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Synthesis and antibacterial activity of some Schiff bases derived from 4-aminobenzoic acid

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Abstract: The following Schiff bases have been synthesized: (1) 4-[(2-chlorobenzylidene)amino]benzoic acid [JP1], (2) 4-[(furan-2-ylmethylene)amino]benzoic acid [JP2], (3) 4-[(3-phenylallylidene)amino]benzoic acid [JP3], (4) 4-[(2-hydroxybenzylidene)amino]benzoic acid [JP4], (5) 4-[(4-hydroxy-3-methoxybenzylidene)amino]benzoic acid [JP5] and (6) 4-[(3-nitrobenzylidene)amino]benzoic acid [JP6]. They were screened as potential antibacterial agents against a number of medically important bacterial strains. The antibacterial activity was studied against *A. faecalis* ATCC 8750, *E. aerogenes* ATCC 13048, *E. coli* ATCC 25922, *K. pneumoniae* NCIM 2719, *S. aureus* ATCC 25923, *P. vulgaris* NCIM 8313, *P. aeruginosa* ATCC 27853 and *S. typhimurium* ATCC 23564. The antibacterial activity was evaluated using the Agar Ditch method. The solvents used were 1,4-dioxane and dimethyl sulfoxide. Different effects of the compounds were found in the bacterial strains investigated and the solvents used, suggesting, once again, that the antibacterial activity is dependent on the molecular structure of the compound, the solvent used and the bacterial strain under consideration. In the present work, 1,4-dioxane proved to be a good solvent in inhibiting the above stated bacterial strains.

Keywords: 4-aminobenzoic acid, Schiff bases, antibacterial activity, DMSO, 1,4-dioxane.

INTRODUCTION

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the increasing microbial resistance to antibiotics in use nowadays necessitates the search for new compounds with potential effects against pathogenic bacteria. The most spectacular advances in medicinal chemistry have been made when heterocyclic compounds played an important role in regulating biological activities.

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Extensive investigations in the field of Schiff bases have been reported.[1,2] Their preparation, chemical and physical properties have been described by various workers.[3,4] Several workers have reported that Schiff bases formed from aromatic aldehydes or aromatic ketones and their derivatives are quite stable. Due to the great flexibility and diverse structural aspects of Schiff bases, a wide range of these compounds have been synthesized and their complexation behavior studied.[5,6] Nitro and halo derivatives of Schiff bases are reported to have antimicrobial and antitumor activities.[7] Antimicrobial and antifungal activities of various Schiff bases have also been reported.[8–10] Sahu *et al.*[11] reported fungi toxicity of some Schiff bases. Gawad *et al.*[12] synthesized some Schiff bases and observed high antimicrobial activities. Many Schiff bases are known to be medicinally important and are used to design medicinal compounds.[13–15]

It this work, the synthesis and characterization of some Schiff bases for pharmacological studies are reported.

EXPERIMENTAL

Synthesis of Schiff bases

The following Schiff bases were synthesized: 1. 4-[(2-chlorobenzylidene)amino]benzoic acid (JP1); 2) 4-[(furan-2-ylmethylene)amino]benzoic acid (JP2); 3) 4-[(3-phenylallylidene)amino]benzoic acid (JP3); 4) 4-[(2-hydroxybenzylidene)amino]benzoic acid (JP4); 5) 4-[(4-hydroxy-3-methoxybenzylidene)amino]benzoic acid (JP5) and 6) 4-[(3-nitrobenzylidene)amino]benzoic acid (JP6).

To the requisite amount of aldehyde dissolved in 200 ml methanol, 0.1 mol of amine and few drops of glacial acetic acid were added and the mixture was refluxed for 10–12 h at 70–80 ºC in a water bath. The resulting solution was cooled to room temperature, and then poured over crushed ice with constant stirring. The precipitate was filtered and washed with sodium bisulfite solution to remove excess of aldehyde. The product was crystallized from hot methanol and dried.

$$\text{R-CHO} + \text{R'-NH}_2 \rightarrow \text{R-CH=N-R'}$$

In this reaction for JP1, R'-NH_2 is 4-aminobenzoic acid, and R is as given in Table I.

Test microorganisms

The bacterial strains studied were identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India: *Alcaligenes faecalis* ATCC 8750, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* NCIM 2719, *Staphylococus aureus* ATCC 25923, *Proteus vulgaris* NCIM 8313, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 23564.

