Design, Synthesis And Spectral Charecterization Of Novel Schiff Base Of Pyridine-Phenylacetamide Derivatives As Anti-Bacterial Agents: An Approach To *In Vitro* And *In Silico* Studies

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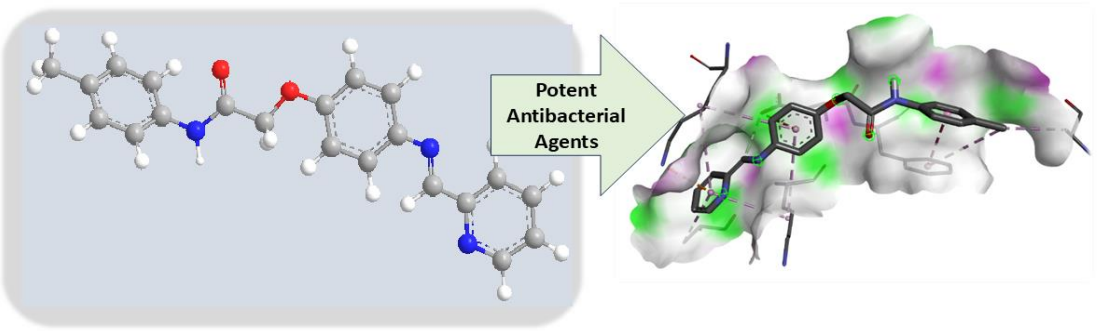
Abstract: *Based on the extensive range of pharmacological actions of Schiff bases and the pyridine moiety, new Schiff base derivatives of phenylacetamide-pyridine analogs were designed and synthesized by multistep synthesis in very good yields. A detailed charecterization of the structure, carried out using various spectroscopic techniques like ^{1}H NMR ^{13}C NMR, FT-IR, and LC-MS/GC-MS. In-vitro antibacterial tests against two bacteria indicated promising antibacterial functionality for final analogs. Further, molecular docking investigation has been achieved against crystal structure of dihydropteroate synthetase from staphylococcus aureus (1AD4) compared to standard drug ciprofloxacin and is in agreement with the in-vitro results. Structure-activity relationship (SAR) studies were conducted to understand how the structure of title analogs affects their biological activity and binding sites of the enzyme.*

Graphical Abstract



Key words
Schiff Bsae ,Pyridine, Synthesis, Antibacterial, Molecular docking

I. Introduction

Pyridine and its derivatives, which are polar and ionizable aromatic chemicals, promote solubility and bioavailability. Pyridine's chemical characteristics, such as basicity, solubility, and capacity to form hydrogen bonds, make it an essential component of most medications. Because of its distinct features, chemists and biochemists are eager to use pyridine and its derivatives in a variety of therapies.[1] Biological consequences of pyridine and it's derivatives include antibacterial[2], antifungal[2], antiviral[3], anticancer[4], anti-inflammatory[5], and antidiabetic[6] properties. Octanidiene is a hybrid derived from pyridine as precursor. It consists of hydrophilic and lipophilic parts and it has been tested for its antimicrobial activity. Some biologically active molecules containing pyridine moiety are shown in **figure 1**.[7]

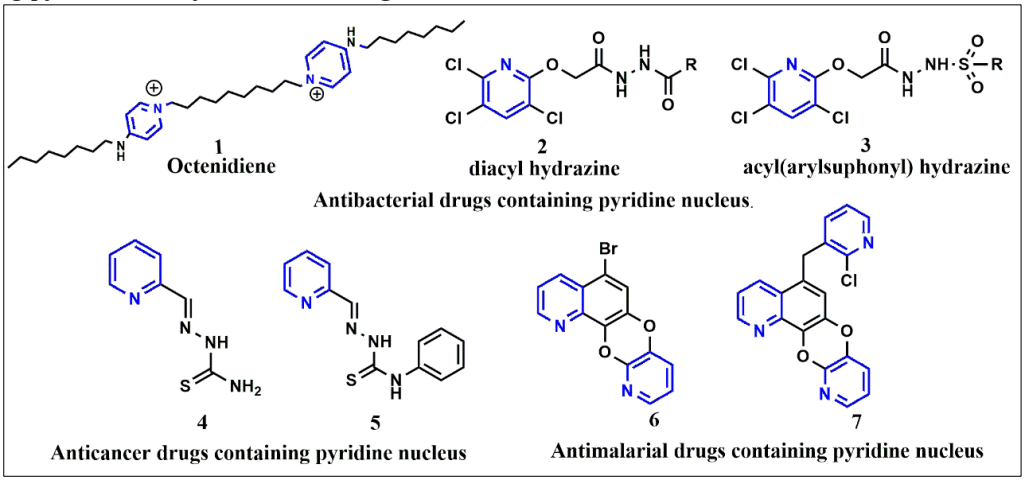


Figure-1 Biologically active molecules containing pyridine moiety.

