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RESEARCH ARTICLE

ONE-POT SYNTHESIS OF BIOACTIVE THIADIAZOLYL-PYRIDINES DERIVATIVES

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ABSTRACT

The rising threat of antimicrobial resistance demands the development of novel therapeutic agents with broad-spectrum efficacy. In this study, a series of thiadiazolyl-pyridine derivatives (5a–5h) was synthesized using a one-pot multicomponent reaction involving acetylthiadiazole, substituted aromatic aldehydes, malononitrile, and ammonium acetate in refluxing acetic acid. This green and efficient synthetic route offered high yields and reduced environmental impact. The structures of the synthesized compounds were confirmed by ^{1}H NMR, LC-MS, and elemental analysis. The antimicrobial potential of the compounds was evaluated against four bacterial strains (*Staphylococcus aureus, Bacillus anthracis, Pseudomonas aeruginosa, Escherichia coli*) and two fungal strains (*Candida albicans, Aspergillus niger*) using the cup plate method. Among the tested compounds, 5d demonstrated the highest antibacterial activity, with inhibition zones comparable to the standard drug streptomycin. Compounds 5c and 5e also exhibited significant antibacterial and antifungal activity. To assess drug-likeness and pharmacokinetic profiles, SwissADME analysis was conducted. All compounds adhered to Lipinski's Rule of Five, indicating favorable physicochemical properties for oral bioavailability. Furthermore, molecular docking studies were performed against microbial protein targets PDB ID: 2EG7 and 5D6P. Compounds 5c, 5e, and 5g exhibited superior binding affinities compared to streptomycin, highlighting their potential as enzyme inhibitors.

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INTRODUCTION

The alarming global increase in antimicrobial resistance (AMR) has escalated the urgency to discover and develop novel, broad-spectrum antibacterial and antifungal agents. Many current antimicrobial therapies are becoming ineffective due to the rapid evolution of resistance mechanisms in pathogenic microbes. In this context, the design and synthesis of new heterocyclic compounds that target essential microbial enzymes or disrupt cellular structures offer a promising strategy to address this critical health challenge. Among heterocyclic pharmacophores, the 1,3,4-thiadiazole moiety has attracted significant attention due to its exceptional pharmacological versatility. This five-membered heterocycle, containing both nitrogen and sulfur atoms, exhibits a wide array of biological activities, including antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory properties. The electron-rich heteroatoms in thiadiazoles facilitate strong binding interactions with biomolecular targets, often leading to high biological potency. Additionally, thiadiazoles are known for their chemical stability, lipophilicity, and ability to cross biological membranes, making them valuable scaffolds in drug design. Similarly, pyridine derivatives are crucial structural motifs in medicinal chemistry and are present in numerous clinically approved drugs. Their planar aromatic structure and nitrogen heteroatom enhance water solubility, receptor binding, and metabolic stability.

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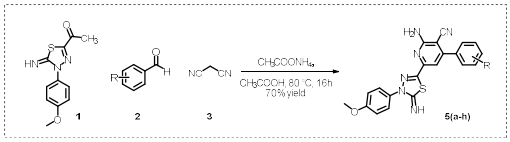
Combining the pharmacophoric potential of thiadiazoles and pyridines into hybrid molecules has been shown to produce compounds with enhanced antimicrobial and therapeutic efficacy. In the present work, we describe a one-pot multicomponent synthetic strategy to prepare a novel series of thiadiazolyl-pyridine derivatives (5a–5h). The one-pot approach not only reduces the number of purification steps and chemical waste but also improves reaction efficiency and overall sustainability—attributes that are increasingly prioritized in green and sustainable chemistry. The synthesis involves a condensation reaction between acetylthiadiazole, substituted benzaldehydes, malononitrile, and ammonium acetate in acetic acid under reflux conditions, yielding structurally diverse derivatives in good yields. To evaluate the biological potential of the synthesized compounds, we conducted in vitro antimicrobial screening using the cup plate method against a panel of clinically relevant microorganisms. These include two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus anthracis*), two Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*), and two fungal strains (*Candida albicans*, *Aspergillus niger*). The standard drugs streptomycin and fluconazole were used as references for antibacterial and antifungal comparisons, respectively. To complement the experimental data, an in silico ADME (Absorption, Distribution, Metabolism, and Excretion) analysis was performed using the SwissADME platform. All synthesized compounds were found to comply with Lipinski's Rule of Five, indicating favorable physicochemical properties for oral bioavailability. Additionally, molecular docking studies were conducted against two bacterial target enzymes—PDB ID: 2EG7 (E. coli Dihydropteroate synthase) and PDB ID: 5D6P (ATP-binding domain of GyrB in Staphylococcus

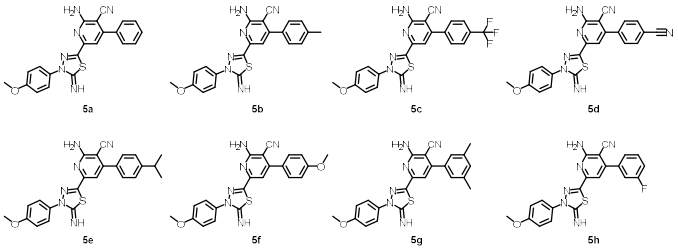
aureus)—to predict the binding modes and interaction affinities of the compounds.

MATERIAL AND METHODS

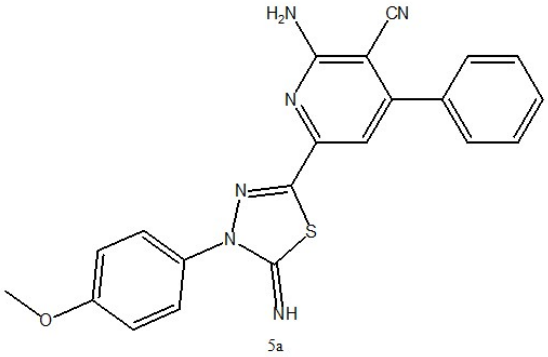
Chemistry: All the melting points reported were determined by open capillary tube method and are uncorrected. The synthesis and analytical studies of the compounds were carried out using laboratory grade and analytical grade reagents as the case may be standard procedure or reported method were followed with or without modification appropriately as and when required. Elemental analysis (C, H and N) was undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within 0.4%) unless indicated. ^{1}H NMR spectra were recorded on the Bruker DPX-400 instrument at 400 MHz. The ^{1}H chemical shifts are reported as parts per million (ppm) downfield from TMS (Me4Si). The LC mass spectra of the compounds were recorded on Shimadzu 8201PC spectrometer. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck)-coated aluminum plates, visualized by iodine vapor.

Scheme of Work



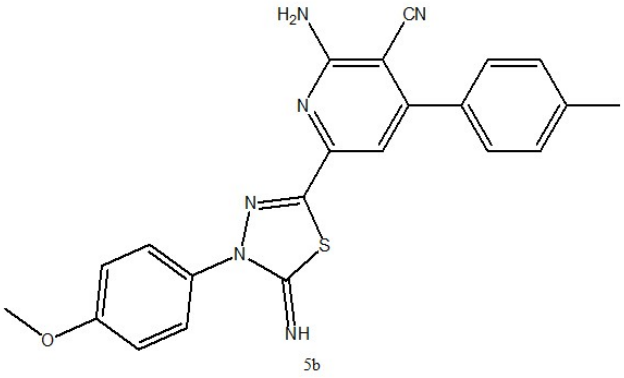


2-amino-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-4-phenylnicotinonitrile (5a)



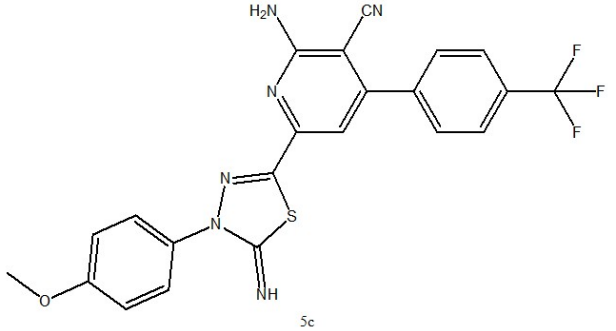
Melting Point: 202-206 ºC
Yield: 78 %
R_f value: 0.65
Solvent system: Benzene: Methanol (9.5: 0.5)
Anal. Calcd. for $C_{21}H16N_6OS$ (400.46): C, 62.98; H, 4.03; N, 20.99.
Found: C, 62.78; H, 4.01; N, 20.89.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.82 (s, 3H, OCH_3), 6.34, 2.27 (s, 1H, NH_2), 6.57-8.59 (m, 10H, Ar-H).
LCMS (ESI): calcd. for $C_{21}H16N_6OS$ $[M+H]^+$: 400.4639, found: 401.1145.

2-amino-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-4-p-tolylnicotinonitrile (5b)



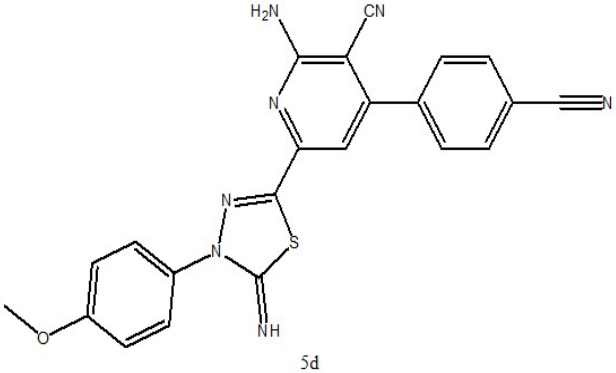
Melting Point: 212-214 ºC
Yield: 81 %
R_f value: 0.58
Solvent system: Benzene: Methanol (9.5: 0.5)
Anal. Calcd. for $C_{22}H18N_6OS$ (414.48): C, 63.75; H, 4.38; N, 20.28.
Found: C, 63.55; H, 4.28; N, 20.08.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.81 (s, 3H, OCH_3), 6.31, 2.18 (s, 1H, NH_2), 2.36 (s, 1H, CH_3), 6.57-8.59 (m, 9H, Ar-H).
LCMS (ESI): calcd. for $C_{22}H18N_6OS$ $[M+H]^+$: 414.4846, found: 415.1385.

2-amino-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-4-(4-(trifluoromethyl)phenyl)nicotinonitrile (5c)



Melting Point: 180-184 ºC
Yield: 84 %
R_f value: 0.62
Solvent system: Benzene: Methanol (9.7: 0.3)
Anal. Calcd. for $C_{22}H_{15}F_3N_6OS$ (468.45): C, 56.41; H, 3.23; N, 17.94.
Found: C, 56.31; H, 3.13; N, 17.74.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.81 (s, 6H, OCH_3), 6.61, 2.11 (s, 1H, NH_2), 6.17-8.59 (m, 9H, Ar-H).
LCMS (ESI): calcd. for $C_{22}H_{15}F_3N_6OS$ $[M+H]^+$: 468.4539, found: 469.1058.

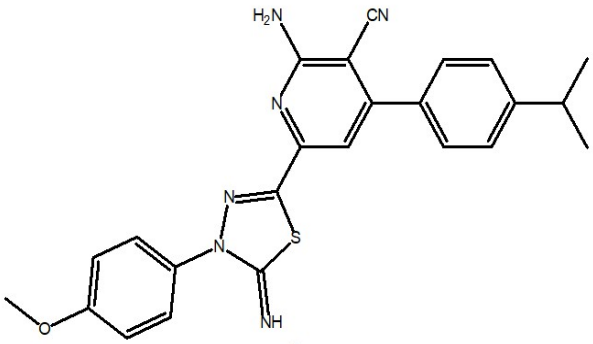
2-amino-4-(4-cyanophenyl)-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)nicotinonitrile (5d)



Melting Point: 224-228ºC
Yield: 85 %

R_f value: 0.58
Solvent system: Benzene: Methanol (9.5: 0.5)
Anal. Calcd. for $C_{22}H_{15}N_7OS$ (425.47): C, 62.10; H, 3.55; N, 23.04.
Found: C, 62.00; H, 3.25; N, 23.04.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.81 (s, 3H, OCH_3), 6.31, 2.11 (s, 1H, NH_2), 6.57-8.59 (m, 9H, Ar-H).
LCMS (ESI): calcd. for $C_{21}H_{19}N_3O_2$ $[M+H]^+$: 425.4761, found: 426.1146.