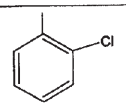
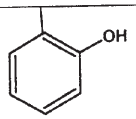
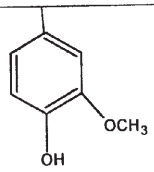
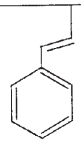
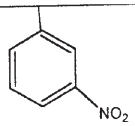
Preparation of the test compound

The compounds were dissolved at a concentration of 10 mg/ml in either of the two solvents (DMSO/1,4-dioxane) in order to obtain a final concentration of 1 mg/0.1 ml. In all, 3 different concentrations of the drug were prepared (1 mg/0.1 ml, 0.1 mg/0.1 ml, 0.01 mg/0.1 ml) for the microbiological assays. The synthesized Schiff bases are soluble only in DMF, 1,4-dioxane and DMSO and from these, two solvents, *i.e*. DMSO and 1,4-dioxane, were selected in the present work.

Preparation of the plates and microbiological assays

A loop full of the given test strain was inoculated in 25 ml of N-broth (Nutrient Broth) and was incubated for 24 h in an incubator at 37 ºC in order to activate the bacterial strain. The plates were

TABLE I.

COMPOUND CODE	R	COMPOUND CODE	R
JP1		JP4	
JP2		JP5	
JP3		JP6	

prepared by dissolving 38 g of Mueller Hinton Agar No. 2 in 1000 ml of distilled water. In order to proceed with the Agar ditch method,[16] 28–30 ml of the autoclaved Mueller Hinton Agar No. 2 media was added into a 100 mm diameter Petri-plate. Inoculation of the test strain was done by the Pour-plate technique. 0.2 ml of the activated strain was inoculated into the media when it reached a temperature of 40–45 ºC. Proper homogenization of the strain was realized by gently shaking the sugar tube followed by gently pouring into a Petri plate. Formation of air bubbles during this procedure of inoculation was strictly avoided. The complete procedure of the preparation of the plate was performed in a Laminar airflow to maintain strict sterile and aseptic conditions. The media was allowed to solidify. After solidification of the media, a ditch/well was made in the plates with the help of a cup-borer (0.85 cm) and then 0.1 ml of the synthetic compound (dissolved in DMSO/1,4-dioxane) was added into the well. The controls were maintained (for each bacterial strain and each solvent), where 0.1 ml of the pure solvent was inoculated into the well.

The antibacterial activities of the synthetic compounds were determined by the inhibition zone formed by these compounds against the particular test bacterial strain.

RESULTS AND DISCUSSION

In all, 6 compounds were synthesized (Table II) and the IR and NMR spectral data confirmed their molecular structure. The IR and NMR analysis data are given below:

JP1: IR (KBr, cm^{-1}): –OH (str.): 3344, C=O (str.): 1685, N=C: 1608, –OH (bend): 1315, C–Cl (str.): 839.
^{1}H-NMR (δ, ppm): 6.62–7.75 (8H, Ar–H), 8.89 (1H, N=CH), 10.45 (1H, –COOH).

JP2: IR (KBr, cm^{-1}): –OH (str.): 3352, C=O (str.): 1681, N=C: 1600.8, C–O–C (str.): 1388, –OH (bend.): 1271.
^{1}H-NMR (δ, ppm): 7.10–7.72 (7H, Ar–H), 8.30 (1H, N=CH), 9.48 (1H, –COOH).

JP3: IR (KBr, cm^{-1}): –OH (str.): 3321, C=O (str.): 1674, N=C: 1600.8, –OH (bend): 1311.

^{1}H-NMR (δ, ppm): 6.89–8.12 (11H, Ar–H), 8.25 (1H, N=CH), 9.81 (1H, –COOH).

JP4: IR (KBr, cm^{-1}): –OH (str.): 3417, –OH (str.): 3013, C=O (str.): 1681, N=C: 1581, –OH (bend): 1419.
^{1}H-NMR (δ, ppm): 6.62–8.06 (8H, Ar–H), 8.82 (1H, N=CH), 10.09 (1H, Ar–OH), 10.75 (1H, –COOH).

