinone and the rest of the tested ligands are comparable. Therefore, compounds V1-V4 have a lot of potential as top candidates for additional pharmacological research targeted at creating therapies for Mycobacterium TB.

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Institute of Chemical Sciences, University of Swat, Swat, Pakistan

Conflict of Interest

The authors declare no conflict of interest

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