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Synthesis, Characterization And Biological Activities Of Novel Methoxy Bezaldehyde Derivatives Of 4-Aminopyrrolo [2, 3-*D*] Pyrimidine

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Abstract
The primary aims of this investigation encompassed the synthesis, characterization, and assessment of antibacterial and antifungal properties of novel pyrrolopyrimidine derivatives. The focus was directed towards the synthesis of methoxy-benzaldehyde derivatives of pyrrolopyrimidine. Spectroscopic techniques were employed for the characterization of synthesized compounds. The antimicrobial efficacy of the synthesized compounds was evaluated against various bacterial and yeast strains, including S. aureus MCC 2010, B. subtilis MCC 2010, E. coli MCC 2412, P. aeruginosa MCC 2080, C. albicans MCC 1439, and S. cerevisiae. Notable peaks were observed in FT(IR), ^{1}H and ^{13}C NMR, and UV spectral analyses for all prepared compounds. Compounds E and g exhibited superior in vitro antibacterial and antifungal activity compared to streptomycin and fluconazole, considered as gold standards.

Keywords: *Methoxy benzaldehyde, pyrrolopyrimidine, antibacterial and antifungal activity*

1. INTRODUCTION:

The significance of pyrimidines and their analogs is well-established within the scientific community. Pyrimidines and purines, heteroaromatic compounds, stand out as fundamental constituents in natural systems. Pyrrolopyrimidine (7H-PP4A) derivatives have garnered attention from researchers due to their potential in chemotherapy applications. Analogous fused heterocycles have piqued interest as they might exhibit bioactive properties. Their biological functionalities span a wide spectrum, encompassing enzyme inhibition [1], cytotoxicity [2], antiviral [3], anti-inflammatory [4-6], anti-allergenic [77], anti-tumor [9-12], as well as antibacterial and antifungal activities [13]. This study presents the synthesis and antibacterial assessment of a novel series of (7H-PP4A) derivatives derived from substituted methoxy benzaldehydes, building upon these insights.

In this paper, we reported the synthesis and antibacterial activity of a novel family of (7H-PP4A) derivatives of substituted methoxy benzaldehydes based on these findings.

Experimental:

All raw materials were obtained from commercial providers and used unaltered unless otherwise noted. Using silica gel plates, thin-layer chromatography (TLC) analysis was performed. Certain chemicals' melting points were not changed for accurate measurement. Potassium bromide (KBr) pellets were used to record Fourier-transform infrared (FT-IR) spectra in the 4000-500 cm^{-1} range using a BRUKER FT-IR spectrophotometer. Using a JASCO V650 spectrophotometer, ultraviolet-visible (UV-Vis) spectra were obtained in methanol at ambient temperature. Using TMS as the internal reference standard, proton nuclear magnetic resonance (^{1}H NMR) spectra in deuterated dimethyl sulfoxide (DMSO-d_6) at 400 MHz were acquired. The concentrations of carbon (C), hydrogen (H), and nitrogen (N) were all within 0.4% of their theoretical levels. There are offered summary statistics for obtained chemicals.

Preparation of (7H-PP4A) derivatives (1e-1h):

A solution containing substituted benzaldehyde (**1-4**) (1.0 mmol) and 4- amino-7H-pyrrolo[2,3-d]pyrimidine (7HPP4A) (1.0 mmol) dissolved in Dimethyl formamide (4.0 mL) was transferred into a microwave reaction vessel and subjected to microwave irradiation at 100 °C for the specified duration. The reaction progress was monitored using thin-layer chromatography (TLC). Upon cooling, the solvent was evaporated under reduced pressure. Subsequently, water (20 mL) was introduced to the reaction mixture, followed by extraction with Dichloromethane (2 × 10 mL). The combined organic phases underwent further purification via column chromatography on silica gel, yielding the desired pure products **1e-1h**

(7H-PP4A) -*o*-methoxybenzaldehyde (1e):

Colour, Brown; M.W., 252.27; Yield (%), 80.25; M.P. (°C), 202; Element content: C, 63.82; H, 5.00; N, 19.85; O, 11.34. FT-IR (cm^{-1}): 3375 (NH), 3022 (-OCH_3), 2836 (C-H), 1587/1459 (>C=C<), 1673 (>C=N-), 1333 (C-N aromatic amine), 767 (DSBR), 693 (MSBR). ^{1}H NMR (ppm): 12.970 (Ar-OH), 8.726 (aro-NH), 8.464 (-CH=), 3.867 (-OCH_3), 7.087-8.009 (aromatic amine), UV spectrum (λ_{nm}): 265 ($\pi\rightarrow\pi^*$), 372 ($n\rightarrow\pi^*$).

(7H-PP4A) -2,4-dimethoxybenzaldehyde (1f):

Colour, Brown; M.W., 282.30; Yield (%), 81.45; M.P. (°C), 205; Element content: C, 63.82; H, 5.00; N, 19.85; O, 11.34. FT-IR (cm^{-1}): 3433 (NH), 3020 (-OCH_3), 2836 (C-H), 1589/1456 (>C=C<), 1655 (>C=N-), 1334 (C-N aromatic amine), 763 (di sub benz ring), 692 (MSBR). ^{1}H NMR (ppm): 12.822 (Ar-NH), 8.902(-CH=), 3.883 (-OCH_3), 7.077-8.579 (aromatic amine), UV spectrum (λ_{nm}): 277 ($\pi\rightarrow\pi^*$), 356 ($n\rightarrow\pi^*$).

(7H-PP4A) -2,5-dimethoxybenzaldehyde (1g):

Colour, Yellow; M.W., 282.30; Yield (%), 83.33; M.P. (°C), 203; Element content: C, 61.41; H, 3.96; N, 22.04; O, 12.59. FT-IR (cm^{-1}): 3115 (-OH), 3355 (NH), 2873 (C-H), 1587/1472 (>C=C<), 1651 (>C=N-), 1335 (C-N aromatic amine), 729 (DSBR), 697 (MSBR). ^{1}H NMR (ppm): 12.963 (Ar-OH), 9.860 (aro-NH), 9.029 (-CH=), 6.741-8.431 (aromatic amine), UV spectrum (λ_{nm}): 289 ($\pi\rightarrow\pi^*$), 350 ($n\rightarrow\pi^*$).

(7H-PP4A) -3,4-dimethoxybenzaldehyde (1h):

Colour, Yellow; M.W., 282.30; Yield (%), 79.30; M.P. (°C), 204; Element content: C, 62.68; H, 4.51; N, 20.88; O, 11.98. FT-IR (cm^{-1}): 3317 (-OH), 3118 (NH), 2910 (C-H), 1583/1477 (>C=C<), 1672 (>C=N-), 1334 (C-N aromatic amine), 722 (DSBR), 690 (MSBR). ^{1}H NMR (ppm): 12.372 (Ar-OH), 10.557 (aro-NH), 7.999 (-CH=), 6.981-7.712 (aromatic amine), UV spectrum (λ_{nm}): 297 ($\pi\rightarrow\pi^*$), 363 ($n\rightarrow\pi^*$).

