Influence of Ferrocene and Transition Metals on the Biological Activities of Schiff Bases

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(Received on 10th February 2016, accepted in revised form 11th August 2016)

Summary: A series of organic and organometallic Schiff bases bearing phenylferrocene and their six transition metal complexes have been prepared and tested for their potential biological applications by using antifungal, antibacterial, antitumor activities, toxicity testing against the brine shrimp and DNA damage analysis. The copper and cobalt complexes of organic Schiff base showed significant antibacterial activity. The antifungal activities tested against six fungal strains revealed that N-(4-hydroxybenzylidene) aniline (A5) had the highest antifungal activity. Most of these compounds showed cytotoxic activity against the brine shrimp. The results of showed that these compounds had significant antitumor activity, up to 97% in the case of N-(4-chlorobenzylidene) aniline (A3). Only two compounds N-(2-hydroxy benzylidene) 4-ferrocenylaniline (F2) and Nickel (II) complex of organic Schiff base (CO2) had DNA damaging activity at 20mg/ml concentration.

Key words: Biological activities, Ferrocene based Schiff bases, Metal complexes, Organic Schiff bases.

Introduction

Schiff bases were first reported by Schiff in 1864 [1]. Since then the interest in these compounds has been increased tremendously because of their applications. Metal Schiff base complexes are known since mid-19th century [2]. Schiff bases occupy an important position as ligand in metal coordination even after a century, since their discovery. Due to the ease of their preparation, diverse properties, medicinal, biochemical and industrial applications, keen interest in the study of these complexes arose in the recent years [3, 4]. A variety of Schiff base derivatives have shown potential biological activities in the recent few years [5-7]. Zhou *et al.* [8] have found that Schiff bases derived from 2-aminothiazole exhibit antibacterial, antifungal and anti-inflammatory activities. Recently, it is found that Schiff base derivatives of metronidazole have much better antigiardial, antifungal and antibacterial activities compared with metronidazole [9]. Similarly, isatin Schiff base derivatives have shown higher cytotoxic effects on HeLa cancer cell lines compared to LS180 [10]. Functionalization of Schiff bases with sulphur, oxygen, nitrogen and fluorine have been used to increase the biological activity and their transition metal complexes have found applications in medicinal chemistry [11]. Some studies reveal that transition metal complexes of Schiff bases have significantly better biological activities compared the corresponding Schiff base ligands [12-14].

Ferrocene, an organometallic compound was discovered in the early 1950s. Interest into the biological activity of ferrocene compounds began when a number of ferrous compounds were patented in 1960s [15]. Ferrocene is often incorporated into organic compounds in order to enhance the biological activity of organic compounds [16]. The presence of iron atom in ferrocene molecule defined in certain extent the direction of these investigations. The biologically active ferrocene derivatives with antianaemic properties were among compounds that attracted the great attention of various research groups [17]. In addition, ferrocene is ideal for use in drug design because of the low toxicity of the molecule containing ferrocenyl moiety. These compounds also exhibit antifungal, antiviral, and anticancer activity [18-24].

In continuation of our interest in biological application of various synthetic compounds [21-27], we investigated the effect of ferrocene and transition metals on the biological activity of Schiff bases. A variety of organic and organometallic Schiff bases bearing phenylferrocene and their transition metal complexes were prepared for this study. The structure and purity of the synthesized compounds was characterized using elemental analysis, FTIR and NMR spectroscopic techniques. The biological activities such as antibacterial, antifungal, antitumor, DNA damage activities and brine shrimp toxicity were thoroughly evaluated.

