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Synthesis and characterization of Schiff base analogues of fluoroaniline and their antibiocidal activity against MRSA

M.V. Santhosh[a,b], H.S. NagendraPrasad[a], S. Nagashree[a], H.M. Manukumar[a], L. Mallesha[c] and P. Mallu[a]*

[a]*Department of Chemistry, Sri Jayachamarajendra College of Engineering, JSS Science and Technology University, Mysuru-570 006, Karnataka, India*
[b]*Department of chemistry, GSSS institute of Engineering and Technology for women, Mysuru-570016, Karnataka, India*
[c]*PG Department of Chemistry, JSS College of Arts, Commerce and Science, Mysuru-570006, Karnataka, India*

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A B S T R A C T

A group of new fluoroaniline Schiff bases **(3a–3f)** were synthesized and structurally characterized by various spectroscopic techniques such as ^{1}H-NMR, LC-MS and FT-IR spectral studies. All compounds were evaluated for *in vitro* antibacterial activity. Compounds exhibited good to moderate antibacterial activity. Compound **3f** (Zone of Inhibition = 10.08±0.06 μM) was found to be the most active one, and comparable to the standard Streptomycin (IC_{50} = 15.95±0.08 μM). The compounds having chloro substituent exhibit good membrane damage property against Methicillin-resistant Staphylococcus aureus (MRSA) confirmed by SEM analysis. Structure-activity relationship (SAR) was rationalized by looking at the varying structural features of the molecules.

1. Introduction

Schiff bases (imine or azomethine, –C=N-), are formed by condensation of precursors of amine and carbonyl groups[1-2]. These Schiff bases are having broad spectrum of applications in various fields such as sensors[3], paints[4] and as polymer stabilizers[5].The Schiff base probes are used for various metal ion detection[6]. Schiff bases have additionally been appeared as biologically potent pharmacophore such as antimicrobial[7], antimalarial, anticonvulsant, antiviral, antioxidant, antiproliferative[8], analgesic and antipyretic properties[9]. Therefore, Schiff base candidate plays a vital role in the medicinal and pharmaceutical chemistry field. The effective bioactivity of these imines is for the most part credited to the alkyl/aryl/heteroaryl gather with multi substituent in the molecule, whereas Schiff base are one center or appended[10,11]. These promoted researchers to design new Schiff base for the desired applications.

Staphylococcus aurous[12] is perceived as a standout amongst the most widely recognized pathogens in charge of nourishment harming and causing different diseases in creature and humans[12,13]. This facultative anaerobe is a characteristic vegetation in 20– 30% of individuals, show inside the front nares and on the skin[14]. Disease happens for the most part by avoidance of invulnerable framework in the host to cause pneumonia, aspiratory tuberculosis, endocarditis, sepsis, delicate tissue contaminations

* Corresponding author.
E-mail address: drmallu66@gmail.com (P. Mallu)

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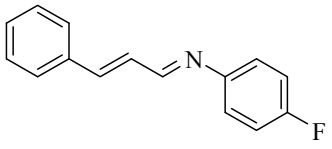
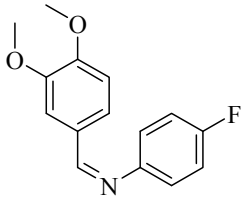
harmful stun sickness, bone and joint diseases, or urinary tract contaminations and even nourishment poisoning[15]. *S. aureus* communicates unmistakable surface proteins that are basic for official to have cells to go about as destructiveness factors[16,17]. These surface proteins normally elevate connection to laminin and fibronectin[18]. Most strains likewise express an amassing factor, coagulase protein response, which elevates connection to blood clusters. When the bacterium gets followed, it duplicates into a biofilm that makes it hard to destroy[19,20]. To overcome from antibiotic resistant bacteria, various strategies were employed[21]. In view of these observation and interest, the present study was investigated on biocidal activity of fluoroaniline Schiff base derivatives against *methicillin Staphylococcus aureus* (MRSA).

