SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF NEW BRIDGED AND MACROCYCLIC SCHIFF BASES

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ABSTRACT

In this work we focused on the synthesis of new Open Schiff bases were prepared by condensation of salicylaldehyde with both 4,4'-diaminodiphenyl methane, 4,4'-diamino diphenyl sulphide, and condensation of O-vanilin with diethyl ester of terephthalic acid respectively, and also were prepared New Macrocyclic Schiff Bases by condensation of 1,6-dibromohexane with thiocarbohydrazid. The Schiff base were checked by different spectral techniques (LC-MS, ¹H-NMR, IR). The Schiff bases have been screened for their antibacterial activity, where compound (II) showed promising results against gram positive and gram negative bacteria.

Keywords: *Open Schiff base, Macrocyclic, Salicylaldehyde, Spectral Technique, Antibacterial Activity.*

1. INTRODUCTION

Azomethine group ($-C=N-$) containing compounds typically known as Schiff bases have been synthesized by the condensation of primary amines with active carbonyls. Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several antibacterial applications that include antibacterial, [1-6] antifungal [3-6] and antitumor activity [7-8]. They have been studied extensively as a class of ligands [9-11] and are known to coordinate with metal ions through the azomethine nitrogen atom. Schiff base complexes play a vital role in designing metal complexes related to synthetic natural oxygen carriers [12]. Metal complexes make these compounds effective as stereospecific catalysts towards oxidation, reduction, hydrolysis, antibacterial activity and other transformations of organic and inorganic chemistry [13]. In organic compounds the presence of $-C=N-$ along with other

functional groups form more stable complexes compared to compounds with only $-C=N-$ coordinating moiety. Similarly coumarin derivatives have a great interest because of their role in natural and synthetic organic chemistry. Many products which contain a coumarin subunit exhibit antibacterial activity such as molluscicides, [14] anthelmintic, hypnotic, insecticidal [15] activity and some are serving as anticoagulant agents [16] and fluorescent brighteners. So coumarins containing a Schiff base are expected to have enhanced antitumor and other antibacterial activities. It is well established that the antibacterial activity associated with the hydrazone compounds attributed to the presence of the active pharmacophore ($-CONH-N=C-$). Hence many hydrazone compounds containing this active moiety showed good anticancer bioactivities according to the literature [17]. Hence, in this paper, we are reporting the synthesis of New open and Macrocyclic Schiff bases from

condensation of Salicylaldehyde with 4, 4'-diamino diphenyl methane, 4, 4'-diaminodiphenyl sulphide, and their characterization by spectral data such as IR, ^1H NMR and Mass spectra was studied. Their antimicrobial activity was also evaluated [18].

2. EXPERIMENTAL

2.1. MATERIALS AND METHOD

Ethanol, dimethyl formamide, salicylaldehyde, 1, 6-dibromohexane, diethylether, 4, 4'-diamino diphenyl methane, 4,4'-diamino diphenyl sulphide, hydrazine hydrate, diethyl ester of terephthalic acid, K_2CO_3 , Nutrient agar, Filter paper discs (Whatman No.1 filter paper, 5mm diameter). All materials obtained from Sigma Aldrich Company.

IR spectra was recorded on Jusco 300 FT-IR Spectrometer using compressed KBr discs. Mass spectra of the ligand were measured on a micro mass Quattro LC-MS/MS Spectrometer. ^1H - NMR spectra were recorded at ambient Broker DT-400 Spectrometer using CDCl_3 with DMSO-DMF as the internal standard.

2.2. SYNTHESIS OF 3, 13-DIAZA-4, 12 DIPHENYLMETHANE, (2',2'-DIHYDROXY-PHENYL) 2, 14-DECA-DIENE. (I)

The compound was prepared by a usual Schiff's base condensation in methanol (50mL) of salicylaldehyde (10 mmol, 1.22 g) with bridging diamine (4,4'-diamino diphenylmethane, 5 mmol, 1.00g). The solutions were stirred and refluxed for 6h. Gold Yellow precipitates were filtered, washed by a small amount of methanol and dried in vacuum. Yield: (87%), m.p. > 300 $^{\circ}\text{C}$, Empirical formula: $(\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_2)$, M.Wt: (406).

