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Antimicrobial Activity of Schiff Bases Substituted by Mannich Side Chain

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Abstract
Two Schiff bases (3-[(1*E*)-*N*-phenylethanimidoyl]phenol(I) and 2,6-bis(dimethylamino)3[(1*E*)phenylethanimidoyl]phenol(II) were synthesized via a general synthesis protocol .The target molecules were identified by spectroscopic tools: UV, IR, NMR and mass spectrometry. The synthesized bases were evaluated for their antimicrobial activity against six standard human pathogens. Both compounds showed significant activity against all tested organisms at a concentrations of 50 , 100 and 200mg/ml .

Keywords: Schiff bases, Synthesis, Antimicrobial activity.

1-Introduction

Studies on the chemistry of Mannich bases are of interest in various areas of applications. A large number of Mannich bases have been synthesized in order to correlate their structure and reactivity with their pharmacological potential. Some Mannich bases possess antibacterial activity(Tamas and Bela,2002; Gabriela et.al.,2009; Prashant and Kapandnis,2004 ; Pernak et.al.,1999 ; Surendra et.al.,2000 ; Pandeya et.al.,1999 ; Pandeya and Nath,1999 . Some were evaluated as novel potential antimalarial agents (Saudrine et.al.,2008 ; Kaylene,1999; Alex and Jirigut,2007; Ying et.al.,2003; Mannich bases with putative cytotoxic activity were reported(Jonathan et.al.,2002; Dimmock et.al.1995; Jonathan et.al.,2000; Inci et.al.2000). Some Mannich bases possess anticonvulsant activity, others exhibit promising antiamoebic potency(Mohammad and Amir,2009; Kiran and Ashok,2004).

Base exchange reactions involving Mannich bases with primary aromatic amines have been reported by a number of workers(Ardashev and Malik,1967; Abdalla and Ramli,1990; Greenhill and Ramli,197; Craig et.al.1964; . Some of these reactions are of particular practical interest , since the resultant secondary amines are not , in general , available by other synthetic routes.

Industrially ,Mannich bases are utilized as dyes for synthetic fabrics(Makra et.al.,2001) , as polymers in treatment of wastewater(Bakes et.al.,1991) as antioxidants and corrosion inhibitors(Farng and Horodysky,1990) as active ingredients in lubricant composition and also as surface active components.

2- Materials and Methods

2.1- Materials

2.1.1-Test organisms

The synthesized compounds were screened for antibacterial and antifungal activities using the standard microorganisms shown in Table(1).

Table 1: Test organisms

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve

4	*Escherichia coli*	G-ve
5	*Salmonella typhi*	G-ve
6	*Aspergillusniger*	fungi
7	*Candida albicans*	fungi

2.1.2- Chemicals and Solvents

Analytical grade reagents (Table .4) were used. They were purchased from Sigma – Aldrich company (UK)

2.2- Methods

2.2.1-Synthesis protocols

2.2.1.1- Synthesis of Schiff bases (I) : 3-[(1*E*)-*N*-phenylethanimidoyl]phenol

3-hydroxyacetophenone (2.72g ,20mmol) was added to aniline (1.86g,20mmol) in methanol (40ml). Then 3drops of concentrated H_2SO_4 were added. The mixture was then stirred for 1 hour at room temperature and left overnight, Removal of the solvent under reduced pressure gave the product.

2.2.1.2- Synthesis of Compound (II) : 2,6-bis(dimethylamino)-3-[(1*E*)-*N*-phenylethanimidoyl]phenol

Formalin(0.4g,5mmol) was added dropwise with stirring to a mixture of compound (I) (1.o55gm,5mmol) and dimethylamine (0.22gm,5mmol) in absolute ethanol (10ml) at 0^0C .The mixture was then stirred for 1 hour and left overnight, Removal of the solvent under reduced pressure gave the product.

