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Antimicrobial Activity of Some Schiff Bases Derived from Benzoin, Salicylaldehyde, Aminophenol and 2,4 Dinitrophenyl Hydrazine

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The antibacterial and antifungal activities of three schiff bases were evaluated against some pathogenic bacteria and fungi. Parallel experiments were also carried out with standard drugs (*Kanamycin* for bacteria and *Nystatin* for fungi). Two compounds [N-(1-phenyl-2-hydroxy-2phenylethylidine)-2',4' dinitrophenyl hydrazine, abbreviated as PDH and N-(2-hydroxy benzylidine)-2'-hydroxy imine, abbreviated as HHP] showed significant antimicrobial activities. The rest one [N-(1-phenyl 2-hydroxy-2 phenyl ethylidine) 2'-hydroxy phenyl imine, abbreviated as PHP] showed moderate activity. All these three compounds were found to possess pronounced cytotoxic effect. These compounds can be considered as potent antimicrobial agents.

KEYWORDS : Antimicrobial activity, Cytotoxicity, Schiff base

A large number of human and animal diseases are caused by pathogenic bacteria and fungi in both developed and developing countries. Consequently a number of chemotherapeutic agents are available to combat such organisms. These treatments however could not completely destroy the organisms, probably (Das *et al.*, 1995) due to the widespread irrational, unscientific and apathetic use of such agents. The survived microorganisms have matched the ingenuity in developing their own defenses. As a result such drugs gradually lose their effectiveness in action. Repetition and overdose of such drugs often cause environmental pollution. In order to get rid of this situation, it has become a common practice to find out safer, more effective and inexpensive new chemical compounds as chemotherapeutic agents. In this context, a series of researches with various schiff bases have been carried out by different workers (Dobek *et al.*, 1980; Chen and Rhodes, 1996). Schiff bases derived from isatin derivatives and N[4-(4' chlorophenyl)thiozole-2-yl] thiosemicarbazide, have already proved to be potent antimicrobial agents (Pandeya *et al.*, 1999). Khanam *et al.* (2002) showed that 2,2 diamino-1-azavinyl aminoamide can be used effectively against a number of both gram positive and gram negative bacteria. The present paper is a continuation of such type of investigations. For the purpose, three schiff bases have been synthesized, characterized and evaluated their capabilities as antimicrobial agents. In addition, cytotoxic effect and minimum inhibitory concentration of these compounds have been evaluated.

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Materials and Methods

The bacteria and fungi used in the subsequent experiments were collected from the Microbiology Laboratory of the Institute of Nutrition and Food Sciences(INFS), University of Dhaka, Bangladesh. All other chemicals used throughout the research work were purchased from BDH (England).

Preparation of 2,4 dinitrophenyl hydrazine (2,4 DNPH) solution. 2,4 DNPH (3 g) was dissolved in concentrated sulphuric acid (15 *ml*). It was diluted with distilled water (20 *ml*) and alcohol (70 *ml*) and finally filtered to get a clear solution.

Synthesis of N(1-phenyl 2-hydroxy-2 phenyl ethylidine) 2',4' dinitrophenyl hydrazone (PDH). Solutions of 2,4 DNPH and benzoin (in alcohol) were mixed together in 1 : 1 molar ratio and warmed on a water bath at 80~90°C for a period of 3~4 hrs. The orange crystalline product was filtered, washed several times with warm water to remove completely the sulphates. The compound was reprecipated from alcoholic medium and dried at 105°C for a period of 6 hours. It was then cooled to room temperature and stored in a desiccator.

Synthesis of N(1-phenyl 2-hydroxy-2 phenyl ethylidine)-2' hydroxy phenyl imine (PHP). Alcoholic solutions of benzoin and 2-aminophenol in 1 : 1 molar ratio were mixed together and refluxed for about 6 hours and then distilled to half of the total volume. The solution was then allowed to stand over night when a grey crystalline

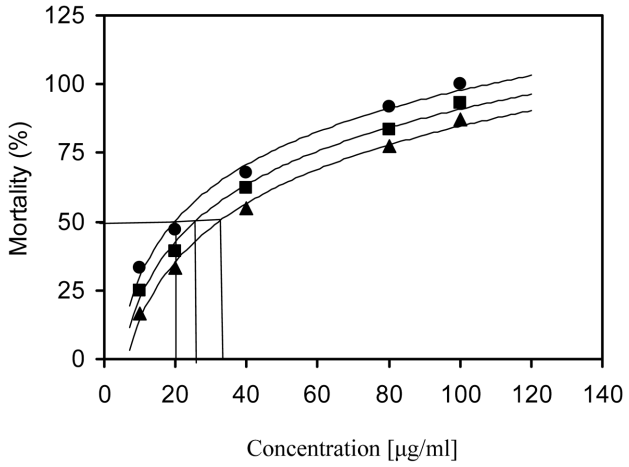


Fig. 1. Brine shrimp lethality bioassay of the compounds. PDH (●), PHP (■), and HHP (▲).

product separated out. The crystals were recrystallised twice, dried in an oven at 50°C and finally stored in a desiccator.