Mass spectra (m/z): 406 $[\text{L}]^+$.

The Elemental analyses: C, 78.76; H, 5.42; N, 6.89; O, 7.87. found, C, 78.79; H, 5.39; N, 6.87; O, 7.84.

IR (KBr disk): 1637.5 cm^{-1} (HC=N), 1284.6 cm^{-1} (C-O), 2850.8 cm^{-1} (C-H alkanes), 1497.2 cm^{-1} (C=C), 3236.4 cm^{-1} (C-H Ar), 3415-3551.7 cm^{-1} (-OH).

^1H NMR (400Hz; d_6 -DMSO) δ 13.33 (2H, s, OH), 6.660-7.422 (m, 16H, Ar), 3.926-4.068 (2H, -CH₂-) 8.65 (s, 2H, CH=N).

2.3. SYNTHESIS OF 3, 13-DIAZA-4, 12 DIPHENYL SULPHIDE, (2', 2'-DIHYDROXY PHENYL) 2, 14-DECA-DIENE (II).

A hot ethanolic solution (50ml) of salicylaldehyde (4.88 g, 0.04mol) was added to a hot ethanolic solution (50ml) of diaminodiphenyl sulphide (4.32g, 0.02 mol) and the reaction mixture was refluxed for 5h. A brown product was formed.

It was filtered, washed with ethanol, diethyl ether and subsequently dried over anhydrous CaCl_2 in desiccators. The Schiff base ligand is soluble in some organic solvents. (dimethylformamide and dimethylsulfoxide). Yield : (75%), m.p>300°C, Empirical formula: ($\text{C}_{26}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$), M.Wt: (424).

Mass spectrum(m/z) : 424[L]⁺

The Elemental analyses : C, 73.56; H, 4.74; N, 6.57; S, 7.56., found, C, 73.58; H, 4.75; N, 6.56; S, 7.61.

IR (KBr disk): 1616.4 cm^{-1} (C=N) , 1284.2 cm^{-1} (C-O), 1456.3-1496.7 cm^{-1} (C=C), 3052 cm^{-1} (C-H Ar), 3414.8-3550.7 cm^{-1} (-OH).

¹H-NMR (DMSO- d_6 vs.TMS) δ =13.147 (s,2H,OH), 6.960-7.451 (m, 16H, Ar), 8.617-8.657 (s,2H,CH=N).

2.4. SYNTHESIS OF 3, 12-DIAZA-4, 11-DICARBONYL PHENYL DIHYDRAZIDE, (2', 2'-DIHYDROXY, 3', 3'-METHOXY PHENYL) 2, 14-DECADIENE. (III)

Synthesis of dihydrazide of terephthalic acid

A mixture of dimethyl ester of terephthalic acid (2.22 g) and hydrazine hydrate (98% 2 cc) in ethanol was refluxed for 4–5 h. The reaction mixture was allowed to cool to room temperature, then the cooled solution was poured onto ice cold water. The dihydrazide of terephthalic acid thus obtained was filtered and recrystallized from ethanol [19].

Yield: (88%), m.p > 300°C, Empirical formula:($\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$), M.Wt: (194).

Mass spectrum(m/z) :194[L]⁺

IR(KBr disk): 3329.50 cm^{-1} (NH_2), 3210, 90(NH), 3036-2961 cm^{-1} , (Ar-CH), 1698,

1628 cm^{-1} (C=O), 1428.60 cm^{-1} (C=C), 765.6 cm^{-1} (substituted benzene).

Synthesis of ligand(III)

o-vanillin (2 mmol) in ethanol (50 mL) was added to an ethanolic solution of 1,4-dicarbonyl phenyl dihydrazide (1 mmol, 30 mL) containing a few drops of concentrated HCl. The reaction mixture was refluxed for 3 h. The mixture was cooled to room temperature. The solid product was formed filtered and washed with cold ethanol and dried under vacuum. Yield : (65%) , m.p>300 °C) , Empirical formula:($\text{C}_{24}\text{H}_{22}\text{N}_4\text{O}_6$), M.Wt:(462).