2.2.2-Antimicrobial Assay

2.2.2.1-Preparation of Bacterial Suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony forming units per ml.The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspensions were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

2.2.2.2-Preparation of fungal suspensions

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

2.2.2.3-Testing for antimicrobial activity

The cup-plate agar diffusion assay was adopted with some minor modifications, to assess the antibacterial activity of synthesized compounds. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divid-

ed into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate Petri dishes were designed for standard antibacterial and antifungal chemotherapeutic agents.

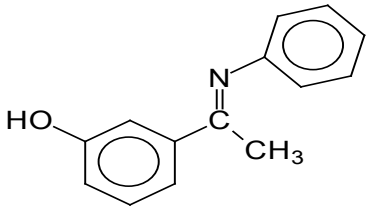
The agar discs were removed, alternate cup were filled with 0.1 ml samples of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentrations of the test compounds and the standard antimicrobial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

3-Results and Discussion

A Schiff base (I) was synthesized via the reaction of 3-hydroxyacetophenone with aniline . Schiff base (I) was then aminomethylated by dimethylamine to obtain (II) . Products were identified by spectroscopic tools (IR, UV, NMR, and MS) and then screened for their antimicrobial activity.

3.1 -Synthesis of the Schiff base (I) : 3-[(1*E*)-*N*-phenylethanimidoyl]phenol



(I)

The Schiff base (I) was synthesized by adding 3-hydroxyacetophenone to aniline in methanol (40ml) .Then 3 drops of concentrated H_2SO_4 were added. The mixture was stirred for 1 hour at room temperature and left overnight, Removal of the solvent under reduced pressure gave the product.

The IR spectrum of (I) (Fig.1) gave υ (KBr) 609,688,794,877 (C-H, Ar. bending) . 1363,1429,1490,1577 (C= C, Ar). 1577,1664(C=N) 2831 (C-H,aliph.). and 3178 (OH stretching).

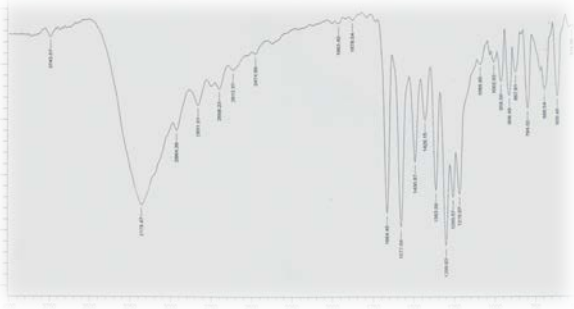


Fig.1: IR spectrum of the Schiff bases (I)

The UV spectrum showed λ_{max}(MeOH) 249 ,309 nm which is due to a benzonitrile chromophore extended by additional phenyl function. The ^{1}HNMR spectrum (Fig. 2) revealed the following signals:

δ 3.41	singlet	3H
δ 7.01	double	4H
δ7.25-7.41	multiplet	5H

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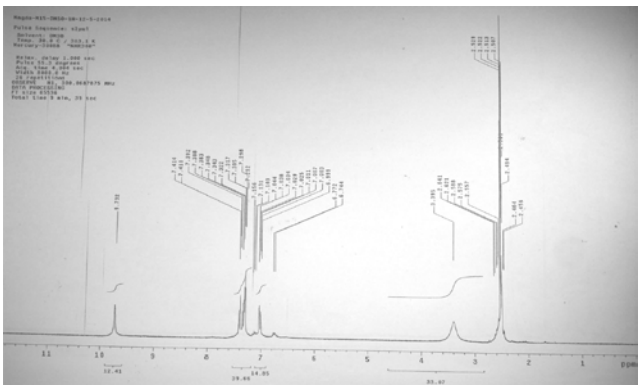


Fig.2: ^{1}HNMR spectrum of the the Schiff bases (I)

The signal at δ3.41(s,3H) was assigned for the methyl group in ($N{=}C{-}CH_3$).The resonances at δ7.01(d,4H) and δ7.25-7.41(m,5H) account for the aromatic protons.The mass spectrum (Fig.3) gave m/z 211 corresponding to M^{+}.

