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Evaluation of Biological Activities of Some Schiff Bases and Metal Complexes

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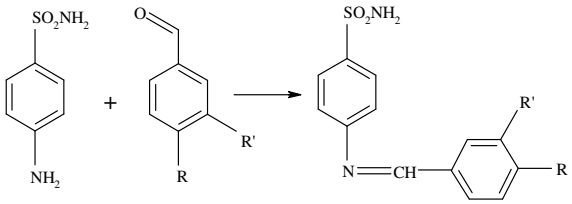
Several Schiff bases were synthesised from sulphonamide and resacetophenone. The characterisation was done by CHN analysis, IR and NMR spectral data. These Schiff bases were evaluated for their antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungi. The antibacterial activity was studied against *B. megaterium, E. coli, B. subtilis, P. fluorescens* and antifungal activity against *A. awamori*. In addition, copper, nickel, cobalt, and iron complexes of two Schiff bases were also synthesised. Their structural characterisation was performed using CHN analysis and IR spectral data and their antibacterial and antifungal activities were also evaluated. The comparison of antimicrobial activities of the ligands and complexes shows that the presence of metal causes more inhibition i.e., more activity. Out of the four metals studied, cobalt and iron were found to have more antimicrobial activity.

Keywords: Antibacterial activity, Metal complexes, Schiff bases

INTRODUCTION

Day by day Schiff bases are more frequentlyapplied for the betterment of human welfare. The importance of the Schiff base is due its versatile nature. Literature survey shows that many Schiff bases exhibit biological activities [1-4] such as antifungal, antibacterial, antitumor, anti-inflammatory, and antipyretic, among others. Some of them have been used as complexing agents [5,6] and powerful corrosion inhibitors [7]. They are synthesised from various compounds [8-11]. The main aim of the present work is to find new molecules such as these by synthesising several Schiff bases from sulphonamide and resacetophenone.



SB1: R = -H, R' = -H
SB2: R = -OCH_3, R' = -H
SB3: R = -SCH_3, R' = -H
SB4: R = -OCH_3, R' = -OH

Scheme 1

EXPERIMENTAL

Synthesis of Schiff Bases Derived from Sulphanilamide (Scheme 1)

Sulphonamide (17.2 g) was dissolved in 200 ml methanol, to which was added 0.01 mole of aldehyde and the mixture was refluxed for 10-12 h at 75-80 °C. The mixture was then poured over crushed ice, filtered and dried.

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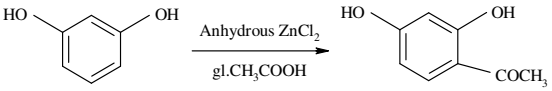
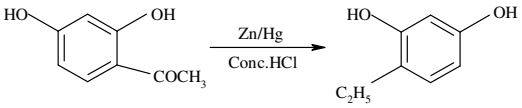
Synthesis of Schiff Bases Derived from Resacetophenone

Preparation of resacetophenone (Scheme 2). Anhydrous zinc chloride (165 g) was dissolved in 158 ml glacial acetic acid. Resorcinol (110 g) was added and the mixture was heated at 110 °C for 3 h. The resulting solution was cooled to room temperature and then poured over crushed ice. The product was filtered and washed with 30% HCl to remove excess zinc chloride. The product was crystallized from methanol, dried and weighed.



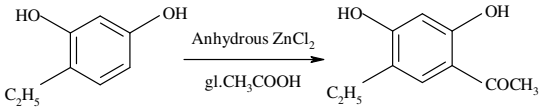
Scheme 2

Synthesis of 4-ethyl resorcinol (Scheme 3). Zinc dust (200 g), 15 g of $HgCl_2$ and 250 ml of water were mixed thoroughly in a round bottom flask (1000 ml). 10 ml of concentrated HCl was added and the mixture was shaken vigorously for 5 min. The aqueous layer was decanted and 70 g of resacetophenone, 100 ml of water and 25 ml of concentrated HCl were added. The mixture was refluxed for 5 h on a sand bath. Concentrated HCl was added in small amounts at fixed intervals, until the zinc amalgam was used up. On cooling, solid crystals of 4-ethyl resorcinol were obtained and recrystallized in hot benzene.