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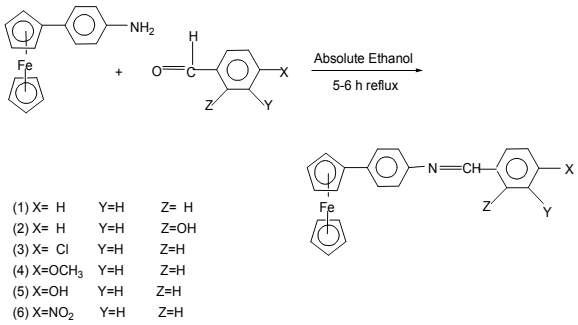
Experimental

Chemicals

Chemicals employed for preparation of these compounds and their complexes were pure and used as purchased. Compounds having phenyl ferrocene (F1-F6) were prepared by the stated method [24] (Scheme-1). Syntheses of these compounds were accompanied under nitrogen that was fashioned by using vacuum line and dry argon gas.



Scheme-1: The synthesis of ferrocenyl Schiff bases **(F1-F6).**

Synthesis

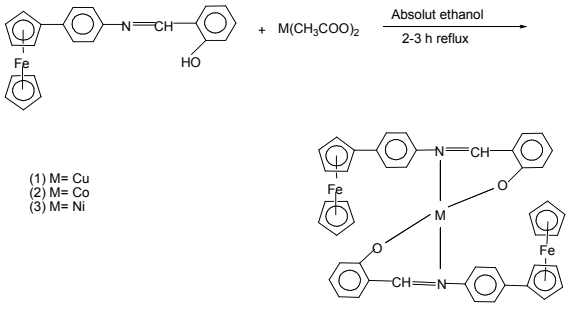
Complexes with Schiff base bearing phenyl ferrocene **(C1-C3)**

General Synthetic Procedure

Two necked round bottom flask was taken and fitted with condenser and magnetic stirrer. N-(2-hydroxybenzylidene)-4-ferrocenylaniline (F2) was suspended in (20-25ml) absolute ethanol and the mixture was brought to boiling temperature. To this hot solution, a solution of the corresponding salt in the same solvent was added. Then the mixture was refluxed for 3-4 hs. The complex was precipitated out on cooling the mixture. After this the product was filtered and then dried. The compounds were recrystallized using warm benzene. It is unstable in hot benzene as well as in $CHCl_3$ where the free ligand is formed slowly (Scheme-2). [25-27]

Copper (II) complex **(C1)**

Copper (II) complex was synthesized by treating N-(2-hydroxybenzylidene)-4-ferocenylaniline 0.2g (0.525mmol) with Copper (II) acetate monohydrate 0.052g (0.262mmol) using high grade ethanol by mean of above procedure. Yield: 82 %, m.p.172˚ C; Anal. Calc. For $C_{46}H_{37}N_2O_2Fe_2Cu$: C, 67.03; H, 4.37%. Found: C, 66.50; H, 4.46%. IR (KBr, υ_{max}/cm^{-1}): 3051, 1611, 1519, 1105, 1022, 500.



Scheme-2: The synthesis of transition metal complexes with ferrocenyl Schiff base **C1-C3.**

Cobalt (II) complex **(C2)**

Cobalt (II) complex was synthesized by treating N-(2-hydroxybenzylidene)-4-ferocenylaniline 0.5g (1.312mmol) with Cobalt (II) acetate 0.163g (0.656mmol) in absolute ethanol as a solvent using the above procedure. Yield: 78 %, m.p. 210˚C, Anal. Calc. For $C_{46}H_{37}N_2O_2Fe_2Co$: C, 67.40; H, 4.39%. Found: C, 65.35; H, 4.42%

IR (KBr, υ_{max}/cm^{-1}): 2925, 1610,1522, 1106, 1025 490.