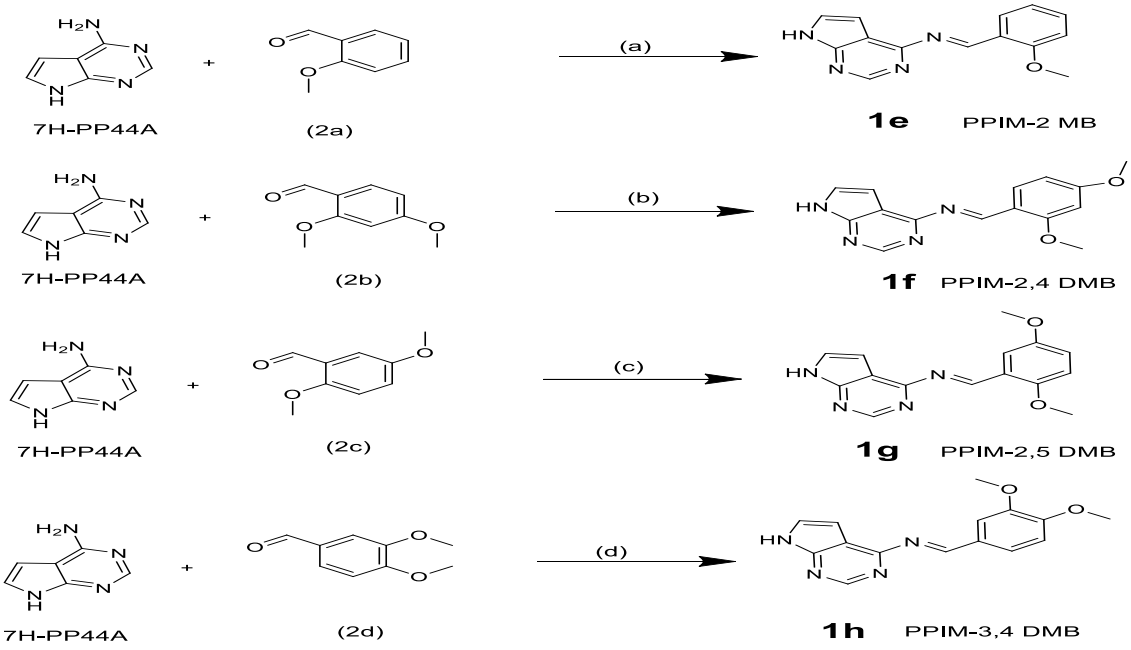

Figure 1: The synthesis of substituted methoxy benzaldehyde-(7H-PP4A) derivatives

Antimicrobial Assay*:*

Test Microorganisms*:*

The antimicrobial efficacy of the synthesized compounds was assessed against two strains of gram-positive bacteria and two strains of gram-negative bacteria. Muller-Hinton agar medium was sterilized via autoclaving at 15 pounds per square inch (psi) for 15 minutes prior to its use in antimicrobial assays. The researchers employed the disc diffusion method to ascertain the antibacterial potential of the newly produced compounds. The inoculum was diluted to approximately 10^8 colony-forming units per milliliter (cfu/mL) by suspending the bacterial culture in sterile distilled water. Subsequently, 20 mL of Muller-Hinton agar medium was inoculated with each microbial strain and incubated for 15 minutes. Wells of 6 mm diameter were aseptically bored into the agar, and 100 microliters of a 4.0 mg/mL solution of each compound were dispensed into the wells. Following incubation at 37°C for 24 hours, the inhibition zones around the wells were measured to evaluate the antibacterial activity of the compounds. Streptomycin served as a positive control, while dimethylformamide (DMF) was utilized as a negative control in the experiments.

Determination of MIC*:*

The minimum inhibitory concentrations (MICs) of all drugs were determined using the adapted disc diffusion method. Synthesized compounds were prepared at concentrations ranging from 10 µg/mL to 1000 µg/mL, derived from a stock solution of 4 mg/mL dissolved in dimethylformamide (DMF). A standardized inoculum of the microorganism, at a concentration of 10^8 colony-forming units per milliliter (cfu/mL), was spread onto agar plates in volumes of 100 µL, with triplicate wells inoculated for each dilution. Following 24 hours of incubation at 37°C, the plates were examined for the presence of inhibitory zones. Streptomycin was utilized as a positive control in the experiments.

Antifungal Activity:

The compounds were assessed against two distinct fungal strains (Candida albicans 1439 and Saccharomyces cerevisiae MCC1033) utilizing the cup-and-plate technique. Test solutions were pipetted onto 5 mm diameter and 1 mm thick discs using micropipettes, followed by incubation of the plates at 37°C for 72 hours. During this incubation period, the test solutions diffused through the agar medium, exerting their effects on the growth of the fungal pathogens. After 36 hours of incubation at 37°C, the size of the inhibition zones was measured. Compounds showing promising antifungal activity underwent further investigation to determine their minimum inhibitory concentrations (MICs). The MIC of an antifungal agent represents the lowest concentration at which visible inhibition of microbial growth is observed following a 24-hour incubation period. Clinical laboratories utilize the minimal inhibitory concentration (MIC) to confirm microbial resistance to antimicrobial agents and to assess the effectiveness of novel antimicrobial compounds.

***In vitro* cytotoxicity:**

The cytotoxicity of the synthesized compounds was assessed using a bioassay employing brine shrimp. Prawn eggs were placed on one side of a tank, while artificial seawater (containing 38 g NaCl per 1000 mL tap water) was provided on the other side. Over a 48-hour period, the shrimp eggs hatched and the nauplii developed. Subsequently, the newly hatched shrimp were collected for testing. Various concentrations (ranging from 2.5 to 12.0 mg/10 mL) of dried complexes were prepared in separate test tubes. DMSO was dissolved in these complexes to assess their cytotoxic potential. Each test tube contained 10 live shrimp, which were transferred using a Pasteur pipette. A control group was included to ensure the reliability of the cytotoxicity assay and the resulting data. After 24 hours of incubation, the contents of the tubes were examined under a microscope, and observations were recorded regarding the survival of nauplii. Each experiment was performed in triplicate, totaling five sets of experiments. Using the obtained data, the LC50, 95% confidence interval (CI), LC90, and chi-square values were determined. Abbott's formula was utilized to adjust the data to compensate for any mortality observed in the control group.

RESULTS AND DISCUSSION:

Chemistry:

(7H-PP4A) underwent a reaction with substituted methoxy benzaldehydes under reflux conditions for 2-5 hours, yielding the corresponding compounds. Desired products were obtained with yields ranging from 79.30% to 83.33%. The synthetic route for obtaining substituted benzaldehyde (7H-PP4A) derivatives is illustrated in **Figure 1**. These synthesized compounds exhibit compatibility with a wide range of organic solvents but are not soluble in water. Elemental analysis data confirms the expected compositions of all derivatives. The compounds appear as colored powders with no tendency to absorb moisture. Thin-layer chromatography (TLC) was employed to assess the purity of the compounds post-synthesis.