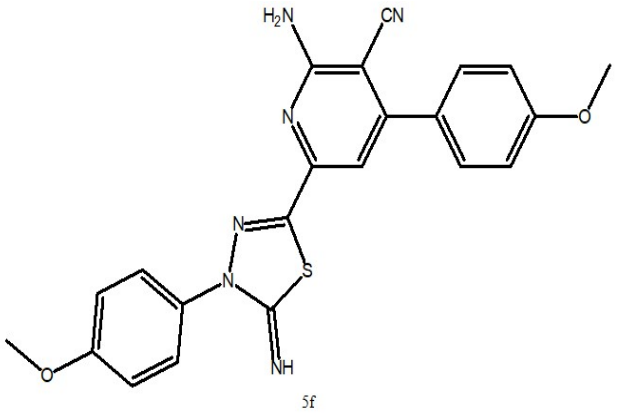
2-amino-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-4-(4-isopropylphenyl)nicotinonitrile (5e)



5e

Melting Point: 236-240ºC
Yield: 77 %
R_f value: 0.72
Solvent system: Benzene: Methanol (9.6: 0.4)
Anal. Calcd. for $C_{24}H_{22}N_6OS$ (442.54): C, 65.14; H, 5.01; N, 18.99.
Found: C, 65.04; H, 5.01; N, 18.79.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.78 (s, 3H, OCH_3), 3.11 (s, 1H, CH), 1.36, 1.35 (s, 6H, $2CH_3$), 6.98 (s, 1H, NH), 6.39, 2.06 (s, 2H, NH_2), 6.57-8.59 (m, 9H, Ar-H).
LCMS (ESI): calcd. for $C_{24}H_{22}N_6OS$ $[M+H]^+$: 442.5435, found: 443.1748.

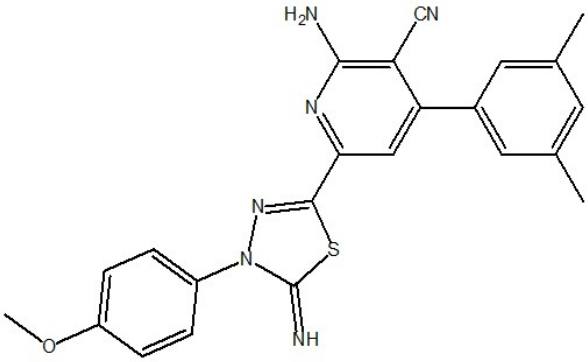
2-amino-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-4-(4-methoxyphenyl)nicotinonitrile (5f)



5f

Melting Point: 194-196 ºC
Yield: 76 %
R_f value: 0.74
Solvent system: Benzene: Methanol (9.5: 0.5)
Anal. Calcd. for $C_{22}H_{18}N_6O_2S$ (430.48): C, 61.38; H, 4.21; N, 19.52.
Found: C, 61.28; H, 4.11; N, 19.32.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.81 (s, 6H, $2OCH_3$), 6.34, 2.28 (s, 1H, NH_2), 6.87 (s, 1H, NH), 2.36 (s, 1H, CH_3), 6.57-8.59 (m, 9H, Ar-H).
LCMS (ESI): calcd. for $C_{22}H_{18}N_6O_2S$ $[M+H]^+$: 430.4894, found: 431.1248.

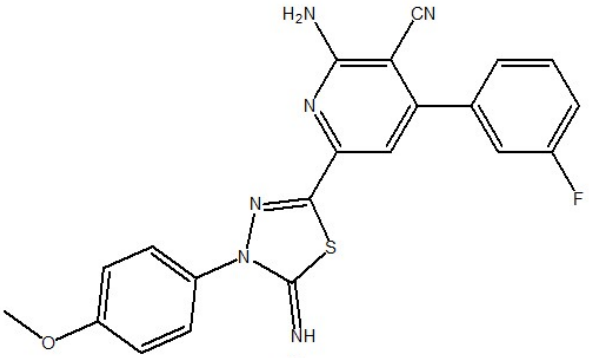
2-amino-4-(3,5-dimethylphenyl)-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)nicotinonitrile (5g)



5g

Melting Point: 176-178 ºC
Yield: 84 %
R_f value: 0.69
Solvent system: Benzene: Methanol (9.6: 0.4)
Anal. Calcd. for $C_{23}H_{20}N_6OS$ (428.51): C, 64.47; H, 4.70; N, 19.61.
Found: C, 64.27; H, 4.50; N, 19.31.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.81 (s, 3H, OCH_3), 2.37 (s, 6H, $2CH_3$), 6.48 (s, 1H, NH), 2.12 (s, 2H, NH_2), 6.57-8.59 (m, 8H, Ar-H).
LCMS (ESI): calcd. for $C_{23}H_{20}N_6OS$ $[M+H]^+$: 428.5134, found: 429.1558.

2-amino-4-(3-fluorophenyl)-6-(5-imino-4-(4-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)nicotinonitrile (5h)



5h

Melting Point: 106-110ºC
Yield: 82 %
R_f value: 0.74
Solvent system: Benzene: Methanol (9.6: 0.4)
Anal. Calcd. for $C_{21}H_{15}FN_6OS$ (418.45): C, 60.28; H, 3.61; N, 20.08.
Found: C, 60.08; H, 3.51; N, 20.08.
^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ 3.82 (s, 3H, OCH_3), 6.33 (s, 1H, NH), 2.10 (s, 2H, NH_2), 6.57-8.59 (m, 9H, Ar-H).
LCMS (ESI): calcd. for $C_{21}H_{15}FN_6OS$ $[M+H]^+$: 418.4539, found: 419.1016.

In vitro Antimicrobial Activity: The synthesized compounds (5a-5h) were screened for antimicrobial activity and cup plate method was used for the determination zone of inhibition. "Two-gram positive bacterial strains *Staphylococcus aureus, Bacillus anthracis,* two gram negative bacterial strains *Pseudomonas aeruginosa and Escherichia coli* were used for determination of antibacterial activity. Two fungal strains *C. albicans* and *A. niger* were used for determination of antifungal activity. Streptomycin and Fluconazole were used as standard for antibacterial and antifungal activity respectively. DMSO was used as solvent control. Nutrient broth and Sabourd dextrose broth were used as Culture Media for bacteria and fungi respectively" [30-32]. "Sterile nutrient broth/ sabourd dextrose broth plates were prepared, by pouring the sterile agar into petri dishes in aseptic conditions. 0.1 ml of each standardized test organism were spreaded into agar plates. Holes were prepared by using a sterile borer of diameter 6 mm. The test drug as well as the standard drug and the solvent control were placed in each hole separately. Then the plates

were maintained at 4 °C for 1 h to allow the diffusion of solution into the medium. All the bacterial plates were incubated at 37 °C for 24 h and fungal plates at 25°C for 48 h. The zone of inhibition was measured in mm" [30-32].

In silico ADME: The calculation of molecular properties like drug likeliness were calculated by Swiss ADME [33-35]. "Lipinski rule or rule of five is like that to be drug-like, a candidate should have less than five hydrogen bond donors (HBD), less than 10 hydrogen bond acceptors (HBA), a molecular weight of less than 500 Da, and a partition coefficient log P of less than 5. The aim of the *rule of five* is to highlight possible bioavailability problems if two or more properties are violated" [36-38].

Molecular Docking Study

Hardware and Software: Windows 10 (64-bit) operating systems with 4 GB RAM and 2.50 GHz Intel(R) Core(TM) i5-7200U processor was used for executing the docking process. PyRx version 0.8, available at https://pyrx.sourceforge.io/ was used to perform the docking in Auto Dock Vina Wizard [39]. Autodock Vina Tools which is made accessible by the Scripps Research Institute at https://autodock.scripps.edu/, was used for preparing the proteins and for grid generation, Ligands were processed using Open babel [40] and PyRx 0.8 and interaction poses of ligands were visualized and analysed using Discovery Studio Visualizer. Selection of Target Proteins: The molecular docking studies were carried out on two microbial proteins for assessing antimicrobial potential. PDB ID 2EG7-*E. coli* Dihydrorootase in complex with HDDP and PDB ID 5D6P-ATP Binding domain of GyrB of *S. aureus* in complex with 57U were chosen [41].

Protein and ligand processing for docking

Protein Preparation: The crystal structures of target proteins (PDB ID 2EG7- *E. coli* Dihydrorootase in complex with HDDP, PDB ID 5D6P-ATP Binding domain of GyrB of *S. aureus* in complex with 57U) were downloaded from the RCSB-Protein Data Bank and the proteins were prepared using Autodock Tools 4.2.6. In this step, attached water molecules and bound heteroatoms/ligand were removed, polar hydrogens and Kollman charges were added, the charge was spread equally over all atoms and residues were checked for missing atoms if any. The prepared PDB files were then converted to the PDBQT format for executing the next step [41].

Ligand Processing: Ligands in smiles format were converted to sdf files and 3D coordinates for all ligands were generated using Open Babel using command line. The 3D structure data files were processed in PyRx using UFF energy minimization and then converted to PDBQT format (autodock detectable format).

Grid Generation: The grid box was first set over attached ligands using AutoDock Tools and then manually adjusted to desired dimensions in PyRx. The grid dimensions were set as 24.946 x 41.236 x 72.500 $Å^3$ keeping number of points as 25 in X, Y, Z direction for PDB ID:2EG7 and -6.093 x 4.440 x 0.210 $Å^3$ keeping number of points as 25 in X, Y, Z direction for PDB ID:5D6P.

Docking and visualization of results: The docking was implemented in Vina Wizard of PyRx Tool, using exhaustiveness of 8 and the resultant out files were split into individual pose files. These files and the protein structure were then taken for visualization of interactions using Maestro Visualizer.

RESULTS AND DISCUSSION

Chemistry: A series of 3-cyanopyridine derivatives 5a–h has been prepared by one-pot condensation of acetylthiadiazole 1, an aldehyde 2a–h, malononitrile 3, and ammonium acetate in refluxing acetic acid (Scheme 1). Structures of compounds 5a–h was confirmed via their spectral data. The infrared (IR) spectra of compound 5a showed CN, NH, and NH_2 groups in their expected locations at υ = 3438–3241 (NH_2 and NH) and 2217 (CN) cm^{-1}. Moreover, an electron impact mass spectroscopic technique gave its correct molecular ion peak at m/z = 370.4 (Experimental section) [27,28].

Antimicrobial Activity

Antibacterial Activity: Compound 5d shows the highest activity against bacterial strains, with inhibition zones of 28 mm against S. aureus, 29 mm against B. anthracis, 28 mm against P. aeruginosa, and 27 mm against E. coli. This activity is comparable to that of streptomycin. Compound 5c and 5f also exhibit moderate to good antibacterial activity across most strains, particularly S. aureus and E. coli. Compound 5g displays weak antibacterial activity against P. aeruginosa (15 mm) and E. coli (13 mm). The least antibacterial activity is observed for 5a and 5b, especially against P. aeruginosa (11–12 mm). The table 5.1 above presents the antimicrobial and antifungal activity of various compounds (5a–5h), streptomycin, and fluconazole, evaluated against six microbial strains: *Staphylococcus aureus (S. aureus), Bacillus anthracis (B. anthracis), Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli), Candida albicans (C. albicans)*, and *Aspergillus niger (A. niger)*. Streptomycin and Fluconazole were used as standard for antibacterial and antifungal activity.

Antifungal Activity: Compound 5e shows the highest antifungal activity, with inhibition zones of 24 mm against C. albicans and 23 mm against A. niger, approaching the activity of fluconazole. Compound 5c and 5d also demonstrate notable antifungal activity, with inhibition zones of 17–20 mm against C. albicans and 19 mm against A. niger. Compound 5a, 5b, and 5h exhibit weak antifungal activity, with inhibition zones not exceeding 15 mm against either fungal strain. Compounds such as 5c, 5d, and 5e show broad-spectrum antimicrobial activity, inhibiting both bacterial and fungal strains effectively. Some compounds (e.g., 5f and 5g) exhibit stronger activity against bacterial strains than fungal strains, while others (5e) are more effective against fungal strains.

In silico drug likeness: Lipinski's Rule of Five is a widely accepted guideline used to evaluate the drug-likeness of compounds, particularly their potential oral bioavailability. All compounds fall well within the acceptable range for molecular weight (<500 g/mol), number of hydrogen bond donors (≤5), and hydrogen bond acceptors (≤10), indicating potential oral bioavailability. Similarly, all compounds possess log P values under 5, which suggests a favorable balance between hydrophilicity and lipophilicity. The number of rotatable bonds (4–5) for all compounds is also within a drug-like range, which is important for conformational flexibility and bioavailability. While Lipinski's original rule does not explicitly include TPSA, a value below 140 $Å^2$ is generally considered favorable for membrane permeability and oral bioavailability. Most compounds (5a–5c, 5e, 5g, 5h) are slightly above or equal to this threshold, and still considered acceptable. (Table 5.2 & Figure 1.1)

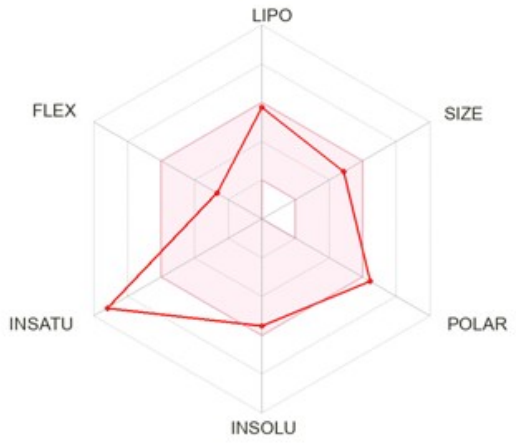
Molecular Docking Study: Molecular docking studies were performed to evaluate the binding affinities and interaction profiles of the synthesized compounds (5a–5h) against the target protein with PDB ID: 2EG7, a bacterial enzyme implicated in resistance mechanisms. Streptomycin, a known antibiotic, was used as a reference standard for comparative purposes. The docking results are summarized in Table 5.3& Figure 5.2.The binding energies of the compounds ranged from -8.0 to -8.7 kcal/mol, indicating a favorable interaction with the active site of the target protein. Notably, compound 5e exhibited the most potent binding energy (-8.7 kcal/mol), surpassing that of the standard drug Streptomycin (-8.3 kcal/mol). Other compounds such as 5c (-8.4 kcal/mol) and 5b and 5g (both -8.3 kcal/mol) also demonstrated strong binding affinities comparable to or better than the reference. These values suggest that the compounds form stable complexes with the target enzyme, implying a potential for strong inhibitory activity.