Nickel (II) complex **(C3)**

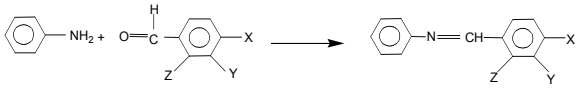
Nickel (II) complex was synthesized by treating N-(2-hydroxybenzylidene)-4-ferocenylaniline 0.5 g (1.312mmol) with Nickel (II) acetate.4 hydrate 0.163 g (0.656 mmol) in absolute ethanol as a solvent using the above procedure. Yield: 76 %, m.p. 270˚C, Anal. Calc. For $C_{46}H_{37}N_2O_2Fe_2Ni$: C, 67.42; H, 4.39%. Found: C, 66.68; H, 4.43 %. IR (KBr, υ_{max}/cm^{-1}): 2924, 1607, 1518,1103, 1020, 480.

Organic Schiff bases **(A1-A6)**

General Synthetic Procedure

To a two necked round bottom flask fitted with Dean and Stark apparatus, a condenser and a magnetic stirrer, the corresponding aldehyde in 30-40 ml toluene was added. This mixture was refluxed for 30-40 mins then aniline was added. The mixture was refluxed for 13-14 hs and the reaction was observed by TLC, using acetone and hexane system (1:3).

After the completion of reaction, indicated by no more water formation, solvent was removed at low pressure and solid residue was recrystallized from warm benzene (Scheme-3).



(1) X= H Y=H Z= H
(2) X= H Y=H Z=OH
(3) X= Cl Y=H Z=H
(4) X=OCH_3 Y=H Z=H
(5) X=OH Y=H Z=H
(6) X=NO_2 Y=H Z=H

Scheme-3: The synthesis of organic Schiff bases **(A1-A6).**

N-(Benzylidene) aniline **(A1)**

A1was synthesized by mixing aniline 5mL (0.054mol) with benzaldehyde 5.6ml (0.054mol) using toluene solvent by mean of above method. Yield: 85 %, m.p. 51˚C, Anal. Calc. For $C_{13}H_{11}N$: C, 86.18; H, 6.07; N, 7.73%. Found: C, 85.41; H, 6.10; N, 7.94 % IR (KBr, υ_{max}/cm^{-1}): 3059, 1626, 1585.). ^{1}H-NMR (300MHz, acetone-d6, Me_4Si): δ 8.4(s, 1H, CH=N), 7.6-7.3(m, Ph)

N-(2-hydroxybenzylidene) aniline **(A2)**

A2 was synthesized by treating aniline 5mL (0.054mol) with 2-hydroxybenzaldehyde 5.8ml (0.054mol) in toluene as a solvent using the aforementioned method. Yield: 86 %, m.p.54˚C. Anal. Calc. For $C_{13}H_{11}NO$: C, 79.18; H, 5.58; N, 7.10%. Found: C, 80.18; H, 5.62; N, 7.20 %. IR (KBr, υ_{max}/cm^{-1}): 3055, 1616, 1571. ^{1}H-NMR (300MHz, Acetone-d6, Me_4Si): δ 8.6(s, 1H, CH=N), 8.0-7.5(m, Ph), 7.5-7.2(m, Ph), 5.0 (s, 1H, OH).

N-(4-chlorobenzylidene) aniline **(A3)**

A3 was synthesized by treating aniline 5mL (0.054mol) with 4-chlorobenzaldehyde 7.56g (0.054mol) in toluene as described above. Yield: 82 %, m.p.69˚ C. Anal. Calc. For $C_{13}H_{11}Cl$: C, 72.39; H, 4.64; N, 6.49%. Found: C,71.48; H,4.59; N, 6.07%. IR (KBr, υ_{max}/cm^{-1}): 3084, 1620, 1585, 1086. ^{1}H-NMR (300MHz, Acetone-d6, Me_4Si): δ 8.01(s,1H, CH=N), 8.00(d, *J*=8.4Hz, Ph), 7.57 (d, *J*=8.4Hz, Ph), 7.45-7.24 (m, Ph).

