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Antibacterial and molecular docking study of Fe (III) metal complex of bidentate hydroxy α-amino phosphonates derivatives

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Abstract
A Fe (III) complex of N, O donor bidentate α-aminophosphonates was synthesized in a 1:2 (ligand: metal) molar ratio and characterized by different analytical and physicochemical methods including FTIR, molar conductance, AAS, elemental analysis and NMR (^{1}H and ^{13}C). The prepared ligand and metal complex were screened for antibacterial activity against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria by disk-diffusion methods using various concentrations of compounds (250, 500, 750, 1000 ppm). Amikacin was used as standard and a comparison of the inhibition zone indicates that the Fe (III) complex showed more antibacterial activity than the ligand. A molecular docking study was also carried out for the prepared ligand and complex against *S aureus* Tyrosyl-tRNA synthetase protein (PDB ID-1JIL) using AutoDock 4.2 software in which both ligand and complex showed good interaction.

Keywords: Metal complex, analytical, spectral characterization, antibacterial, molecular docking

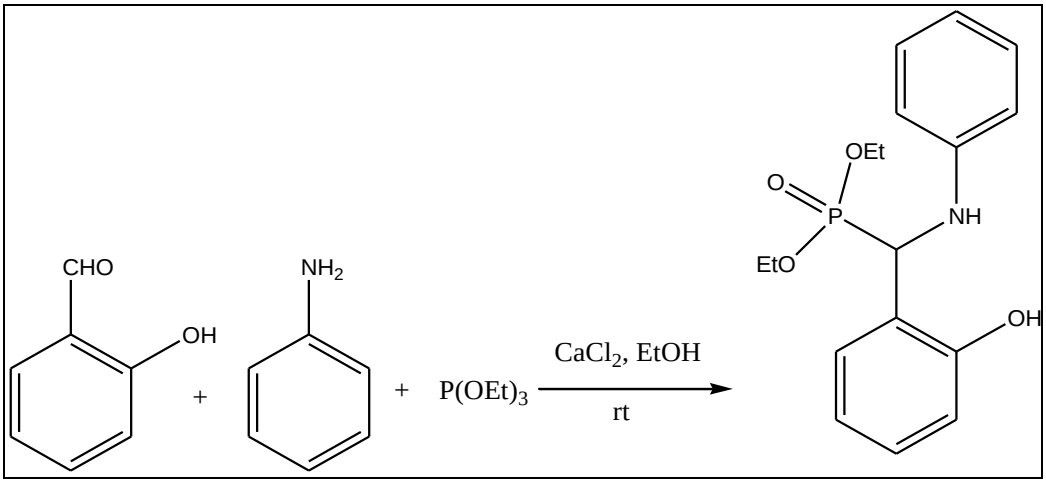
Introduction
A-Aminophosphonates are the phosphorous analogous to α-amino acids which explain its biological activities and various applications [1, 2], which range from pharma to agriculture such as enzyme inhibitors [3], antibiotics [4], herbicides [5] and anticancer agents [6]. Phosphonate containing heterocycles showed inhibition of various enzymes like HIV-protease and human collagenase [7]. The Kabachnik-Fields [8] and Pudovik [9] reactions are the main synthetic path for the synthesis of α-aminophosphonates, which are nothing but special cases of Mannich reaction, where phosphorus atom takes the place of the enolate moieties or enamine in the Mannich reaction. In addition, aminophosphonates are versatile intermediate in synthesis because of their application in Wadsworth-Emmons and related reactions [10]. Chelation with different metals influences the properties and activities of aminophosphonates and hence, metal complexes of heterocyclic α-aminophosphonates are becoming a subject of growing interest. In recent years, transition metal complexes with phosphonate [11, 12] and phosphate [13, 14] dieters bearing N-heterocyclic donor atom have been the object of intensive investigations, because of their potential or significant antitumor activity [15, 16].

