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Synthesis and Antimicrobial Activity of New Schiff Base Compounds Containing Methylene Derivatives

Divyanshu D. Patel[1], Keshav C. Patel[2]

Department of Chemistry, Veer Narmad South Gujarat University, Surat-395 007, Gujarat, India

Abstract: ***Acid Catalysed condensation of 4,4'-methylene bis (2,5-difluoro aniline) with different various substituted aromatic aldehyde yielded corresponding Schiff base series. All these compounds were characterized by means of their IR, ^{1}H NMR spectroscopic data. All the synthesized compounds are tested for their antimicrobial activities by the broth dilution method.***

Keywords: 4,4'-methylene bis (2,5-difluoro aniline), aldehyde, acetic acid, methanol, antimicrobial activity.

1. Introduction

Schiff base have been synthesized from the condensation of amine and aldehyde in presence of acid as catalyst. Azomethine group (-C=N-)-containing compounds, typically known as Schiff's bases. We have prepared 4,4'-methylene bis (*N*- substituted benzylidene-2,5-difluoro aniline) from various substituted aromatic aldehydes. Schiff base also possessed various biological activities like antibacterial [1 - 3], antifungal [4], antitumor [5], anti-inflammatory [6], antiviral [7], antioxidant [8-10] and anticancer [11]. The study of Schiff derivatives is of considerable current interest as a result of their important biological, biophysical properties and their industrial applications.

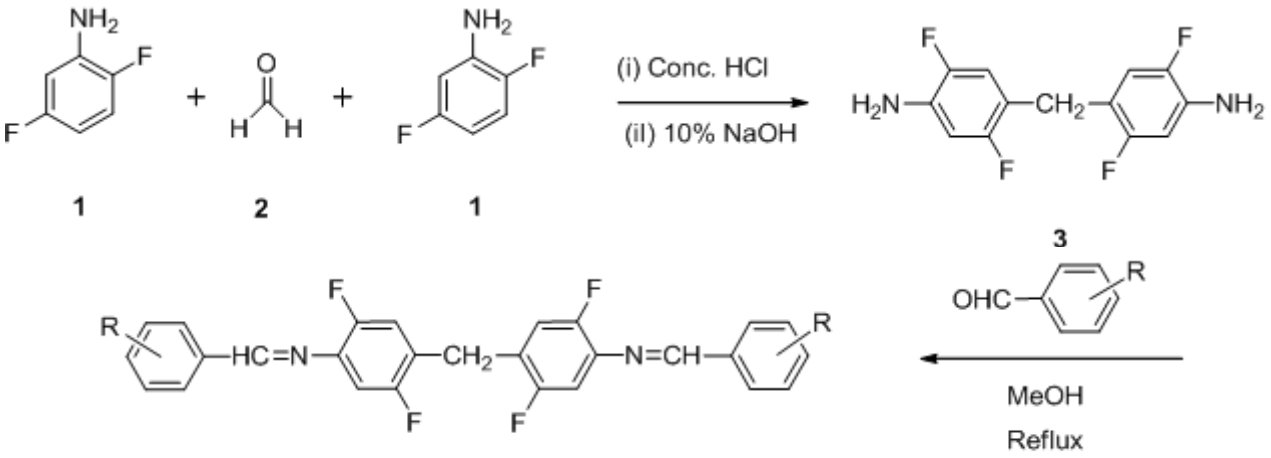
2. Results and discussions

All the synthesized compounds were recrystallization and successive purified by using ethanol. The purity of the synthesized compounds was checked by performing TLC. The structures of the newly synthesized compounds were determined on the basis of their FTIR and ^{1}HNMR data. The IR spectra of the synthesized compounds showed the presence of C=N stretching bands at 1600-1670 cm^{-1} (Schiff base). The ^{1}HNMR spectra of the synthesized compound showed chemical shifts, which are characteristics of the anticipated structure of compounds.

Experimental

Procedure for the synthesis of Synthesis of 4,4'-methylene bis (2,5-difluoro aniline) (3)
4-4'-methylene bis (2,5-difluoro aniline) (3) was synthesized by the method described in the literature [12].

General preparation of the compounds (4a- j)
The title compounds were synthesized by reaction between 4,4'-methylene bis 2,5-difluoro aniline (2.70 g, 0.01 mol) and various substituted aromatic aldehydes (0.02 mol). Each reactant was dissolved in a minimum amount of methanol, then mixed together and followed by addition of few drops of glacial acetic acid as a catalyst. The solution was refluxed for 4 hrs. It was then poured into ice cold water to give solid product. It was filtered, washed with water, dried and recrystallized from ethanol [13].



