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Synthesis and evaluation of antimicrobial activity of some new Schiff bases and formazans

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ABSTRACT

New halogeno Schiff bases and formazans were synthesized and evaluated for antimicrobial activity. Newly synthesized Schiff bases and formazans showed potent activity.

Keywords: Synthesis, Schiff bases, formazans, antimicrobial activity.

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INTRODUCTION

The Schiff bases constitute one of the most active classes of the compounds possessing diversified biological applications. The Schiff bases have been reported to possess higher degree of antitubercular [1], anticancer [2], antibacterial [3], anti-inflammatory [4], antifungal [5]. Schiff bases belong to a widely used group of organic intermediates used for synthesis of pharmaceutical or rubber [6] additives and amino protective group in organic synthesis [7-10]. Various formazans occupy an important role in medicinal chemistry. Formazans have been reported to possess antiviral [11-13], antimicrobial [14,15] and anti-inflammatory [16], anticancer [17], anti-HIV [18] activities.

An environmentally benign synthesis method has received considerable attention. Verma *et al*. [19] reported synthesis of Schiff bases using water as a solvent. Jarrahpour *et al*. [20] has prepared bis-Schiff bases of isatin by conventional method using ethanol. Tania *et al.* [21] reported synthesis of bis-imine Schiff bases under solvent free conditions and also in polypropylene glycol (PPG) as a recyclable reaction medium. Naqvi *et al.* [22] have synthesized Schiff bases using (A) water based synthesis, (B) Microwave synthesis and (C) Grindstone synthesis. Laulloo *etal.* [23] synthesised bis-Schiff bases under solvent free conditions. Jarrahpour and khaili [24] reported synthesis of bis-Schiff bases of isatin and 5-Fluoroisatin in a water suspension medium. All these reported synthetic protocols are simple and giving high yield of products.

Considering wide range of biological activities in Schiff bases and formazans, in the present communication we have synthesized some new Schiff bases by conventional as well as by using grindstone technique further formazans were synthesized by conventional method. All the newly synthesized Schiff bases and formazans were evaluated for antimicrobial acitivities.

Experimental

Synthesis of Schiff bases by conventional method. Method A

Equimolar quantities of halogeno substituted benzaldehyde and substituted aromatic amines were dissolved methanol (15 ml) acetic acid (0.5 ml) was added and refluxed for 2 hr. After completion of reaction (monitored on TLC), the reaction mixture was cooled and poured in water, solid separated out. Solid was filtered, washed with water and crystallized from ethanol to give corresponding Schiff bases **1a-i**. M.P. yield and analytical data of Schiff bases are given in Table-1.

Synthesis of Schiff bases by grindstone technique. Method B

Substituted benzaldehyde and substituted aromatic amine (0.01 mol) each were taken in a mortar, acetic acid (0.5 ml) and water (5 ml) added and reaction mixture grounded for 15-30 min (monitored on TLC). On completion of reaction, water 25 ml added and stirred. Separated solid was filtered, washed with water and crystallized from ethanol to corresponding

Schiff bases **1a-f**. M.P. and mixed M.P. with Schiff bases obtained by method A was not depressed.

Spectral Data of some Schiff Bases

Compound 1e

IR (ν max) cm^{-1} : 3078, 2960, 1597, 1618, 1506, 1209, 1093, 827
^1H NMR ($CDCl_3$) : δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 7.25-7.62 (m, 6H, Ar-H), 8.63 (s, 1H, =CH)
MS: m/z : 355 (M^+), 338, 274, 188, 138, 111, 75, 50

Compound 1g

IR (ν max) cm^{-1} : 3076, 2978, 1778, 1666, 1506, 1444, 1388, 1155, 868.
^1H NMR ($CDCl_3$) : δ 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 7.14-8.31 (m, 5H, Ar-H), 8.62 (s,1H, =CH)
MS: m/z : 490 (M^+), 366, 267, 245, 138, 91, 50.

Compound 1h

^1H NMR ($CDCl_3$) : δ 3.81 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 7.14-7.87 (m, 5H, Ar-H), 8.56 (s, 1H, =CH)
MS: m/z : 481(M^+) 446, 420, 400, 264, 187, 110, 75, 50

General Procedure for Synthesis of Formazans

P-nitroaniline (0.01 mol) dissolved in acetic acid (2 ml) and added hydrochloric acid (2ml, 0.5N). The solution was cooled to 0-5°C and sodium nitrite (0.015mmol) dissolved in water was added drop wise within 10 min. Schiff base (0.01 mol) was dissolved in pyridine (5 ml) and cooled to 0-5°C. To this cold solution, diazotized amine solution was added drop wise with shaking within 10 min and solution left at room temperature for 3 hr. Solid separated out. Solid was filtered, washed with water and crystallized from ethanol to give corresponding formazans **2a-f**. M.P., yield and analytical data are given Table-2.