N-(4-methoxybenzylidene) aniline **(A4)**

A4 was synthesized by treating aniline 5mL (0.054mol) with 4-methoxybenzaldehyde 6.6ml (0.054mol) in toluene solvent by the stated method. Yield: 80 %, m.p. 64˚ C. Anal. Calc. For $C_{14}H_{13}NO$: C, 79.62; H, 6.16; N, 6.63 %. Found: C,78.05; H,6.15; N, 6.82%. IR (KBr, υ_{max}/cm^{-1}): 3050, 1601, 1566, 2843. ^{1}H-NMR (300MHz, Acetone-d6, Me_4Si): δ 8.51(s,1H, CH=N), 7.93(d, *J*=8.4Hz, Ph), 7.07 (d, *J*=8.4Hz, Ph), 7.43-7.19 (m, Ph).

N-(4-hydroxybenzylidene) aniline **(A5)**

A5 was synthesized by treating aniline 3ml (0.032mol) with 4-hydroxybenzaldehyde 4.013g (0.032mol) in toluene using the method given above. Yield: 78 %. m.p. 201˚ C. Anal. Calc. For $C_{13}H_{11}NO$: C, 79.18; H,5.58; N, 7.10%. Found: C,78.38; H,5.64; N, 7.06%. IR (KBr, υ_{max}/cm^{-1}): 3048, 1601, 1575. ^{1}H-NMR (300MHz, Acetone-d6, Me_4Si): δ 9.04(s, 1H, OH), 8.45(s,1H, CH=N), 7.85(d, *J*=8.7Hz, Ph), 6.98(d, *J*=8.7Hz, Ph), 7.42-7.17 (m, Ph).

N-(4-nitrobenzylidene) aniline **(A6)**

A6 was synthesized by treating aniline 1ml (0.0109mol) with 4-nitrobenzaldehyde 1.64g (0.0109mol) in toluene as a solvent using the above procedure. Yield: 76 %. m.p. 94˚ C. Anal. Calc. For $C_{13}H_{10}N_2O_2$: C, 69.02; H,4.42; N, 12.38%. Found: C,67.13; H,4.44; N, 12.36%. IR (KBr, υ_{max}/cm^{-1}): 3121, 1595, 1514, 1341. ^{1}H-NMR (300MHz, Acetone-d6, Me_4Si): δ 8.36(s, 1H, CH=N), 8.38(d, *J*=8.7Hz, Ph), 8.23(d, *J*=8.7Hz, Ph), 7.48-7.29 (m, Ph).

Complexes with organic schiff base **(CO1, CO2, CO3)**

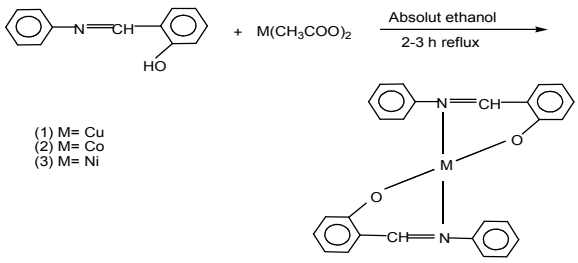
General Synthetic Procedure

In the two necked flask equipped with condenser and magnetic stirrer, the A2 suspended in absolute ethanol (25-30ml) and the mixture was refluxed for 15 minutes. To this hot reaction mixture, a solution of the corresponding salt in the same solvent was added. Then the reaction mixture was refluxed for 5-6 hours. On cooling to room temperature, the complex was precipitated out. The precipitate was filtered, dried and recrystallized from warm benzene (Scheme-4).

Copper (II) complex **(CO1)**

This complex was synthesized by treating N-(2-hydroxybenzylidene) aniline 0.5g (2.53mmol) with Copper (II) acetate monohydrate 0.25g (1.26mmol) in absolute ethanol as a solvent using the above procedure. Yield: 84 %. m.p. 206˚ C Calc. For

$C_{26}H_{24}N_2O_4Cu$: C, 63.54; H, 4.88; N, 5.70%. Found: C, 62.84; H, 4.02; N, 5.10%. IR (KBr, υ $_{max}$/cm^{-1}): 3044, 1606, 1516, 1103, 1018.