Amide linkage is an extremely significant feature in drug design. Its presence in the molecule promotes drug binding8. Amide linkage is seen in a number of medications. Figure 2 shows examples of physiologically active amides.[8]. Some examples of biologically active amides are given in **Figure 2**[9]

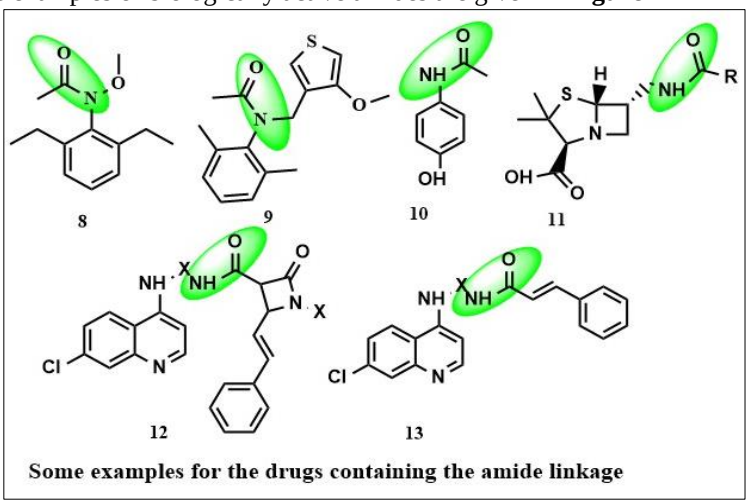


Figure-2: Some examples of the bio-active molecules containing the amide linkage.

A Schiff base is an organic molecule that has an imine or azomethine (-C=N-) functional group. These compounds are generally generated via a condensation reaction between the carbonyl group of an aldehyde or ketone and a primary amine. Hugo Schiff, the first to describe Schiff bases, gave them their name Their versatility and reactivity make Schiff bases valuable tools in research and industrial applications.[10] Schiff bases have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like anti-inflammatory[11], antimicrobial[12], anticonvulsant[13], antitubercular[14], anticancer[15], anthelmintic[16], and so forth. Apart from biological activities, Schiff bases are also used as catalysts, intermediates in organic synthesis, dyes, pigments, polymer stabilizers,[10] and corrosion inhibitors.[17] Using ring closure, cycloaddition, and replacement processes, schiff bases have been used as synthons to create a variety of industrial and physiologically active chemicals, such as formazans, 4-thiazolidinines, benzoxazines, and so on[18]. In a variety of procedures, Schiff base derivatives encouraged researchers to build new heterocyclic/aryl Schiff bases for the creation of environmentally beneficial technologies[19].

Ether linkages in heterocyclic compounds also enhance biological activities. For example, in **Figure-3**

coumarin clubbed carbonodithioate compound **17** display much stronger antimicrobial efficacy which is equal to standard drugs Ciprofloxacin and Fluconazole i.e., 0.5-1 µg/m[20]. There are many naturally occurring coumarins possessing anti-inflammatory activity as a structural core of coumarin **16**. Coumarin derivatives flanked by ether linkage have shown a very good anti-microbial activity[21]. **18** Synthesis of coumarin clubbed indole via ether linkage shows good activity and also the compound is fluorescent in nature. Numerous analogues bearing ether substituent at C-4 position have been reported as excellent bioactive pharmacophore **19-21**[22–24].

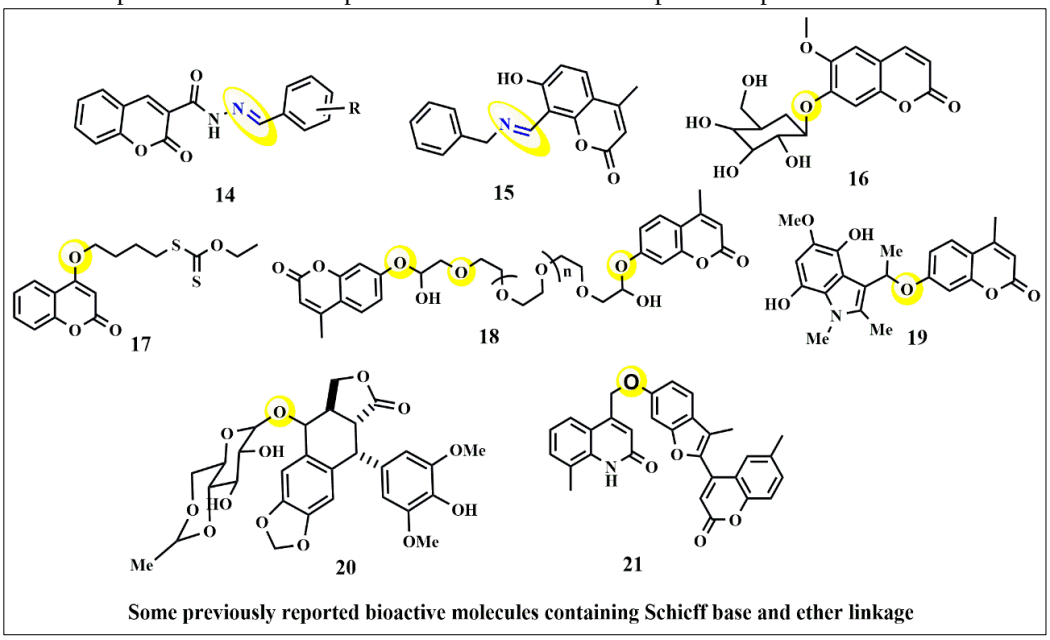


Figure-3: Some bio-active molecules containing Schiff base and ether linkage.

There are several cases in the literature where combining two biodynamic heterocyclic systems results in increased activity. The synthesis of Schiff base of pyridine with phenylacetamide derivatives with an amide bond is the focus of this work. This was motivated by the literature survey and our ongoing research. The structures of the produced compounds have been confirmed using methods such as IR, NMR, mass spectrometry, and computer-based molecular docking, in addition to drug-likeness and ADME-Tox assessments.