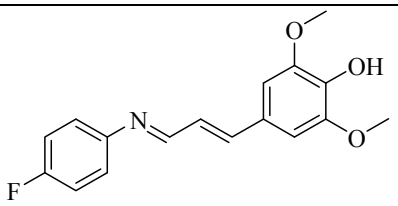
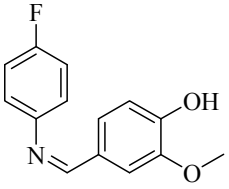
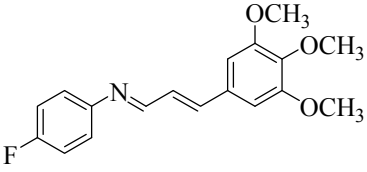
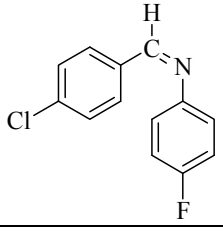
2. Results and Discussion

2.1 Chemistry

In the present work, a series of fluroaniline derivatives were synthesized in good yield. Structures of the synthesized compounds were established on the basis of spectral studies. The UV spectra of **3**(**a-f**) were recorded using suitable solvents in the range of 200 - 800 nm. The electronic absorption spectra of compounds show new bands and appearance of wavelength absorption band in the UV region in UV-visible spectrum owing to confirms the formation of new compounds. The FT-IR spectra of **3**(**a-f**) were recorded in the range of 4000 - 400 cm^{-1}. The absence of NH_2 and C=O absorption bands in the IR spectra confirmed that the synthesized compounds. The absorption bands at 1600-1502 cm^{-1} are assigned to the aromatic C=C stretch. The appearance of a medium to strong absorption new bands at 1626-1590 cm^{-1} due to a stretching vibration of the azomethine (HC=N) bond formation in the synthesized compound. The bands appeared at 1400-1202 cm^{-1} (**3a-f**) corresponding to C-F stretching frequency. The characteristic resonance peaks in ^1H NMR for the new compounds were reported using DMSO-d_6. The expected resonances were assigned by their peak multiplicity and integration. The integration of spectra shows good agreement with the synthesized compounds. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. In all the synthesized compounds **3**(**a-f**) the new resonances assigned to the –CH=N (δ 8.27 – 8.97 ppm) were observed which confirmed the product and good agreement with structure. Mass spectra of all the newly synthesized compounds showed M^+ fragmentation peak in agreement with their molecular formula. The mass spectra of **3a** showed molecular ion peak at m/z 225.45 which is in agreement with the molecular formula $C_8H_{12}FN$. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ± 0.4 %.

Table 1. Physical data of synthesized compounds **3(a-f)**

Compound	Structure	Mol. Formula	Mol. Wt.	Yield (g)	UV-Visible (λ_{max})	Melting Point
3a		$C_8H_{12}NF$	225.4	0.56	360 nm	119 ^oC
3b		$C_{15}H_{14}FNO_2$	259.3	0.61	350 nm	101 ^oC

3c		$C_{17}H_{16}FNO_3$	301.3	0.31	248 nm	98 °C
3d		$C_{14}H_{12}O_2NF$	245.3	0.70	320 nm	105 °C
3e		$C_{18}H_{18}FNO_3$	315.1	0.54	317 nm	145 °C
3f		$C_{14}H_9ClNF$	233.7	0.42	268 nm	89 °C

The synthesized compounds were evaluated for the biocidal potency against both Gram-positive and Gram-negative bacteria in agar diffusion method. The compounds assessed the antibacterial property and showed that the compounds **3f** and **3c** are the highly potent compared to the other synthesized analogs carryout in the present investigations. These results were depicted in the **Table 2**, and the promising lead molecule was further used to confirm the dose depended action against one model organism in the further study. As expected, the **3f** exhibited significant zone of inhibition against assessed bacteria compared to standard antibiotic streptomycin. The highest antibacterial activity observed at concentration of 100 µg/mL against Gram-positive *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis* and Gram-negative *Enterobacter aerogenes, Escherichia coli, Salmonella typhimurium, Shigella flexneri, Vibrio cholera, V. parahaemolyticus* and *Pseudomonas aeruginosa*

Table 2. Bactericidal activity of synthesized compounds against perilous pathogens

Test Microorganisms	3a	3b	3c	3d	3e	3f
Gram-positive						
Staphylococcus aureus	-	-	-	-	-	+
Bacillus cereus	-	-	-	-	-	+
B. substilis	-	-	-	-	-	+
Gram-negative						
Enterobacter aerogenes	-	-	+	-	-	+
Escherichia coli	-	-	+	-	-	+
Pseudomonas aeruginosa	-	-	+	-	-	+
Shigella flexneri	-	-	+	-	-	+
Salmonella typhimurium	-	-	+	-	-	+
Vibrio cholera	-	-	+	-	-	+
V. parahaemolyticus	-	-	+	-	-	+