Scheme-4: The synthesis of transition metal complexes with organic Schiff base **(CO1-CO3).**

Cobalt (II) complex **(CO2)**

Cobalt (II) complex was synthesized by treating N-(2-hydroxybenzylidene) aniline 0.5g (2.53mmol) with Cobalt (II) acetate 0.223g (1.26mmol) in absolute ethanol as a solvent using the above procedure. Yield: 81 %. m.p.113˚ C Calc. For $C_{26}H_{24}N_2O_4Co$: C, 64.06; H,4.92; N, 5.74%. Found: C,63.01; H,4.96; N, 5.43%.IR (KBr, υ $_{max}$/cm^{-1}): 3389, 2933, 1611,1526, 1112, 1021.

Nickel (II) complex **(CO3)**

Nickel (II) complex was synthesized by treating N-(2-hydroxybenzylidene) aniline 0.5g (2.53mmol) with Nickel (II) acetate.4 hydrate 0.313g (1.26mmol) in absolute ethanol as a solvent using the above procedure. Yield: 78 %. m.p.296˚ C . Calc. For $C_{26}H_{20}N_2O_2Ni$: C, 69.22; H, 4.43; N, 6.21%. Found: C, 68.18; H, 4.35; N, 6.50%.IR (KBr, υ $_{max}$/cm^{-1}): 2942, 1609, 1511, 1108, 1024, 481.

Physical Characterization

Melting temperature of the compounds was determined on melting point apparatus, Mel-Temp, Mitamura Riken Kogyo, Inc. Tokyo Japan.

The solid state Fourrier transform infrared spectra (KBr pallets, 4000-400 cm^{-1}) were recorded on Bio-Rad Excalibur FTIR, Model 3000 MX.

^{1}H-NMR spectral analysis was performed in acetone and recorded on a Bruker 300 MHz. Tetramethylsilane was used as an internal reference.

Biological Evaluation

Antibacterial Assay

Agar well diffusion method [20] was used for the determination of inhibition zone. Single colony from each bacterial culture plate was transferred to nutrient broth (pH 7) and incubated at 37ºC for 24 hours. A volume of 0.75 mL of broth culture containing ca.10^6 colony forming units per mL of test strain was added to the 75 mL of nutrient agar medium, mixed well, and then poured into a 14 cm sterile agar plate. Reaction was performed in triplicate. Wells were prepared by using 8 mm sterilized metallic borer, sealed with medium and filled with 100 µL of 1 mg/mL solution of each compound. Roxythromycine (1 mg/mL) and Cefixime (1 mg/mL) were used as standard drugs while DMSO was used as negative control. Plates were incubated at 37ºC aerobically and zone of inhibition was measured after 24 hours.

Antifungal Assay

Antifungal behavior was evaluated against six fungal strains *[Fusarium moniliformes, Fusarium solani, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus* and *Mucor specie*] by mean of agar tube dilution method [21]. Fungal strains were grown on 6.5 % SDA (Sabouraud dextrose agar, pH 5.7) at 28ºC and preserved at 4ºC in refrigerator. Screw capped test tubes comprising Sabouraud dextrose agar (SDA) medium (4ml) was autoclaved at 121ºC for 15 minutes. Tubes were then cool at 50ºC and non-solidified SDA was loaded with 66.6 µl of compound pipeted from the stock solution (12mg/ml in DMSO) to make 200 µl/ml final concentration. Tubes were allowed to solidify at room temperature in slanting position. Each slant was inoculated with 4 mm piece of respective fungal strain and incubated at 28ºC for 7-10 days. The media supplemented with DMSO and Turbinafine (200 µl/ml) were employed as negative and positive control respectively. Fungal growth was studied by evaluating linear growth (mm) and matched with negative control to get the % age inhibition by mean of the following formula.