Material and Methods
Material: All chemicals and reagents used were in the highest pure state (AR grade). Aniline, salicylaldehyde, triethyl phosphonate and $FeCl_3.6H_2O$ were purchased from sigma Aldrich Company and used without further purification. Solvents were purchased from Molychem Company and were used without further purification. The percentage amount of C, H and N was analyzed by CHNS analyzer (Elementar Analysensysteme Germany Model: Vario Micro Cube), the metal percentage was analyzed by AAS instrument (Element AS AAS4141), FTIR spectra were obtained from Perkin Elmer FTIR spectrophotometer, 1H NMR and 13 CNMR by 400 MHz JEOL JNM ECS400, XRD by Bruker D8-Advance model and VSM by Lake Shore, 7410 Series VSM. The antibacterial activity was carried out in the department of microbiology, College of Veterinary & Animal Sciences.

Synthesis of hydroxy α-amino phosphonate derivative ligand and its metal complexes
Synthesis of hydroxy α-amino phosphonate derivative ligand: The ligand was synthesized by reacting aniline (10 mmol), salicylaldehyde (10 mmol) and triethyl phosphonate (10 mmol) in ethanol using $CaCl_2$ as a catalyst. The reaction mixture was refluxed for 7-8 hours and reaction progress was checked by TLC using hexane: ethyl acetate (20:80) as a mobile phase.

The precipitate was obtained and recrystallized with ethanol and dried in the oven at 40 ^{0}C (Scheme 1).

Ligand (L): Anal. Calcd. for $C_{17}H_{22}NPO_4$: Yield: 56%; Yellow; M.P. 92-94^{0}C; Λ_m 9.67 mho cm^2 mol^{-1}; C, 60.8%; H, 6.5%; N, 4.2%; Found: C, 58.48%; H, 6.1%; N, 5.1%.^{1}HNMR (400 MHz, DMSO-d^6) δ(ppm): 1.6 (t, 3H), 3.6 (NH), 5.7 (s, OH), 6.51-7.04 (m, 4H). ^{13}CNMR (400 MHz, DMSO-d^6) δ(ppm): 14.8 (CH_3), 46.1 (CH_2), 122 (Ar-CH), 130 (Ar-CH), 149.3 (C-O). FTIR (KBr, cm^{-1}): 3667 (N-H), 3261 (O-H), 1242 (P=O), 1026 cm^{-1} (P-O-C).



Scheme 1: Synthesis of hydroxy α-amino phosphonate derivative ligand.

Synthesis of hydroxy α-amino phosphonate derivative Fe (III) complex: The metal complex was synthesized by refluxing the prepared ligand (10 mmol) with a metabolic solution of $FeCl_3.6H_2O$ metal salts (20 mmol). The reaction mixture was refluxed at 80 ^{0}C and reaction progress was checked by TLC using hexane: ethyl acetate (20:80) as a mobile phase. The precipitate was obtained and recrystallized with ethanol and dried in the oven at 80 ^{0}C.

Fe (III) Complex: Anal. Calcd. for $((C_{17}H_{20}NPO_4)_2Fe\ Cl_2)$: Yield: 74%; Dark Brown; 295-298^{0}C; μ 1.74 BM; Λ_m 16.78 mho cm^2 mol^{-1}; C, 51.4%; H, 5.04%; N, 3.5%; Cl, 8.9%; Fe, 7.1%; Found: C, 42.8%; H, 5.2%; N, 3.6%; Cl, 9.1%; Fe, 6.8%. ^{1}HNMR (400 MHz, DMSO-d^6) δ(ppm): 1.5 (t, 3H), 4.09 (q, 2H), 6.4 – 6.8 (m, 4H), 7.24-7.28 (d, 2H). ^{13}CNMR (400 MHz, DMSO-d^6) δ(ppm): 37.3 (-CH_2-), 40.1 (-CH_2-), 121.2 (Ar-CH), 137.6 (C-O), 161.9 (C-N). FTIR (KBr, cm^{-1}) 1242 (P=O), 1026 cm^{-1} (P-O-C), 1039 (Fe-O), 514 (Fe-N).

Biological activities

Antibacterial assay: Antibacterial activity was evaluated against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria by disk diffusion methods. The different concentration (250 ppm, 500 ppm, 750 ppm, 1000 ppm) of ligand and Fe complex in DMSO was used and amikacin was used as a standard. The zone of inhibition was measured and compared with the standard.