(4a- j)
R = 2-F, 4-F, 2-OH, 2-CH_3, 2,4-Cl, 4-Cl, 4-CH_3, 2-Cl, 4-OH, 2-NO_2.

4,4'-Methylene bis-(N-(2-fluorobenzylidine)-2,5-difluoro aniline) 4a

Brown colour solid powder, mp 179 ^{0}C, yield 77 %; IR (KBr, cm^{-1}): 3030 (C-H stretching, aromatic), 2935, 2820 (C-H stretching, -CH_2- group), 1628 (C=N stretching, Schiff base), 1505 (C=C stretching, aromatic), 1430 (C-H bending, -CH_2- group), 1051 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.96 (S, 2H, CH_2), 6.93-7.81 (m, 12H, Ar-H), 8.66 (S, 2H, HC=N); Anal. Calcd for: $C_{27}H_{16}F_6N_2$ (482.42); Found (C, 67.29); requires (C, 67.22), Found (H, 3.28); requires (H, 3.34), Found (N, 5.82); requires (N, 5.81).

4,4'-Methylene bis-(N-4-fluorobenzylidine)-2,5-difluoro aniline) 4b

Brown colour solid powder, mp 160 ^{0}C, yield 80 %; IR (KBr, cm^{-1}): 3035 (C-H stretching, aromatic), 2940, 2815 (C-H stretching, -CH_2- group), 1620 (C=N stretching, Schiff base), 1510 (C=C stretching, aromatic), 1425 (C-H bending, -CH_2- group), 1060 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.97 (S, 2H, CH_2), 6.90-7.75 (m, 12H, Ar-H), 8.70 (S, 2H, HC=N); Anal. Calcd for: $C_{27}H_{16}F_6N_2$ (482.42); Found (C, 67.27); requires (C, 67.22), Found (H, 3.41); requires (H, 3.34), Found (N, 5.88); requires (N, 5.81).

4,4'-Methylene bis-(N-(2-hydroxybenzylidine)-2,5-difluoro aniline) 4c

Brown colour solid powder, mp 172 ^{0}C, yield 65 %; IR (KBr, cm^{-1}): 3400 (O-H stretching, Ar-OH), 3060 (C-H stretching, aromatic), 2935, 2825 (C-H stretching, -CH_2- group), 1630 (C=N stretching, Schiff base), 1520 (C=C stretching, aromatic), 1435 (C-H bending, -CH_2- group), 1065 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.90 (S, 2H, CH_2), 6.97-7.72 (m, 12H, Ar-H), 8.60 (S, 2H, HC=N), 12.86 (S, 2H, OH); Anal. Calcd for: $C_{27}H_{18}F_2N_2O_2$ (478.44); Found (C, 67.85); requires (C, 67.78), Found (H, 3.71); requires (H, 3.78), Found (N, 5.79); requires (N, 5.86).

4,4'-Methylene bis-(N-(2-methylbenzylidine)-2,5-difluoro aniline) 4d

Yellow colour solid powder, mp 161 ^{0}C, yield 75%; IR (KBr, cm^{-1}): 3050 (C-H stretching, aromatic), 2940, 2825 (C-H stretching, -CH_2- group), 2920, 2870 (C-H stretching, -CH_3), 1635 (C=N stretching, Schiff base), 1525 (C=C stretching, aromatic), 1470,1380 (C-H bending, -CH_3), 1435 (C-H bending, -CH_2- group), 1075 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 2.23 (S, 6H, CH_3), 3.92 (S, 2H, CH_2), 7.23-7.72 (m, 12H, Ar-H), 8.55 (S, 2H, HC=N); Anal. Calcd for: $C_{29}H_{22}F_4N_2$ (474.49); Found (C, 73.47); requires (C, 73.41), Found (H, 4.59); requires (H, 4.67), Found (N, 5.98); requires (N, 5.90).

4,4'-Methylene bis-(N-(2,4-dichlorobenzylidine)-2,5-difluoro aniline) 4e

Light yellow colour solid powder, mp 184 ^{0}C, yield 80%; IR (KBr, cm^{-1}): 3045 (C-H stretching, aromatic), 2940, 2828 (C-H stretching, -CH_2- group), 1635 (C=N stretching, Schiff base), 1525 (C=C stretching, aromatic), 1435 (C-H bending, -CH_2- group), 1070 (C-F stretching, Fluoro), 725 (C-Cl stretching, Chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.99 (S, 2H, CH_2), 7.40-7.98 (m, 10H, Ar-H), 8.65 (S, 2H, HC=N); Anal. Calcd for: $C_{27}H_{14}Cl_4F_4N_2$ (584.22); Found (C, 55.59); requires (C, 55.51), Found (H, 2.36); requires (H, 2.42), Found (N, 4.87); requires (N, 4.80).