The **3f** showed strong inhibition of 10.08 mm zone of inhibition (ZOI) for *S. aureus* (**Fig. 1A and B)** and 9.28, 9.33, 12.03 mm ZOI for *S. typhimurium, P. aeruginosa* and *S. flexneri* found at 150 µg/mL compared to standard antibiotic streptomycin (ZOI 125.95, 10.50, 12.57 and 13.40 mm respectively). The effective inhibition was observed for **3f** compared to different Gram classes of bacteria, while no

ZOI was observed for negative control (sterile distilled water) against all tested pathogens. This shows that, the molecular moiety in the synthesized analog was responsible for the outcome of the observation was compared. Hence, the active agent **3f** against different tested pathogens has advantage over studied different pathogens in the present study **(Table 3)**. The antibacterial activity of the compound **3f** was further confirmed by the membrane damaging activity in SEM analysis. The membrane damage was confirmed in the SEM analysis showed compared to the control in the **Fig. 1 C and D** respectively. The membrane damage was observed by the alterations in the membrane structure indicated by the arrow present in the figure.

Table 3. Bactericidal activity of synthesized 3f compound against perilous pathogens

Test Microorganisms	Zone of inhibition (in mm)± SD (n = 3)							
	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	Standard Drug-Streptomycin (10 µg)	Negative control
Gram-positive								
Staphylococcus aureus	1.07±0.09	3.07±0.09	5.17±0.13	6.25±0.11	7.12±0.12	10.08±0.06	15.95±0.08	NS
Bacillus cereus	5.35±0.10	5.97±0.17	6.30±0.12	7.13±0.16	7.47±0.11	9.20±0.09	14.33±0.34	NS
Gram-negative								
Enterobacter aerogenes	3.97±0.20	5.27±0.10	6.07±0.03	6.20±0.12	7.38±0.06	7.10±0.04	11.10±0.16	NS
Escherichia coli	3.97±0.26	4.73±0.30	5.23±0.23	6.20±0.24	7.32±0.16	8.03±0.09	13.03±0.16	NS
Pseudomonas aeruginosa	5.25±0.11	6.83±0.07	8.20±0.08	8.80±0.23	9.13±0.05	9.33±0.07	12.57±0.27	NS
Shigella flexneri	4.93±0.18	6.53±0.14	7.03±0.16	8.10±0.04	10.20±0.09	12.03±0.11	13.40±0.16	NS
Salmonella typhimurium	3.57±0.10	3.97±0.07	5.17±0.09	6.37±0.16	7.23±0.11	9.28±0.13	10.50±0.19	NS

Values are means of three independent replicates (n=3). ± standard errors. Values followed by the same letter(s) within the same column are not significantly ($p \leq 0.05$) different according to Tukey's HSD. NS: Not sensitive

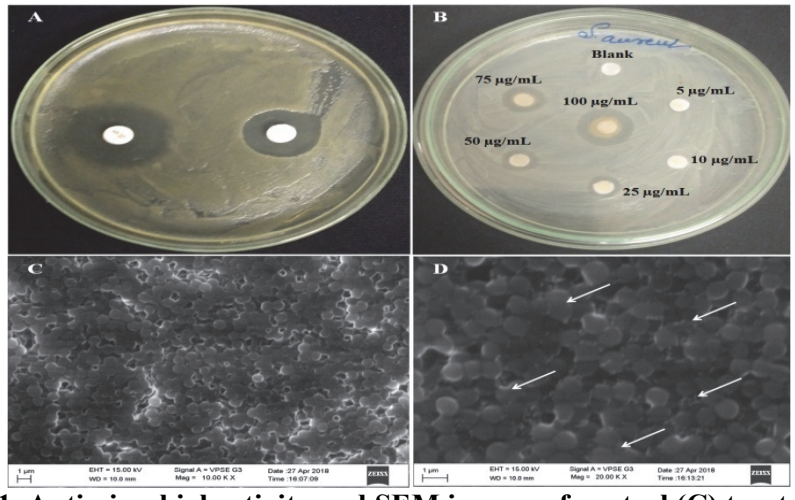


Fig. 1. Antimicrobial activity and SEM images of control (C),treated(D)

SAR for test compounds **3a** to **3f** can be drawn from the above antibacterial bioassay assessment as takes after. The greater part of the moieties at 100mg/mL indicated direct to great antibacterial activity against MRSA. The information in above table demonstrates that the Schiff base compound **3f** shows good activity against MRSA contrasted with **3a**, **3b**, **3c**, **3d**, and **3e**. The present investigation of antibacterial outcome and spectral information and structure of Schiff base candidates revealed that **3f** compounds have electron withdrawing group Cl substituted phenyl ring exhibit more potent against to

MRSA. **3f** compound exhibit 100% inhibition rate at 100 mg/ML against MRSA. The presence of the -Br, -I,–F and -Cl, groups increases the antibacterial property of the compounds. The most interesting thing found by the SAR of **3f** compounds containing chloro substitution at aromatic ring shows better activity compared to other compounds. In this connection electron withdrawing and electron donating groups were introduced at different positions on phenyl ring to study the antibacterial efficacy.