Zone of Inhibition = (a – b) / 2

Where, a = the diameter of the inhibition zone and b = the diameter of disk used.

Molecular Docking: A molecular docking study of test samples with the protein of *S. aureus* Tyrosyl-tRNA synthetase (PDB ID- 1JIL) was performed. The structure of the complex was obtained from Chem Draw ultra 8.0. The docking studies were carried out on the prepared ligand and receptor molecules using AutoDock 4.2, Discovery Studio 2021 client and Cygwin 64 terminal. Among the three different search algorithms performed by AutoDock 4.2, the Lamarckian Genetic Algorithm (LGA) was applied.

Result and discussion

Anti-bacterial Activities: The antibacterial activity was tested and demonstrated in Table 1. The data revealed that both the ligand and metal complexes showed antibacterial activity but the metal complex exhibited better inhibition than the ligand (Figs. 1, 2, 3, and 4). The increased activity of the metal complex can be explained by the chelation theory. Chelation tends to make the complex more powerful bactericidal agents as the +ve charge of the metal partly shared with the ligand and p-electron delocalization take place all over[17]. This enhanced the lipophilic character of the metal complex and its permeation through the membrane's lipid layer become easier. The antibacterial screening data showed agreement with the fact that the complex possessed more activity than the ligand. The highest inhibition zone was found against *S. aureus*. Both the complex and ligand showed lesser activities than the standard (Amikacin).

Table 1: Antibacterial Screening of ligand and metal complex with different bacterial strains

Sample	Conc. (ppm)	Zone of inhibition (mm)			
		Gram+ve Bacteria		Gram-ve Bacteria	
		S Aureus	B Subtilis	E Coli	S Typhi
Ligand	250	1	0.5	0.5	0.5
	500	1.25	0.75	1	1
	750	1.5	1	1.5	1.5
	1000	1.75	1.25	1.75	2
Fe (III) complex	250	1.25	0.5	1	0.5
	500	1.5	1	1.25	1
	750	1.75	1.5	1.5	1.75
	1000	2	1.75	1.75	2
Amikacin		7.5	7.5	5	8
DMSO		-	-	-	-