4,4'-Methylene bis-(N-(4-chlorobenzylidine)-2,5-difluoro aniline) 4f

Light yellow colour solid powder, mp 180 ^{0}C, yield 75%; IR (KBr, cm^{-1}): 3060 (C-H stretching, aromatic), 2936, 2825 (C-H stretching, -CH_2- group), 1635 (C=N stretching, Schiff base), 1530 (C=C stretching, aromatic), 1430 (C-H bending, -CH_2- group), 1090 (C-F stretching, Fluoro), 740 (C-Cl stretching, Chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.90 (S, 2H, CH_2), 6.93-7.77 (m, 12H, Ar-H), 8.60 (S, 2H, HC=N); Anal. Calcd for: $C_{27}H_{16}Cl_2F_4N_2$ (515.33); Found (C, 62.99); requires (C, 62.93), Found (H, 3.05); requires (H, 3.13), Found (N, 5.37); requires (N, 5.44).

4,4'-Methylene bis-(N-(4-methylbenzylidine)-2,5-difluoro aniline) 4g

Yellow colour solid powder, mp 161 ^{0}C, yield 75%; IR (KBr, cm^{-1}): 3058 (C-H stretching, aromatic), 2940, 2840 (C-H stretching, -CH_2- group), 2925, 2877 (C-H stretching, -CH_3), 1628 (C=N stretching, Schiff base), 1530 (C=C stretching, aromatic), 1475,1365 (C-H bending, -CH_3), 1440 (C-H bending, -CH_2- group), 1085 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 2.27 (S, 6H, CH_3), 3.90 (S, 2H, CH_2), 6.76-7.72 (m, 12H, Ar-H), 8.50 (S, 2H, HC=N); Anal. Calcd for: $C_{29}H_{22}F_4N_2$ (474.49); Found (C, 73.46); requires (C, 73.41), Found (H, 4.73); requires (H, 4.67), Found (N, 5.93); requires (N, 5.90).

4,4'-Methylene bis-(N-(2-chlorobenzylidine)-2,5-difluoro aniline) 4h

Light yellow colour solid powder, mp 155 ^{0}C, yield 75%; IR (KBr, cm^{-1}): 3065 (C-H stretching, aromatic), 2936, 2830 (C-H stretching, -CH_2- group), 1618 (C=N stretching, Schiff base), 1530 (C=C stretching, aromatic), 1437 (C-H bending, -CH_2- group), 1090 (C-F stretching, Fluoro), 745 (C-Cl stretching, Chloro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.99 (S, 2H, CH_2), 6.93-7.80 (m, 12H, Ar-H), 8.60 (S, 2H, HC=N); Anal. Calcd for: $C_{27}H_{16}Cl_2F_4N_2$ (515.33); Found (C, 62.97); requires (C, 62.93), Found (H, 3.20); requires (H, 3.13), Found (N, 5.52); requires (N, 5.44).

4,4'-Methylene bis-(N-(4-hydroxybenzylidine)-2,5-difluoro aniline) 4i

Brown colour solid powder, mp 185 ^{0}C, yield 65 %; IR (KBr, cm^{-1}): 3415 (O-H stretching, Ar-OH), 3065 (C-H stretching, aromatic), 2935, 2825 (C-H stretching, -CH_2- group), 1625 (C=N stretching, Schiff base), 1525 (C=C stretching, aromatic), 1445 (C-H bending, -CH_2- group), 1080 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.90 (S, 2H, CH_2), 6.90-7.65 (m, 12H, Ar-H), 8.55 (S, 2H, HC=N), 12.80 (S, 2H, OH); Anal. Calcd for: $C_{27}H_{18}F_2N_2O_2$ (478.44); Found (C, 67.72); requires (C, 67.78), Found (H, 3.85); requires (H, 3.78), Found (N, 5.92); requires (N, 5.86).