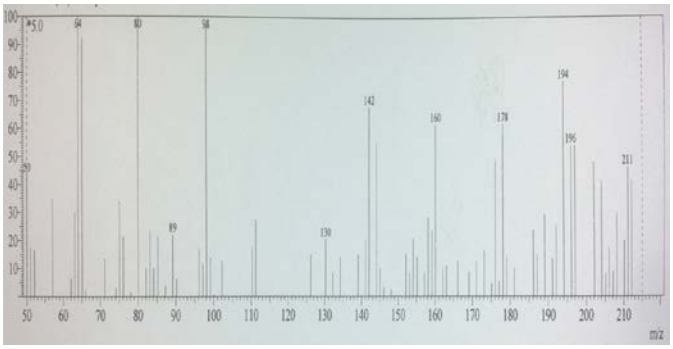
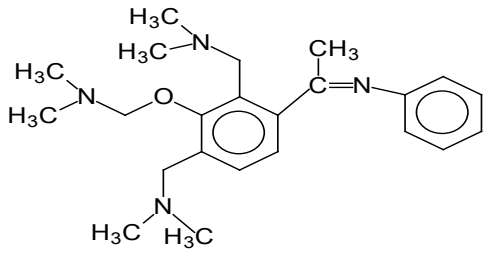


Fig.3: Mass spectrum of the Schiff bases (I)

3.2 -Synthesis of the Mannich base (II) : 2,6-bis(dimethylamino)-3-[(1*E*)-*N*-phenylethanimidoyl]phenol



(II)

The Mannich base (II) was synthesized by adding formalin dropwise to a mixture of compound (I) and dimethylamine in absolute ethanol (10ml) at room temperature.The IR spectrum (Fig.4) gave υ (KBr) 684, ,790,871 (C-H, Ar. bending). 1299 (C-N). 1363,1436,1492,1575 (C= C, Ar.). 1575,1666 (C=N). 2827 (C-H,aliph.) and 3178 cm^{-1} (OH stretching).

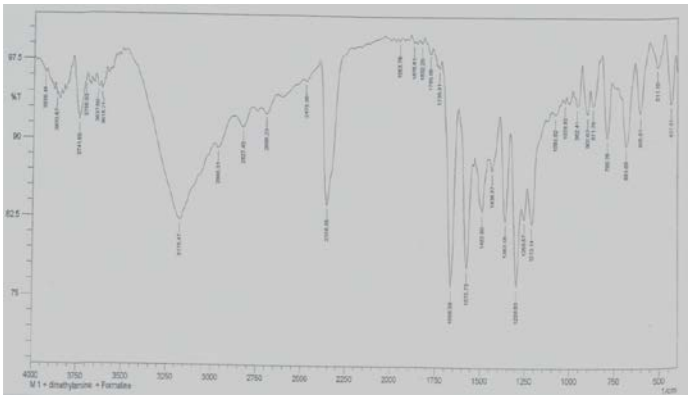


Fig.4: IR spectrum of the Mannich base (II)

The UV spectrum showed λ_{max}(MeOH) 249, 308 nm which corresponds to benzonitrile chromophore extended by a phenyl function. The ^{1}HNMR spectrum of (II) (Fig. 5) revealed the following signals:

δ 3.15	singlet	6H
δ 3.27	singlet	12H
δ 3.52	doublet	3H
δ7.40	doublet	5H
δ7.85	doublet	2H