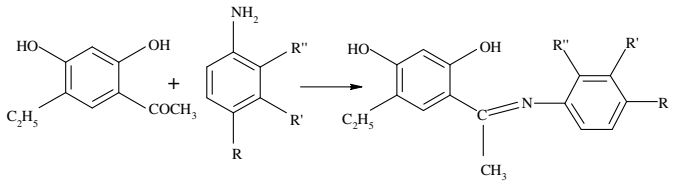
Scheme 3

Synthesis of 5-ethyl resacetophenone (Scheme 4). Anhydrous zinc chloride (140 g) was dissolved in 210 ml glacial acetic acid and 52.5 g of 4-ethyl resorcinol was added to it. The mixture was boiled at 110 °C for 3-4 h and allowed to cool to room temperature. The resulting solution was poured over crushed ice with constant stirring. The precipitate was filtered, dried and crystallized from hot benzene.



Scheme 4

Condensation of 5-ethyl resacetophenone with aniline derivatives (Scheme 5). 5-Ethyl resacetophenone (0.01mol) was dissolved in methanol. Anhydrous $ZnCl_2$ (approx. 1 g) and 0.01 mole of aniline derivative were added and the solution was refluxed in an oil bath at 140-160 °C for 4-5 h. The resulting solution was poured over crushed ice. The product was filtered, dried and crystallized from methanol.



SB5: R = -NO_2, R’ = -H, R” = -H
SB6: R = -CH_3, R’ = -H, R” = -CH_3
SB7: R = -H, R’ = -NO_2, R” = -H
SB8: R = -H, R’ = -CH_3, R” = -H

Scheme 5

Preparation of Plates and Microbiological Assays

The *in vitro* antibacterial activity of the synthesized compounds was tested against some clinically important bacteria by the well diffusion method using Mueller-Hinton agar No. 2 as the nutrient medium. The solutions of Schiff bases (10 mg ml^{-1}) were prepared in DMF. The bacterial strains were activated by inoculating a loop full of the test strain into 25 ml of nutrient broth and incubated for 24 h in an incubator at 37 °C. The activated strain (0.2 ml) was inoculated in Mueller-Hinton agar at 45 °C. It was then poured into Petri plates and allowed to solidify. After

Table 1. Characteristics and Yields of Synthesised Schiff Bases

Compound code	Molecular formula	Molecular weight (g mol^{-1})	M.P. (°C)	Yield (%)	R_f[a] Value
SB1	$C_{13}H_{12}N_2O_2S$	260	190	68.59	0.66
SB2	$C_{14}H_{14}N_2O_3S$	290	206	59.84	0.69
SB3	$C_{14}H_{14}N_2O_3S_2$	306	222	63.14	0.60
SB4	$C_{14}H_{14}N_2O_4S$	306	202	55.25	0.57
SB5	$C_{16}H_{16}N_2O_4$	300	78	52.15	0.51
SB6	$C_{18}H_{21}N_2O_2$	283	108	49.06	0.41
SB7	$C_{16}H_{16}N_2O_4$	300	62	54.38	0.71
SB8	$C_{17}H_{14}NO_4$	269	110	60.72	0.72

[a]Solvent Systems. Acetone:Benzene (4:6): Comps. SB1-SB6. Acetone:Benzene (1:9): Comp. SB7. Acetone:Benzene (2:8): Comp. SB8.

solidification of the media, a 0.85 cm ditch was made in the plates using a sterile cork borer and these were completely filled with the test solution. The plates were incubated for 24 h at 37 °C. The experiment was repeated three times simultaneously under the same conditions for each compound and the mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain with the solvent, where pure solvent was inoculated into the well. The inhibition zone, formed by the Schiff bases, against the particular bacterial strain were subtracted from the control, thereby determining the antibacterial activities of the Schiff bases.