II. Materials And Methods

Neutral alumina silica gel was used for TLC. The mobile phase was a 1:1 mixture of ethyl acetate and n-hexane, and UV light was used for detection. Unless otherwise noted, all chemicals used were analytically pure and did not require any extra purification. Using KBr pellets, IR spectra were recorded on a Nicolet 5700 FT-IR. 1 H and 13 C NMR spectra were obtained using CDCl 3 and DMSO-6 as solvents and TMS as an internal standard on a Bruker 400 MHz and JEOL 400 MHz spectrometer, respectively. Chemical changes were reported as δ values (ppm). Mass spectra were collected using a GCMS-TQ8040 NX, an LC-MS/ESI-MS-Waters USS model-Micromass ZQ, and a Shimadzu 2010. Melting points were measured using a Buchi apparatus using the open capillary technique.

Preparation of ligands, molecular docking

The ligand structures were drawn with ChemDraw Professional 17.0 software, and their energy was minimized using UCSF Chimera [25] and Chem3D 17.0.0 tools. Docking of the generated receptors and ligands were performed using CB-Dock 2[26]. The Discovery Studio Visualizer was used to create a depiction of the best docked protein-ligand interaction as well as to describe the complex interactions between the produced pyridine-phenylacetamide analogs and the three receptors.

Synthesis

Substituted *2-chloro-N-phenylacetamide* derivatives (1a-1c)

Substituted aniline (1mmol), chloroacetyl chloride (1.5mmol) and triethylamine(1.5mmol) were placed in to a 100 ml R.B flask equipped magnetic stir bar and condenser and the mixture refluxed on oil bath for about 4-5 hours. The reaction is monitored by TLC; the reaction mixture was quenched into ice with constant stirring. The product was collected by filtration to yield substituted 2-chloro-N-phenylacetamide. Yield 90-95% which was employed in the next step without further purification.

4-((pyridin-2-ylmethylene)amino)phenol (2)
Schiff base is formed reacting 1 mole of 2-formyl pyridine with the 1 mole of 4-amino phenol in methanol as solvent and refluxed for 2 to 3 h. The reaction was monitored using thin-layer chromatography (TLC). Then reaction mixture containing product was filtered, dried and recrystallised to get pure product.Yield, 92%.

Substituted N-phenyl-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide (3a-3c)
1.5mol of activated potassium carbonate in dry acetone was taken in round bottom flask. One mole of Schiff base was added to the mixture then stirred for 15 min. One mole of substituted 2-chloro-N-phenylacetamide derivatives (1a-1c) added to mixture. The whole mixture was stirred for about 12 h. The reaction mixture containing product was filtered, dried and recrystallised to get pure product.Yield, 68-87%.

N-phenyl-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide (3a)
Physical state: Off white amorphous solid, M.P.= 206-208^{0}C. IR (KBr) (vmax/cm^{-1}): 1676.18 cm^{-1} (amide carbonyl stretching), 3418.29cm^{-1} (-NH stretching frequency); Analytical calculation for $C_{20}H_{17}N_3O_2$: C, 66.65; H, 4.40, N, 11.49; 1H-NMR (400 MHz, DMSO-D6) δ 10.23 (s, 1H), 8.707 (s, 1H), 8.58 (s, 1H), 8.51 (s, 1H), 8.02 (d, J = 6.9 Hz, 1H), 7.82 (s, 1H), 7.58 (d, J = 6.9 Hz, 2H), 7.28-7.06 (m, 3H), 6.96 (d, J = 7.6 Hz, 2H), 4.64 (s, 2H):13C-NMR (101 MHz, DMSO-D6) δ 165.7, 158.4, 150.6, 147.4, 146.3, 138.7, 133.3, 132.8, 130.3, 127.2, 126.6, 125.1, 123.5, 117.3, 113.0, 65.2; m/z at 331.84;

N-(4-chlorophenyl)-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide (3b)
Physical state: brownish amorphous solid, M.P. 190-192 ^{0}C. IR: (KBr) (vmax/cm^{-1}): 1677.43 cm^{-1} (amide carbonyl stretching), 3356.13cm^{-1} (-NH stretching frequency); Analytical calculation for $C_{20}H_{16}ClN_3O_2$: 65.67; H, 4.41; Cl, 9.69; N, 11.49; O, 8.75; 1H-NMR (400 MHz, DMSO-D6) δ 10.33 (s, 1H), 8.69 (s, 1H), 8.61 (s, 1H), 8.12 (d, J = 6.9 Hz, 1H), 7.92 (s, 1H), 7.68 (d, J = 6.9 Hz, 2H), 7.44 (m, 5H), 7.06 (d, J = 7.6 Hz, 2H), 4.75 (s, 2H); 13C-NMR (101 MHz, DMSO-D6) δ 165.9, 160.0, 154.1, 150.7, 149.0, 143.6, 139.1, 136.9, 133.8, 127.6, 125.9, 122.5, 120.3, 115.5, 113.0, 66.5; and m/z. at 365;

N-(2-chlorophenyl)-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide (3c)
Physical state: brownish amorphous solid, M.P. 190-192 ^{0}C. IR: (KBr) (vmax/cm^{-1}): 1685.91 cm^{-1} (amide carbonyl stretching), 3429.77cm^{-1} (-NH stretching frequency); Analytical calculation for $C_{20}H_{16}ClN_3O_2$: C, 65.67; H, 4.41; Cl, 9.69; N, 11.49; O, 8.75; 1H-NMR (400 MHz, DMSO-D6) δ 10.30 (s, 1H), 8.62 (d, 2H), 8.26 (d, 1H), 8.09 (d, J = 6.9 Hz, 1H), 7.89 (s, 1H), 7.65 (d, J = 6.9 Hz, 1H), 7.41 (m, 4H), 7.03 (d, J = 7.6 Hz, 2H), 4.72 (s, 2H); 13C-NMR (101 MHz, DMSO-D6) δ 164.9, 155.1, 154.1, 151.5, 151.3, 143.4, 141.5, 139.4, 138.1, 136.9, 136.6, 131.2, 130.4, 126.4, 125.0, 123.5, 122.1, 116.1, 115.2, 66.2; and m/z. at 365.