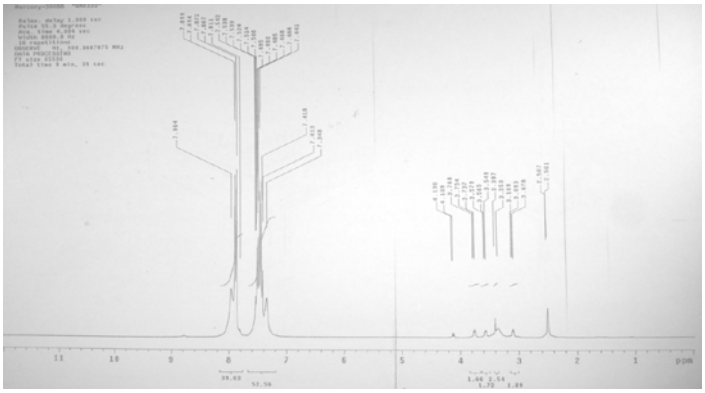


Fig.5: ^{1}HNMR spectrum of the Schiff base (II)

The signal at δ 3.15(6H) was assigned for two methyl functions. The resonance at δ3.27 (12H) accounts for the four methyl functions of dimethylamino moiety, while the signal at δ 3.52(3H) was attributed to one methyl group.The resonances at δ7.40(d,5H) and δ7.85(m,2H) account for the aromatic protons. The mass spectrum (Fig.6) gave the ion m/z382corresponding to M^{+}+1.

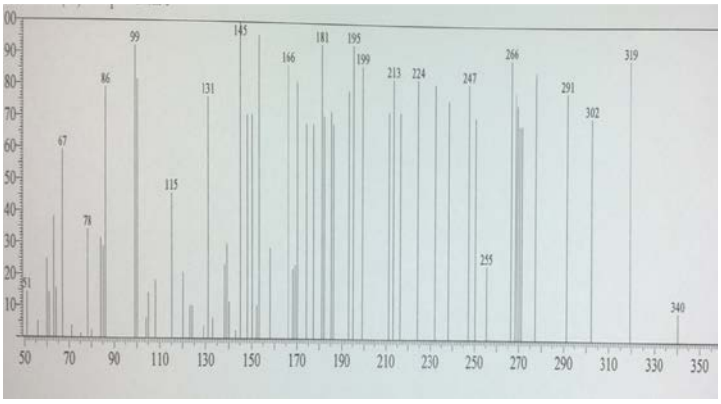


Fig.6: Mass spectrum of Schiff bases (II)

3.3- Antimicrobial activity

The synthesized compounds were screened for their antimicrobial activity against six standard organisms. The average of the diameters of the growth inhibition zones are displayed in Table (2) .The results were interpreted in terms of the commonly used terms. Compounds resulting in 13mm or more growth inhibition zones were considered to be active and those resulting in less than 9mm were considered inactive ; 9-12mm being partially active. Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively. Both compounds showed significant activity against all tested organisms at a concentrations of 50 , 100 and 200mg/ml

Table (2) : Antimicrobial activity of synthesized compounds

Sample	Conc	E.C	PS	B.S	Sal	S.A	Ca	A.n
Compound(I)	200	35	27	35	40	30	31	28
	100	30	21	30	30	26	27	25
	50	25	18	25	25	22	25	20
Compound(II)	200	28	26	30	30	25	26	35
	100	21	25	25	24	21	20	25
	50	17	20	20	20	18	16	18

Table (3) : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	B.s.	S.a.	E.c.	P.a.	S.t
Ampicillin	40	15	30	-	-	-
	20	14	25	-	-	-
	10	11	15	-	-	-
Gentamycin	40	25	19	22	21	22
	20	22	18	18	15	17
	10	17	14	15	12	14

S.a*: Staphylococcus aureus*
E.c*: Escherichia coli*
P.a: *Pseudomonas aeruginosa*
A.n: *Aspergillus niger*
C.a: *Candida albicans*
S.t: *Salmonella typhi*
B.a: Bacillus subtilis

Table (4) : Antifungal activity of standard chemotherapeutic agent

Drug	Conc. mg/ml	A.n	C.a
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

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