FT(IR) spectra:

FT-IR spectra were analyzed to investigate the bonding efficiency of (7H-PP4A) with substituted methoxy benzaldehydes. By selecting relevant bands, we explored the impact of (7H-PP4A) vibration on substituted methoxy benzaldehydes. The emergence of a distinct new band in the range of 1651-1673 cm^{-1} corresponding to the azomethine (HC=NN-) group confirmed the formation of all synthesized compounds and indicated that the aldehyde (CHO) and amino (NH_2) functionalities of the amino derivatives hinder their stretching vibrations. The presence of a continuous band spanning 3317-3433 cm^{-1}, attributed to aromatic (NH) groups, suggested the presence of these compounds in their processed forms. Bands within the range of 2836-2910 cm^{-1} were characteristic of aldehydic substances. Two separate bands, observed at 1583-1587 and 1456-1477 cm^{-1}, were apparent in the infrared spectra of compounds **1e-1h**, both corresponding to the >C=C group of an aromatic ring. In the FT-IR spectra of compounds **1e-1h**, the aromatic (C-N) band was detected at 1334-1335 cm^{-1}, the di/trisubstituted benzene ring at 722-767 cm^{-1}, and the monosubstituted benzene ring at 690-697 cm^{-1}. A band around 3020-3118 cm^{-1} observed in the FT-IR spectrum of compound **1e-1h** was attributed to the aromatic -OCH_3 group.

^{1}H NMR spectra:

All compounds were characterized by their ^{1}H NMR spectra, wherein large singlet signals appeared in the range of 12.372-12.976 ppm, indicative of the presence of a -NH- group within the pyrrolyl ring. Singlet peaks corresponding to aromatic -CH= groups were observed in the ranges of 7.999-9.029 ppm. To verify the successful substitution of the amino group by Schiff base, the ^{1}H NMR spectra of all synthesized derivatives were examined, revealing a broad singlet signal at 9.84 ppm (2H), corresponding to the -NH_2 of 4-chloro-7H-pyrrolo[2,3-d]pyrimidine. Additionally, singlet signals at 3.854-3.913 ppm in the ^{1}H NMR spectra of compounds **1e-1h** were indicative of the -OCH_3 group on the aromatic ring. The observed ^{1}H NMR spectra are consistent with previously published data [23,24].

UV-Visible spectra:

The compounds **1e-1h**, which were synthesized, were dissolved in dimethylformamide (DMF), and their UV spectra were recorded at room temperature. The aromatic band observed in compounds **1e-1h**, ranging from 233 to 298 nm in wavelength, arises from the $\pi \rightarrow \pi^*$ transition within the benzene ring. Additionally, in compounds 2a-h, the $n \rightarrow \pi^*$ transition of the non-bonding electrons located on the nitrogen of the azomethine groups extends the band to 327-370 nm.

Biological studies:

Antibacterial studies:

The synthesized compounds were subjected to in vitro testing to evaluate their antibacterial activity against a range of bacterial and fungal strains. During the antibacterial screening, the minimum inhibitory concentration (MIC) values of all compounds against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa ranged from 7.5 to 29 mm. Notably, all synthesized compounds exhibited

superior inhibitory effects (ranging from 20.5 to 25.5 mm) against Staphylococcus aureus compared to streptomycin.

Table 3: Antibacterial studies of **1e-1h** compounds

Compound	Antibacterial Activity (zone of inhibition)			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1e	21.0	29.0	21.5	17.5
1f	24.0	25.0	19.5	19.5
1g	24.5	12.0	20.0	20.0
1h	24.5	14.0	18.5	19.0
Streptomycin	15.5	14.5	12.0	14.0

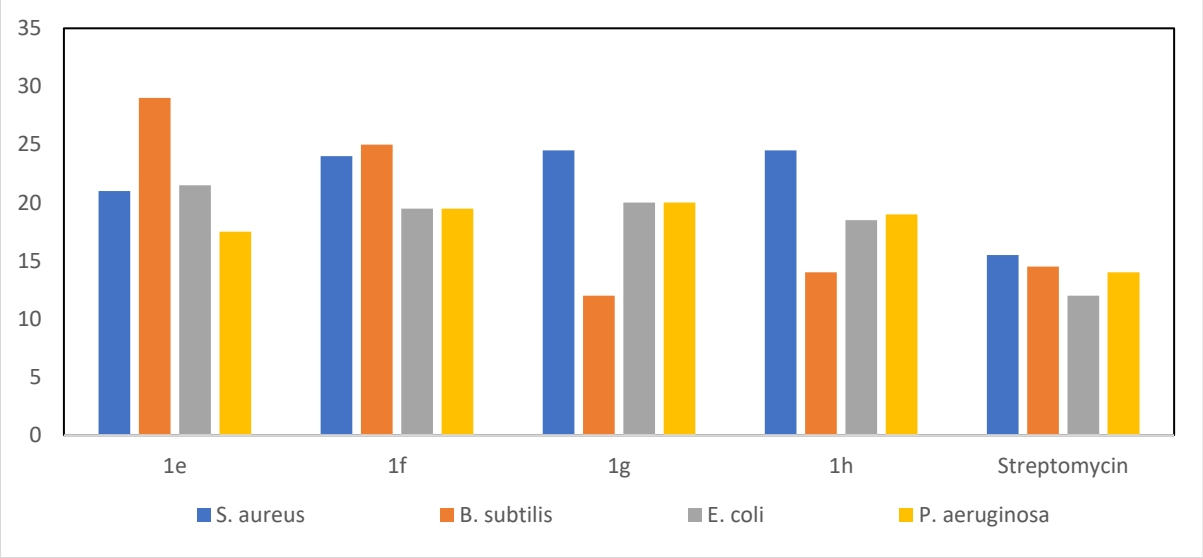

Figure-1: Antibacterial activities of **1e-1h** compounds and standard drug

Antifungal studies:

In antifungal experiments against the fungi Candida albicans MCC1439 and Saccharomyces cerevisiae MCC1033, the synthesized compounds exhibited approximately 2.5-fold greater efficacy compared to the reference drug fluconazole (with inhibition zones ranging from 10.5 to 14.0 mm)

Table 2: Antifungal studies of **1e-1h** compounds

Compound	***Candida albicans***	***Saccharomyces cerevisiae***
1e	14.5	12.5
1f	18.5	18.0
1g	14.0.	17.5
1h	17.0	11.0
Fluconazole	10.5	14.0

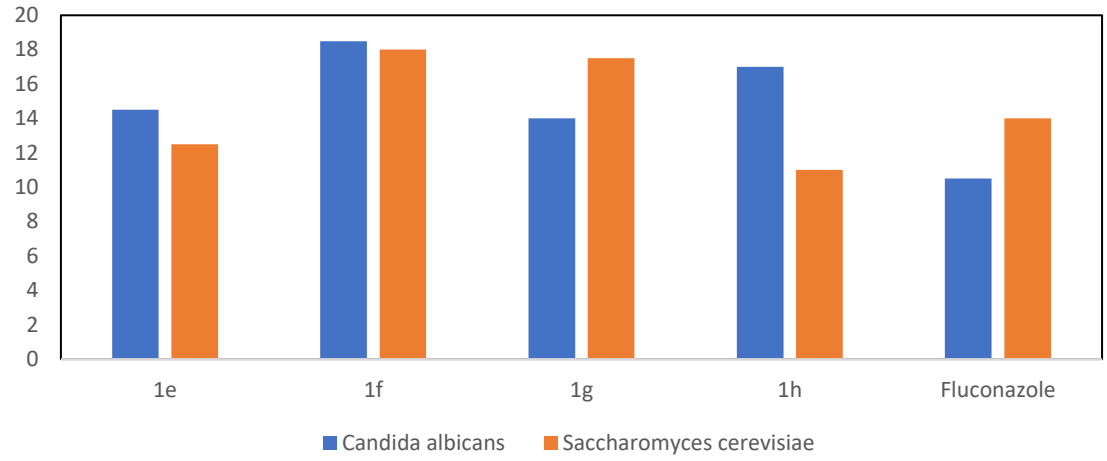

Figure-2: Antifungal activities of **1e-1h** compounds and standard drug

***In vitro cytotoxicity*:**

All synthesized compounds demonstrated cytotoxic activity against Artemia salina, with LD50 values ranging from 3.50 to 8.50 x10^{-4} M/mL.