%age of fungal inhibition =

$$100 - \frac{\text{Fungal growth (mm) in sample}}{\text{Fungal growth (mm) in control}} \times 100$$

Toxicity testing against the brine shrimp

The brine shrimp lethality bioassay was carried out by previously reported method [27]. Brine shrimp (*Artemia salina*) eggs were hatched in artificial seawater (40g sea salts/L) at room temperature (22-29ºC). After two days these shrimps were shifted to vials (10 shrimps per vial) comprising artificial sea water (5 mL) with 1000, 100 and 10ppm final concentrations of each compound taken from

their stock solution of 10000ppm. After 24 hours numbers of surviving shrimps were counted. Data was analyzed with a finny computer programme (Probit analysis [28]) to determine LD_{50} values.

Antitumor Assay

The potato disc method was used for antitumor activity of organic compounds as reported [29]. *Agrobacterium tumefaciens* (At10) was grown on Luria broth (Miller's LB broth) medium for 48 hours at 28ºC in shaking incubator. Three controls were used. Positive control contained 0.5mL DMSO and 4.5mL distilled water. Negative control consisted of 0.5mL DMSO and 2mL bacterial culture and 2.5mL distilled water and third control consisted of blank. Sample was prepared by taking 2mL bacterial culture, 0.5mL organic compound (1000ppm) and 2.5mL distilled water. Cylinders of surface sterilized red skinned potato were made with the help of sterilized borer. The 5mm thick discs of these potato cylinders were made and placed on solidified agar plates (10 discs per plate). 50µL of inoculum was poured on the surface of each disc of respective concentration as well as controls. The discs were examined under dissecting microscope after staining with Lugol's solution (10% KI, 5% Iodine in distilled water). Number of tumors per disc was counted. Percentage inhibition was determined by using the following formula.

Percentage inhibition = 100- No of tumor with sample / No of tumor with control× 100

DNA Damage Analysis

DNA of pBR322 was treated with three different concentrations of compounds dissolved in DMSO i.e 25mg/ml, 10mg/ml and 1mg/ml. In this assay restriction endonuclease enzyme (Eco R1) was used as the positive control. Untreated plasmid DNA was used as negative control. In order to check DNA damaging effect of DMSO, DNA was also treated with DMSO. These reaction mixtures were kept at 37°C for 1 hour for the complete digestion of plasmid DNA. After incubation plasmid DNA was observed on 1% agarose gel. The gel was allowed to run for 150 minutes and then observed under UV light.

Results and Discussion

Synthesis and Characterization

The synthesis, structure and characterization of Schiff bases bearing phenyl ferrocene (F1-F6) (Scheme-1) have been reported earlier [24]. The N-(2-hydroxybenzylidene)-4-ferrocenylaniline (F2) was selected for metal complexation, as the OH group is expected to be coordinated with transition metals. Three metal complexes (C1-C3) were synthesized (Scheme-2) by reacting F2 with the corresponding metal acetate using absolute ethanol as a solvent. These metal complexes were characterized by their physical properties, elemental analyses and FTIR spectral studies. The elemental analysis data is in good agreement with the calculated values confirming the purity of these complexes. The FTIR spectra of these complexes showed all the expected characteristics bands e.g. bands were observed at 3000-3100 cm^{-1}, which can be assigned to aromatic CH stretching and around 1520 cm^{-1} for aromatic C=C stretching. These complexes showed a sharp band around 1610 cm^{-1} for azomethine C=N which is slightly shifted as compared with the ligand. This shift is probably due to the co-ordination of the Schiff base ligand with metal through nitrogen atom.