Table 5.1. Antimicrobial Activity of Title compounds (5a-5h)

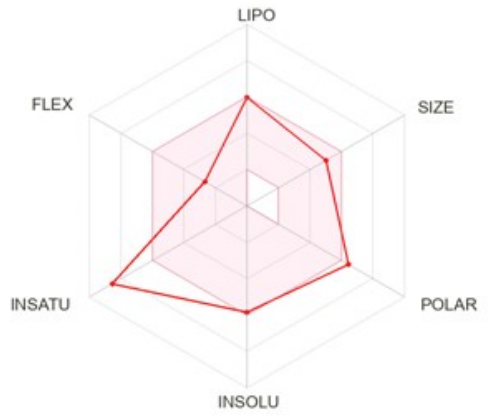
Compound (1000 µg/ml)	Zone of Inhibition (mm)					
	S. aureus	*B. anthracis*	*P. aeruginosa*	*E. coli*	*C. albicans*	*A. niger*
Streptomycin	33	34	31	32	-	-
Fluconazole	-	-	-	-	28	30
5a	17	19	12	18	12	11
5b	21	21	11	17	13	15
5c	23	18	16	21	20	19
5d	28	29	28	27	17	19
5e	21	19	16	22	24	23
5f	25	13	14	22	18	15
5g	24	19	15	13	15	16
5h	23	21	13	16	12	10

Table 5.2. *In silico* Drug Likeness and absorption

Comp	Molecular Weight (g/mol)	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	TPSA (Å²)	Log Po/w (iLOGP)
5a	400.46	4	5	2	141.84	2.96
5b	414.48	4	5	2	141.84	3.22
5c	468.45	5	8	2	141.84	3.11
5d	425.47	4	6	2	165.63	2.83
5e	442.54	5	5	2	141.84	3.49
5f	430.48	5	6	2	151.07	3.27
5g	428.51	4	5	2	141.84	3.38
5h	418.45	4	6	2	141.84	3.04



5a



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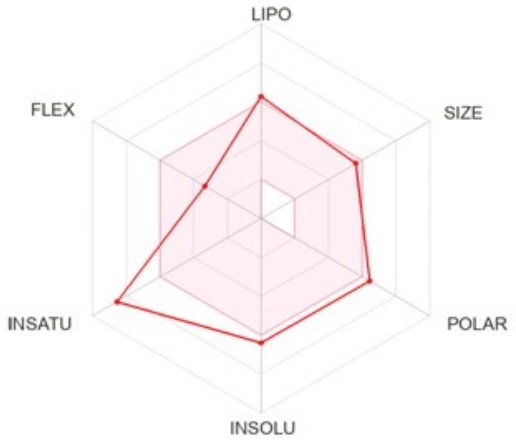

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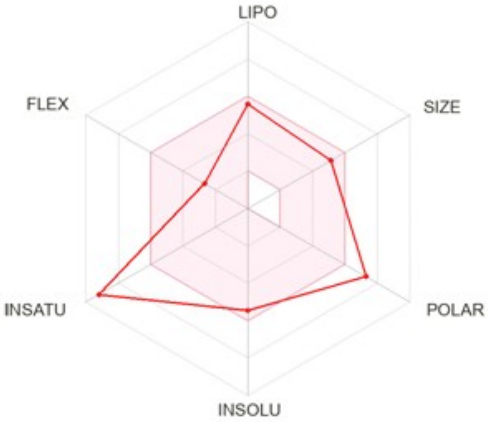


5d

Figure 5.1. In silico ADME of Synthesized compounds (5a-5h)

Table 5.3. Molecular Docking of Compounds (5a-5h and Streptomycin) with PDB 2EG7

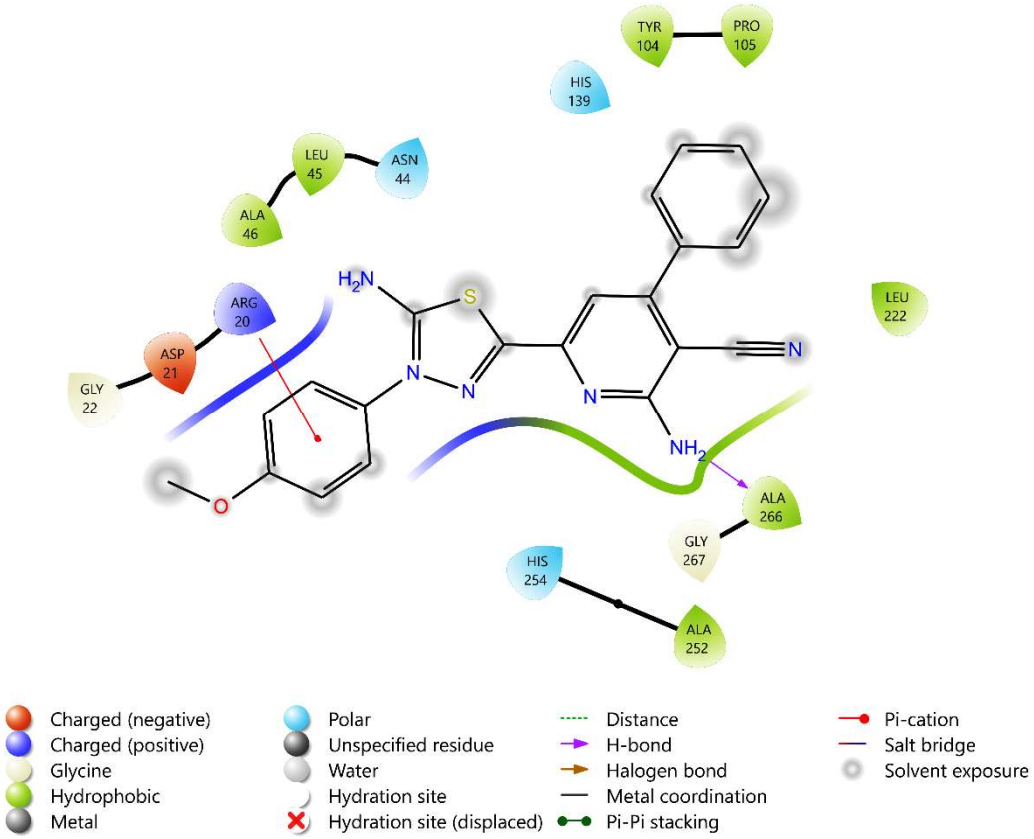
Compound	Binding Energy (Kcal/mol)	Hydrogen Bonding Interactions (Conventional)	Receptor Ligand Interactions
5a	-8	ALA266	GLY2, ASP21, ARG20, ALA46, LEU45, ASB44, HIS139, TYR104, PRO105, LEU222, GLY267, ALA252, HIS254
5b	-8.3	ALA266	GLY267, ALA252, HIS254, LEU222, ASN44, LEU45, ALA46, ARG20, ASP21, GLY22, GLU141, HIS139, PRO105, TYR104
5c	-8.4	-	ALA46, LEU45, ASN44, TYR104, PRO105, MET24, ASP21, ARG24, HIS254, ALA252, LEU22, ALA266, THR143, GLU141, HIS139
5d	-8.1	LEU222, TYR79	HIS114, PRO48, ALA46, LEU45, ASN44, PRO105, TYR104, KCX102, HIS18, HIS139, CYS221, ALA266, GLY267
5e	-8.7	-	ALA266, GLY267, LEU222, ALA252, HIS254, GLY22, ASP21, ARG20, ALA46, LEU45, ASN44, TYR104, PRO105, HIS139, GLU141, THR143
5f	-8.2	-	MET24, ASP21, ARG20, ALA46, LEU45, ASN44, GLY267, ALA266, HIS254, ALA252, LEU222, TYR104, PRO105, HIS139, GLU141, THR143
5g	-8.3	THR143	THR110, HIS114, GLY115, VAL116, TYR79, LEU80, KCX102, TYR104, PRO105, GLU141, HIS139, ARG20, HIS18, LEU222, CYS221, ALA266, GLY267, CYS268,ASP250, GLN201, ALA252, HIS254, ASN44
5h	-8.2	HIS139	TYR79, PRO105, TYR104, KCX102, GLU141, LEU222, HIS177, ASP250, ALA252, HIS254, HIS16, HIS18, ARG258, ARG20, ASP21, ALA46, LEU45, ASN44
Streptomycin	-8.3	ASN44, ALA266, KCX102, HIS177	TRY79, PRO48, HIS114, ALA46, LEU45, TYR104, PRO105, HIS139, HIS16, HIS18, ARG20, ASP250, ALA252, HIS254, GLY267, LEU222, ASP250, ALA252, HIS254, ARG258

Hydrogen bonding plays a crucial role in ligand-receptor stabilization. Most compounds showed at least one conventional hydrogen bond interaction, enhancing their binding specificity. For instance:

- 5a and 5b interacted through ALA266, a residue also involved in streptomycin binding, suggesting a similar binding pattern.
- 5d formed two hydrogen bonds with LEU222 and TYR79, while 5g and 5h showed hydrogen bonding with THR143 and HIS139, respectively.
- 5c, 5e, and 5f did not display any conventional hydrogen bonding, yet maintained strong binding energies, likely due to extensive van der Waals and hydrophobic interactions.

Interestingly, Streptomycin formed multiple hydrogen bonds including with ASN44, ALA266, and KCX102, contributing to its strong binding affinity. The replication of these key residues in ligands 5a, 5b, 5e, and 5g reinforces their therapeutic relevance.

Detailed interaction analysis revealed that all compounds commonly interacted with critical residues such as ALA46, LEU45, ASN44, TYR104, PRO105, HIS139, ALA252, HIS254, GLY267, and LEU222.



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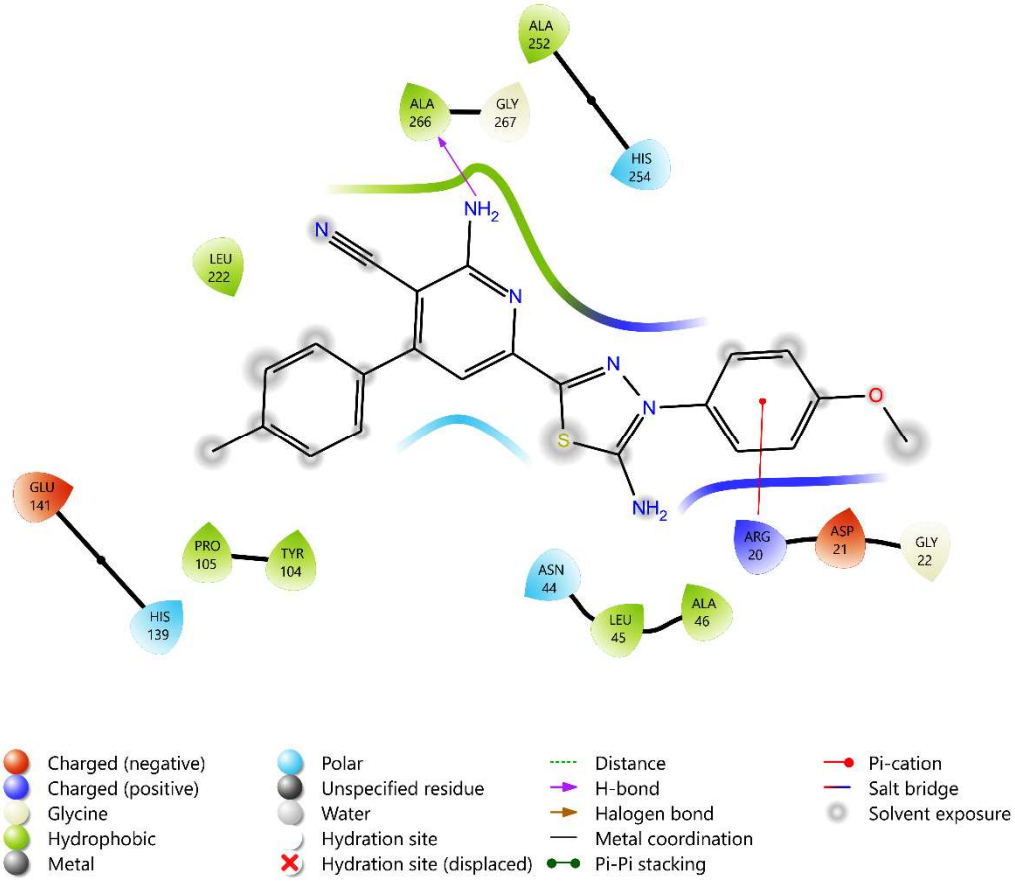