3. Conclusion

The present investigation, the six new Schiff base containing fluoroaniline moiety were synthesized and antibacterial activity assessed *in vitro* **3f** compound showed more grounded antibacterial property against MRSA contrasted and other integrated Schiff base compounds. Primary SAR investigation demonstrates that the, - Br,- I,- F and – Cl on the phenyl ring enhance the antibacterial property of the Schiff bases. The system fundamental their upgraded antibacterial action, ought to be performed in future investigation.

4. Acknowledgment

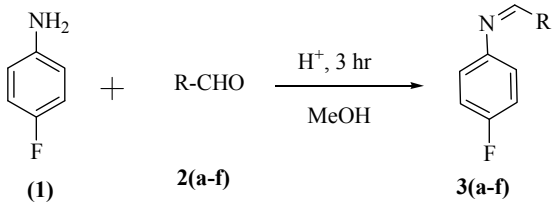
We grateful to Department of chemistry JSSS and TU Mysore for providing laboratory facilities and university of Mysore for NMR and Mass spectroscopic analysis.

5. Materials and Methods

All solvents were of reagent grade quality and purchased commercially. Aldehydes and 4-fluoroaniline were obtained from Merck and was used without further purification. Characterization of synthesized compounds will be carried out using spectral techniques such as UV-visible spectroscopy, FT-IR, LCMS and ^{1}H NMR was recorded. All lyophilized reference bacterial strains were procured from Microbial Typing Culture Collection (MTCC), Chandigarh, India and American Tissue Culture Collection (ATCC) with the help of Mr. Anand, Ganesh Analytical Services and Consultancy, Mysuru. Salmonella typhimurium-98, Escherichia coli-1610, Staphylococcus aureus-96, Bacillus cereus-430, Shigella flexneri-1457, Vibrio cholera-3904, Vibrio paraheamolyticus-451, Pseudomonas aeruginosa-1688 and Enterobacter faecalis-439 strains were cultured in recommended broth as per the revival procedure provided by MTCC and Enterobacter aerogenes-13048 ATCC. Culture media was purchased from Hi-Media Laboratories

5.1 Synthesis of Schiff base compounds **3(a-f)**

Equimolar concentrations of fluoroaniline 1 and aryl aldehyde/ketones 2(a-f) were stirred for 3 hrs at 60 ^{o}C using methanol solvent and then 2-3 drops of glacial acetic acid was added to the reaction mixture. The progress of the reaction was followed by TLC until the reaction was complete. The product **3(a-f)** was cooled to 0 °C, the precipitate was filtered, washed with diethyl ether and the residue was recrystallized from suitable solvent.



2a. R=$C_6H_4N(CH_3)_2$
2b. R=$(OCH_3)_2C_6H_3$
2c. R=C_8H_7
2d. R=$(OH)OCH_3C_6H_3$
2e. R=$(OCH_3)_3C_6H_2$
2f. R=ClC_6H_4

Scheme 1. Synthetic route for compounds 3(a-f)

*4-Fluoro-N-(3-phenylallylidene)aniline (**3a**)*

^{1}H-NMR (500MHz,DMSO-d_6) δ in ppm : 7.21(d,2H,C-H), 7.44(d,2H,C-H), 8.54(s,1H,CH=N), 7.73(d,2H,Ar-H),6.92(d,2H,Ar-H), 7.13(t,1H,Ar-H), 6.87(s,2H,NH_2). IR ν_{max}(cm^{-1}): 1610(C=N), 1578(C=C), 1365(C-F). LCMS m/z Calculated for $C_8H_{12}FN$: 225.26, found is 225.45

*N-(3,4-Dimethoxybenzylidene)-4-fluoroaniline (**3b**)*

^{1}H-NMR(500MHz,DMSO-d_6) δ in ppm : 8.97(s,1H,C=N), 7.71(s,1H,C-H), 7.45(d, 1H, C-H), 6.84(d,1H,C-H), 7.33(d,2H,Ar-H),7.28(d,2H,Ar-H), 3.87(s,6H,CH_3). IR ν_{max}(cm^{-1}): 1619(C=N), 1579(C=C), 1400(C-F), 1200(C-O). LCMS m/z Calculated for $C_{15}H_{14}FNO_2$: 259.28, found is 259.37.