Synthesis of N-(2hydroxybenzylidine) 2' hydroxyl phenyl imine (HHP). Alcoholic solutions of salicylaldehyde and 2-aminophenol were mixed together in 1 : 1 molar ratio and the red crystalline product (HHP) was obtained by the same procedure as described for PHP.

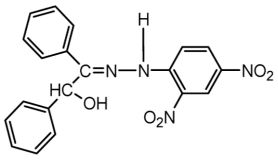
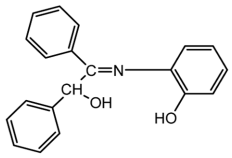
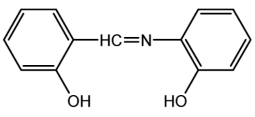
Characterization of schiff bases. The synthesized compounds were characterized by taking melting point, IR spectra (as KBr disc by a Shimadzu FTIR) and elemental analysis data (by a Perkin 240C analyzer).

Preparation of stock solution of schiff bases. These solutions were prepared by dissolving 75 mg substance in 25 *ml* methanol.

Antimicrobial screening. *In vitro* antimicrobial screening was performed by disc diffusion method (Beur *et al.*, 1966; Rois *et al.*, 1988). The compounds PDH, PHP and HHP were tested against some bacteria (*S. aureus*, *B. megaterium*, *E. coli*, *S. dysenteriae*, *S. sonnei* and *P. aeruginosa*) and fungi (*A. flavus*, *A. fumigatus*, *A. niger*, *Candida albicans* and *Mucor* sp). The activity was determined after 24 hours of incubation at room temperature (37°C) and was expressed in terms of mm by measuring the diameter of zone of inhibition. Standard drugs viz. *kanamycin* (30 µg/disc) for bacteria and *nystatin* (100 µg/disc) for fungi were used in parallel experiments for comparing the results.

Minimum inhibitory concentration (MIC). The MIC of the test compounds was determined by serial dilution technique (Jawetz *et al.*, 1980) against the same bacteria as used for antibacterial screening. Nutrient broth media were used here for this purpose. Decreasing concentrations of the test compounds were prepared in serial two fold dilution using the stock solution. Bacterial suspension (10 µl) containing 10^7 cells ml^{-1} was inoculated in to all tubes. After incubation for 24 hours at 37°C, the test

Table 1. Physical constants and IR spectral data of the schiff bases

Test compound	Formula	Yield (%)	Physical form	Melting point, °C	Solubilty	Elemental analytical data found (calculated)	IR spectra, cm^{-1}
PDH		78	Orange crystalline	220°C	Ethanol, Methanol, DMSO	C=60.80 (60.22) H=4.19 (4.08) N=13.98 (14.28)	3413w (also OH) 3230s (-NH-) 1618s, 1577s (C=N) 1515s, 1490sh (NO_2) 1425m, 1334s (also CH) 1309s (C-N)
PHP		60	Gray crystalline	69°C	Ethanol, Methanol, DMSO	C=78.96 (79.21) H=5.46 (5.61) N=4.55 (4.62)	3400-3115w (Phenol & also OH), 3062s (C-H), 1660s, 1593s and 1577s (C=N), 1450s (C-H), 1325m, 1315sh and 1292sh (C-N)
HHP		76	Red crystalline	175°C	Ethanol, Methanol, DMSO	C=72.87 (73.24) H=5.01 (5.16) N=6.44 (6.57)	3429w (phenol OH), 2923m (C-H), 1629s, 1616sh and 1593sh (C=N), 1463s, 1305m (C-H); 1274s, 1244m (C-N)

s = strong, m = medium, w = wide, sh = showder

tube with no visible growth of the microorganism was taken to represent the MIC value of the sample in $\mu g\ ml^{-1}$.