N-(4-methoxyphenyl)-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide (3d)
Physical state: Colourless amorphous solid, M.P. 208-210 ^{0}C. IR: (KBr) (vmax/cm^{-1}): 1660.65 cm^{-1} (amide carbonyl stretching), 3304.96cm^{-1} (-NH stretching frequency); Analytical calculation for $C_{21}H_{19}N_3O_3$: C, 69.77; H, 5.29; N, 11.63 ; 1H-NMR (400 MHz, DMSO-D6) δ 10.41 (s, 1H), 8.51 (s, 1H), 8.41 (s, 1H), δ 6.92-8.07 (Aromatic protons), δ 5.01 (s, 2H), δ 3.84 (s, 3H); 13C-NMR (101 MHz, DMSO-D6) δ 164.7, 161.5, 156.9, 153.2, 148.5, 147.6, 145.8, 136.5, 130.1, 127.4, 122.7, 120.8, 116.4, 115.9, 65.7,54.7; and m/z. at 362.2

N-(2,6-dimethylphenyl)-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide (3e)
Physical state: colourless amorphous solid, M.P. 210-212 ^{0}C. IR: (KBr) (vmax/cm^{-1}); 1673.07 cm^{-1} (amide carbonyl stretching), 3276.84cm^{-1} (-NH stretching frequency); Analytical calculation for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69; O, 8.90; 1H-NMR (400 MHz, DMSO-D6) δ 10.54 (s, 1H), 8.69 (d, 2H), δ 7.06-8.21 (Aromatic protons), δ 4.6 (s, 2H), δ 2.20 (s, 6H); 13C-NMR (101 MHz, DMSO-D6) δ 165.0, 155.2, 151.5, 147.2, 141.6, 137.0, 135.3, 130.5, 126.3, 124.4, 122.7, 120.5, 116.2, 65.8, 21.0; and m/z. at 359.88.

2-(4-((pyridin-2-ylmethylene)amino)phenoxy)-N-(p-tolyl)acetamide (3f)
Physical state: Colourless amorphous solid, M.P. 198-200 ^{0}C. IR: (KBr) (vmax/cm^{-1}): 1672.55 cm^{-1} (amide carbonyl stretching), 3414.13cm^{-1} (-NH stretching frequency); Analytical calculation for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17; O, 9.26: 1H-NMR (400 MHz, CHLOROFORM-D) δ 10.11 (s, 1H), 8.60 (d, 2H), 8.53-8.25 (m, 2H), 8.12-8.00 (m, 3H), δ 7.0-8.0 (Aromatic protons), 4.63 (s, 2H), 2.25 (s, 3H); 13C-NMR (101 MHz, CHLOROFORM-D) δ 167.2, 157.9, 153.4, 149.8, 148.3, 145.1, 139.1, 135.7, 134.4, 133.1, 127.7, 125.8, 123.6, 121.5, 114.4, 67.2, 25.3; and m/z. at 346.18.

III. RESULTS AND DISCUSSION

Chemistry

The target compounds were synthesized in accordance with **Scheme 1**. The present study describes the literature survey and facile synthesis of Schiff base of pyridine aldehyde with phenylacetamide derivatives and their applications in pharmaceutical field. Different solvents were tried to synthesize Schiff base of 2-formyl pyridine(**Table-1)**.

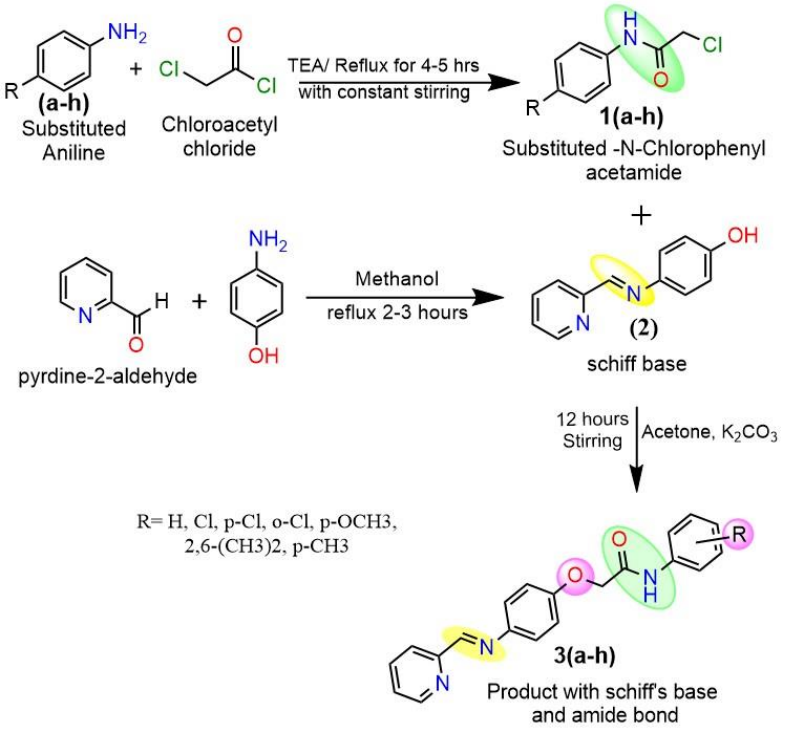
Sl No.	Solvents	Time (h)[a]	Yield[b]
1	1,4-Dioxane	10	57%
2	Ethanol	5	75%
3	DMF	6	68%
4	THF	5	45%
5	DMSO	4	65%
6	Methanol	2	92%

Table-1 Solvent effect on the synthesis of Schiff base.