Table 3: Brine shrimp bioassay of 1e-**1h** compounds

Compound	**LD_{50} (M)**
1e	$>7.00 \times 10^{-4}$
1f	$>4.00 \times 10^{-4}$
1g	$>3.50 \times 10^{-4}$
1h	$>7.50 \times 10^{-4}$
Vincristine Sulphate	$>5.28 \times 10^{-4}$

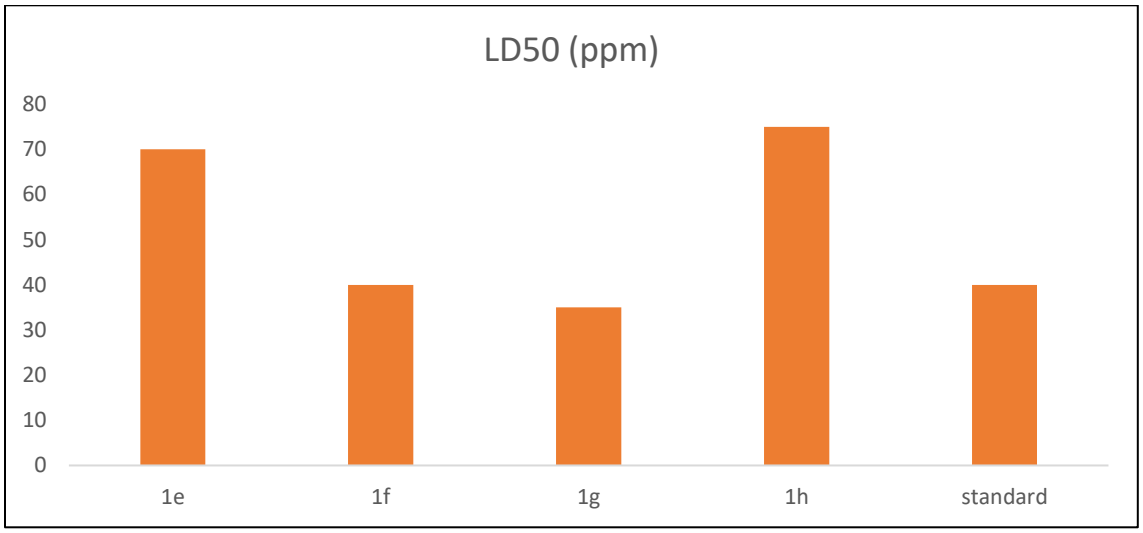

Figure-3: Brine shrimp bioassay of 1e**-1h** compounds and standard drug

Dcking data:

Drug-Likeness and Pharmacokinetic Profiles:

The drug-likeness and pharmacokinetic properties of compounds 1a to 1b were comprehensively evaluated using the SwissADME platform, with all compounds demonstrating favorable attributes within established thresholds for drug-like molecules. Molecular weights ranged from 252.27 Da to 282.30 Da, well below the 500 Da limit, indicating excellent potential for membrane permeability and gastrointestinal absorption. The

counts of hydrogen bond acceptors (HBA) and donors (HBD) adhered to the recommended limits of ≤ 10 and ≤ 5, respectively, supporting effective molecular interactions with biological targets. Topological polar surface area (TPSA) values, ranging from 63.16 Å 2 to 72.39 Å 2, remained significantly below the 130 Å 2 threshold, suggesting strong passive membrane permeability. Lipophilicity, assessed via Log P values, fell between 2.42 and 2.48, aligning with the optimal range of -0.7 to 5.0, which indicates a balanced profile for solubility and permeability. Aqueous solubility, represented by Log S values from -3.22 to -3.27, confirmed acceptable solubility characteristics. All compounds exhibited high gastrointestinal absorption, a critical factor for oral bioavailability. Compliance with Lipinski's Rule of Five was observed across all compounds, with no violations, and each compound achieved a bioavailability score of 0.55, suggesting a reasonable likelihood of systemic exposure. Synthetic accessibility scores, ranging from 2.66 to 2.85, indicated that these compounds are feasible for laboratory synthesis. Additionally, a root-mean-square Deviation (RMSD) value of 0.897 Å validated the reliability of binding pose alignments, reflecting high docking accuracy. Collectively, these results underscore the potential of 1a-1b as promising candidates for further drug development due to their robust drug-like properties and favorable pharmacokinetic profiles. Molecular Docking Studies Molecular docking studies were conducted to assess the binding affinities and interaction profiles of compounds 1a-1b against selected bacterial and fungal protein targets, with streptomycin and fluconazole serving as reference standards. The docking scores of the test compounds were compared to evaluate their potential as antimicrobial agents.

Gram-Positive Bacteria: Staphylococcus aureus (PDB: 2W9H):

Docking simulations against the Staphylococcus aureus protein (PDB: 2W9H) revealed binding energies for 1a-1b ranging from -8.4 to -8.8 kcal/mol, indicating strong interactions with the target. Streptomycin, the reference drug, exhibited a binding energy of -8.4 kcal/mol, forming hydrogen bonds with Leu24, Ser49, Ile14, and Trp22. In comparison, 1a demonstrated a slightly improved binding energy of -8.7 kcal/mol, forming hydrogen bonds with Thr96 and Thr46, and additional interactions with Lys45. 1b achieved the highest binding energy of -8.8 kcal/mol, driven by hydrophobic interactions with Ile50, Leu20, Ile14, and Phe98, despite lacking hydrogen bonds. 1c, with a binding energy of -8.5 kcal/mol, interacted with Ile14 and Lys45, while 1b matched streptomycin's binding energy (-8.4 kcal/mol) through multiple hydrogen bonds with Gly94, Thr46, Thr96, Thr121, and Gln95, supplemented by interactions with Ile14, Leu20, and Lys45. An RMSD value of 0.767 Å confirmed the precision of the docking poses. These findings highlight 1a and 1b as particularly promising candidates for further exploration due to their superior binding affinities.