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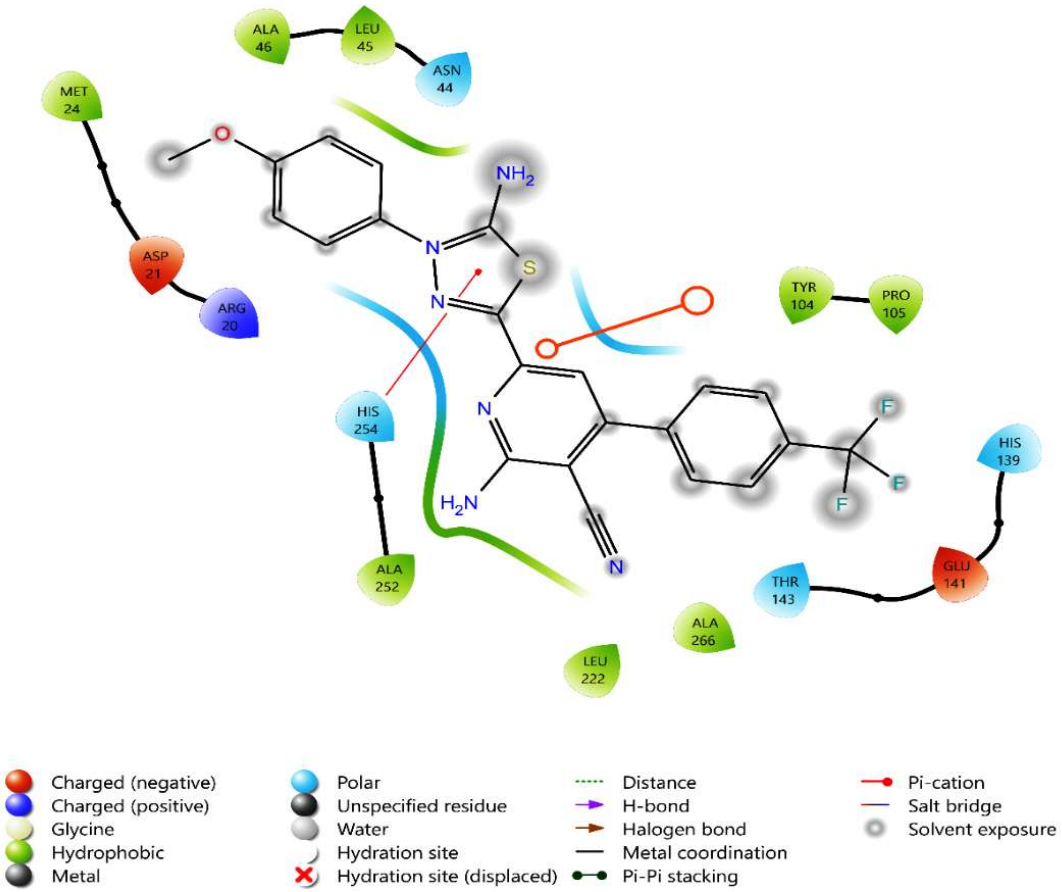
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MET 24
ASP 21
ARG 20
HIS 254
ALA 252
TYR 104
PRO 105
HIS 139
GLU 141
THR 143
ALA 266
LEU 222
Charged (negative)
Charged (positive)
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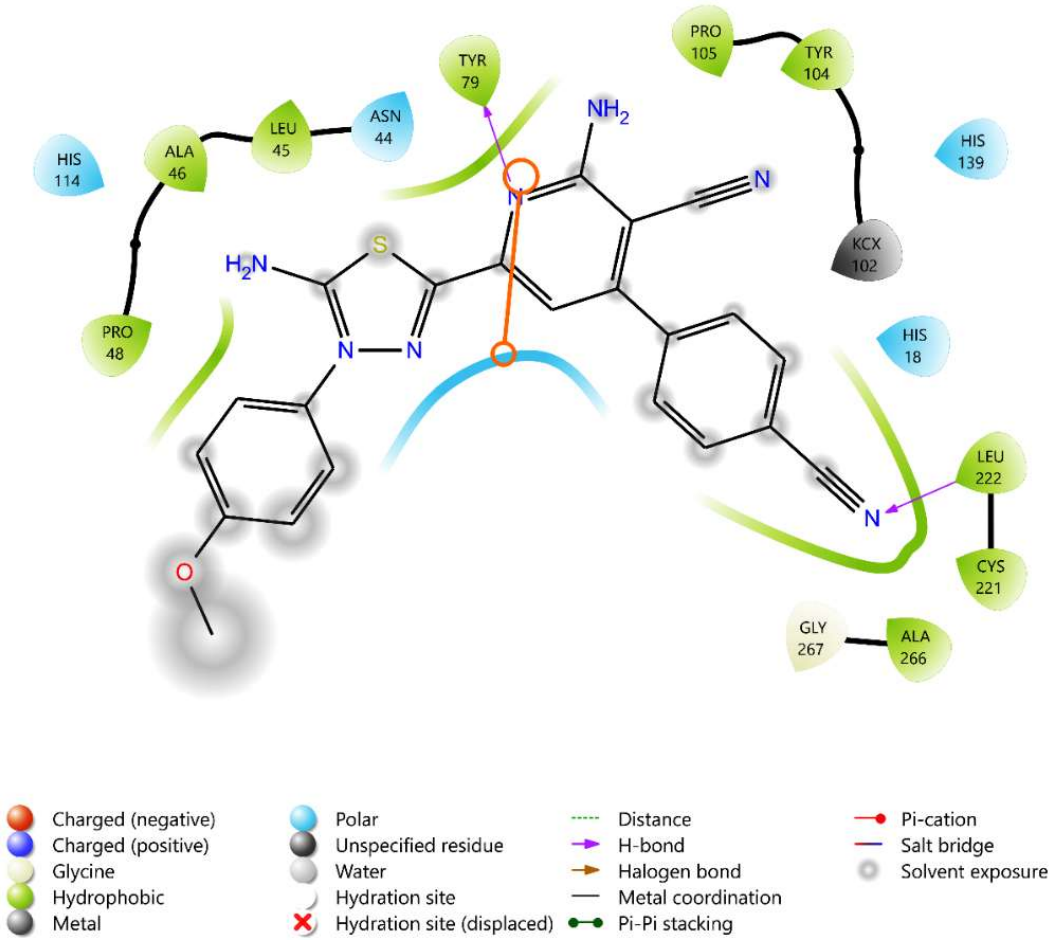
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5c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TYR 79
HIS 139
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LEU 45
HIS 114
ALA 46
KCX 102
PRO 48
HIS 18
LEU 222
CYS 221
GLY 267
ALA 266
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
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Hydration site
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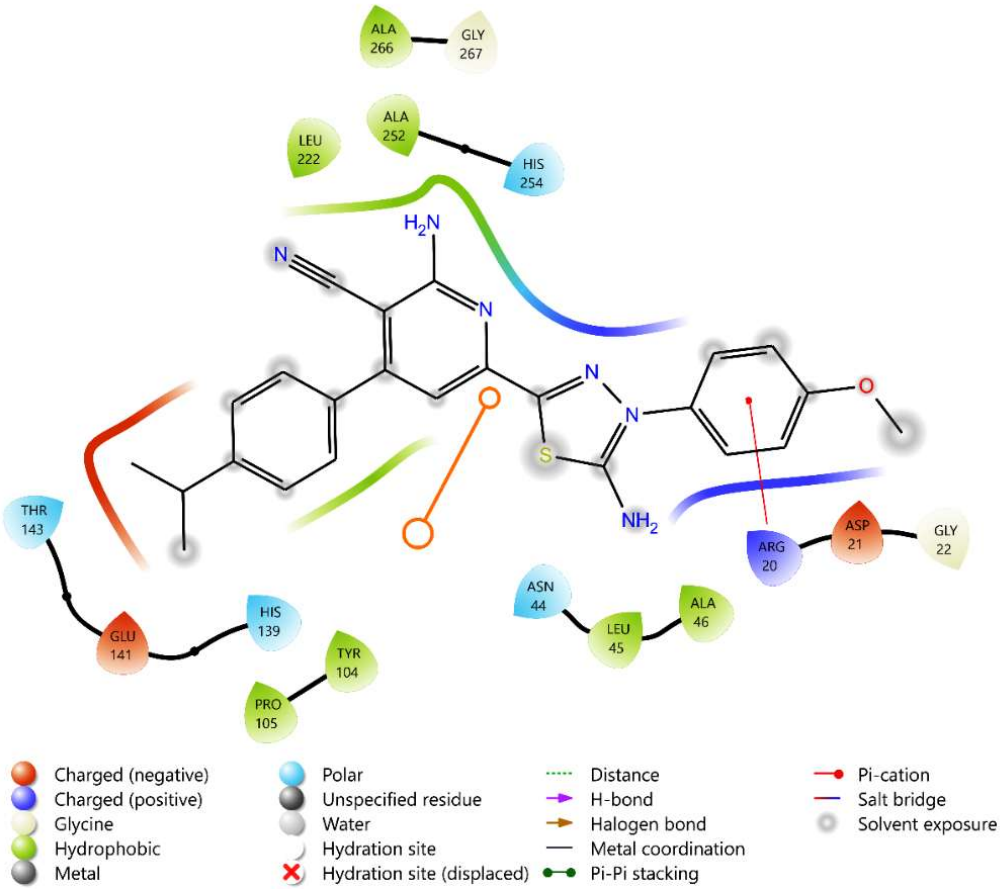
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5d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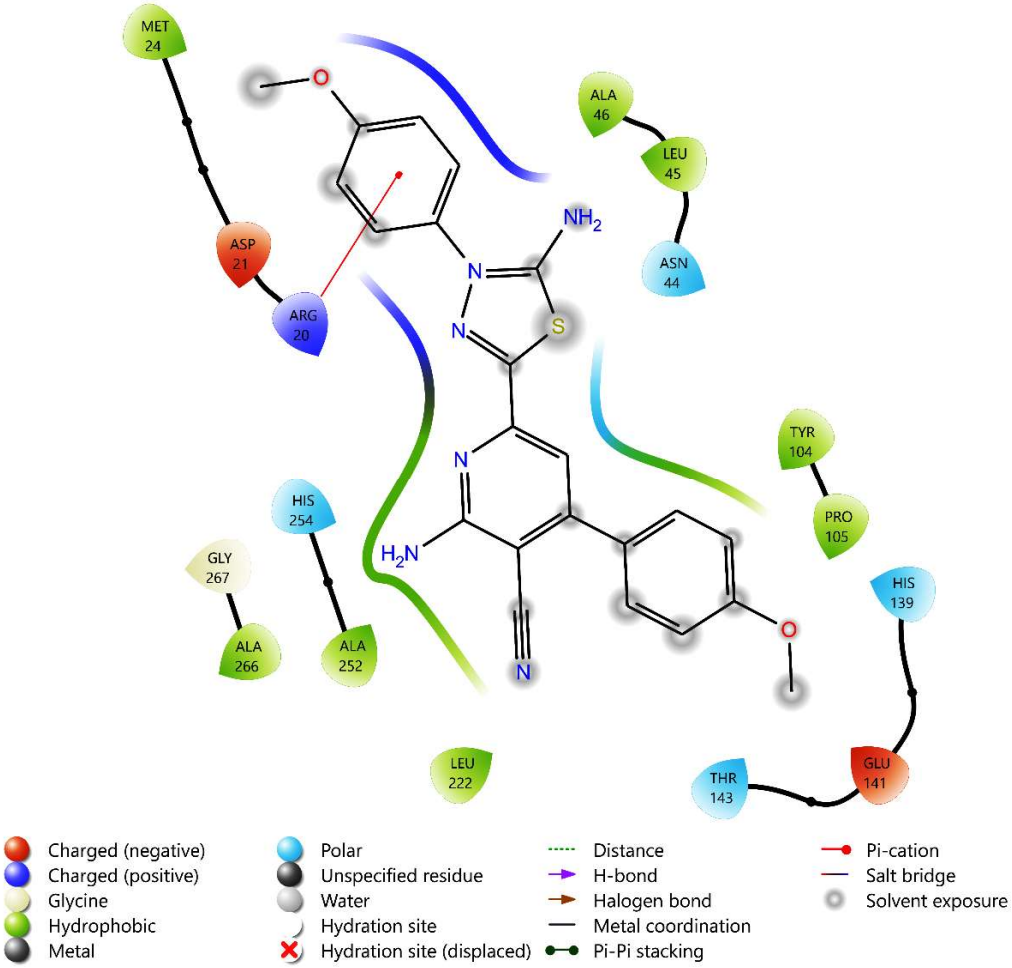
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5e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HIS 254
HIS 139
GLY 267
ALA 266
ALA 252
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LEU 222
THR 143
GLU 141
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
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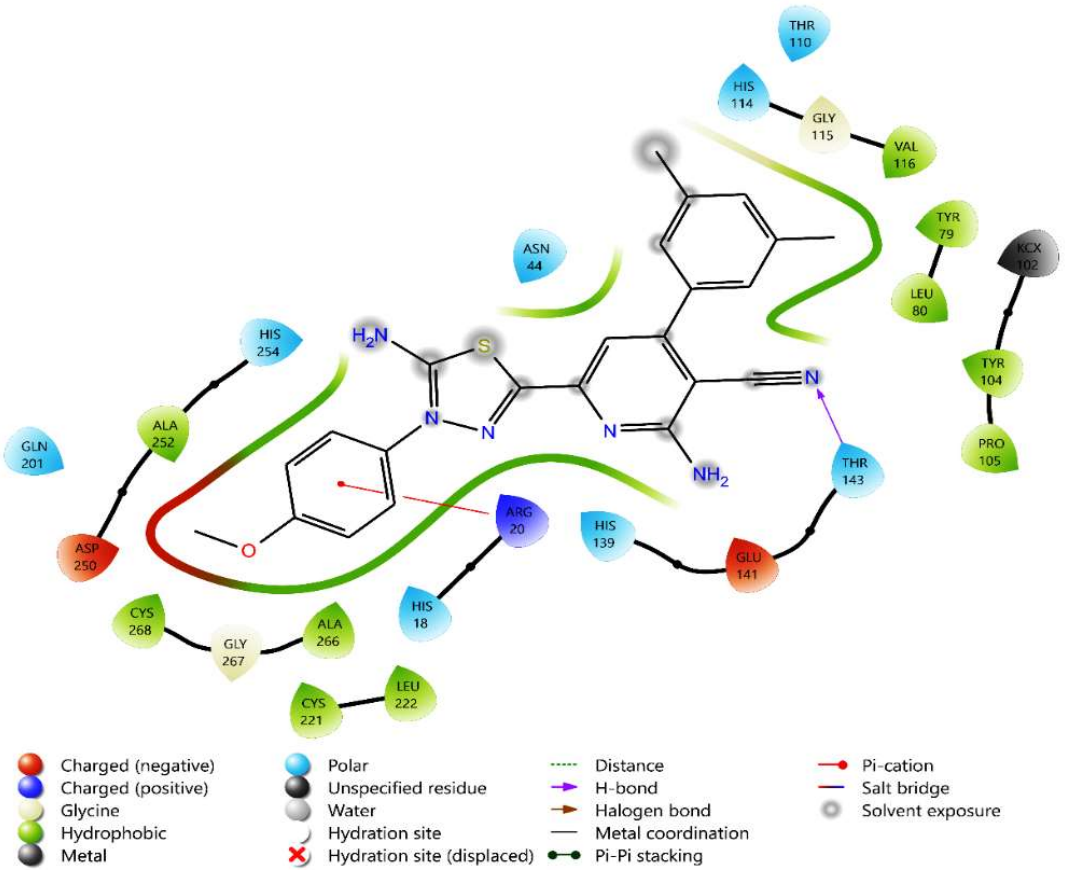
Hydration site (displaced)
Distance
H-bond
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5f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ASP 250
ARG 20
HIS 139
GLU 141
THR 143
HIS 18
CYS 268
GLY 267
ALA 266
CYS 221
LEU 222
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
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Distance
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Halogen bond
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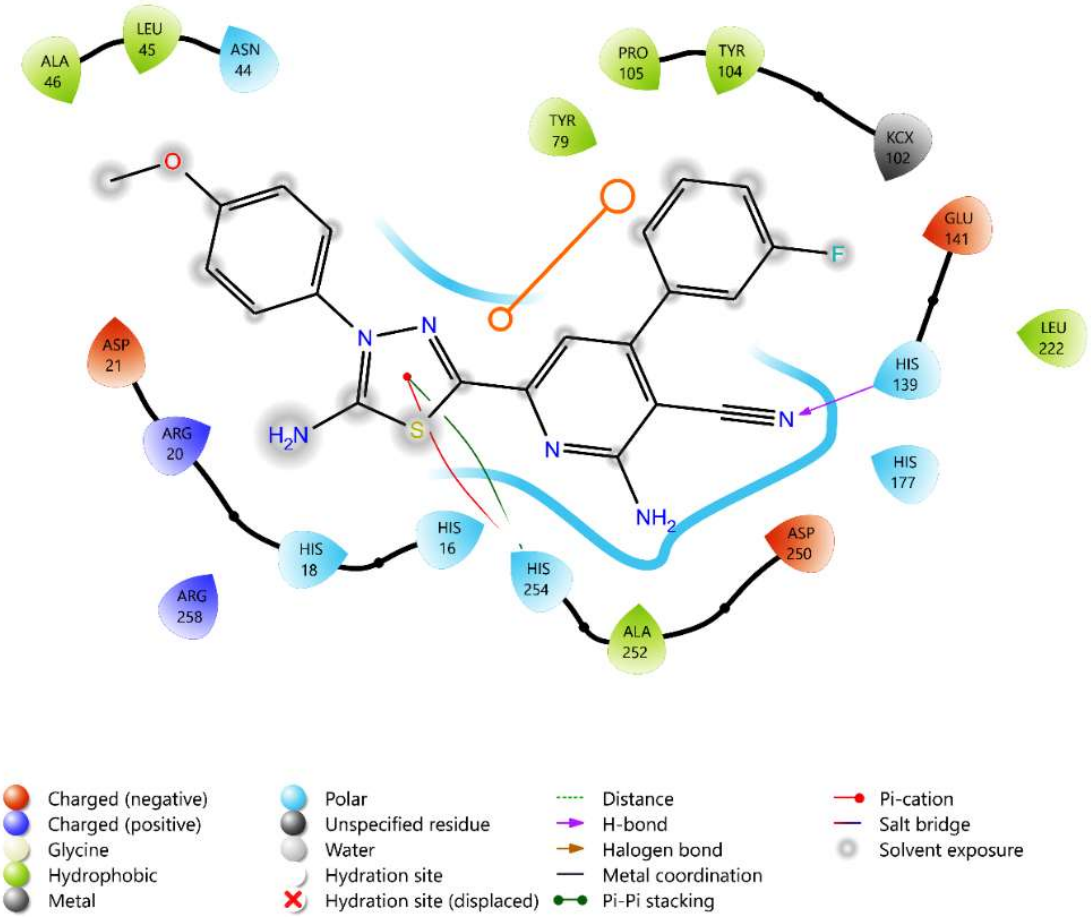
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5g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Polar
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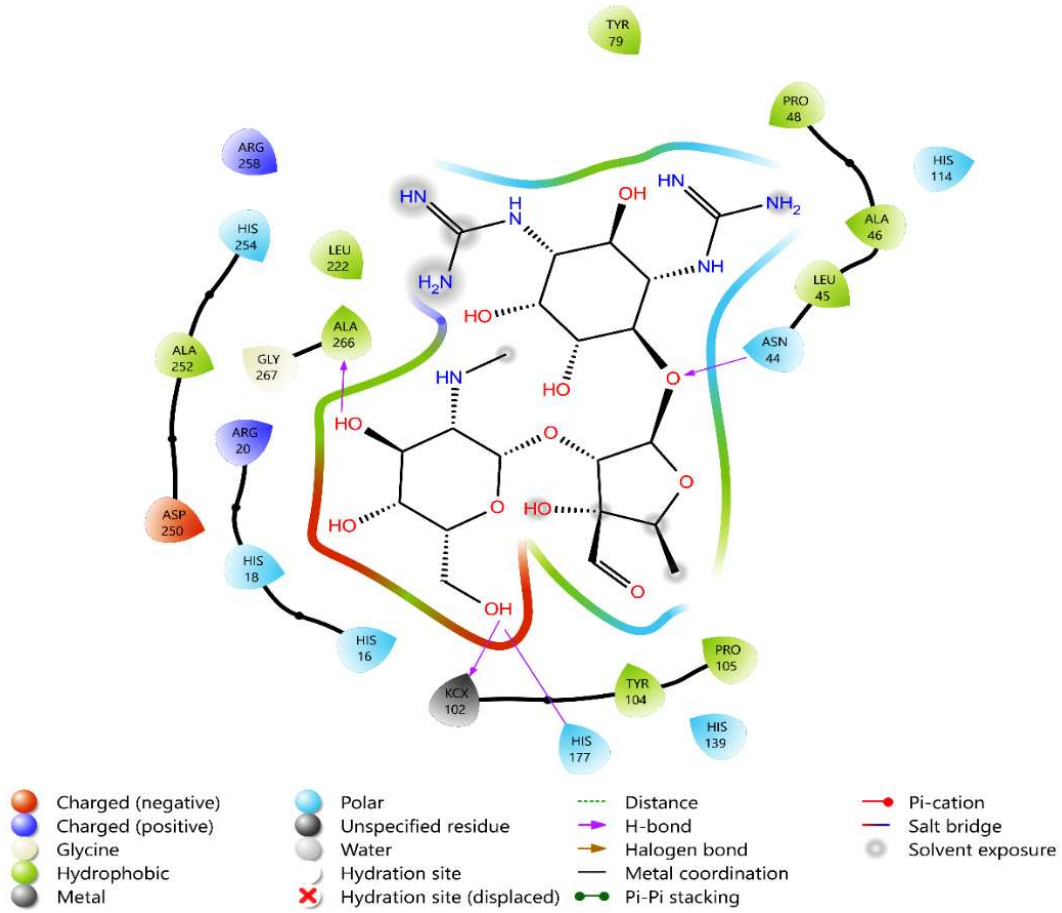
Pi-cation
Salt bridge
Solvent exposure