Out of all the solvents, methanol was chosen as the solvent because it provided the best yield and purity for the further reaction as solvent choice can significantly impact reaction outcomes. The synthesis of titled compounds (E)-N-substituted-2-(4-(pyridin-2-ylmethyleneamino) phenoxy) acetamide**(3a-3f)** was carried out by condensation of (E)-4-(pyridin-2-ylmethyleneamino) phenol **(2)** and substituted N-phenyl acetamide(**1a-1f**) using K_2CO_3 and acetone as a solvent. (**Table-2**)

Compounds	Mol. formula	R	M.P in ^oC	Yield	Time in hours
3a.	$C_{20}H_{17}N_3O_2$	-H	206-208	87%	8-10
3b	$C_{20}H_{16}ClN_3O_2$	p-Cl	194-196	75%	10-12
3c	$C_{20}H_{16}ClN_3O_2$	o-Cl	190-192	68%	10-12
3d	$C_{21}H_{19}N_3O_3$	p-OCH_3	206-208	80%	10-12
3e	$C_{22}H_{21}N_3O_2$	2,6-$(CH_3)_2$	210-212	75%	8-10
3f	$C_{21}H_{19}N_3O_2$	p-CH_3	188-190	80%	10-12

Table-2 Schiff's base of substituted pyridine-phenylacetamide derivatives.



Scheme-1 Synthetic path way of novel Schiff base of pyridine -phenylacetamide derivatives

R= H, Cl, p-Cl, o-Cl, p-OCH_3, 2,6-$(CH_3)_2$, p-CH_3

Characterization of the synthesized compounds has been carried out by using

spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and GC/LC MS. The presented spectral data of the compounds **3(a–f)** were witnessed to be in accordance with the assigned structures of the compounds. The IR spectrum of compound **3b** exhibited characteristic bands at 3356 cm^{-1} for -NH group of amide and another stretching band at 1677 cm^{-1} due to the carbonyl group of amide. Further, the formation of the product was approved by ^{1}H NMR spectrum. The -NH of the amide group is resonated at 10.33 ppm as a broad singlet, methylene linker C_{16}-proton resonated at 4.75ppm as singlet. C_7-proton of C=N group resonated at 8.61ppm. Aromatic protons resonated as multiplets between 7.06 and 8.65 ppm. The numbering of the compound **3b** is represented in **Figure-4**. The ^{13}C NMR spectrum provides additional support for the structure of the compound 3b, wherein the amide linked -CH2 ie C_{16} resonated at δ 66.5 ppm, the carbon of amide carbonyl group C_{17} resonated at δ 165.9 ppm, The mass spectra further supports the structure of the compound **3b**, wherein the molecular ion peak at 365$[M]^{+}$ in the GC-MS spectrum confirms the expected structure for compound **3b**.

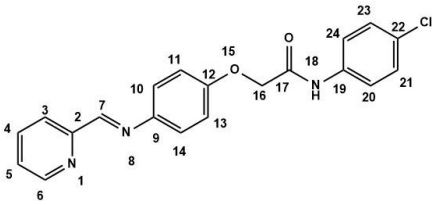


Figure-4 The structure of the compound **3b** for spectral analysis.

Antibacterial Susceptibility

All the newly synthesized novel Schiff base of pyridine-phenylacetamide derivatives derivatives were initially screened for their in-vitro antibacterial activity against *Staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative), at 50 mg/disk concentration using DMF as control, ciprofloxacin as standard. Results were represented in **Table-3**. p-Cl and 2,6-$(CH_3)_2$ were found to be highly active with zone of inhibition with 19mm, 22mm and 20mm, 22mm against *Staphylococcus aureus* and *Escherichia coli* respectively. The antibacterial results reveal that the efficiency of the compounds was decreased when the position of the –Cl atom is substituted at ortho position. The least activity was exhibited with group -CH_3 at para positions whereas others were moderately active compared to standard. Larger the zone of inhibition higher the susceptibility, and lower the MIC. Whereas, a small zone of inhibition higher MIC value and indicate that bacteria are resistant to antimicrobial agents.