Spectral Data of some Formazans

Compound 2d

IR (ν max) cm^{-1} : 3105, 3005, 2314, 1668, 1504, 1039, 738.
^1H NMR ($CDCl_3$) : δ 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 7.30-8.34 (m, 9H, Ar-H)
MS: m/z : 640 (M^+), 579, 551, 442, 408, 359, 244, 229, 173, 122, 107, 94, 79.

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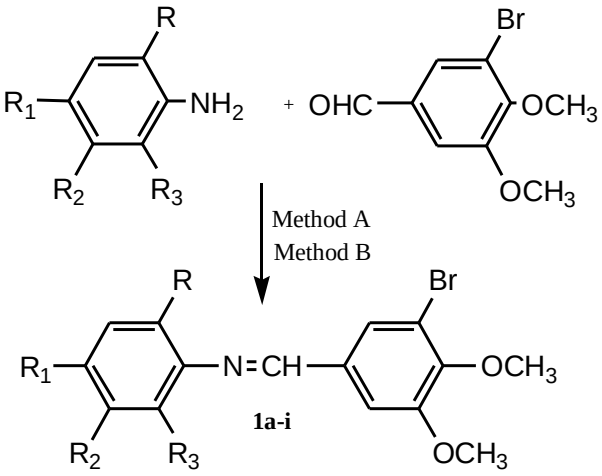
Compound 2f

IR (v max) cm^{-1} : 3076, 3007, 2438, 1670, 1506, 1010, 742.

^1H NMR ($CDCl_3$) : δ 3.80 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3) 7.30-8.25 (m, 10H, Ar-H).

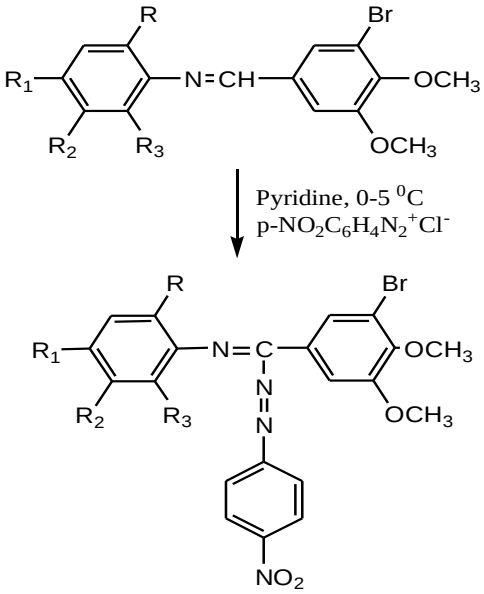
Scheme-1: Synthesis of Schiff bases 1a-i:



Method A:Two drops of CH3COOH, EtOH, Reflux

Method B:Two drops of CH_3COOH, Grinding

Scheme-2: Synthesis of Formazans 2a-f:



2a-f

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Table-1 Physical and Analytical Data of Schiff Bases. (1a-i)

Entry	R	R_1	R_2	R_3	R_4	R_5	M.P. ^{0}C	Yield (%)		Halogen Analysis (%)	
								Meth A	Meth B	Calcd.	Found
1a	H	Cl	H	H	OMe	OMe	80	78	86	12.88	12.97
1b	H	Cl	H	OMe	OMe	OMe	105	80	87	11.62	11.50
1c	I	NO_2	H	H	OMe	OMe	248	76	87	30.82	30.94
1d	I	NO_2	H	OMe	OMe	OMe	250	74	85	28.73	28.85
1e	H	Cl	H	Br	OMe	OMe	158	76	90	32.58	32.44
1f	Cl	I	Cl	Br	OMe	OMe	148	73	84	53.98	54.09
1g	NO_2	I	H	Br	OMe	OMe	138	75	90	42.15	42.26
1h	Cl	I	H	Br	OMe	OMe	158	77	89	50.46	50.37
1i*	H	NO_2	H	OMe	OMe	OMe	247	72	84	-----	-----

1i*: Calcd. C, 67.36; H, 4.21. **Found**: C, 67.53; H, 4.31.

Table-2 Physical and Analytical Data of Formazans. (2a-f)

Entry	R	R_1	R_2	R_3	M.P.^{0}C	Yield %	Halogen Analysis (%)	
							Calcd.	Found
2a	I	H	I	NO_2	164	85	32.34	32.49
2b	I	H	I	Cl	177	79	37.68	37.52
2c	Cl	I	H	Cl	130	82	41.86	41.99
2d	H	NO_2	H	I	151	86	32.34	32.45
2e	Cl	H	H	Cl	170	88	28.06	27.93
2f	H	Cl	H	H	134	80	22.94	23.07

RESULTS AND DISCUSSION

Schiff bases (**1a-i**) were synthesized by condensation of substituted aldehydes with aromatic amines. Substituted benzaldehydes and aromatic amines were dissolved in methanol, few drops of acetic acid added and refluxed for 2 hr (monitored on TLC). Reaction mixture on cooling to room temperature or on pouring in cold water solid separated. Solid was filtered, washed with cold water and crystallized from ethanol (scheme-**1**).