5h



Streptomycin

Figure 5.2. Binding Interactions of (5a-5h and Streptomycin) with PDB 2EG7

Table 5.4. Molecular Docking of Compounds (5a-5h and Streptomycin) with PDB 5D6P

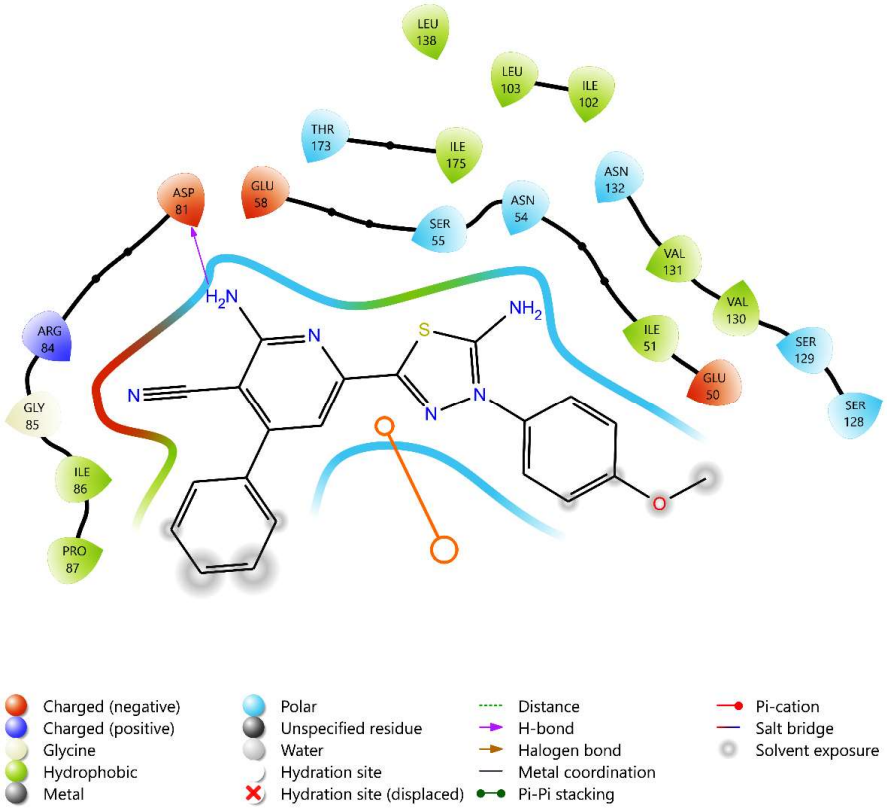
Compound	Binding Energy (Kcal/mol)	H-Bond Interactions	Receptor Ligand Interactions
5a	-8.9	ASP81	PRO87, ILE86, GLY85, ARG84, GLU58, SER55, ASN54, ILE51, GLU50, THR173, ILE175, LEU138, LEU103, ILE102, ASN132, VAL131, VAL130, SER129, SER128
5b	-9.1	ASP81	PRO87, ILE86, GLY85, ARG84, GLU58, SER55, ASN54, ILE51, GLU50, ASN132, VAL131, VAL130, SER129, SER128, THR173, ILE175, LEU138, LEU103, ILE102
5c	-9.8	GLY85, GLU58, GLN91	GLY172, THR173, ILE175, ILE51, ASN54, SER55, ASP81, GLY83, ARG84, ILE86, PRO87, ARG44, LEU103, ILE102, VAL101, ALA98
5d	-9.1	-	PRO87, ILE86, GLY85, ARG84, ASP81, GLU58, ASP57, SER55, ASN54, ILE51, GLU50, THR173, ILE175, LEU138, ASN132, VAL131, VAL130, SER129, SER128, LEU103, ILE102
5e	-9.1	-	ARG144, PRO87, ILE86, GLY85, ARG84, ASP81, GLU58, SER55, ASN54, ILE51, GLU50, THR173, ILE175, LEU138, ASN132, VAL131, VAL130, SER129, SER128, LEU103, ILE102
5f	-8.7	ASP81	PRO87, ILE86, GLY85, ARG84, ARG144, GLU58, ASP57, SER55, ASN54, ILE51, GLU50, THR173, ILE175, LEU138, ASN132, VAL131, VAL130, SER129, SER128, LEU103, ILE102
5g	-9.5	GLY85	ARG144, PRO87, ILE86, ARG84, GLY83, ASP81, THR173, ILE175, GLU58, SER55, ASN54, ILE51, LEU103, ILE102, VAL101, ALA98, GLN91
5h	-9.1	ASP81	PRO87, ILE86, GLY85, ARG84, THR173, ILE175, GLU58, SER55, ASN54, ILE51, GLU50, LEU138, LEU103, ILE102, ASN132, VAL131, VAL130, SER129, SER128
Streptomycin	-6.7	ARG84	ARG144, ALA61, GLU58, ASP57, SER55, ASN54, ILE51, GLY172, THR173, ILE175, PRO87, ILE86, GLY85, GLY83, ASP81, ILE102

These residues are crucial for active site stabilization and may play significant roles in the inhibitory activity of these ligands.