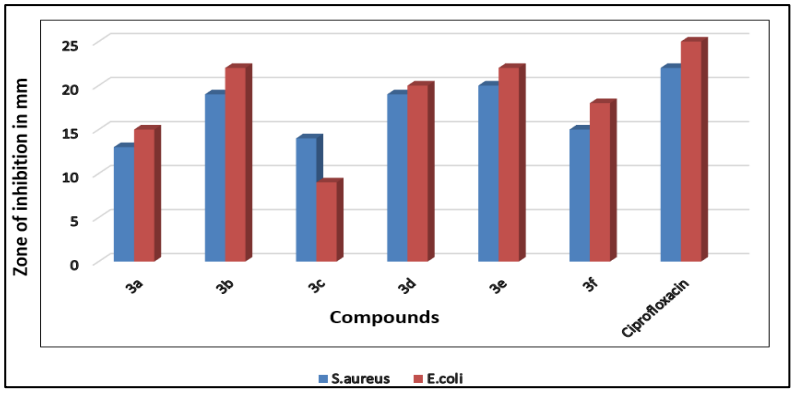


Figure-5 Antibacterial activity of **3(a-f)** in comparison with standard drug

Code	R	Zone of inhibition in mm	
		Staphylococcus aureus	*Escherichia. coli*
3a	H	13	15
3b	p-Cl	**19**	**22**
3c	o-Cl	14	09
3d	p-OCH_3	19	20
3e	2,6-$(CH_3)_2$	**20**	**22**
3f	p-CH_3	15	18
Ciprofloxacin		22	25

Table-3 Mean zone of inhibition in (mm) of Schiff's base of phenylacetamide derivatives **3(a-f)**

Molecular docking

The primary objective of molecular docking is to predict the likely binding geometries of a potential ligand of a known three-dimensional structure with a target protein. In this investigation, a series of Schiff base of pyridine-phenylacetamide derivatives (3a-3f) were studied *in silico* to highlight their possible binding energy and interaction modes against crystal structure of dihydropteroate synthetase from staphylococcus aureus (PDB code -1AD4) The binding affinities calculated varied in the range of (-7.5 to -8.2 kcal/mol) (**Table 4**).

Table-4 Selective nonbonding interactions data of Schiff base of pyridine-phenylacetamide derivatives against

Ligand ID	**Substituted functional groups at R**	**Docking Score (kcal/mol)**	**Type of Interactions**	**Receptor Residues Binded to Ligands**
3a.	No Substitution	-7.5	Hydrophobic Hydrogen Bonding Electrostatic	PHE B:255 ASP B:254 LYS B:251 LYS B:3 ALA B:247
3b.	**p-Cl**	**-8.0**	Hydrophobic Hydrogen Bonding Electrostatic	ALA A:180 PHE B:255 LYS B:251 ALA B:247 LYS B:3
3c	o-Cl	-7.8	Hydrophobic Hydrogen Bonding Electrostatic Van der waals	LYS B:3 LYS B:251 ALA B:247 ALA B:250 ILE B:6 THR B:4 ASP B:254
3d	p-OCH_3	-7.8	Hydrophobic Hydrogen Bonding Electrostatic Van der waals	ALA B:180 ASN B:259 PHE B:255 LYS B:3 LYS B:251 ALA B:247
3e	**2,6-$(CH_3)_2$**	**-8.0**	Hydrophobic Hydrogen Bonding Electrostatic Van der waals	LYS B:3 LYS B:251 ALA B:247 ASP B:254 PHE B:255 ALA B:262
3f	**p-CH_3**	**-8.2**	Hydrophobic Hydrogen Bonding Electrostatic Van der waals	ALA B:180 PHE B:255 ASP B:254 LYS B:251 LYS B:3 ALA B:247

Staphylococcus aureus 1AD4

According to the outcomes obtained from docking screening, all the analogs exhibited very good binding score with the receptor. Graph demonstrated in **(Figure-6)** shows that superior results were obtained compared to the standard drug ciprofloxacin (CP). The analogs 3b,3e and 3f were found to be strongly binded to the receptor through hydrogen and hydrophobic Van der waals and electrostatic interactions suggesting that the synthesized molecules can spontaneously interact within the binding site of the staphylococcus aureus. Although the blind docking studies revealed that all the molecules can act as potential agents for the establishment of e.coli inhibitors, from the estimated free energy of binding values, it may be inferred that the analog 3f with the highest negative minimum binding energy value amongst all the studied analogs could be the best possible staphylococcus aureus inhibitors.(**Figure7 & 8**)

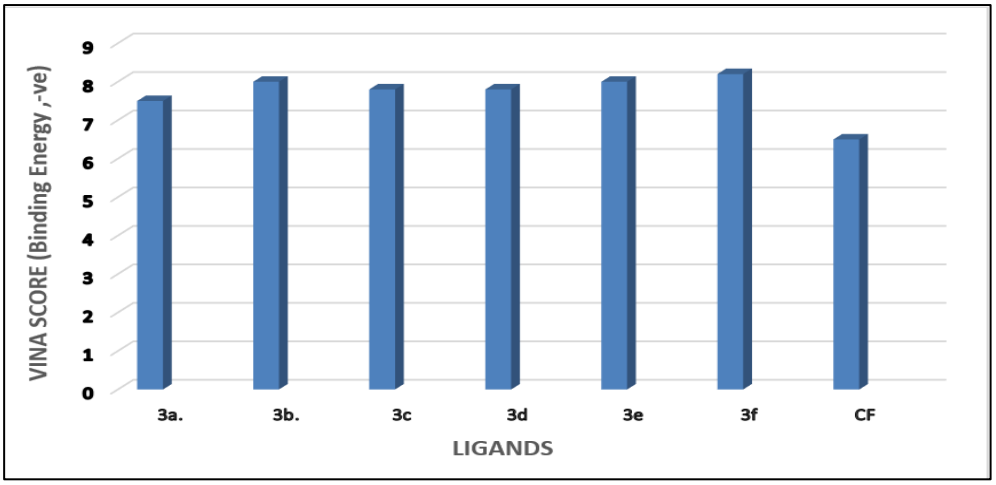


Figure-6 Representation of binding vina scores of (3a-3f) in comparison with with Ciprofloxacin