Ligands	H-bond	Other Interactions	Binding Energy (kcal/mol)
Streptomycin	Leu 24, Ser 49, Ile 14, Trp 22	--------	-8.4
1e	Thr 96, Thr 46	Lys 45	-8.7
1f	----	Ile 50, Leu 20, Ile14, Phe98	-8.8

1g	----	Ile 14, Lys 45	-8.5
1h	Gly 94, Thr 46, Thr 96, Thr 121, Gln 95	Ile 14, Leu 20, Lys 45	-8.4

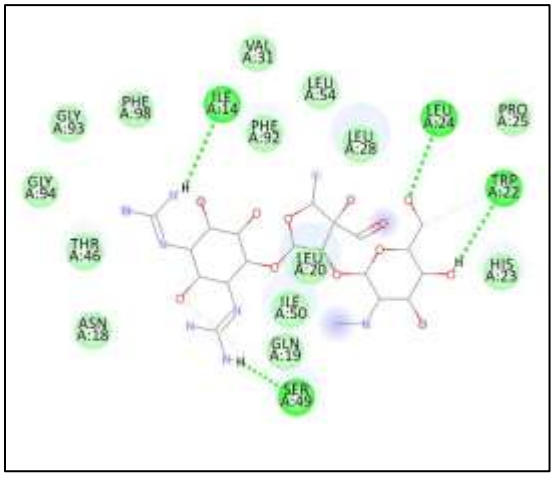

Fig. 1: Streptomycin 2D interactions with 2W9H

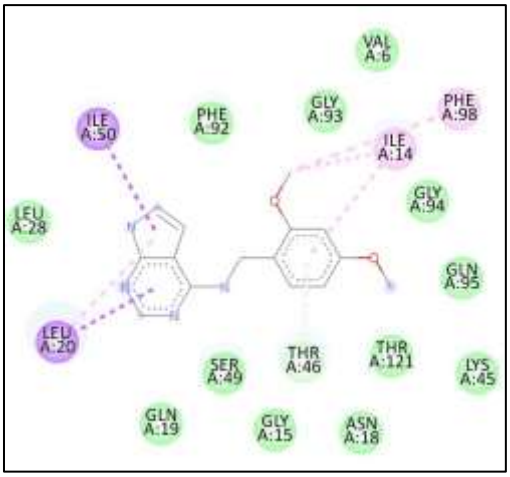

Fig. 2: CC 1 2D interactions with 2W9H

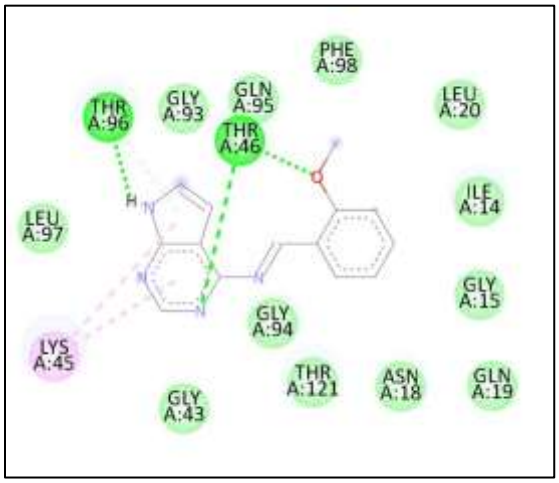

Fig. 3: CC 2 2D interactions with 2W9H

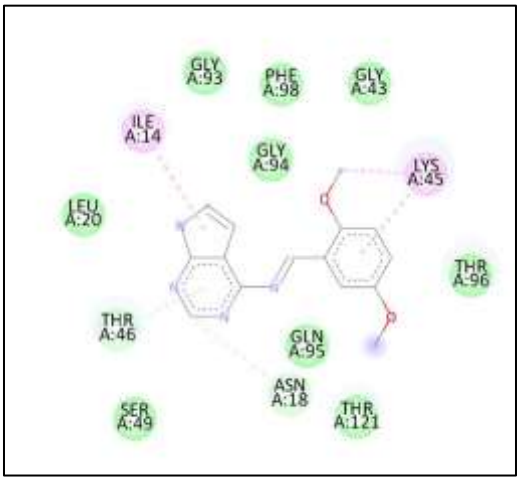

Fig. 4: CC 3 2D interactions with 2W9H

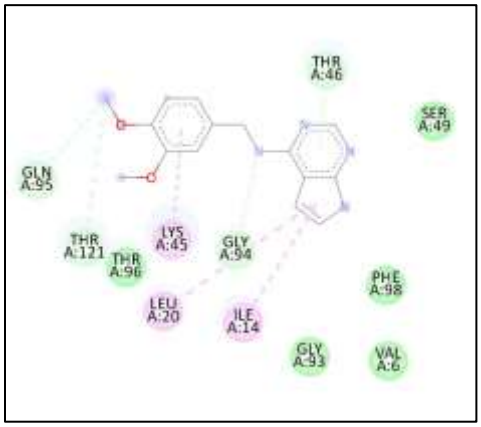

Fig. 5: CC 4 2D interactions with 2W9H

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Gram-Positive Bacteria: Bacillus subtilis (PDB: 2HQU)

For the Bacillus subtilis protein (PDB: 2HQU), streptomycin displayed a binding energy of -6.3 kcal/mol, forming hydrogen bonds with Gly110, Asp102, Gly156, Gln131, Ser86, Arg85, Arg153, Gly159, Gly87, and Asp49, and additional interactions with Gly47, Gly41, Gly157, and Mg^{2}ff (Mg998). Among the test compounds, 1c exhibited the highest binding affinity at -7.4 kcal/mol, forming hydrogen bonds with Phe158 and Arg85, and further interactions with Mg999, Thr161, Arg153, Tyr105, Ile101, Ala98, and Gly156. 1b and 1a showed binding energies of -6.5 kcal/mol and -6.3 kcal/mol, respectively, with 1a matching streptomycin's affinity through hydrogen bonds with Gly110 and Ser86, and interactions with Arg85, Mg997, Ala98, Ile101, Asn108, and Tyr105. 1b had the lowest binding energy at -5.6 kcal/mol, interacting with Phe158, Arg85, and other residues. An RMSD value of 0.772 Å affirmed the docking reliability. 1c's superior binding energy positions it as a leading candidate for further development.

Ligands	H-bond	Other Interactions	Binding Energy (kcal/mol)
Streptomycin	GLY 110, Asp 102, Gly 156, Gln 131, Ser 86, Arg 85, Arg 153, Gly 159, Gly 87, Asp 49	Gly 47, Gly 41, Mg 998, ,Gly 157,	-6.3
1e	Gly 110, Ser 86,	Arg 85, Mg 997, Ala 98, Ile 101, Asn 108, Tyr 105	-6.3
1f	Phe 158, Arg 85,	Thr 161, Arg 153, Gly 156, Asp 102, Ile 101, Tyr 105, Val 109, Gly 110	-5.6
1g	Phe 158, Arg 85,	Mg 999, Thr 161, Arg 153, Tyr 105, Ile 101, Ala 98, Gly 156	-7.4
1h	Ser 86, Gly 110	Asp 102, Arg 85, Mg 997, Ala 98, Ile 101, Phe 15, Tyr 105, Asn 108	-6.5