Mass spectrum(m/z) : 462[L]⁺

The Elemental analyses :C,62.34; H, 4.77; N, 12.10; O, 20.75. found.,C,62.41; H, 4.78; N, 12.12; O, 20.79.

IR (KBr disk): 1601.9 cm^{-1} (C=N) , 1645.5 cm^{-1} (C=O), 1285.7 cm^{-1} (C-O), 2950 cm^{-1} (C-H alkanes), 3000 cm^{-1} (C-H Ar), 1451.5-1515.7 cm^{-1} (C=C), 3230.8 cm^{-1} (-C-NH-), 3432.5 cm^{-1} (-OH).

¹H-NMR (DMSO- d_6 vs.TMS) δ = 13.327 (s,2H,OH), 7.285-8.285 (m,12H,Ar), 3.713-3.763 (s, 6H, O-CH₃) 8.601 (s, 2H, C=NH), 9.845(NH-C=O).

2.5.SYNTHESIS OF 1,16-DIAZA-(3,4;13,14-DIBENZO)-17,19-THIOCARBOHYDRAZIDE - 5,8,11,14-TETRA-OXACYCLO HEPTACOSINE-1,15-DIENE(IV)

Synthesis of 1,6- bis (2- formyl phenel) hexane .

To a stirred solution of salicylaldehyde (2.44 g - 0.02mol) and K_2CO_3 (1.38g- 0.01mol) in DMF (50ml) 1,6-dibromohexane (1.22 g - 0.01mol) in DMF(10ml) was added drop wise,

the reaction was continued with stirring for 10h at 150-155 °C and then 5h at room temperature [20]. After the addition was completed, 20ml distilled water was added it was put in a refrigerator, 1h later the precipitate was filtered washed by 50ml water, dried in air, recrystallized from ethanol. Yield:(88%), m.p.=75 °C), Empirical formula: (C₂₀H₂₂O₄), M.Wt:(326).

Mass spectra(m/z) :326[L]⁺

IR(KBr disk) : 3071, 3038 cm⁻¹(Ar-CH), 2949-2853cm⁻¹(Alk.-CH), 1683cm⁻¹ (C=O), 1485-1455 cm⁻¹ (Ar-C=C), 1288.22-1246.75 cm⁻¹(Ar-O), 1188-1036 cm⁻¹ (R-O) ,757.89 cm⁻¹ (substituted benzene).

Synthesis of the macrocyclic Schiff bases(IV)

The macrocyclic ligand(IV-B) was prepared by dropwise addition of a solution of the thiocarbohydrazide(2 mmol) in methanol(40 ml)to a stirred solution of 1,6-bis(2-formylphenyl) hexane(2mmol) in methanol (60 ml). After the addition was completed, stirring was continued for 4 h. yellow colored precipitate was filtered and washed with methanol. Yield:(65%), m.p.>300°C), Empirical formula: (C₂₁H₂₄N₄O₂S), M.Wt:(396).

Mass spectra(m/z) :396[L]⁺

The Elemental analyses : C, 63.58; H, 6.11; N, 14.11; O, 8.04;S, 8.08. found., C, 63.54; H, 6.10; N, 14.11; O, 8.02; S, 8.03

IR (KBr disk): 1637.2cm⁻¹ (C=N), 1616.8cm⁻¹ (C=S), 1245.2cm⁻¹ (C-O), 1106.5-1160.5(R-O), 2869cm⁻¹(C-H alkanes), 1455.3-1486.2 cm⁻¹(C=C), 2939.3 cm⁻¹(C-H Ar), 3414.3-3477 cm⁻¹ (H₂O), 3236.8 (C-NH-).

¹H-NMR (DMSO-d₆vs.TMS) δ= 1.9082.103 (8H,CH₂CH₂), 6.987-7.663 (m,8H,Ar-H) , 3.262-3.491 (4H,O-CH₂) 8.3 (2H,HC=N), 2.954 (NH-N).