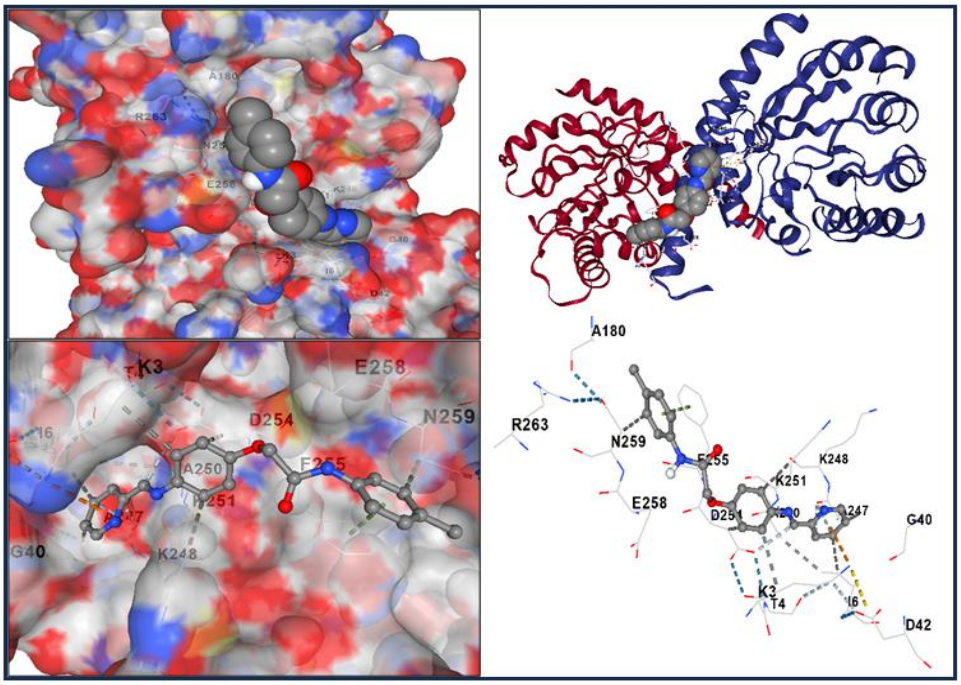


Figure-7 Surface (3D) interactions between compound **3f** with staphylococcus aureus 1AD4

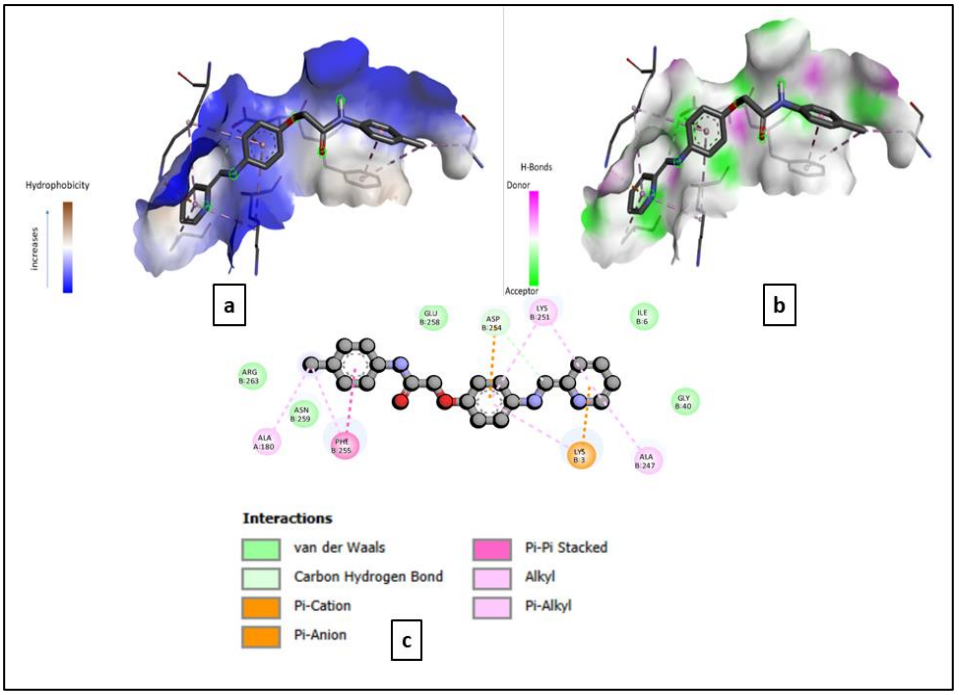


Figure-8 Interactions between compound **3f** with staphylococcus aureus (1AD4) **a-** Hydophobicity **b-** Hydrogen bonding **c-** 2D represention of ligand-enzyme interaction.