JP5: IR (KBr, cm^{-1}): –OH (str.): 3367, C–H (asym. str.): 2922, C=O (str.): 1685, N=C: 1600.8, C–H (sym. str.): 1454, C–O–C (str.): 1396, –OH (bend.): 1315.
^{1}H-NMR (δ, ppm): 3.90 (3H, –OCH_3), 6.95–8.00 (7H, Ar–H), 8.34 (1H, N=CH), 9.07 (1H, Ar–OH), 10.60 (1H, –COOH).

JP6: IR (KBr, cm^{-1}): –OH (str.): 3315, C=O (str.): 1691.5, N=C: 1602, Ar–NO_2: 1571, –OH (bend.): 1315.
^{1}H-NMR (δ, ppm): 6.68–8.26 (8H, Ar–H), 8.60 (1H, N=CH), 10.10 (1H, –COOH).

TABLE II. Compound code, molecular formula, molecular weight, melting point, percentage yield and R_f values for the stated solvent system

Compd. Code	Molecular formula	Molecular weight/g mol^{-1}	M.p./ºC	Yield/%	R_f*
JP1	$C_{14}H_{10}NO_2Cl$	256.68	217	52	0.45*
JP2	$C_{12}H_9NO_3$	215.20	>300	59	0.48
JP3	$C_{16}H_{13}NO_2$	251.28	172	63	0.58
JP4	$C_{14}H_{11}NO_3$	241.24	260	57	0.41*
JP5	$C_{15}H_{13}NO_4$	271.26	115	55	0.52
JP6	$C_{13}H_9N_2O_4$	257.22	250	65	0.34

*Ethyl acetate + hexane (2.5 + 7.5); Acetone + benzene (4.0 + 6.0)

The 6 synthetic compounds and their respective controls produced different inhibition zones against the tested bacterial strains. The controls were deducted from the tested compounds; their effect was noticeably different depending on the type of solvent used. Of the three concentrations evaluated, the lowest concentration had little effect while the compounds were slightly effective at a concentration of 0.2 mg/0.1 ml (data is not shown). The third concentration (*i.e.*, 1.0 mg/0.1 ml) was effective and only this data will be presented.

The *in vitro* antibacterial activity of the six Schiff bases in DMSO and DMF against medically important Gram positive and Gram negative bacteria is shown in Table II.

In *A. faecalis* none of the compounds in DMSO showed any antibacterial activity while in 1,4-dioxane, antibacterial activity was observed to a certain extent; JP4 and JP6 showed comparatively more activity followed by JP3 and JP5; JP1 and JP2

showed the least activity (Table III). An entirely different trend was observed when the same compounds were tested against *E. aerogenes*. In *E. aerogenes* in DMSO, JP4 showed considerable inhibitory activity, followed by JP1; JP2 and JP3 were inactive while JP5 and JP6 showed intermediate activity. These Schiff bases in 1,4-dioxane showed considerably less antibacterial activity than in DMSO in *E. aerogenes*. JP2, JP3 and JP5 in 1,4-dioxane were not effective at all. Comparing the antibacterial activity of the six Schiff bases against *A. faecalis* and *E. aerogenes*, a differential effect of both of the solvents could be observed. All the compounds showed better antibacterial activity against *A. faecalis* when 1,4-dioxane was used as the solvent, while the same compounds showed no activity or less activity against *E. aerogenes*. Also, all the compounds showed no activity at all in *A. faecalis* when DMSO was used, while in *E. aerogenes* these compounds showed antibacterial activity to a certain extent.

TABLE II. The *in vitro* antibacterial activity of the synthesized Schiff bases (10 mg/ml)

Microorganisms	Inhibition zone (mm/100 µl)											
	JP1		JP2		JP3		JP4		JP5		JP6	
	A	B	A	B	A	B	A	B	A	B	A	B
A. faecalis	0	0.7	0	0.3	0	1.5	0	2.2	0	1.5	0	2.2
E. aerogenes	4.3	0.5	0.6	0	0.3	0	8.6	1.5	1.3	0	2	1.5
E. coli	0	0	0	0	0	0	0	0.5	0	1	0	1.3
K. pneumoniae	2.5	1.6	0	1.3	0	0	0	2.6	0	2	12.6	2.6
P. vulgaris	3	0.1	0	0	0	0	0	0.5	0	0	1.3	0
P. aeruginosa	0	0	0	0	0	0	0	0	0	0	0	0
S. typhimurium	0	1.3	0	1.7	0	0.8	0	1	0	0.2	0	0.3
S. aureus	0	1.2	0	0	4.1	3	0	0	0	0	15.8	18

Extraction solvent (100 µl) A: dimethyl sulfoxide (DMSO); B: 1,4-dioxane

This different effect of the compounds against bacteria may be because of the structure of the compound and also the solvent used. The diffusion capacity of the compounds varies with the employed solvent, which may be because of the polarity of the solvent. In all the six compounds, the central ligand is 4-aminobenzoic acid with different side chains. In JP1 it is *o*-chlorobenzaldehyde, in JP2 it is furfuraldehyde, in JP3 it is cinnamaldehyde, in JP4 it is salicylaldehyde, in JP5 it is vanillin and in JP6 it is *m*-nitrobenzaldehyde.