*3-((4-Fluorophenyl)imino)prop-1-en-1-yl)-2,6-dimethoxypheno (**3c**)*

^{1}H-NMR(500MHz,DMSO-d_6) δ in ppm : 8.27(d,1H,N=CH), 7.98(d,2H,Ar-H), 7.51(d,2H, Ar-H), 7.35(t,1H,CH), 7.24(d,1H,C-H), 6.83(t,2H,C-H), 5.37(s,1H,OH), 3.83(s,6H,O-CH_3). IR ν_{max}(cm^{-1}): 3028(O-H),1626(C=N), 1600(C=C), 1311(C-F), 1216(C-O). LCMS m/z Calculated for $C_{17}H_{16}FNO_3$: 301.31, found is 301.57.

*4-(4-Fluorophenyl)imino)methyl)-2-methoxyphenol (**3d**)*

^{1}H-NMR(500MHz,DMSO-d_6) δ in ppm :8.89(s,1H, N=CH) 7.58(d,2H,Ar-H), 7.41(d,2H, Ar-H), 7.35(d,1H, Ar-H), 7.24(s,1H,Ar-H), 6.89(d,1H,Ar-H), 7.37(d,2H,Ar-H), 7.28(d,2H,Ar-H) IR ν_{max}(cm^{-1}): 2946(O-H), 1590(C=N), 1502(C=C), 1202(C-F), 1153(C-O). LCMS m/z Calculated for $C_{14}H_{12}O_2NF$: 245.25, found is 245.67.

*4-Fluoro-N-(3-(3,4,5-trimethoxyphenyl)allylidene)aniline (**3e**)*

^{1}H-NMR(500MHz,DMSO-d_6) δ in ppm :8.58(s,1H, N=CH) 7.52(d,2H,Ar-H), 7.31(d,2H, Ar-H), 7.15(d,2H,Ar-H), 6.15(d,1H,C-H), 6.36(d,1H,C-H), 3.24(s,9H,CH_3). IR ν_{max}(cm^{-1}): 1619(C=N), 1578(C=C), 1332(C-F), 1205(C-O). LCMS m/z Calculated for $C_{18}H_{18}FNO_3$: 315.14, found is 316.10

*(4-Chlorobenzylidene)-4-fluoroaniline (**3f**)*
^{1}H-NMR(500MHz,DMSO-d_6) δ in ppm :8.48(s,1H, N=CH) 7.32(d,2H,Ar-H), 7.21(d,2H, Ar-H), 7.79(d,2H,Ar-H),7.89(d,2H,Ar-H). IR ν_{max}(cm^{-1}): 1623(C=N), 1589(C=C), 1213(C-F), 821(C-O) LCMS m/z Calculated for $C_{14}H_9ClNF$: 233.67, found is 233.77.

5.2. Antimicrobial activity

5.2.1. Disc diffusion assay

The MRSA strain was subjected to agar dilution method to deduce the compounds minimum inhibitory concentration (MIC). Along with the reference, bacterial strain *Staphylococcus aureus* (96) was received from Microbial Typing Culture Collection (MTCC), Chandigarh, India, as a positive control. The bacterial suspension was prepared from the overnight culture and $1x10^6$ CFU/mL cells were inoculated on to Mueller-Hinton agar, then plates were bored using cork borer (6 mm) to create wells, to which 5 µL of different serial dilutions of fluoroaniline Schiff bases were added. Control was performed without any test sample and incubated at 37 ^oC for 24 h to examine zone of inhibition. Assay performed in triplicates and repeated thrice (22-23).

5.2.2. Membrane damage study

The scanning electron microscopy (SEM) carried out to study MRSA membrane damage by treating 1 mg/mL concentration of compound for 2 h, then cells were pelleted by centrifugation (10,000 rpm for 5 min) at 4 ^oC. Cells were fixed by using glutaraldehyde (2.5%) in PBS, pelleted and deposited on glass

slide followed by stepwise treatment of 30% to 100% ethanol drying. After, 2 days drying under room temperature used for SEM analysis (23).

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