SAR study

SAR analysis reveals novel structural linkages in the new compounds. The structure–activity relationship (SAR) of Schiff base of pyridine-phenylacetamide derivatives (3a-3f) can be established from the results of the antibacterial activities reported in **Table-3**. Substitutions in the skeleton had a significant effect on antibacterial activity. It can be seen that there is a trend for lowering influence of zone of inhibition in the following order: 4b=4e>4d>4f>4a>4c for gram-nagative bacteria and 4e>*4d=4b>4f>4c>4a* for gram-positive bacteria respectively. For two bacteria tested, 3b and 3e having -Cl and 2,6-$(CH_3)_2$ substitutions found to be more active than all other derivatives. Adding chloro and methyl groups at para position of the phenyl ring (3b) improved binding aginst 1AD4 by enhancing hydrophobic interactions. **(Figure-9)**

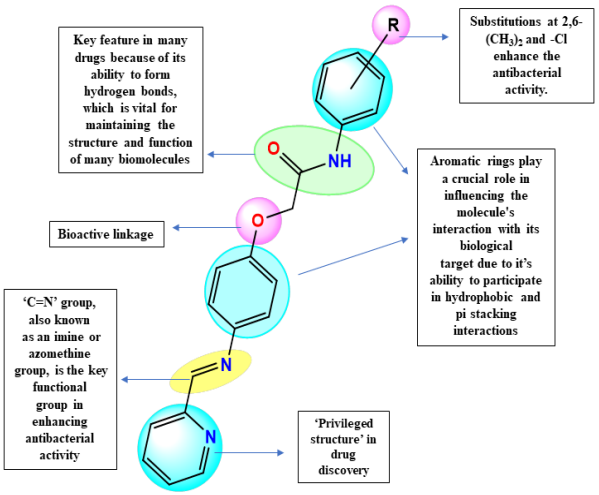


Figure-9. SAR model

ADME properties prediction

The pharmacokinetic (ADME) properties of the bio-active compounds of this series were estimated by using the SwissADME web server[27]. **Table-5** displays the calculated values for different physicochemical, ADME descriptors. The numbers of rotatable bonds (NRB ≤10), the hydrogen-bond acceptor (NHBA ≤ 10), and the hydrogen-bond donor (NHBD ≤5) were found to be between 7 and 8, between 4 and 5, and 1, respectively for the all new analogues (**3a-3f**). The log *P* (≤5) values were predicted as 2.99–3.68. The molecular weights of all the Schiff base of pyridine-phenylacetamide derivatives (3a-3f) were less than 500($M_W \leq 500$). Since none of the analogs deviated from Lipinski's rule of five, they were all anticipated to be orally active compounds with good gastrointestinal absorption.

Ligands	Formulae	MW	NRB	NHBA	NHBD	log P	BAS	SA	LV
3a	$C_{20}H_{17}N_3O_2$	331.37	7	4	1	3.06	0.55	2.64	0
3b	$C_{20}H_{16}ClN_3O_2$	365.81	7	4	1	3.55	0.55	2.66	0
3c	$C_{20}H_{16}ClN_3O_2$	365.81	7	4	1	3.6	0.55	2.71	0
3d	$C_{21}H_{19}N_3O_3$	361.39	8	5	1	2.99	0.55	2.79	0
3e	$C_{22}H_{21}N_3O_2$	359.42	7	4	1	3.68	0.55	2.9	0
3f	$C_{21}H_{19}N_3O_2$	345.39	7	4	1	3.34	0.55	2.72	0

Table-5: Table 5: SwissADME-Tox predicted pharmacokinetic profile for pyridine-phenylacetamide derivatives.

MF – molecular formula; MW – molecular weight; NRB – number of rotatable bonds; HBA – number of H-bond acceptors; NHBD – number of H-bond donors; log P – partition coefficient between n-octanol and water; BAS – bioavailability score; SA – synthetic accessibility; LV – Lipinski's violation

Prediction of toxicity

Apart from ADME prediction, toxicity prediction was also studied for all synthesized (**3a-4f**) derivatives and their results are given in **Table-6**. ProTox-II[28] online tool was used for this work. All of the compounds were predicted to be class IV compounds in terms of toxicity and possessed slight neurotoxicity and respiratory toxicity, while most of the compounds were predicted as inactive in terms of cytotoxicity, Hepatotoxicity, Nephrotoxicity and cardiotoxicity.

Ligands	Tox.	CT	LD50	HT	Neu	NT	RT	Car.T	CG	IT	MG
3a	4	-ve	687	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
3b	4	-ve	1000	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
3c	4	-ve	1000	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
3d	4	-ve	687	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
3e	4	-ve	687	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
3f	4	-ve	687	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve

Table-6: ProTox-II predicted pharmacokinetic profile for Schiff base of pyridine-phenylacetamide derivatives (3a-3f) {+ve=active; -ve=Inactive}

IV. CONCLUSION

We have successfully synthesized six novel compounds, each in a three-step synthetic pathway with high yield. The final reaction of this route yielded the novel substituted (E)-N-phenyl-2-(4-((pyridin-2-ylmethylene)amino)phenoxy)acetamide **(3a-3f)** in excellent yields and were analysed *in vitro* and *in silico* for their antibacterial, molecular docking and drug-likeness properties. the investigation showed that the substituted analogs of -chloro (3b) and -2,6-$(CH3)_2$ (3e) had more potential against bacteria with better pharmacokinetic and biological spectra. These observations were concluded by conducting molecular docking, which revealed promising antibacterial efficacy for all the all the analogs compared to standard drug. Surprisingly, 3f, 3b and 3e had encouraging binding energy and interactions with the receptor compared to standard drug. According to the Veber and Lipinski criteria, the ADME/Tox analysis showed drug-likeness.

Credit authorship contribution statement

Swati S. Bhat: Synthesis, purification, data validation, writing–original draft, methodology, computational studies. **K.M. Hosamani**: Research Supervision and Validation of work.

Declaration of Competing Interest

The authors declare that they have no conflict of interest

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