2.6. ANTIBACTERIAL ACTIVITY

The compounds were tested for their antimicrobial activity against four speices of bacteria (klebcella, Escherichia coli, Staphylococcus aureus , Salmonella typhi) using filter paper disc method [21].The screened compounds were dissolved individually in DMSO(dimethyl sulfoxide) in order to make up a solution of 1000 µg/ml concentration for each of these compounds. Filter paper discs (Whitman No.1 filter paper,5mm diameter) were saturated with the solution of these compounds. The discs were placed on the surface of solidified Nutrient agar dishes seeded by the tested bacteria. The diameters of inhibition zones (mm) were measured at the end of an incubation period,which was 24 h at 37C for bacteria. Discs saturated with DMSO are used as solvent control. Ampicillin 25 µg/ml was used as reference substance for bacteria [22].

3. RESULT AND DISCUSSION

The prepared organic Ligands (I,II, III,IV) were soluble in DMF,DMSO and partially soluble in acetone, chloroform, carbon tetra chloride. All the ligands are characterized by LC-MS (Figures 1,4,7,10),IR, ¹H-NMR spectra, which help in elucidating their empirical formula (Table 1).

TABLE 1: COLOR,MOLECULAR WEIGHT,MELTING POINT OF (I,II,III,IV).

Ligand	Color	M.Wt	Melting point °C
I	Gold Yellow	406	> 300 °C dec.
II	Brown	424	> 300 °C dec.
III	Yellowish white	462	> 300 °C dec.
IV	yellow	396	> 300 °C dec.

dec = decomposition

3.1. IR SPECTRA

IR Spectra of Schiff bases (I,II,III,IV) (Figures 1,3,5,7) are shown in table (2) the significant IR absorption bands. The four bands at 1637.5, 1616.4, 1601.9, 1637.2 cm^{-1} are attributed to imine group ($-\text{HC}=\text{N}-$) for (I,II,III,IV), respectively. The bands in the spectra at 1497.2, 1456.3-1496.7, 1451.5-1515.7 and 1455.3-1486.2 is due to ($\text{C}=\text{C}$) of aromatic rings. The $\nu(\text{OH})$ stretching frequencies bands are observed at 3415-3551.7, 3414.8-3550.7, 3432.5 cm^{-1} for (I,II,III), respectively. Also, a strong $\nu(\text{H}_2\text{O})$ band of ligand (IV) at 3414-3477 about is observed because of hydrated water molecule. The IR spectra of (III,IV) show characteristic absorption bands at 3230.8 and 3236.8 cm^{-1} due to $\nu(-\text{NH}-)$ stretching vibrations, respectively. The bands which observed for all Ligands at 1284.6, 1284.2, 1285.7 and

1245.2 cm^{-1} due to $\nu(\text{C}-\text{O})$ vibration, while the bands at 2850.8, 2950 and 2869-2939 cm^{-1} are attributed to ($\text{C}-\text{H}$ alkanes) for (I,III,IV) respectively. Also, the bands at 3236.4, 3052, 2939 and 3000 cm^{-1} about are attributed to ($\text{C}-\text{H}$ Ar) for (I,II,IV,III) respectively.

3.2. ^1H -NMR SPECTRA

The data of ^1H -NMR Spectra of prepared compounds are shown in table (3). The ^1H -NMR spectra of (I,II,III,IV) ligands in d_6 -DMSO (Figures 2,4,6,8) shows a singlet signal at 13.339, 13.147 and 13.327 ppm assigned to the protons OH groups of the Ligands (I,II,III) respectively. The multiplet signals 6.660-7.422, 6.960-7.451, 7.285-8.285 and 6.987-7.663 ppm are due to the aromatic protons for (I,II,III,IV) respectively. Also the signals at 8.65, 8.617, 8.601 and 8.3 ppm are assigned to the 2H, ($\text{HC}=\text{N}$) protons of the Ligands (I,II,III), respectively.

