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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL SCHIFF BASES AND THEIR DERIVATIVES AS ANTIMICROBIAL AGENTS

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ABSTRACT
In the present study, Schiff bases of anthranilic acid have been synthesized by reaction with different aromatic aldehydes and the Azetidinones have been synthesized by cyclocondensation of the Schiff's base with chloroacetyl chloride in the presence of triethylamine. The structures of the newly synthesized compounds were confirmed by IR, ^{1}H NMR, Mass Spectroscopic and Elemental analysis. The synthesized compounds were screened for their *in vitro* growth inhibitory activity against different strains of bacteria and fungi viz., *Staphylococcus aureus*, *Escheria coli* and *Aspergillus niger* were compared with the standard such as gentamycin (50 µg/ml) and fluconazole (50 µg/ml) by cup plate method. Compounds **Ib** and **IIa** exhibits highest antibacterial activity and compound **Ia** and **IIa** showed better antifungal activity.

KEYWORDS: Azetidinone, Schiff's base, Antibacterial, Antifungal.

INTRODUCTION

Schiff bases, a versatile lead molecule for potential bioactive agents and its derivatives were reported to possess antibacterial and antimicobacterial activity.[1,2] Azetidinones derivatives are also reported to have powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and antitubercular activity.[2] In view of the versatile activity of schiff bases and azetidinones we aimed at synthesizing schiff bases of anthranilic acid and azetidinone of synthesized schiff bases, in the hope of getting potent biodynamic agents and evaluate their antibacterial and antifungal activity.

MATERIALS AND METHOD

Anthranilic acid of A. R. grade was purchased from LOBA Chemical, Mumbai and all other chemicals used were of G. R. grade. Melting points were determined using DBK programmed melting point apparatus. The proton nuclear magnetic spectra [^{1}H-NMR] were recorded on Bruker NMR 300 MHz using DMSO as a solvent. Chemical shifts are given in parts per million (ppm). Mass spectra (MS) were recorded on Agilent 1100 Series Iontrap mass spectrometer. The elemental analysis for carbon, hydrogen and nitrogen was performed by HRMS 1200 Series Q/ToF. Infrared spectra were recorded on FTIR spectrophotometer 8400 S, Shimadzu Corporation, Kyoto, Japan. Ultra violet spectra were taken on U.V. 2401(PC) S 220V double beam U.V. spectrophotometer, Shimadzu Corporation, Kyoto, Japan. Purity of the compounds was checked using TLC technique using pre-coated TLC plate (Silica gel 60 F_{254} pre-coated 20x20cm, MERK, Germany), visualizing the spots under iodine vapors and/or by ultraviolet light.

GENERAL PROCEDURE FOR SCHIFF BASES[3] (I):

Ethanolic solution of anthranilic acid (30 ml), (1.3714 g; 0.01 mol) was added in 30 ml ethanolic solution of aldehyde (0.01 mol). The mixture was reflux for 2 h at 100^0 C with continuous stirring. Then the resulting solution was evaporated to remove solvent. The product was washed several times with methanol. It was re-crystallized from hot ethanol and then dried. The reaction was monitored by TLC using toluene: ethyl acetate: methanol as mobile phase in the ratio of 3:6:1. The physicochemical data and elemental analysis data for synthesized Schiff base are given in Table 1.

GENERAL PROCEDURE FOR SYNTHESIS OF AZETIDINONES[3,4] (II):

To a mixture of compound **I** (0.01 mol) and triethylamine (3.49 ml, 0.025 mol) in dioxane (10 ml), chloroacetyl chloride (1.99 ml, 0.025 mol) was added drop-wise at 5-10^0C. The reaction mixture was stirred for 6 h. The reaction was monitored by TLC using toluene: ethyl acetate: methanol as mobile phase in the ratio of 3:6:1. After the completion of reaction, the reaction mixture was poured into crushed ice to get solid, which was filtered and dried. This synthesized product was purified by column chromatography on silica gel {elute: chloroform: n-hexane/benzene= 40:60}. The physicochemical data and elemental analysis data for synthesized Azetidinones are given in Table 1.

ANTIBACTERIAL ACTIVITY[5,6]:

The cup plate method using Hi-Media agar was employed to study the antimicrobial activity of **Ia-d** and **IIa-c** against *S. aureus*, *E. coli* and *A. niger*. Preparation of culture medium and subculture was done as per the standard procedure. Each test compound (5 mg) was dissolved in dimethylformamide (10 ml, 500 µg/ml), which was used as sample solution. Sample size for all the compounds was fixed at 0.1 ml. Using a sterilized cork borer, cups were scooped out of agar medium contained in a petri dish which was previously inoculated with the microorganisms. The test compounds solutions (0.1 ml) were added in the cups and for antibacterial and antifungal activity, the petridishes were subsequently incubated at 37°C for 48 h and 72 h respectively. gentamycin and fluconazole were used as standard drugs for antibacterial and antifungal activity respectively, where as dimethylformamide is used as negative control. Zones of inhibition produced by each compound were measured in mm, and the results are listed in Table 2.