RESULTS AND DISCUSSION

Characterization of Compounds SB1-SB8

Table 1 shows the molecular formula, molecular weight, melting points, percent yield and R_f values of all the synthesised compounds. The IR and NMR data of all the Schiff bases are given below.

SB1: Found (Calcd.): C, 60.10 (60.00); H, 4.50 (4.61); N, 10.72 (10.77); IR (KBr, cm^{-1}): 3298 (-NH_2 str.), 1578 (-NH_2 bend.), 1335 (-S=O sym.), 1155 (-S=O asym.), 1624 (N=C); ^{1}H NMR (ppm): 5.77 (1H, singlet, =CH), 6.55-8.00 (9H, multiplet, Ar-H), 10.00 (2H, -NH_2).

SB2: Found (Calcd.): C: 58.00 (57.93), H: 4.75 (4.83), N: 9.60 (9.66); IR (KBr, cm^{-1}): 3279 (-NH_2 str.), 1584 (-NH_2 bend.), 1318 (-S=O sym.), 1172 (-S=O asym.), 1610 (N=C), 1411 (-C-O-C); ^{1}H NMR (ppm): 3.86 (3H, singlet, -OCH_3), 5.78(1H, singlet, =CH), 6.57 -7.92 (8H, multiplet, Ar-H), 9.87 (2H, -NH_2).

SB3: Found (Calcd.): C: 54.96 (54.90), H: 4.55 (4.57), N: 9.10 (9.15); IR (KBr, cm^{-1}): 3290 (-NH_2 str.), 1582 (-NH_2 bend.), 1333 (-S=O sym.), 1154 (-S=O asym.), 1619 (N=C), 665 (-C-S); ^{1}H NMR (ppm): 2.50 (3H, singlet, -SCH_3), 5.78 (1H, singlet =CH), 6.55-7.89 (8H, multiplet, Ar-H), 9.92 (2H, -NH_2).

SB4: Found (Calcd.): C: 54.98 (54.90), H: 4.52 (4.57), N: 9.05 (9.15); IR (KBr, cm^{-1}): 3465 (-OH str.), 1277 (-OH bend.), 3347 (-NH_2 str.), 1597 (-NH_2 bend.), 1320 (-S=O sym), 1153 (-S=O asym.), 1646 (N=C); ^{1}H NMR (ppm): 3.84 (3H, singlet, -OCH_3), 5.78 (1H, singlet =CH), 6.58 -7.45 (7H, multiplet, Ar-H), 9.77 (2H, singlet, -NH_2), 10.22 (1H, -OH).

SB5: Found (Calcd.): C: 63.87 (64.00), H: 5.60 (5.53), N: 9.05 (9.13); IR (KBr, cm^{-1}): 3500-3350 (-OH str.), 1328 (-OH bend.), 1632 (-C=N), 1506 (-N=O), 1301 (-N=O); ^{1}H NMR (ppm): 1.19-1.26 (3H, triplet, -CH_2-CH_3), 2.50-2.60 (2H, quartet, CH3-CH_2), 2.67 (3H, singlet, C-CH_3), 6.30-8.30 (6H, multiplet, Ar-H), 12.53 (1H, singlet, -OH), 13.19 (1H, singlet, -OH).

SB6: Found (Calcd.): C: 76.94 (76.83), H: 5.69 (5.42), N: 6.55 (6.59); IR (KBr, cm^{-1}): 3300-3250 (-OH str.), 1333 (-OH bend.), 1584 (-C=N); ^{1}H NMR (ppm): 1.20-1.28 (3H, triplet,

-CH_2-CH_3), 2.54-2.62 (2H, quartet CH_3-CH_2), 2.65 (3H, singlet, C-CH_3), 6.90-7.50 (5H, multiplet, Ar-H), 12.50 (1H, singlet, -OH), 13.20 (1H, singlet, -OH).