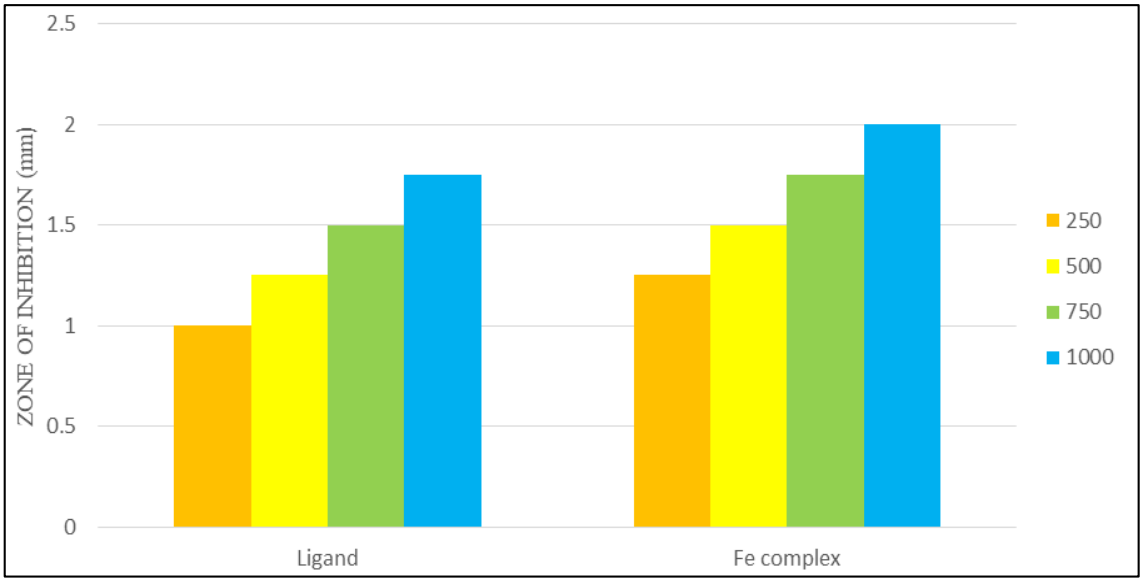


Fig 1: Antibacterial activity of Ligand and Fe complex against *S Aureus*

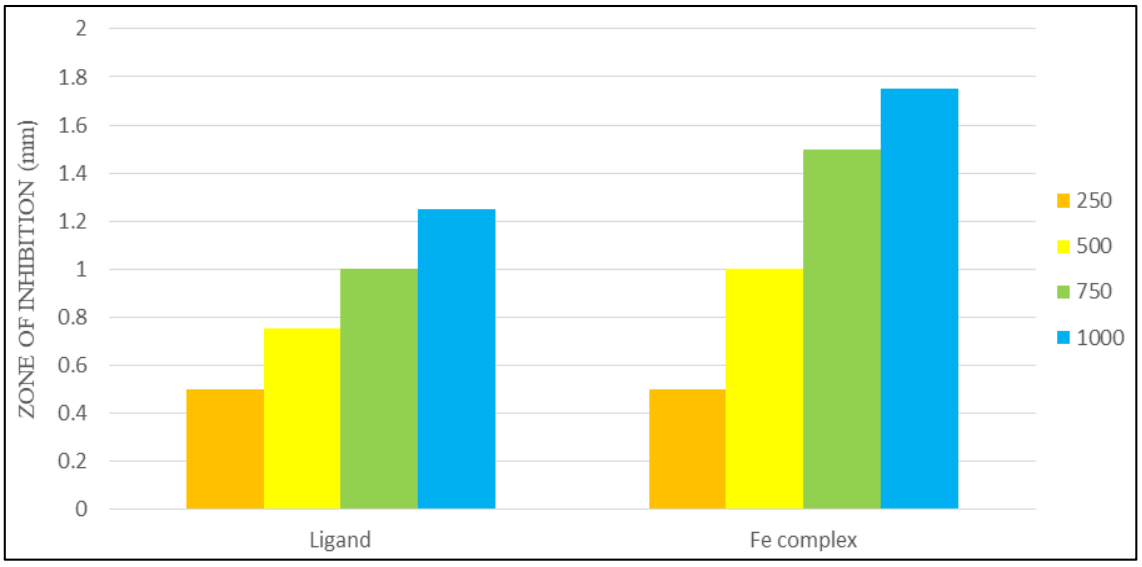


Fig 2: Antibacterial activity of Ligand and Fe complex against *B Subtilis*

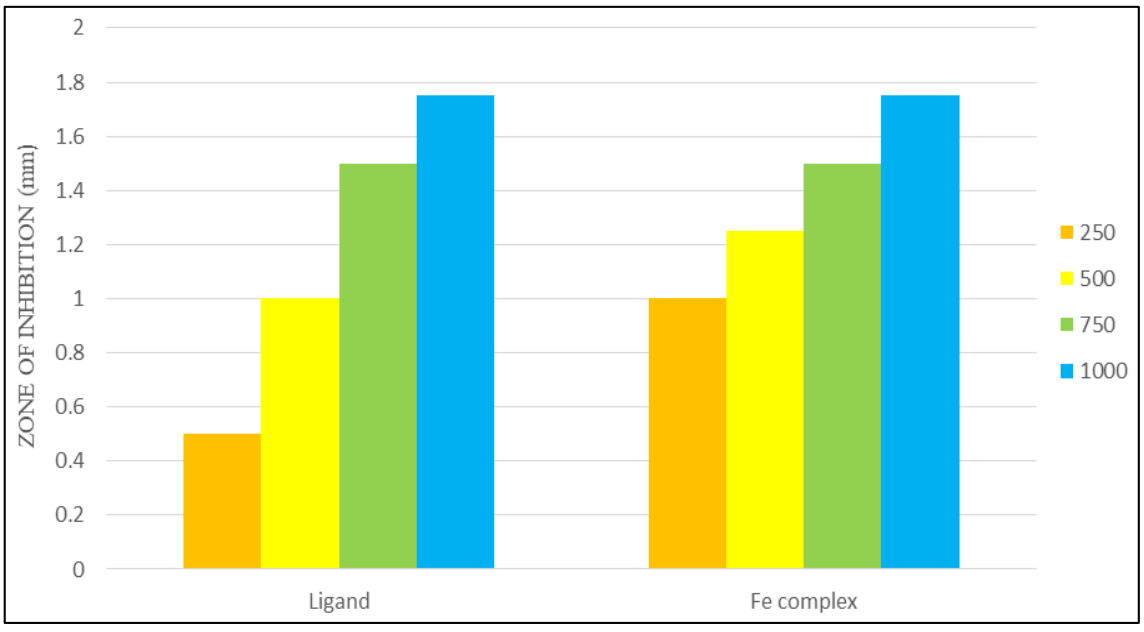


Fig 3: Antibacterial activity of Ligand and Fe complex against *E Coli*

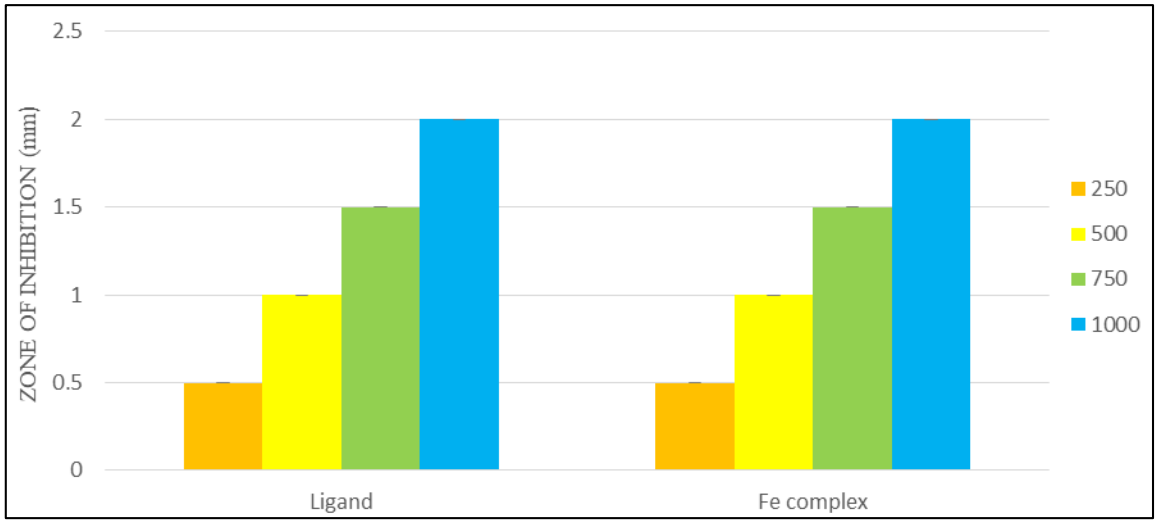


Fig 4: Antibacterial activity of Ligand and Fe complex against *S Typhi*

Molecular docking study

The best binding mode of docked compounds with protein of *S. aureus* Tyrosyl-tRNA synthetase (PDB ID- 1JIL) is shown in fig 5 and Fig 6. Respectively and it was analyzed to find out various types of interactions like hydrophobic, H-bonding and electrostatic interactions. The ligand shows interaction with Gly74, Ser132, Leu173, Leu128, Leu133, Ile131, Gly129 and Phe136 amino acid residue of *S Aureus* Tyrosyl-tRNA synthetase. The Fe complex shows interaction with Ser132, Ile78, Ile176, Ile131, Gly74, Leu128, Leu133, Leu173 and Phe136 amino acid residue of *S Aureus* Tyrosyl-tRNA synthetase. These interactions reveal significant binding of the synthesized ligand and its Fe (III) complex with the protein receptor molecule contributing to a favorable free binding energy. The results obtained by molecular docking of synthesized compounds with the receptor molecules are summarized in Table 2. The Fe (III) complex showed better activity than the ligand as Fe complex showed low binding energy (-9.27 Kcal mol^{-1}) as compared to the ligand (-4.43 Kcal mol^{-1}).