RESULTS AND DISCUSSION

Schiff base of anthranilic acid and Azetidinone were synthesized by reported method in good yield. The purity of the compounds was confirmed by TLC and the synthesized

compounds were analyzed by IR, ^{1}H NMR, Mass and Elemental analysis.

The spectral data of the synthesized compounds are as follows

2-(2'-Hydroxybenzylidine amine) benzoic acid (Ia), Yield, 79 %; Colour, Orange; Melting Point, 196-198; R_f Value, 0.52; λmax, 335.00 nm; IR (KBr, cm^{-1}), 3000-3100 (O-H, str.), 1616.24 (C=N, str.), 1685.67 (C=O, str.); ^{1}H NMR ppm, 6.4-7.1 (m, Ar-H, 8H), 10.1 (s, N=CH, 1H); MS m/z, 242 (M+1); Anal. calcd. for $C_{14}H_{11}O_3N$, C 69.70, H 4.56, N 5.80 %; found, C 69.68, H 4.56, N 5.80 %.

2-(4'-Hydroxybenzylidine amine) benzoic acid (Ib), Yield, (76%); Color, Yellow; Melting Point, 220-222; R_f Value, 0.56; λmax, 284.80 nm; IR (KBr, cm^{-1}), 3330.84 (O-H, str.), 1608.52 (C=N, str.), 1708.81 (C=O, str.); ^{1}H NMR ppm, 6.5-7.2 (m, Ar-H, 8H), 9.7 (s, N=CH, 1H); MS m/z, 242 (M+1); Anal. calcd. for $C_{14}H_{11}O_3N$, C 69.70, H 4.56, N 5.80 %; found, C 69.68, H 4.56, N 5.80 %.

2-(2'-Furfurylidine amine) benzoic acid (Ic), Yield, 67%; Color, Ash; Melting Point, 175-177; R_f Value, 0.38; λmax, 340.80 nm; IR (KBr, cm^{-1}), 2700-3200 (OH, str.), 1618.17 (C=N, str.), 1731.96 (C=O, str.); ^{1}H NMR ppm, 6.6-7.8 (m, Ar-H, 8H), 8.4 (s, N=CH, 1H); MS m/z, 216 (M+1); Anal. calcd. for $C_{13}H_9O_3N$, C 72.55, H 4.18, N 6.51 %; found, C 72.53, H 4.18, N 6.50 %.

2-(4'-Nitrobenzylidine amine) benzoic acid (Id), Yield,70%; Color, Buff; Melting Point, 160-162; R_f Value, 0.58; λmax, 270.80 nm; IR (KBr, cm^{-1}), 3334.69 (OH, str.), 1610.45 (C=N, str.), 1610.45 (C=O, str.); ^{1}H NMR ppm, 6.7-8.4 (m, Ar-H, 7H), 10.1 (s, N=CH, 1H); MS m/z, 271 (M+1); Anal. calcd. for $C_{14}H_{10}O_4N$, C 62.22, H 3.70, N 5.18 %; found, C 62.20, H 3.70, N 5.18 %.

2-(3'-Chloro-4'-(2''-hydroxy phenyl)-2'-oxo azetidin-1'-yl) benzoic acid (IIa), Yield, 73 %; Color, White; Melting Point, 180-182 ; R_f Value, 0.30; λmax, 253.00 nm; IR (KBr, cm^{-1}), 3292.26-3500 (OH, str.), 1631.67 (C=OCOO, str.), 1691.46 (C=Oβ-lactam, str.); ^{1}H NMR ppm, 4.2 (s, CH-Cl, 1H), 7.1 (d, ArCHCHCl, 1H), 7.5-7.8 (m, Ar-H, 8H); MS m/z, 318 (M+1), 300, 271; Anal. calcd. for $C_{16}H_{12}O_4NCl$, C 60.47, H 3.77, N 4.40 %; found, C 60.55, H 3.78, N 4.41 %.

2-(3'-Chloro-4'-(4''-hydroxy phenyl)-2'-oxo azetidin-1'-yl) benzoic acid (IIb), Yield, 70 %; Color, White; Melting Point, 184-186; R_f Value, 0.34; λmax, 253.60 nm; IR (KBr, cm^{-1}), 2750-3100 (OH, str.), 1641.31 (C=OCOO, str.), 1693.38 (C=Oβ-lactam, str.); ^{1}H NMR ppm, 4.3 (s, CH-Cl, 1H), 7.1 (d, ArCHCHCl, 1H), 7.6-8.2 (m, Ar-H, 8H); MS m/z, 318 (M+1), 300, 272; Anal. calcd. for $C_{16}H_{12}O_4NCl$, C 60.47, H 3.77, N 4.40 %; found, C 60.56, H 3.79, N 4.42 %.