SB7: Found (Calcd.): C: 63.95 (64.00), H: 5.60 (5.53), N: 8.95 (9.03); IR (KBr, cm^{-1}): 3500-3350 (-OH str.), 1349 (-OH bend.), 1625 (-C=N), 1523 (-N=O), 1323 (-N=O); ^{1}H NMR (ppm): 1.18-1.30 (3H, triplet, -CH_2-CH_3), 2.52-2.63 (2H, quartet, CH3-CH_2), 2.66 (3H, singlet, C-CH_3), 6.30-8.30 (6H, multiplet, Ar-H), 12.53 (1H, singlet, -OH), 13.19 (1H, singlet, -OH).

SB8: Found (Calcd.): C: 75.95 (75.84), H: 7.10 (7.06), N: 5.10 (5.20); IR (KBr, cm^{-1}): 3300-3200 (-OH str.), 1373 (-OH bend.), 1618 (-C=N); ^{1}H NMR (ppm): 1.20-1.29 (3H, triplet, -CH_2-CH_3), 2.30 (s, 3H, CH_3), 2.55 (2H, quartet CH_3-CH_2), 2.66 (3H, singlet, C-CH_3), 6.30-8.30 (6H, multiplet, Ar-H), 12.50 (1H, singlet, -OH), 13.19 (1H, singlet, -OH).

SB5-Cu: Found (Calcd.): C: 52.83 (52.96), H: 4.10 (4.14), N: 7.80 (7.72), metal: 17.48 (17.52); IR (KBr, cm^{-1}): 3329 (-OH str.), 1321 (-OH bend.), 1626 (-C=N), 559 (M -N), 455 (M-O).

SB5-Ni: Found (Calcd.): C: 53.56 (53.66), H: 4.10 (4.19), N: 7.90 (7.83), metal: 16.48 (16.41); IR (KBr, cm^{-1}): 3300 (-OH str.), 1310 (-OH bend.), 1620 (-C=N), 469 (M-N), 426 (M-O).

SB5-Co: Found (Calcd.): C: 53.54 (53.65), H: 4.12 (4.19), N: 7.93 (7.82), metal: 16.40 (16.45); IR (KBr, cm^{-1}): 3364 (-OH str.), 1308 (-OH bend.), 1603 (-C=N), 528 (M-N), 455 (M-O).

SB5-Fe: Found (Calcd.): C: 54.15 (54.11), H: 4.26 (4.23), N: 7.82 (7.89), metal: 15.66 (15.73); IR (KBr, cm^{-1}): 3350 (-OH str.), 1309 (-OH bend.), 1601(-C=N), 538(M-N), 455 (M-O).

SB6-Cu: Found (Calcd.): C: 60.15 (60.08), H: 5.50 (5.56), N: 7.70 (7.79), metal: 17.62 (17.66); IR (KBr, cm^{-1}): 3327 (-OH str.), 1321 (-OH bend.), 1601(-C=N), 552 (M-N), 455 (M-O).

SB6-Ni: Found (Calcd.): C: 60.94 (60.89), H: 5.60 (5.64), N: 7.96 (7.89), metal: 16.63 (16.55); IR (KBr, cm^{-1}): 3323 (-OH str.), 1321 (-OH bend.), 1601(-C=N), 470 (M-N), 430 (M-O).

SB6-Co: Found (Calcd.): C: 60.82 (60.86), H: 5.68 (5.63), N: 7.76 (7.88), metal: 16.50 (16.59); IR (KBr, cm^{-1}): 3423 (-OH str.), 1321 (-OH bend.), 1624 (-C=N), 580 (M-N), 455 (M-O).

SB6-Fe: Found (Calcd.): C: 61.30 (61.39), H: 5.62 (5.68), N: 7.92 (7.96), metal: 15.90 (15.86); IR (KBr, cm^{-1}): 3357 (-OH str.), 1350 (-OH bend.), 1624 (-C=N), 546 (M-N), 455 (M-O).