Brine shrimp lethality bioassay. Twelve vials were taken (two for each concentration) for this study. Sea water (prepared by dissolving 38 g sodium chloride in 1 *l* distilled water) was given to each of the vials. Test compounds from the stock solutions were added to the vials (with the aid of a micropipette) in such a way so as to get final concentrations of 10, 20, 40, 80 and 100 $\mu g\ ml^{-1}$. No sample solution was added to those of the control. Using pasteur pipette 10 living shrimps were taken to each of these vials. After 24 hours, the survived nauplii in each vials were counted and the results were noted. The full description of the method has been described else where (Atta-ur-Rahman *et al.*, 1999; Meye *et al.*, 1982).

Results and Discussion

The physical constants and the data obtained from IR spectral and elemental analyses have been furnished in Table 1. The formation of schiff bases has been confirmed by the appearance of absorption bands at 1,660~1,570 cm^{-1} assigned for a C=N bond. The formation is also supported with the absence of any band at 1,730~1,690 cm^{-1} characteristic for an aldehyde (salicylaldehyde) or a ketone (benzoin). The molecular weights and hence the formulae of these compounds have been verified from elemental analysis data.

The results for the antibacterial activity of the test compounds have been presented in Table 2. The results obtained with PDH at 30 μg/disc are almost analogous to those obtained with the standard drug *kanamycin* at this same dose. However at higher doses, the diameters of zone of inhibition were found to be increased slightly. The other two compounds (PHP and HHP) showed moderate activity even at high doses. PHP did not show any activity against some of the microorganisms studied (*S. aureus*, *B. megaterium*, *P. aeruginosa*).

The results for antifungal activity of these three compounds have been presented in Table 3. The activity of these compounds showed the same type of results as responded against the bacteria studied. The activity of HHP is quite identical to that obtained with PDH. In comparison with the standard drug *nystatin*, PDH and HHP with the same dose showed their effectiveness to almost 80~85%. PHP, however, showed modest activities against only two fungi out of five studied.

Evidently it can be concluded that phenolic -OH, $-NO_2$ and -NH- groups enhanced the antimicrobial activities of these schiff bases. PHP containing only one phenolic OH group showed moderate activity. Probably alcoholic OH group might have a little or no such effects. Again since these compounds showed pronounced cytotoxic activity

Table 2. Antibacterial activity of the test compounds

Test organisms	PDH (μg/disc)			PHP (μg/disc)			HHP (μg/disc)			*Kanamycin* (μg/disc)
	30	60	90	30	100	200	30	100	200	30
	Diameter of zone of inhibition (in mm)									
Gram (+)										
Staphylococcus aureus	25	26	27	R	R	R	13	18	21	29
Bacillus megaterium	23	25	28	R	R	R	10	13	17	33
Gram (−)										
Escherichia coli	23	25	30	12	15	20	10	14	16	31
Shigella dysenteriae	19	22	25	12	16	19	08	13	15	27
Shigella sonnei	20	23	27	09	11	13	09	14	18	29
Pseudomonas aeruginosa	24	26	28	R	R	R	07	14	20	32

R = Resistance

Table 3. Antifugal activity of the test compounds

Test organisms	PDH ($\mu g/ml$)		PHP ($\mu g/ml$)		HHP ($\mu g/ml$)		*Nystatin* ($\mu g/ml$)
	100	200	100	200	100	200	100
	Diameter of zone of inhibition (in mm)						
Aspergillus flavus	R	R	R	R	R	R	23
Aspergillus fumigatus	17	21	R	R	16	19	21
Aspergillus niger	18	20	R	11	15	17	26
Candida albicans	23	26	08	15	25	28	30
Mucor sp.	20	24	R	R	19	25	28

R = Resistance

Table 4. Minimum Inhibitory Concentration (MIC) of test compounds

Test organisms	PDH ($\mu g/ml$)	PHP ($\mu g/ml$)	HHP ($\mu g/ml$)
Gram (+)			
Staphylococcus aureus	64	256	32
Bacillus megaterium	32	256	128
Gram (−)			
Escherichia coli	32	32	64
Shigella dysenteriae	16	64	128
Shigella sonnei	32	64	64
Pseudomonas aeruginosa	16	512	64

(for PDH $LC_{50} = 20.1\ \mu g/ml$, for PHP $LC_{50} = 27.0\ \mu g/ml$ and for HHP $LC_{50} = 34.1\ \mu g/ml$), it is expected that compounds of this type might also possess antineoplastic activities. Further experiments are required to investigate the actual mechanism of cytotoxicity and their probable effects on higher animal model on cancer cell line.

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