Schiff bases (**1a-i**) were also synthesized by grindstone technique. Substituted benzaldehydes and substituted aromatic amines were taken in mortar, water (2 ml) and acetic acid (0.5 ml) added and grounded for 20-30 min (reaction monitored on TLC). The color of the reaction mixture was changes to pale yellow. Solid separated was filtered, washed with water and crystallized from ethanol (scheme-**1**). Both these methods gave unambiguous product, reaction conditions are simple giving excellent yield, without any side products. Grindstone method is eco-friendly in absence of organic solvent, or any costly catalyst.

The structures of the Schiff bases were assigned on the bases of elemental analysis and spectral data. IR spectra of Schiff bases showed characteristic band in the region 1610-1635 cm^{-1} due to C=N. Stretching vibration and band around 1600-1480 cm^{-1} due to aromatic stretching. ^1H NMR spectra of Schiff bases showed multiplet in the region 7.2-8.2 due to aromatic protons. A singlet of azomethine was observed at δ 8.5-9 and phenolic hydroxy group as a singlet at δ 12-13. Mass spectra of the Schiff bases were in agreement with its suggested structure.

Formazans **2a-f** were synthesized from Schiff bases. Schiff bases were dissolved in pyridine and cooled up to 0-5^{0}C and diazotized. The cold solution of diazotized p-nitro aniline was added with stirring into cold solution of Schiff bases within 10 minutes. The reaction mixture was allows to stand for 3 hrs at room temperature. A dark colored solid separated which was filtered, washed with water and crystallized from ethyl alcohol (scheme-**2**). Purity of synthesized formazans was checked by TLC and structures were assigned on the basis of halogen analysis and spectral data.

Formazans showed characteristics band at near 1558 cm^{-1} due to N=N stretching vibration. ^1H NMR showed a singlet peak at δ 8-9 due to azomethine proton in precursor, did not appeared in formazans. Mass spectrum of formazans was in good agreement with their suggested structure.

Antimicrobial activity

The antibacterial activity of newly synthesized compounds (**1a-i**) and (**2a-f**) was determined by cup plate agar diffusion method [25]. The compounds were evaluated for antibacterial activity against *B.subtillus (Bs)* and *E.coli.* The antibiotic *Streptomycin* was used as standard antibiotic and 5% DMF was used as solvent control.

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5 ^{0}C for 24 hr. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 ml of respective bacterial culture strain prepared in sterile saline (0.85%) of CFU/Ml of compound solution at fixed concentration 25 µg/ml separately for each bacterial strain. All the plates were incubated at 37±0.5 ^{0}C for 24 hr. The activity is reported by measuring the diameter of zone of inhibition in mm scale. Table- **3**.

The *A.niger (An)* and *A.flavus (Af)* fungi were used for antifungal activity and *Fluconazole* was used as standard fungicide by poison plate method [26]. The fungal culture was maintained on Potato Dextrose Agar (PDA) and Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27± 0.2 ^{0}C for 12 h. After incubation well prepared using sterile cork borer and each agar well was filled with 0.1 mL of compound solution at fixed concentration 25 µ/mL. The plates were kept in refrigerator for 20 min for diffusion and then incubated at 27± 0.2 ^{0}C for 5 days. After incubation, zone of inhibition of compounds were measured in mm along with standard, Table-**4.**

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Table: 3 Antimicrobial activity of Schiff bases (1a-i)

Entry	Zone of inhibition in mm			
	Ba	E.coli	A.niger	A.flavus
1a	20	15	15	-------
1b	26	17	12	12
1c	28	26	21	22
1d	27	16	22	25
1e	24	15	14	13
1f	28	24	19	26
1g	17	10	09	13
1h	25	18	24	21
1i	19	27	12	18
Streptomycin	**26**	**24**	-------	-------
Fluconazole	-------	-------	**25**	**27**

Table: 4 Antimicrobial activity of formazans. (2a-f)

Entry	Zone of inhibition in mm			
	Ba	E.coli	A.niger	A.flavus
2a	13	24	11	16
2b	17	10	09	13
2c	24	`20	21	25
2d	12	10	14	10
2e	22	15	12	07
2f	29	17	12	12
Streptomycin	**26**	**24**	-------	-------
Fluconazole	-------	-------	**25**	**27**

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