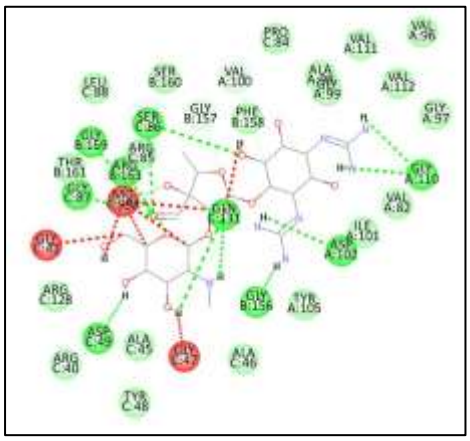

Fig. 6: Streptomycin 2D interactions with 2HQU

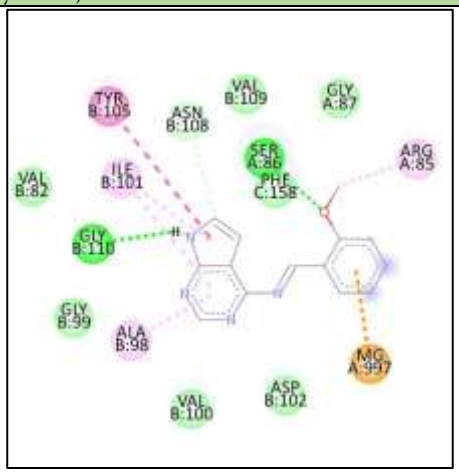

Fig. 7: CC 1 2D interactions with 2HQU

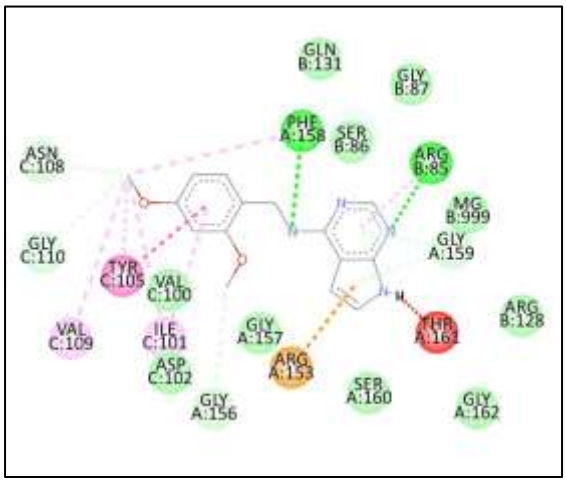

Fig. 8: CC 2 2D interactions with 2HQU

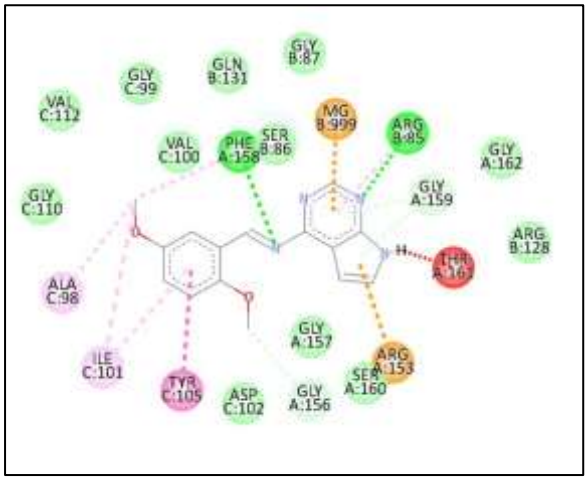

Fig. 9: CC 3 2D interactions with 2HQU

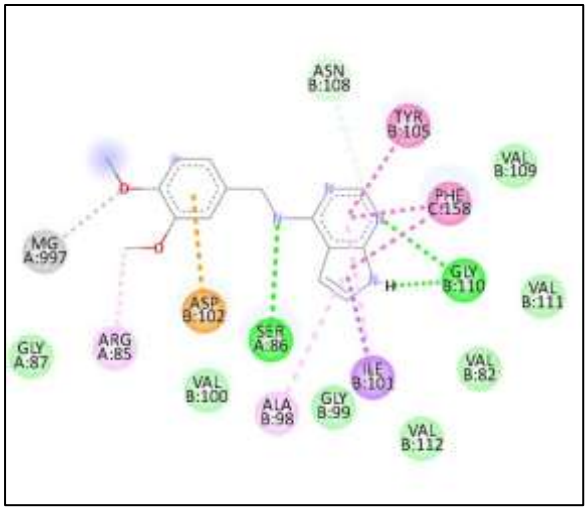

Fig. 10: CC 4 2D interactions with 2HQU

Gram-Negative Bacteria: Pseudomonas aeruginosa (PDB: 4DFR):

Docking against the Pseudomonas aeruginosa protein (PDB: 4DFR) showed streptomycin with a binding energy of -9.6 kcal/mol, forming hydrogen bonds with Ala145, Asn1, Trp22, Met20, Thr123, Ile14, Tyr100, Ala7, and Ile94, and additional interactions with Met16, Ser49, and His45. Among the

test compounds, 1b exhibited the highest binding energy at -7.7 kcal/mol, forming a hydrogen bond with Thr123 and interacting with Gly15, Met16, Cl160, Ile14, and Thr123. 1b and 1c both achieved -7.6 kcal/mol, with 1b forming a hydrogen bond with Asn23 and 1c with Thr123, alongside extensive interactions with key residues. 1a had the lowest binding energy at -7.5 kcal/mol, forming hydrogen bonds with Thr46 and Thr123. An RMSD value of 0.789 Å validated the docking accuracy. While streptomycin outperformed the test compounds, 1b and 1c showed competitive binding profiles, warranting further investigation.

Ligands	H-bond	Other Interactions	Binding Energy (kcal/mol)
Streptomycin	Ala 145, Asn 1, Trp 22, Met 20, Thr 123, Ile 14, Tyr 100, Ala 7, Ile 94, Ala 145	Met 16, Ser 49, His 45,	-8.6
1e	Thr 46, Thr 123	Ile 14, Gly 96, Met 16, His 45, Cl 160	-8.5
1f	Asn 23	Ala 19, Trp 22, Ser 49, Met 20	-8.6
1g	Thr 123	Ala 7, Ala 6, Tyr 100, Ile 14, Gly 15, Met 16, His 45, Cl 160, Thr 123	-8.6
1h	Thr 123	Gly 15, Met 16, Cl 160, Ile 14 Thr 123	-8.7