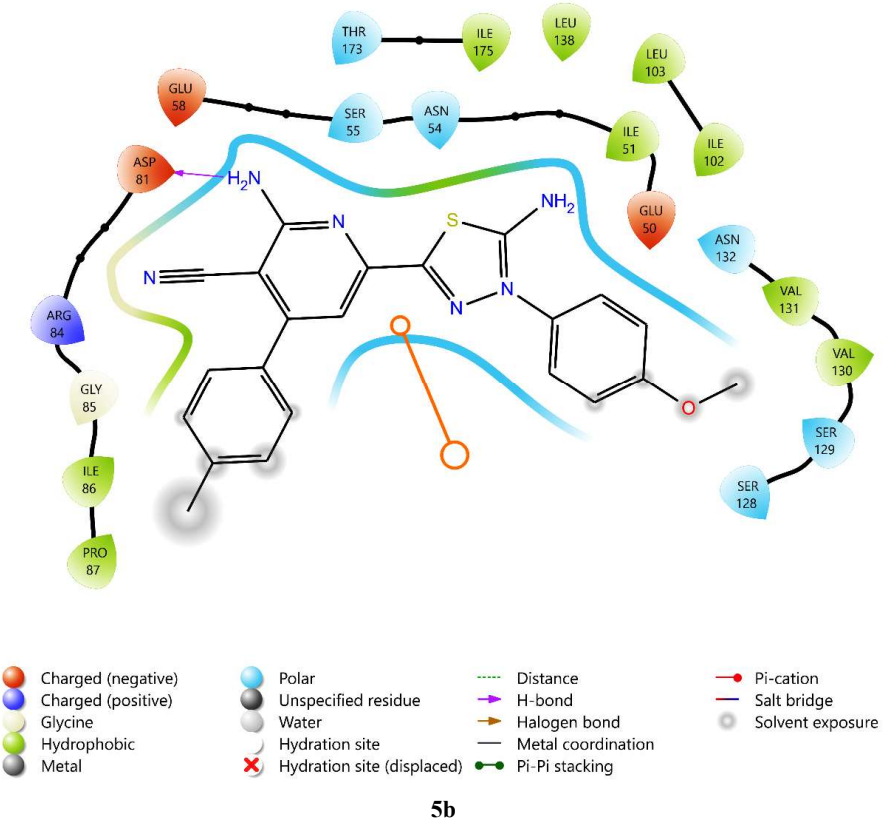
- Compound 5e exhibited the broadest interaction spectrum, including 16 active site residues, which could explain its superior binding affinity.
- Residues like ARG20, ASP21, and GLU141 were frequently involved across multiple ligands, suggesting their significance in maintaining ligand binding orientation.
- The consistency of interaction with ALA266 and HIS254 across most compounds and Streptomycin confirms these as potential pharmacophoric hotspots.

The molecular docking results suggest that the synthesized derivatives, particularly 5e, 5c, 5b, and 5g, show promising interaction profiles and binding affinities, potentially rivaling or exceeding that of the standard antibiotic Streptomycin. To assess the potential antibacterial activity of synthesized compounds 5a–5h, molecular docking was performed against the bacterial target enzyme PDB ID: 5D6P. The docking outcomes—specifically binding energies, hydrogen bond interactions, and key receptor-ligand interactions—are outlined in Table 5.4& Figure 5.3.

The docking results revealed that all test compounds exhibited stronger binding affinities than Streptomycin, which recorded a binding energy of -6.7 kcal/mol. Compound 5c demonstrated the highest binding affinity with a score of -9.8 kcal/mol, followed by 5g (-9.5 kcal/mol) and a cluster of compounds (5b, 5d, 5e, and 5h) at -9.1 kcal/mol. Even the lowest-scoring test compound, 5f, showed a better binding affinity (-8.7 kcal/mol) compared to Streptomycin. Hydrogen bonding is crucial in maintaining strong and specific ligand-receptor interactions.



5a



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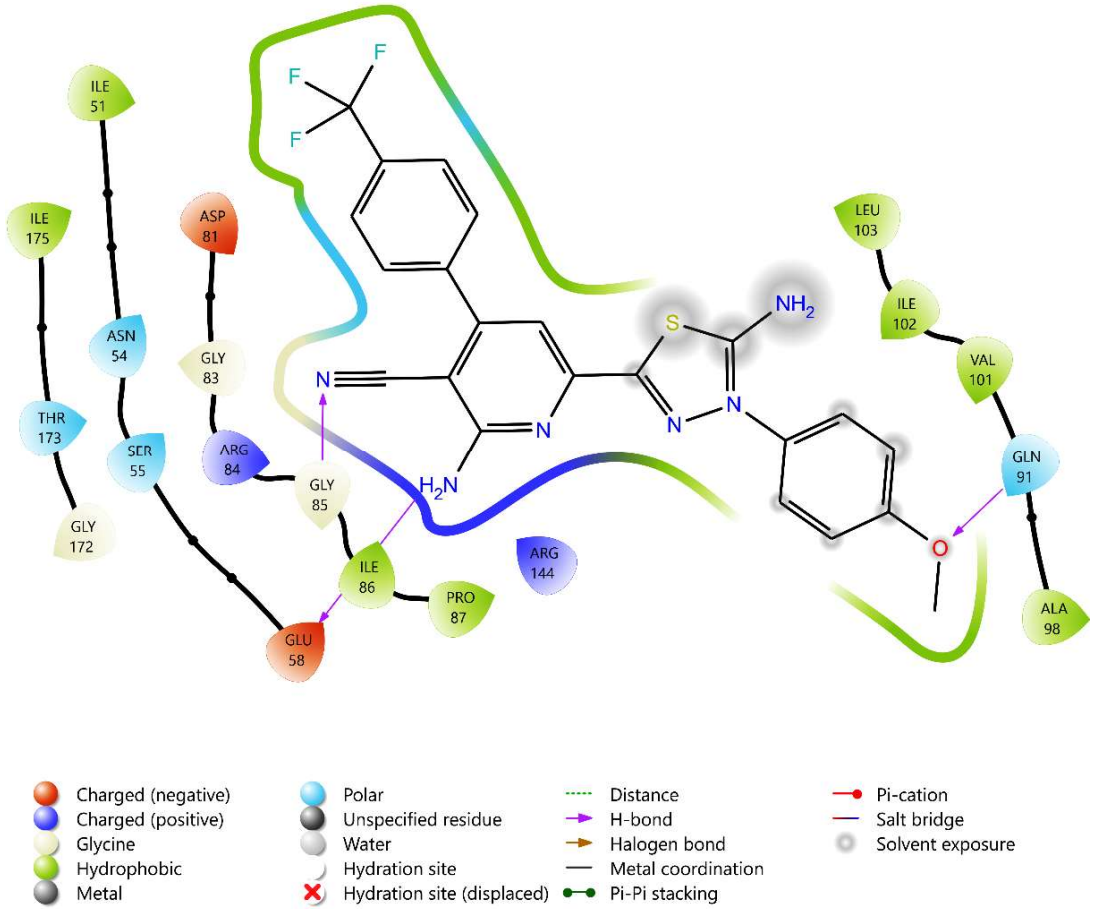
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Metal
Polar
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Halogen bond
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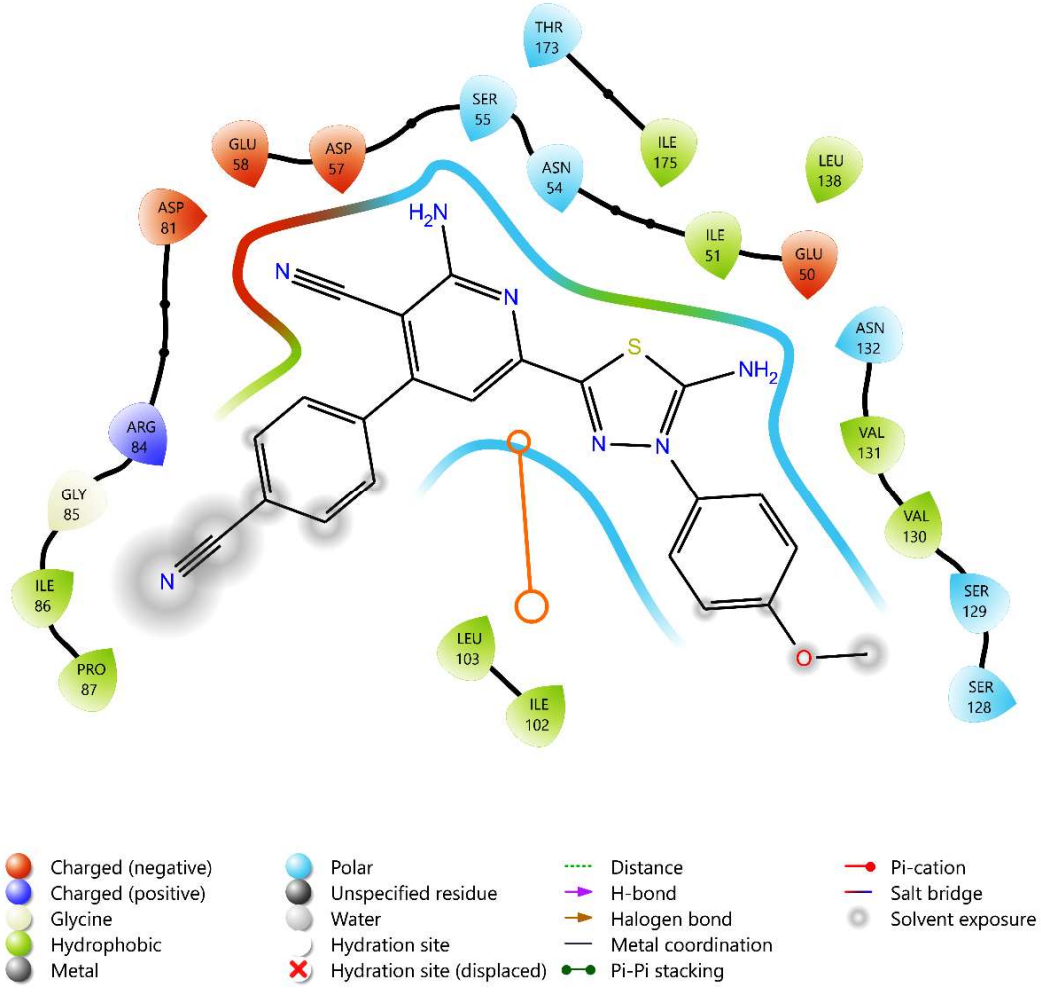
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5c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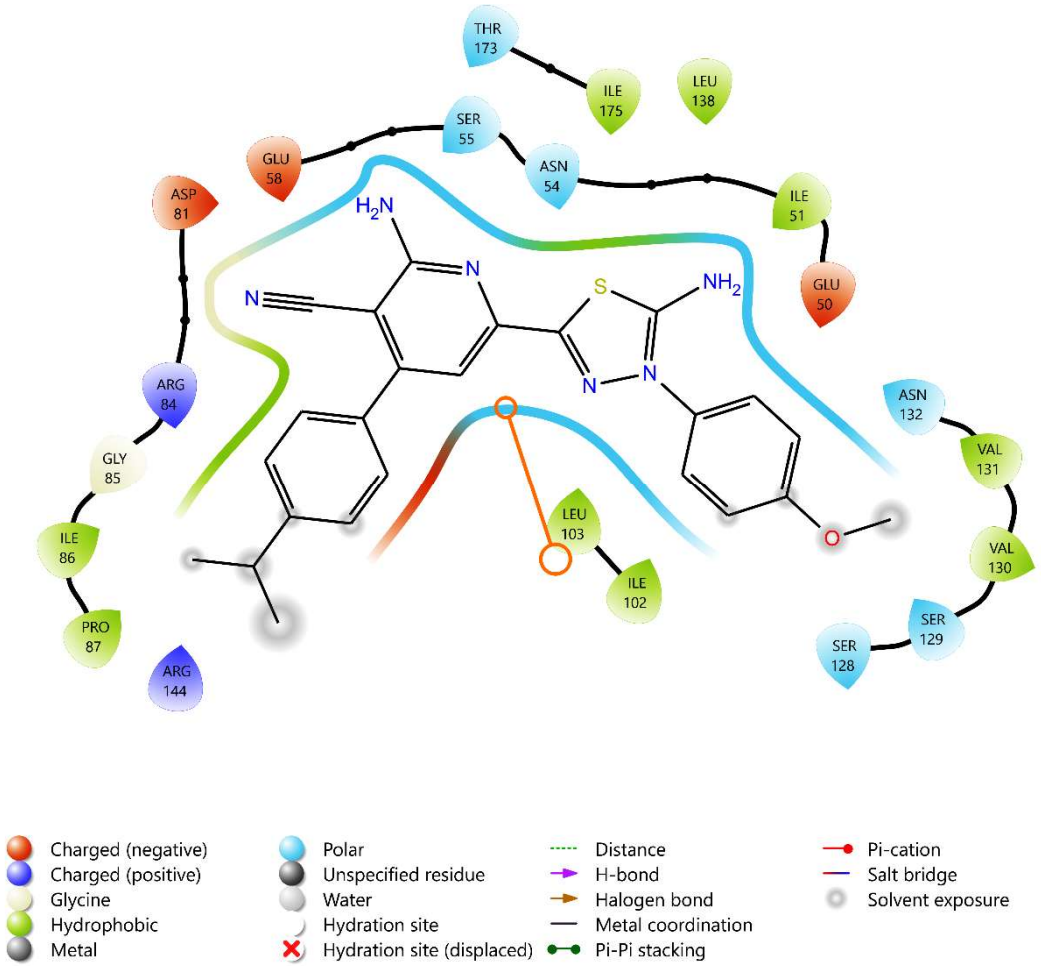
Salt bridge
Solvent exposure