TABLE 3: ^1H NMR SPECTRA OF THE PREPARED SCHIFF BASES I-IV SPECTRA OF LIGANDS(I-IV)

Ligand	(s,2H,OH)	(m,H,Ar)	(s,2H,C=NH)
I	13.339	6.660-7.422	8.65
II	13.147	6.960-7.451	8.617
III	13.327	7.285-8.285	8.601
IV	-	6.987-7.663	8.3

TABLE 2: IR SPECTRA OF LIGANDS(I,II,III-B,IV-B)

Ligand	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{O})$	$\nu(\text{OH})$	$\nu(\text{CH}-\text{Ar})$	$\nu(\text{NH})$	$\nu(\text{CH}-\text{Alka})$	$\nu(\text{C}=\text{O})$
I	1637.5	1284.6	3415-3551.7	3236.4	-	2850.8	-
II	1616.4	1284.2	3414.8-3550.7	3052	-	-	-
III	1601.9	1285.7	3432.5	3000	3230.8	2950	1723.88
IV	1637.2	1245.2	-	2939	3236.8	2869	-

Fig.1. IR spectrum of Schiff base (I)

Fig.2. ¹H NMR spectrum of Schiff base(I)

Fig.3. IR spectrum of Schiff base(II)

Fig.4. ¹H NMR spectrum of Schiff base(II)

Fig.5. IR spectrum of Schiff base(III)

Fig.6. ¹H NMR spectrum of Schiff base(III)

Fig.7. IR spectrum of Schiff base(IV)

Fig.8. ^1H NMR spectrum of Schiff base(IV)

3.3. ANTIBACTERIAL ACTIVITY

During the last two or three decades, attention has been increasingly paid to the synthesis of Schiff bases Ligands which exhibits various antibacterial activities including antibacteria, fungicidal, tuberculostatic and plant growth regulative properties [21, 22]. It was judicious to investigate the synthesis of various new types of Schiff bases and studied their antibacterial activity against four strains of bacteria

(klebcella, Escherischa coli, Staphylococcus aureus, Salmonella typhimurium). The concentration used for the screened compounds is 1000 $\mu\text{g}/\text{ml}$. Control discs were preformed using DMSO solvent and inhibition zones are measured in mm. The results of antibacterial activity were compared with 25 $\mu\text{g}/\text{ml}$ Ampicilline [23]. The results of the antibacterial activity are summarized in Table (4).

TABLE 4: EFFECT OF THE LIGANDS(I,II,III,IV) ON THE GROWTH OF BACTERIA (ZONE OF INHIBITION IN MM)

Ligand (1000 µg/ml)	Bacteria			
	Gram- negative			Gram-positive
	Escherichia coli	Salmonella typhi	klebcella	Staphylococcus aureus
I	+	+	+	++
II	++	++	+	+++
III	+	+	+	+++
IV	-	-	-	++
Ampicillin (25 µg/ml)	+	+	+	+

(-)No zones of inhibition were observed. Moderately sensitive,(+)Inhibition zones of 7-10mm. Sensitive,(++)Inhibition zones of 11-14mm. High sensitive,(+++Inhibition zones of 15-20mm.

The ligands(I,III) show moderate activity against the gram-negative bacteria,except (II) against Escherichia coli , Salmonella typhi is high. Also, the Ligands(I,IV) show moderate activity against the gram- positive bacteria,except (II,III) against Staphylococcus aureus is high.

The ligand (IV) does not have significant activity against the gram-negative bacteria.

4. CONCLUSION

The compounds are new and are prepared for the first time . Also were characterization by ¹H-NMR,IR,LC-MS spectral methods. Some the prepared ligands have been antibacterially screened i.e. studying their effects against gram-positive, three gram-negative bacteria. The results show that their activities were found to vary from moderate to very strong.

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تحضير ودراسة خواص قواعد شيف مفتوحة وحلقة ضخمة جديدة ودراسة فعاليتها الحيوية

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الملخص:

يركز هذا البحث على اصطناع أسس شيف مفتوحة جديدة تم تحضيرها من تكاثف الساليسل الدهيد مع كلٍ من ثنائي امينو ثنائي فينيل الميثان، وثنائي امينو ثنائي فينيل سلفيد، ومن تكاثف الاورثو فانيلين مع ثنائي ايتيل استر لحمض النيرفتاليك، وايضاً من حضر اساس شيف حلقي ضخمة من تكاثف 6,1- ثنائي برومو حلقي الهكسان مع ثيوكربوهيدرازيد. وشُخصت هذه المركبات بالتقنيات الطيفية المختلفة (LC-MS, ¹H-NMR, IR). كما دُرست الفعالية البكتيرية لهذه المركبات المصنعة.