4,4'-Methylene bis-(N-(2-nitrobenzylidine)-2,5-difluoro aniline) 4j

Brown colour solid powder, mp 197^{0}C, yield 75%; IR (KBr) cm^{-1}: 3060 (C-H stretching, aromatic), 2930, 2835 (C-H stretching, -CH_2- group), 1618 (C=N stretching, Schiff

base), 1580, 1350 (N=O stretching, Nitro), 1525 (C=C stretching, aromatic), 1445 (C-H bending, -CH_2- group), 1080 (C-F stretching, Fluoro); ^{1}H NMR (400.1 MHz, DMSO): δ_H 3.90 (S, 2H, CH_2), 7.59-8.09 (m, 12H, Ar-H), 8.60 (S, 2H, HC=N); Anal. Calcd for: $C_{27}H_{16}F_4N_4O_4$ (536.43); Found (C, 60.51); requires (C, 60.51), Found (H, 3.07); requires (H, 3.01), Found (N, 10.51); requires (N, 10.44).

3. Antimicrobial Activity

3.1 Antibacterial Activity

For the antibacterial activity, the newly synthesized compounds were screened for their antibacterial activity against gram positive bacteria *S. aureus* (MTCC-96) and *Streptococcus pyogenes* (MTCC-443) and gram negative *E.Coli* (MTCC-442) and *Pseudomonas aeruginosa* (MTCC-2488)]. Antibacterial activity was carried out by serial broth dilution method [14-15]. The standard strains used for the antimicrobial activity was procured from Institute of Microbial Technology, Chandigarh. The compounds (4a–j) were screened for their antibacterial activity in triplicate against *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. pyogenes* at different concentrations of 1000, 500, 250, 125, 62.5 µg/ml as shown in **(Table 1)**. The drugs which were found to be active in primary screening were similarly diluted to obtain 125, 62.5 µg/ml concentrations.

The lowest concentration, which showed no growth after spot subculture was considered as MIC for each drug. The highest dilution showing at least 99% inhibition is taken as (MIC). The test mixture should contain 10^8 cells/ml. The standard drug used in this study was 'Norfloxacin, Ciprofloxacin and Chloramphenicol' for evaluating antibacterial activity which showed (100, 50, 25, and 12.5 µg/ml) MIC against *E. coli*, *P. aeruginosa*, *S. aureus*, and S. *pyogenes* respectively.

Table 1: Antibacterial activity of compounds [4a to 4j]

Compound	Minimum Inhibitory			
	Gram negative		Gram positive	
	E.			
4a	250	62.5	125	250
4b	62.	250	250	125
4c	500	250	250	250
4d	125	500	250	500
4e	250	500	125	500
4f	250	500	62.5	1000
4g	250	125	125	500
4h	125	250	250	250
4i	125	125	250	62.5
4j	125	250	250	250
Norfloxacin	50	50	50	50
Ciprofloxacin	50	50	50	50
Chloramphenicol	50	50	50	50

3.2 Antifungal activity

While for the antifungal activity, same compounds were tested for antifungal activity in triplicate against *C. albicans*, *S. Cervecieaceae* and *A. clavatus* at various concentrations of 1000, 500, 200 and 100 µg/ml as shown in **(Table 2)**. The results were recorded in the form of primary and secondary screening. The synthesized compounds were diluted at 1000 µg/ml concentration, as a stock solution. The synthesized compounds which were found to be active in this primary screening were further tested in a second set of dilution against all microorganisms. The lowest concentration, which showed no growth after spot subculture was considered as (MIC) for each drug. The highest dilution showing at least 99% inhibition is taken as MIC. The test mixture should contain 10^8 spores/ml MIC. "Nystatin-B" and "grisiofulvin" was used as a standard drug for antifungal activity, which showed MIC against *C. albicans*, *S. cervecieaceae*, and *A. clavatus*, respectively. The results of antimicrobial evaluation of derivatives (4a–j) are collected in (Table 2).

Table 2: Antifungal activity of compounds [4a to 4j].

Compound	Minimum Inhibitory		
	Fungus		
	C.	*S.*	*A.*
4a	500	250	500
4b	500	500	500
4c	500	1000	1000
4d	1000	1000	1000
4e	500	250	1000
4f	500	1000	1000
4g	500	1000	1000
4h	250	500	500
4i	500	500	500
4j	1000	500	500
Nystatin-B	100	100	100
Gresiofulvin	100	100	100

4. Conclusions

A variety of Schiff base have been successfully synthesized in excellent appreciable yields and screened in vitro for their antimicrobial activities against both strains of Gram-positive, Gram-negative bacteria and fungal strains. All spectral analysis data confirmed the proposed structures for these newly synthesized compounds.

5. Acknowledgement

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Author Profile

Divyanshu D. Patel is Research student, Department of Chemistry, Veer Narmad South Gujarat University, Surat, Gujarat, India.