5d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VAL 131
VAL 130
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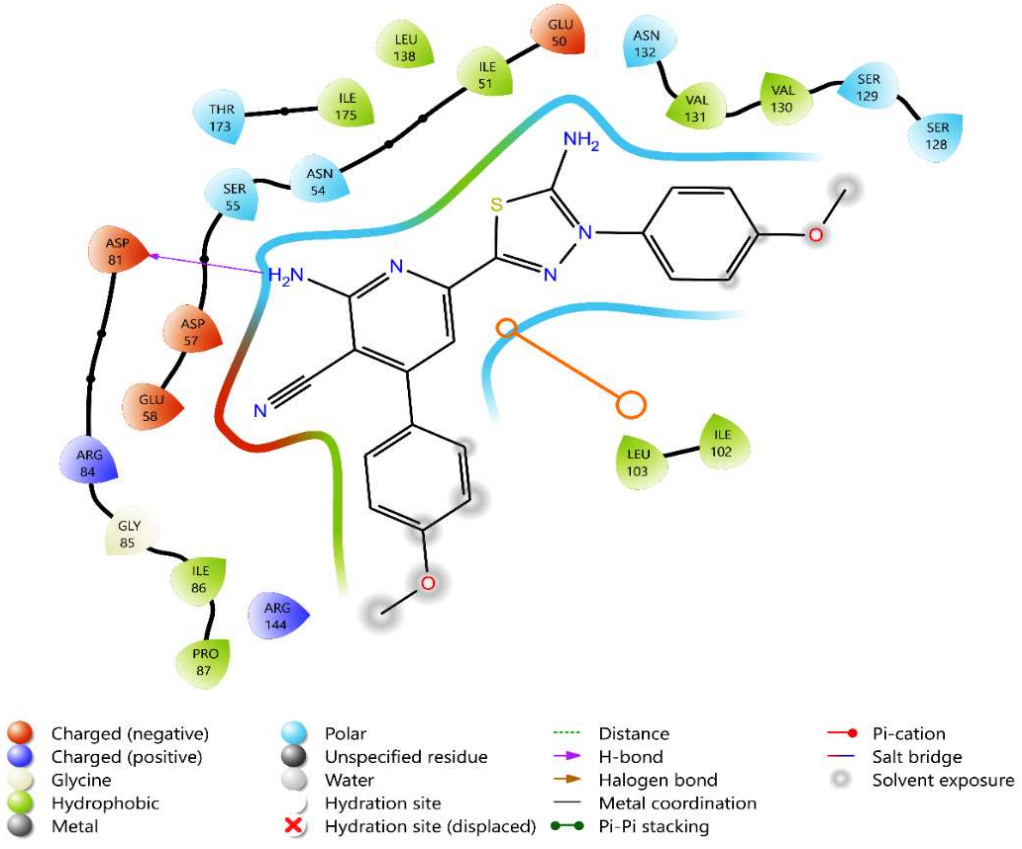
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5e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GLU 58
ARG 84
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ARG 144
LEU 103
ILE 102
Charged (negative)
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Glycine
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Metal
Polar
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Distance
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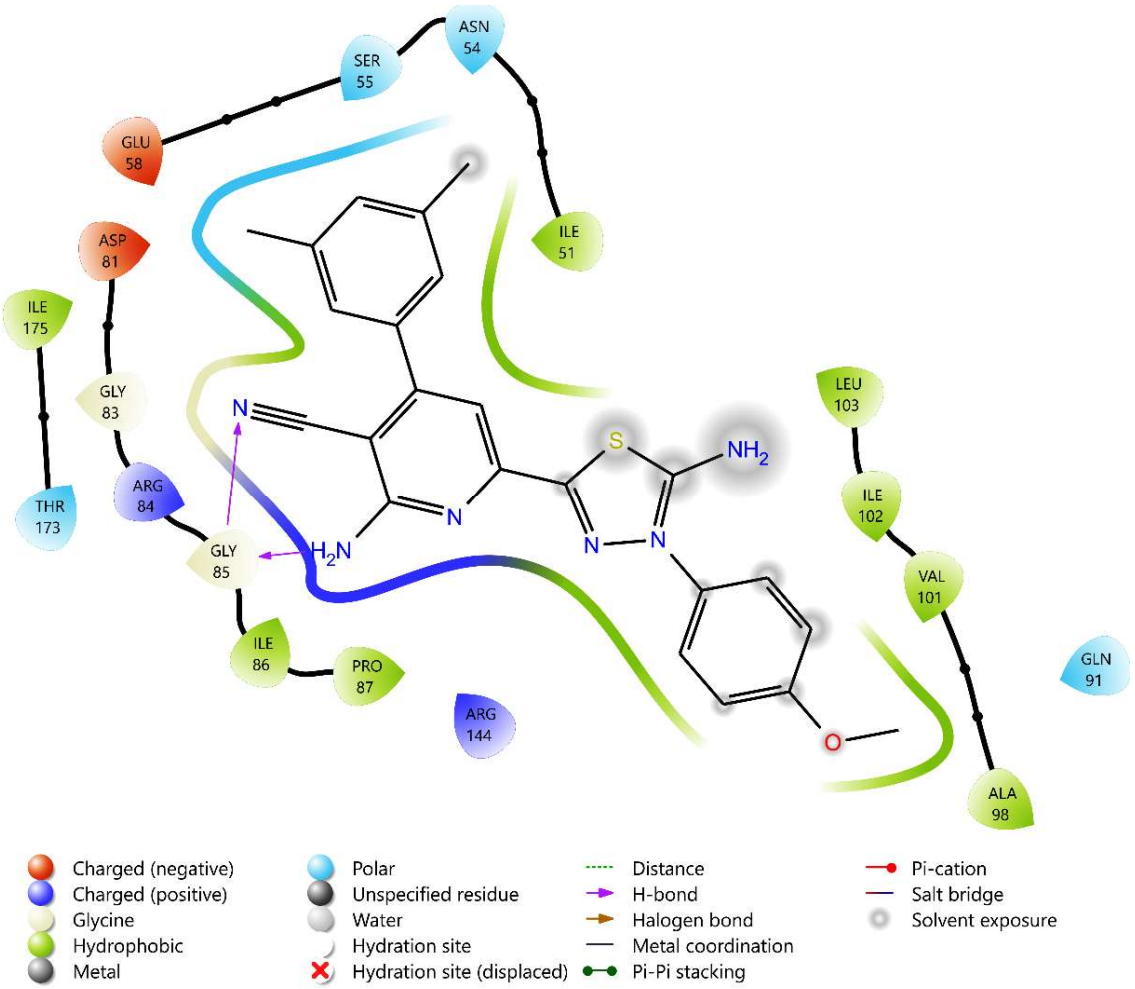
Halogen bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

5f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GLN 91
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Polar
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Metal coordination
Pi-Pi stacking
Pi-cation
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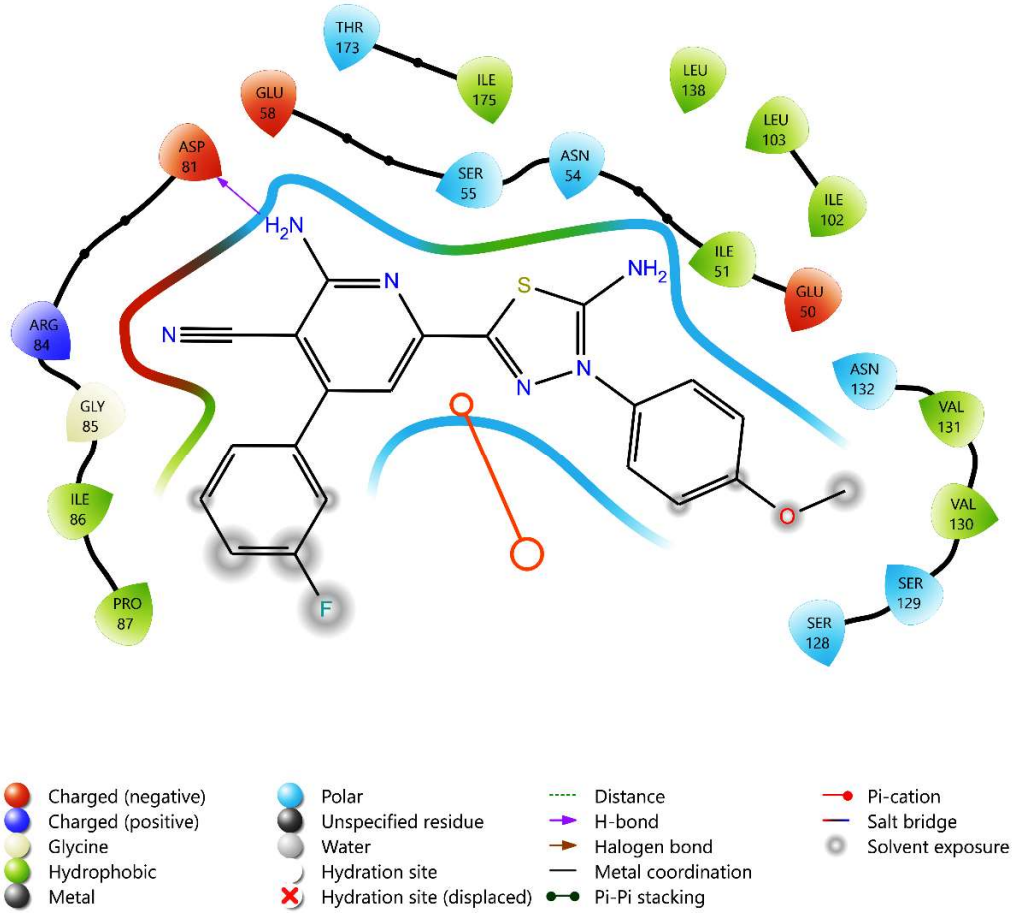
Solvent exposure

5g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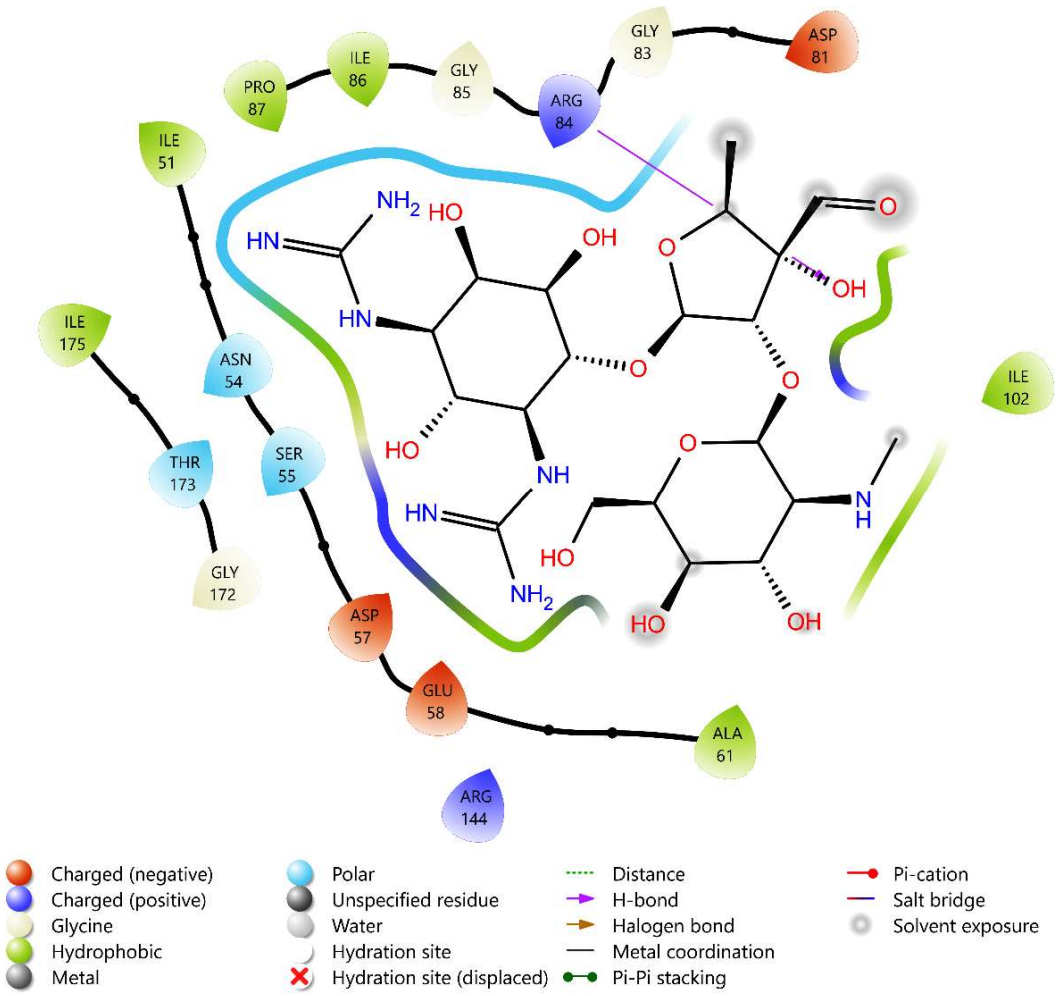
Salt bridge
Solvent exposure

5h



Streptomycin

Figure 5.3. Binding Interactions of (5a-5h and Streptomycin) with PDB 5D6P

Most of the synthesized compounds formed at least one conventional hydrogen bond, reinforcing their binding stability.