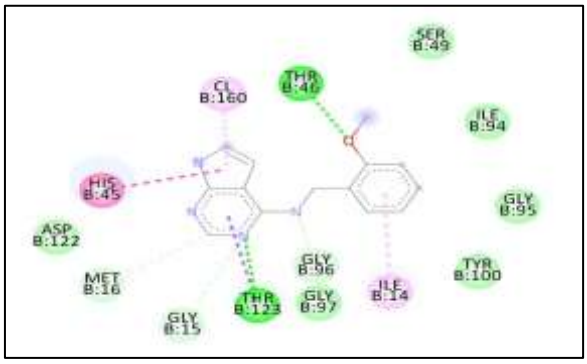

Fig. 11: Streptomycin 2D interactions with 4DFR

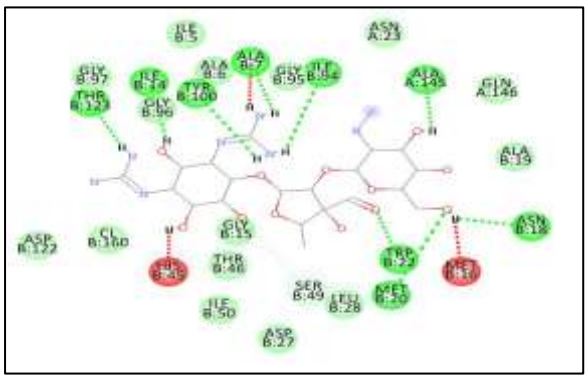

Fig.12: CC 1 2D interactions with 4DFR

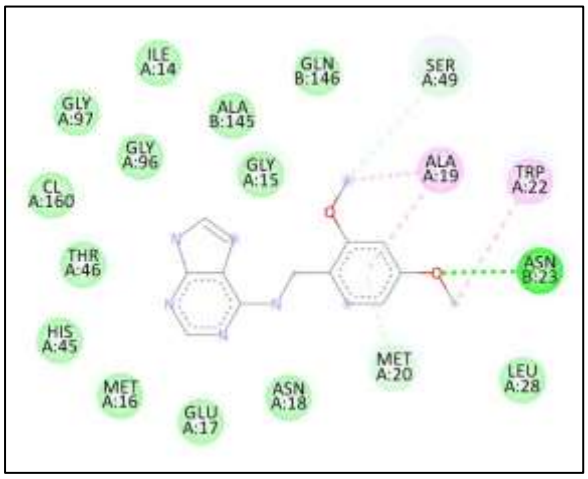

Fig.13: CC 2 2D interactions with 4DFR

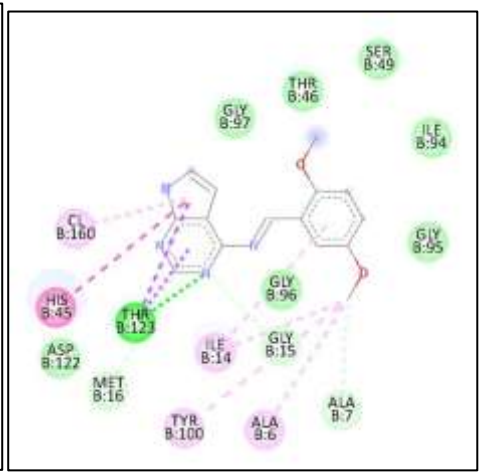

Fig.14: CC 3 2D interactions with 4DFR

Gram-Negative Bacteria: Escherichia coli (PDB: 1RX2)

For the Escherichia coli protein (PDB: 1RX2), streptomycin exhibited a binding energy of -7.1 kcal/mol, forming hydrogen bonds with Ala145, Asn18, Trp22, Met20, Thr123, Ile14, Tyr100, Ala7, and Ile94. 1b led the test compounds with a binding energy of -8.0 kcal/mol, forming a hydrogen bond with Asn23 and interacting with Ala19, Trp22, and Ser49. 1c followed closely at -7.9 kcal/mol, forming a hydrogen bond with Thr123 and extensive interactions with multiple residues. 1a and 1b recorded binding energies of 7.5 kcal/mol and -7.7 kcal/mol, respectively, with consistent interactions with key residues like Thr123 and Ile14. An RMSD value of 0.687 Å validated the docking precision. 1b and 1c's enhanced binding affinities highlight their potential for further optimization.

Ligands	H-bond	Other Interactions	Binding Energy (kcal/mol)
Streptomycin	Ala 145, asn 18, trp 22, met 20, thr 123, ile 14, tyr 100, ala 7, ile 94, ala 145	Met 16, ser 49, ser 49, his 45, gly 96, ala 7,	-7.1
1e	Thr 46, thr 123	Cl 160, his 45, thr 123, ile 14, met 16	-7.5
1f	Asn 23,	Ala 19, trp 22, ser 49	-8.0
1g	Thr 123	Cl 160, his 45, thr 123, ile 14, tyr 100, ala 6, met 16, gly 15, ala 7	-7.9
1h	Thr 123	Cl 160, Ile14, gly 15, met 16,thr 123,	7.7

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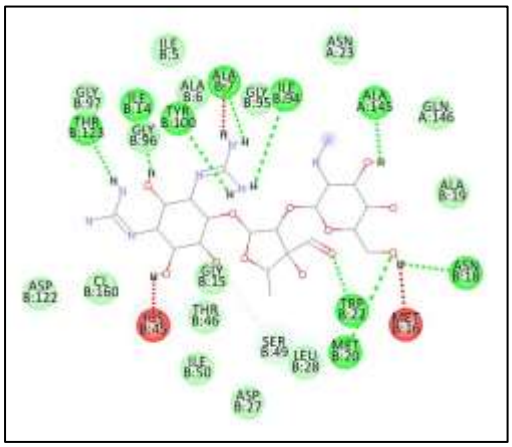

Fig. 15: Streptomycin 2D interactions with 1RX2

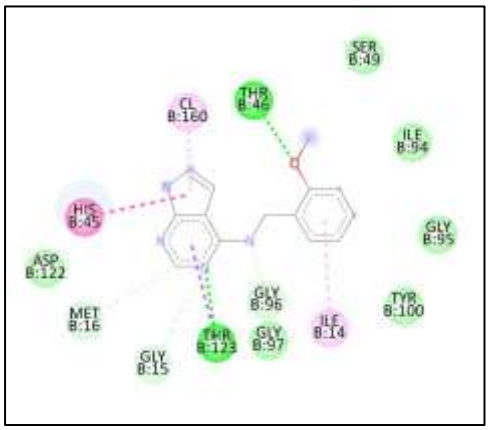

Fig. 16: CC 1 2D interactions with 1RX2

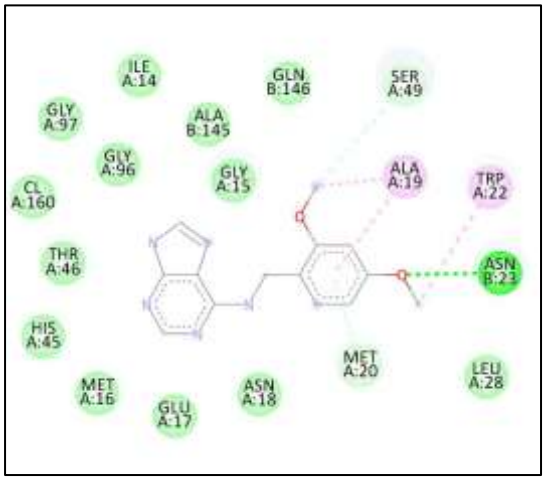

Fig. 17: CC 2 2D interactions with 1RX2

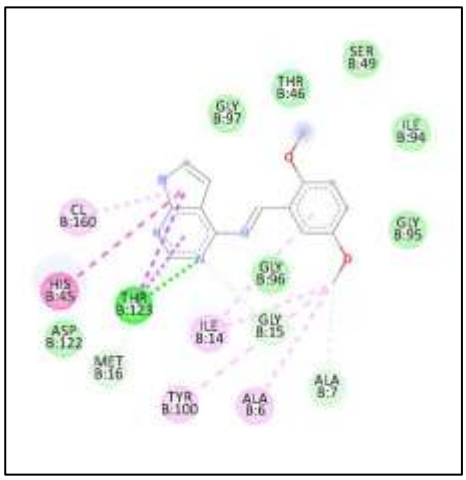

Fig. 18: CC 32D interactions with 1RX2

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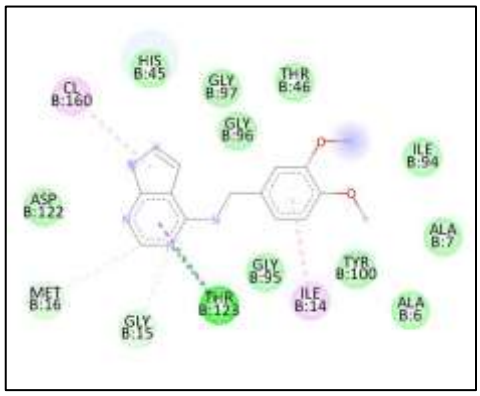

Fig. 19: CC 4 2D interactions with 1RX2

Fungi: Candida albicans and Saccharomyces cerevisiae (PDB: 5TZ1):

Docking against the fungal protein (PDB: 5TZ1) revealed fluconazole as the reference with a binding energy of -7.6 kcal/mol, forming hydrogen bonds with Phe52, Ser63, Ser74, and Glu70. Among the test compounds, 1a exhibited the highest binding affinity at -8.0 kcal/mol, forming a hydrogen bond with Trp54 and extensive interactions with Phe52, Lys75, Ala62, and Trp54. 1b followed with -7.8 kcal/mol, forming hydrogen bonds with Phe52, Trp54, and Lys78. 1c and 1b recorded binding energies of -7.7 kcal/mol and -7.5 kcal/mol, respectively, with 1c forming a hydrogen bond with Ser63 and 1b with Ile55. An RMSD value of 0.567 Å confirmed the reliability of the docking poses. 1a and 1b's superior binding energies suggest their potential as antifungal agents.