Antibacterial Activity

The zones of inhibition for all Schiff bases are shown in Table 2. It is evident from Table 1 that all Schiff bases are moderately active against all tested bacteria and fungi. For *E. coli,* SB6 showed maximum activity followed by SB4 and SB8. Against *B. megaterium*, SB4 showed maximum activity. Minimum inhibition was observed by SB7. For *B. subtilis*, SB6 showed maximum inhibition. Again, SB4 showed good activity against *P. fluorescens*.

From SB1 to SB4, the central moiety was sulphonamide whereas from SB5 to SB8, central moiety is 5-ethyl resacetophenone. To these central moieties, different groups are attached. It is observed that SB4 and SB6 showed maximum activity. SB4 contained a vanilline side chain with a sulphonamide Moiety, whereas SB6 had two methyl groups in the side chain of 5-ethyl resacetophenone.

All Schiff bases, except SB1 showed inhibition against the fungi *A. awamori.* This suggests that substitution to the benzene ring increases antimicrobial activity. As there is no group attached to the benzene ring in SB1, it appears to be less active than other bases. However, SB3 showed maximum inhibition followed by SB2 and SB4. This suggests that SCH_3 group is more effective than OCH_3 and OH groups. The Schiff bases SB5 to SB8 showed minimum activity. This proves that the 5-ethyl resacetophenone moiety, along with different side chains, are not effective for this fungi.

Furthermore, the metal complexes of SB5 and SB6 were also studied for their antibacterial and antifungal behaviour. The inhibition zones for the complexes are given in Table 3 along with the ligands. The metal complexes are much more toxic compared to the parent ligand itself. In general, the toxicity is in the order cobalt > iron > nickel > copper.

The increased toxicity of metal complexes may be due to the effect of the metal ion configuration and charge on the normal cell. A possible mode of toxicity may be specified by the chelation theory.

Chelation reduces considerably the polarity of the metal

Table 2. Antimicrobial Activity of Synthesised Schiff Bases

Compound	Antibacterial activity zone of inhibition (mm)				Antifungal activity zone of inhibition (mm)
	E. coli	*B. megaterium*	*B. subtilis*	*P. fluorescens*	*A. awamori*
DMF	10	12	11	10	11
SB1	13	15	14	14	11
SB2	11	16	13	15	18
SB3	12	17	11	13	19
SB4	15	18	16	19	16
SB5	12	16	13	11	10
SB6	20	12	22	15	12
SB7	11	11	12	12	11
SB8	15	16	17	16	10

Table 3. Antimicrobial Activity of Ligands and their Complexes

Compound	Antibacterial activity zone of inhibition (mm)				Antifungal activity zone of inhibition (mm)
	E. coli	*B. megaterium*	*B. subtilis*	*P. flourescens*	*A. awamori*
DMF	10	12	11	10	11
SB5	12	16	13	11	10
SB5-Cu	17	21	13	18	19
SB5-Ni	20	23	15	17	20
SB5-Co	23	26	19	21	22
SB5-Fe	22	25	16	19	21
SB6	20	12	22	15	12
SB6-Cu	20	13	13	17	20
SB6-Ni	21	15	15	18	21
SB6-Co	23	20	18	20	23
SB6-Fe	22	19	16	19	22

ion mainly because of partial sharing of its π electrons and delocalization over the whole chelate ring. Such chelation increases the lipophilic character of the metal chelate, which probably tends to break down the permeability barrier of cells, resulting in interference with the normal cell process (Mishra and Singh, 1993). Thus, the results suggest that the variation in structure affects the growth of microorganisms and may result in an inhibitory effect, stimulatory effect or reduction in the toxicity of metal ions toward some microorganisms. Thus, iron and cobalt complexes are excellent bactericides and fungicides.

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