- ASP81 emerged as a common hydrogen bonding residue in multiple compounds (5a, 5b, 5f, 5h), indicating its key role in ligand anchoring.
- Compound 5c formed multiple hydrogen bonds with GLY85, GLU58, and GLN91, explaining its exceptionally high binding affinity.
- Surprisingly, compounds 5d and 5e did not show any conventional hydrogen bonds but still maintained strong binding energies, likely due to robust hydrophobic interactions and van der Waals forces.

In contrast, Streptomycin formed only a single hydrogen bond with ARG84, suggesting weaker stabilization within the active site compared to the novel derivatives. All test compounds demonstrated extensive interactions with multiple amino acid residues, suggesting comprehensive engagement with the active site. Some key findings include:

- Residues such as PRO87, ILE86, GLY85, ARG84, SER55, ASN54, ILE51, GLU50, THR173, ILE175, LEU103, and ILE102 were consistently involved across all ligands, marking them as crucial points of contact.
- Compound 5c interacted with ARG44, ALA98, VAL101, and GLY172, indicating deeper or slightly altered binding site engagement compared to other ligands.
- Compounds 5g and 5e also showed interactions with GLN91 and ARG144, residues involved in the Streptomycin complex, further affirming their potential to mimic or surpass the reference drug's activity.

Overall, the docking analysis with PDB ID: 5D6P suggests that the synthesized compounds 5a–5h have superior binding affinities and interaction profiles compared to Streptomycin. Compound 5c, in particular, emerged as the most promising candidate due to its highest binding energy and multiple hydrogen bonding interactions. These results strongly suggest that all synthesized derivatives have greater potential for enzyme inhibition than the reference drug.

CONCLUSION

Compounds 5d, 5c, and 5e could serve as promising candidates for further development as broad-spectrum antimicrobial agents. Structural optimization of weaker compounds (e.g., 5a and 5b) might enhance their activity spectrum.Based on Lipinski's parameters, all synthesized compounds (5a–5h) exhibit favorable physicochemical profiles that align well with drug-likeness criteria. The results suggest that combining compounds or optimizing their formulations could yield enhanced activity across a broader microbial spectrum. The results highlight that the test compounds, particularly 5d, 5c, and 5e, possess significant antimicrobial and antifungal potential. Their performance relative to standard drugs like streptomycin and fluconazole underscores their potential as versatile therapeutic agents. The molecular docking investigations of synthesized compounds 5a–5h against two crucial bacterial target proteins, 2EG7 and 5D6P, demonstrated promising inhibitory potential compared to the reference drug Streptomycin. All compounds exhibited strong binding affinities, with values consistently better than Streptomycin. Notably, compound 5c emerged as the top performer with a binding energy of -

9.8 kcal/mol (5D6P) and -8.4 kcal/mol (2EG7). Common residues such as ALA266, HIS254, ARG84, ASP81, GLU58, SER55, and ILE51 were frequently involved in ligand interactions, indicating that the test compounds are effectively engaging the active sites of both enzymes. Hydrogen bonding analysis revealed that ASP81 (in 5D6P) and ALA266/LEU222 (in 2EG7) are key stabilizing anchors for most compounds, particularly 5a, 5b, and 5h. Interestingly, even in the absence of conventional hydrogen bonding, compounds such as 5d and 5e still maintained high binding energies, suggesting a significant contribution from hydrophobic and van der Waals interactions. Overall, the docking profiles indicate that the test compounds not only mimic the interaction behavior of Streptomycin but also exhibit enhanced binding energies and broader receptor engagement. Further studies, including mechanism exploration and in vivo evaluation, are recommended to establish their efficacy and safety profiles.

Spectra

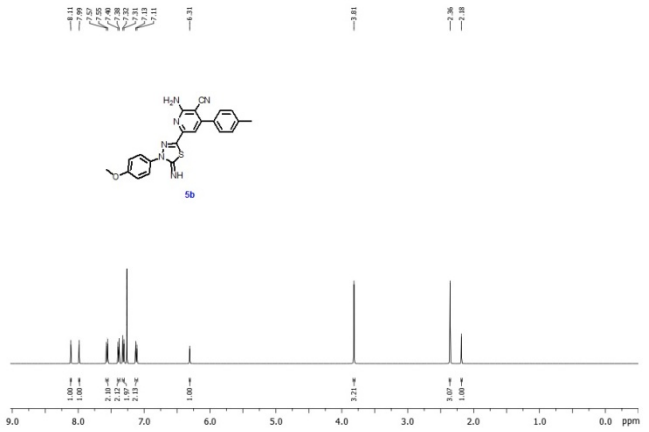


Figure 5.4: ^{1}H NMR spectrum of Compound 5b

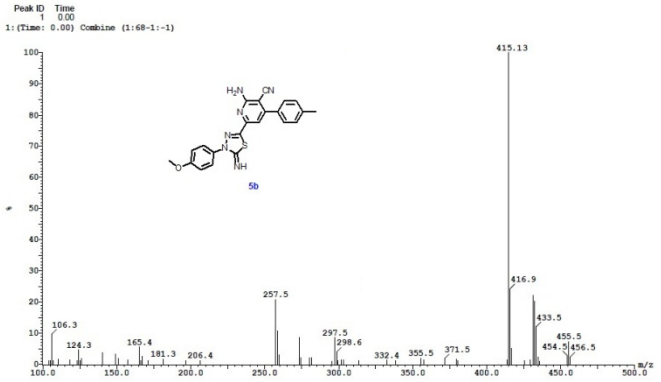


Figure 5.5: Mass spectrum of Compound 5b

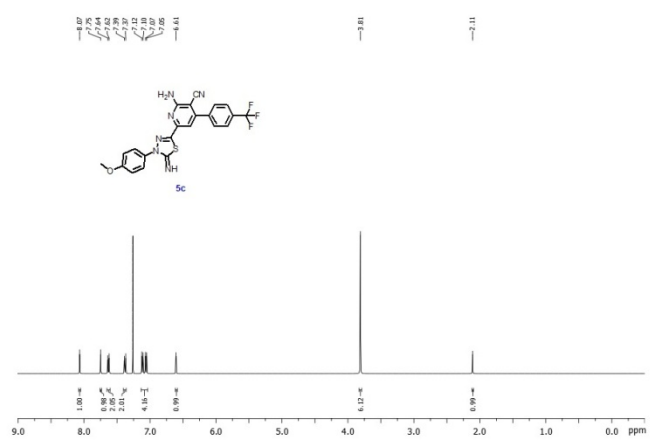


Figure 5.6: ^{1}H NMR spectrum of Compound 5c

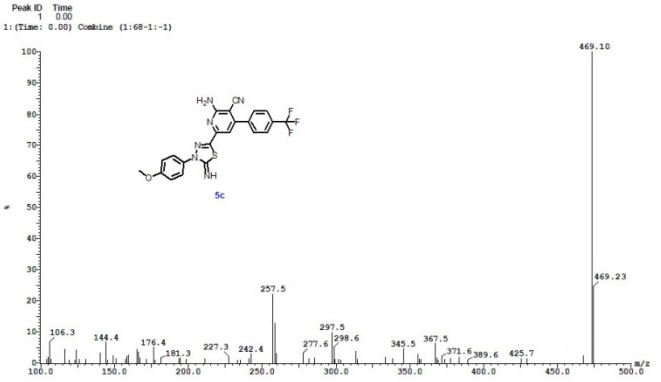


Figure 5.7: Mass spectrum of Compound 5c

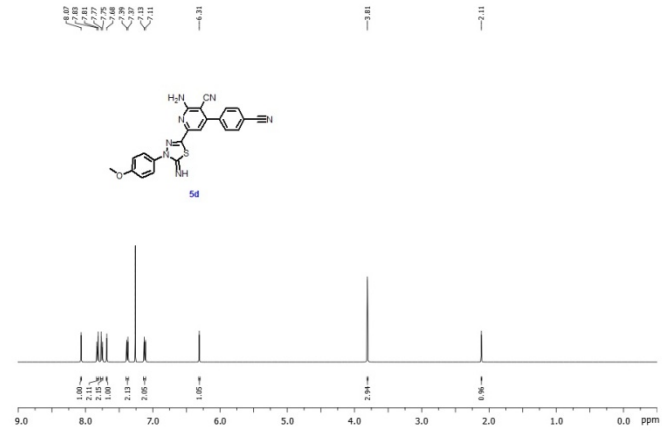


Figure 5.8: ^{1}H NMR spectrum of Compound 5d

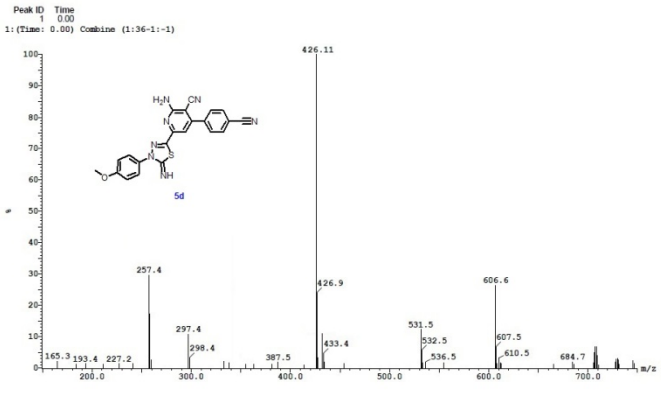


Figure 5.9: Mass spectrum of Compound 5d

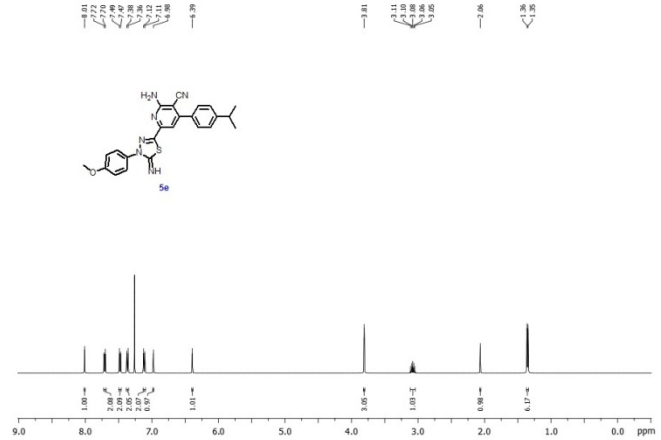


Figure 5.10: ^{1}H NMR spectrum of Compound 5e

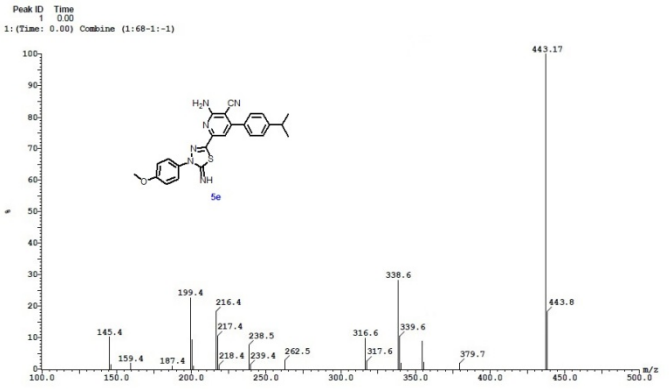


Figure 5.11: Mass spectrum of Compound 5e

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