Ligands	H-bond	Other Interactions	Binding Energy (kcal/mol)
Fluconazole	Phe 52, Ser 63, Ser 74, Glu 70, Ser 63,	Ser 63,	-7.6
1e	Trp 54,	Phe 52, Lys 75, Ala 62, Phe 52, Phe 52, Trp 54	-8.0
1f	Ile55,	Phe 52, Trp 54, Val 51	-7.5
1g	Ser 63,	Trp 54, Trp 54, Ala 62, Phe 52	-7.7
1h	Phe 52, Trp 54, Lys 78	Ala 62, Phe 71, Trp 54	-7.8

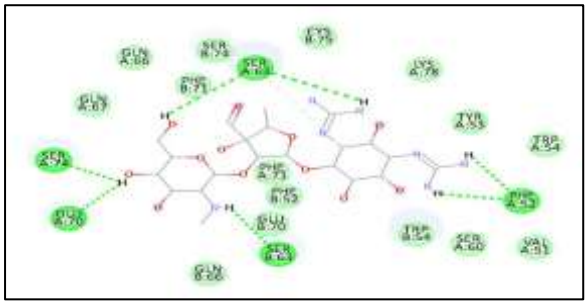

Fig. 20: Streptomycin 2D interactions with 5TZ1

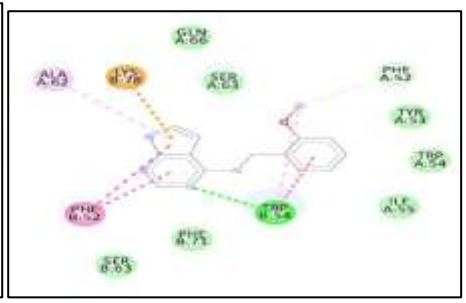

Fig. 21: CC 1 2D interactions with 4DFR

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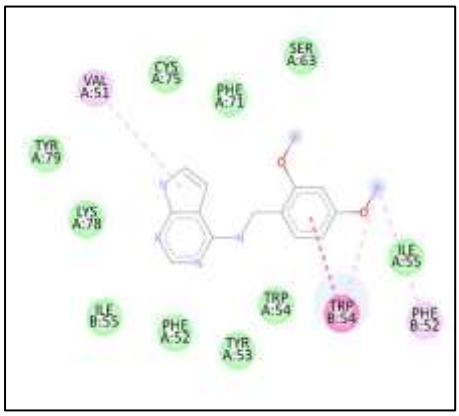

Fig. 22: CC 2 2D interactions with 4DFR

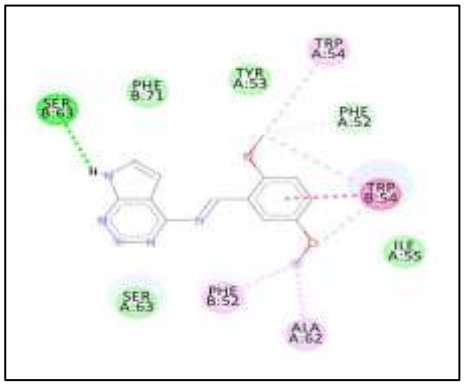

Fig. 23: CC 3 2D interactions with 4DFR

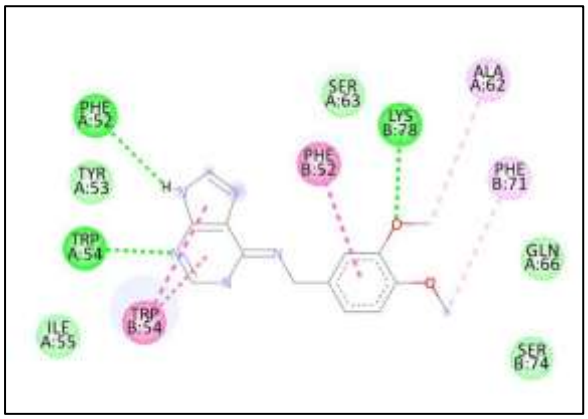

Fig. 24 CC 4 2D interactions with 4DFR

Pharmacokinetic and Drug-Likeness Evaluation:

The pharmacokinetic and drug-likeness properties of compounds 1a to 1d were assessed using the SwissADME web-based tool. This platform was employed to compute key physicochemical descriptors, including molecular weight (MW), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), topological polar surface area (TPSA), lipophilicity (Log P), and aqueous solubility (Log S). Additionally, parameters such as gastrointestinal (GI) absorption, bioavailability score, and synthetic accessibility were evaluated to determine the drug-like characteristics of the compounds. Compliance with Lipinski's Rule of Five was analyzed to ensure the compounds meet standard criteria for oral bioavailability. Molecular docking studies were performed to calculate the root mean square deviation (RMSD) of binding poses, with results indicating the accuracy of docking alignments. All calculations were conducted using default settings of the SwissADME server, and the resulting data were compiled for comparative analysis.

ADME Analysis (Absorption, Distribution, Metabolism, and Excretion)

Molecule	MW	HBA	HBD	TPSA	Log P	Solubility	GI absorption	Lipinski violations	Bioavailability Score	Synthetic Accessibility
Swiss ADME Ranges	<500	≤10	≤5	130 Å	-0.7 - 5	-6 - 0	-	0 - 2	0 – 1	1 - 10
1a	252.27	4	1	63.16	2.47	-3.22	High	0	0.55	2.66
1b	282.3	5	1	72.39	2.48	-3.27	High	0	0.55	2.85
1c	282.3	5	1	72.39	2.42	-3.27	High	0	0.55	2.85
1d	282.3	5	1	72.39	2.42	-3.27	High	0	0.55	2.75

CONCLUSION

In this study, we synthesized several novel derivatives of substituted methoxy benzaldehydes (1e-**1h**) utilizing (7H-PP4A) as a precursor. Analytical, spectral, and electrochemical data collectively support the successful synthesis of the proposed compounds. Spectral studies and elemental analysis (C, H, N, O) were employed to characterize the produced benzaldehyde-based compounds. Our findings indicate that (7H-PP4A) and substituted benzaldehydes should be combined in a 1:1 ratio. Each synthesized molecule exhibited potent antibacterial properties, and when tested on susceptible cell lines, all produced compounds displayed significant cytotoxicity.

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