Table 2: Molecular docking data for Ligand and Fe complex against Tyrosyl-tRNA synthetase protein of *S Aureus*

S.N	Compound	Binding Energy (ΔG)	Inhibition Constant (Ki) µM	Interactive Amino Acids
1.	Ligand	-4.43	570.46	Gly74, Ser132, Leu173, Leu128, Leu133, Ile131, Gly129, Phe136
2.	Fe complex	-9.27	159.82	Ser132, Ile78, Ile176, Ile131, Gly74, Leu128, Leu133, Leu173, Phe136

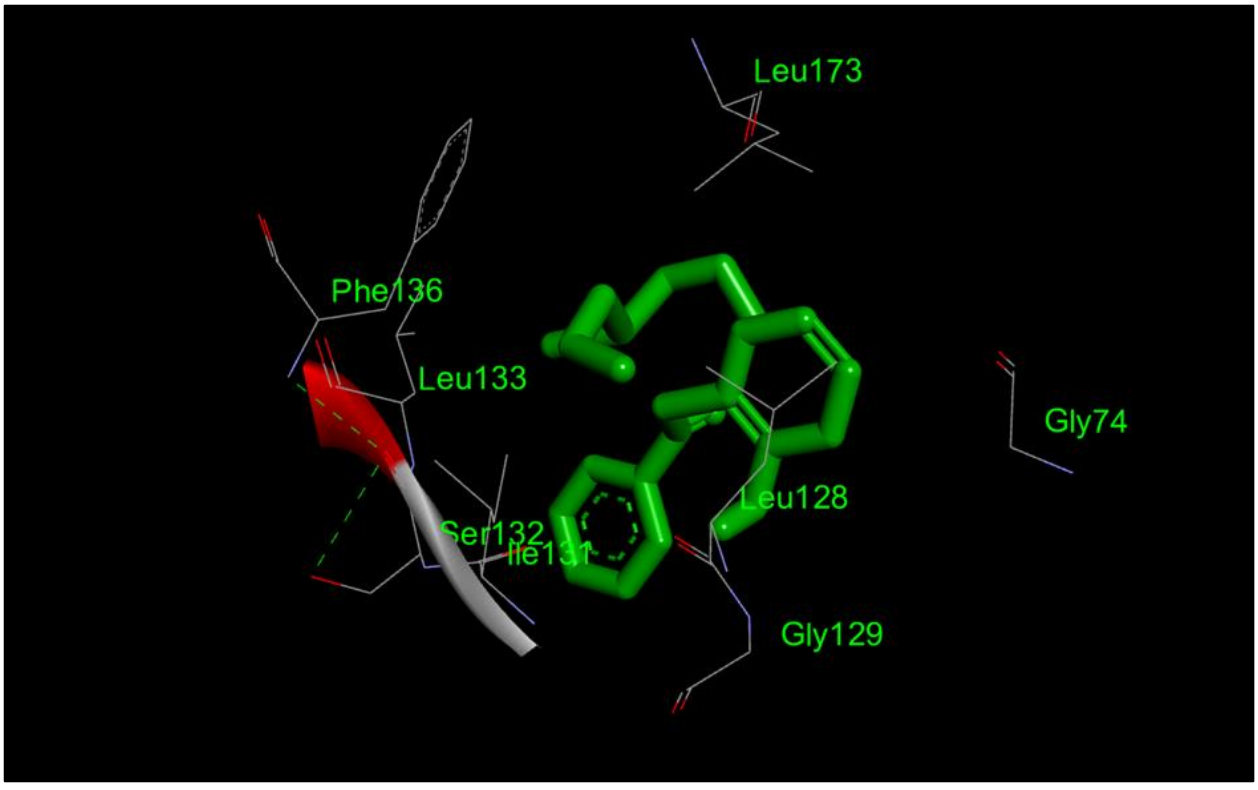


Fig 5: Protein-Ligand 3D interaction of ligand with Tyrosyl-tRNA synthetase protein of *S Aureus*