The antibacterial activity of the synthetic compounds against *E. coli* were similar to that shown in *A. faecalis*. None of the compounds in DMSO showed any antibacterial activity, while in 1,4-dioxane, JP1, JP2 and JP3 showed no activity while the other three compounds showed some antibacterial activity. This different response is because of the difference in their molecular structures. The compound JP6 in DMSO showed high inhibitory activity against *K. pneumoniae*, while JP1 showed little and the

other four compounds showed no inhibitory activity against these Gram negative bacteria. The same compounds in 1,4-dioxane showed antibacterial activity against *K. pneumoniae*, except JP3. In *P. vulgaris*, only two compounds (JP1 and JP6) showed inhibitory activity, while the compounds showed no antibacterial activity when 1,4-dioxane was the solvent used. None of the compounds in either of the solvents could inhibit *P. aeruginosa*; thus this bacterium appears to be the most resistant bacterium. In *S. typhimurium*, the compounds extracted in DMSO were inactive while the 1,4-dioxane extracted compounds showed low inhibitory activity. JP3 and JP6 were the only compounds which showed antibacterial activity against the Gram positive bacteria *S. aureus*. These two compounds showed inhibitory zones in both solvents. JP3 showed less activity while the maximum was shown by JP6.

It can be deduced from these results that the different response of the synthesized Schiff bases arise because of their structural differences and are also solvent dependent, *i.e.*, the polarity of the solvent is also responsible for inhibition of the bacteria under investigation.

From this study, it can be concluded that it cannot be assumed that one solvent is better than the other. It is dependent on the molecular structure and the particular bacterial strain considered. However, with the studied compounds, 1,4-dioxane appears to be a better solvent than DMSO since it has a broad spectrum (though less) of inhibitory activity. In an earlier study,[17] it was shown that cinnamaldehyde as a side chain and sulfonamide as the central ligand exhibited considerable antibacterial activity. In the present study, the central ligand being 4-aminobenzoic acid, the same inhibition against these medically important bacteria could not be produced.

ИЗВОД

СИНТЕЗА И АНТИБАКТЕРИЈСКА АКТИВНОСТ НЕКИХ ШИФОВИХ БАЗА ИЗВЕДЕНИХ ОД 4-АМИНОБЕНЗОЕВЕ КИСЕЛИНЕ

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Синтетизоване су следеће Шифове базе: (1) 4-[(2-хлоро-бензилиден)-амино]-бензоева киселина [JP1], (2) 4-[(фуран-2-илметилен)-амино]-бензоева киселина [JP2], (3) 4-[(3-фенил-алилиден)-амино]-бензоева киселина [JP3], (4) 4-[(2-хидрокси-бензилиден)-амино]-бензоева киселина [JP4], (5) 4-[(4-хидрокси-3-метокси-бензилиден)-амино]-бензоева киселина [JP5] и (6) 4-[(3-нитро-бензилиден)-амино]-бензоева киселина [JP6]. Њихова потенцијална антибактеријска активност испитана је на низу медицинских важних бактеријских врста. То су: *A. faecalis* ATCC 8750, *E. aerogenes* ATCC 13048, *E. coli A*TCC 25922, *K. pneumoniae* NCIM 2719, *S. aureus* ATCC 25923, *P. vulgaris* NCIM 8313, *P. aeruginosa* ATCC 27853 и *S. typhimurium* ATCC 23564**.** Испитивање је вршено коришћењем методе бунарчића у агару**.** Растварачи су били 1,4-диоксан и диметил-сулфоксид. Различити запажени ефекти указују на значај молекулских структура једињења и врсте растварача на испитивану бактеријску врсту. Показано је да је 1,4-диоксан добар растварач за инхибицију испитиваних бактеријских врста.

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