2-(3'-Chloro-4'-(2''-furfuryl)-2'-oxo azetidin-1'-yl) benzoic acid (IIc), Yield, 65 %; Color, Brown; Melting Point, 150-152; R_f Value, 0.36; λmax, 254.60 nm; IR (KBr, cm^{-1}), 2750-3250(OH, str.), 1664.45 (C=OCOO, str.), 1681.81 (C=Oβ-lactam, str.); ^{1}H NMR ppm; 3.6 (s, CH-Cl, 1H), 7.0 (d, ArCHCHCl, 1H), 7.5-7.9 (m, Ar-H, 7H); MS m/z, 292 (M+1), 274; Anal. calcd. for $C_{14}H_{10}O_4NCl$, C 57.63, H 3.43, N 4.80 %; found, C 57.71, H 3.43, N 4.80 %.

The results of the biological evaluation of synthesized compounds are given in Table 2. The *in-vitro* microbial study revealed that compounds **Ib** and **IIa** possesses highest antibacterial and the compounds **Ia** and **IIa** showed better antifungal activity. The other synthesized compounds have moderate antibacterial and antifungal activity.

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TABLE 1: PHYSICAL AND ANALYTICAL DATA OF COMPOUNDS Ia-d AND IIa-c

Compound	Mol. Formula	Mol. Wt.	Yield %	mp	% of C, H, N calculated (found)		
					C	H	N
Ia	$C_{14}H_{11}O_3N$	241	79	196-198	69.70(69.68)	4.56 (4.56)	5.80(5.80)
Ib	$C_{14}H_{11}O_3N$	241	76	220-222	69.70(69.68)	4.56 (4.56)	5.80(5.80)
Ic	$C_{13}H_9O_3N$	215	67	175-177	72.55(72.53)	4.18 (4.18)	6.51(6.50)
Id	$C_{14}H_{10}O_4N$	270	70	160-162	62.22(62.20)	3.70 (3.70)	5.18(5.18)
IIa	$C_{16}H_{12}O_4NCl$	317.5	73	180-182	60.47(60.55)	3.77 (3.78)	4.40(4.41)
IIb	$C_{16}H_{12}O_4NCl$	317.5	70	184-186	60.47(60.56)	3.77 (3.79)	4.40(4.42)
IIc	$C_{14}H_{10}O_4NCl$	291.5	65	150-152	57.63(57.71)	3.43 (3.43)	4.80(4.80)

TABLE 2: ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUND Ia-d AND IIa-c

Compound	Bacteria and fungi along with zone of inhibition (mm)								
	S. aureus			*E. coli*			*A. niger*		
	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
Ia	-	-	16	10	11	14	-	14	16
Ib	-	-	18	5	11	17	10	11	14
Ic	-	13	14	-	10	12	-	12	14
Id	-	-	14	5	12	14	-	5	13
IIa	12	14	19	5	11	16	11	13	16
IIb	-	-	13	5	10	13	5	11	12
IIc	-	-	-	-	10	12	-	12	14
Gentamycin	19	23	24	16	19	23	-	-	-
Fluconazol	-	-	-	-	-	-	12	15	16

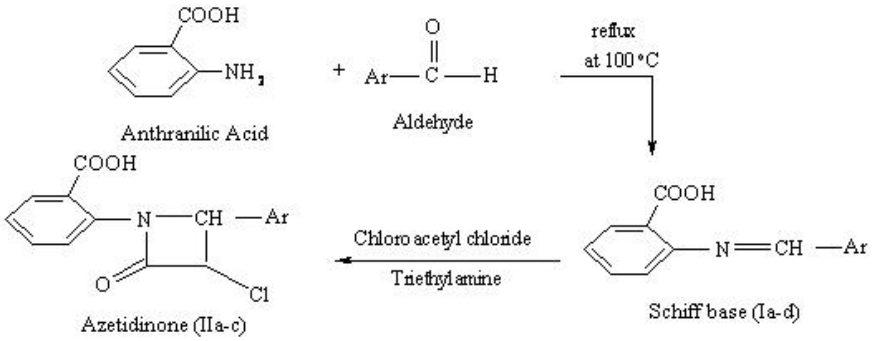

Fig. 1: Scheme of the synthesized compound.
For compound Ia and IIa, Ar is o-hydroxy phenyl; for compound Ib and IIb, Ar is p-hydroxy phenyl; for compound Ic and IIc, Ar is furfuryl and for compound Id, Ar is p-nitro phenyl.

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