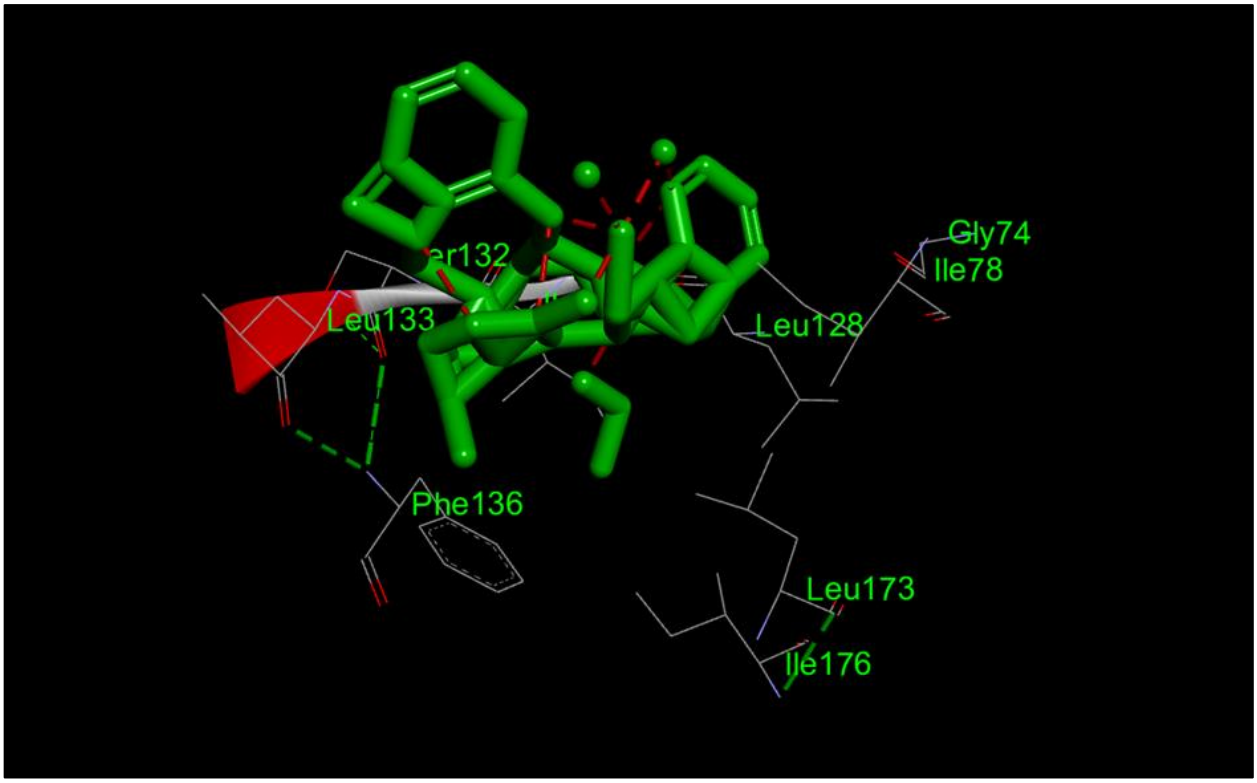


Fig 6: Protein-Ligand 3D interaction of Fe (III) metal complex with Tyrosyl-tRNA synthetase protein of *S Aureus*

Conclusion

The Ligand, hydroxy derivative of α-aminophosphonates and its Fe (III) metal complex were synthesized and characterized by different spectral and physico-chemical methods. The synthesized ligand and its Fe (III) metal complex showed good to medium activity against all tested bacterial strains and the docking study confirms its activity. The Fe (III) complex showed better activity than the ligand against all the bacterial strains which is also confirmed by its docking study where the Fe complex showed low binding energy (-9.27 Kcal mol^{-1}) as compared to the ligand (-4.43 Kcal mol^{-1}).

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