Organic Schiff bases (A1-A6) were synthesized by reacting aniline with the corresponding aromatic aldehydes (Scheme-3). These compounds were characterized by their physical properties, elemental analysis, FTIR and ^{1}H-NMR spectroscopy. The elemental analysis data correspond to the calculated values. The presence of all characteristic bands in their FTIR spectra confirmed their formation. A band around 1610 cm^{-1} is due to C=N stretching vibration which is the characteristic peak for Schiff bases. In the FTIR spectra of all these Schiff bases, the absence of band at 3500-3300 for NH stretching vibration and at 1720-1660 cm^{-1} for C=O indicates the formation of these Schiff bases. ^{1}H-NMR spectra of all synthesized organic Schiff bases show a sharp singlet around 8.5 ppm characteristic for azomethine proton.

The N-(2-hydroxybenzylidene) aniline (A2) was selected for metal complexation and three metal complexes (CO1, CO2 and CO3) were synthesized following the same procedure as used for F2 (scheme-4). The elemental analysis data confirmed the formation and purity of these compounds. These complexes were further characterized by FTIR spectroscopy. The shifting of the absorption band due to azomethine C=N stretching vibration confirmed the formation of these complexes.

Biological Evaluation

Antibacterial Activity

Results of antibacterial activity against six different bacterial strains are presented in Table-1. The compounds were dissolved in DMSO and their

MIC (minimum inhibitory concentration) was determined. The organic Schiff bases (A1-A6), ferrocenyl Schiff bases (F1-F6) and the metal complexes of ferrocenyl Schiff bases with Cu, Co and Ni (C1-C3) did not exhibit any significant antibacterial activity. This has been observed previously for similar compounds [25]. However, it should be noted that metal complexes of the organic Schiff bases (CO1-CO3) showed significant activity with highest inhibitory activity against *S. setubal* (MIC=0.05mg/ml). CO1 containing copper was the most active compound showing antibacterial activity against all the six tested strains followed by CO2 that was active against the five strains. CO2 contains cobalt as central atom in the complex of Schiff bases. The MIC values of CO1 and CO2 are comparable to roxithromycin and these two complexes can be used as potential antibacterial drugs because of their inhibitory activity against bacteria at low concentrations. This data reveal that incorporation of transition metals into the organic Schiff bases in the form of complexes might boost their antibacterial activity.

Antifungal Activity

The antifungal activity of the Schiff bases and their complexes was evaluated against six fungal strains. The percentage inhibition determined is summarized in Table-2. The screened data revealed that A5 (an organic Schiff base) showed relatively higher activity among all the 21 compounds tested with maximum inhibition against *F. solani* (63.5%). Some compounds like A2, C1, F1, F2, F4, F5, F6, and CO1 showed moderate activity against *F. moniliformes, F. solani* and *A. flavus,*. Similar results were obtained in experiments performed with ferrocene derivatives [26]. Few compounds like A1, A2 and A3 showed stimulatory effect i.e. they promoted linear growth of fungi rather than inhibiting it.

Toxicity testing against the brine shrimp

These 21 Schiff bases were also assayed for cytotoxic activity. The results obtained are tabulated Table-3. This data showed that a copper complex of the organic Schiff base (CO1) has the highest activity against brine shrimps (LD_{50} value = 2.95ppm) as compared to all others. Five compounds C1, C2, C3, F5 and F6 showed LD_{50} value more than 1000ppm, while all the rest of the compounds showed LD_{50} between 2.95 ppm to 909.9 ppm.

Antitumor Assay

The antitumor activity of all these compounds was evaluated. The results showed that all the studied compounds exhibit significant antitumor activity varying from 37% to 97.10%. Schiff bases are known to have some antitumor activities [27-30]. N-(4-chlorobenzylidene) aniline A3 showed highest i.e., 97.10% tumor inhibition. The results are tabulated in Table-4. It is interesting to note that with changes in the chemical structure of these Schiff bases can significantly change the antitumor activity.

Table-1: Antibacterial activity of Schiff bases and their complexes.

Sample code	Mean zone of Inhibition (in mm) ± S.E.					
	M. leuteus	*B. subtilis*	*S. aureus*	*E. coli*	*S. Setubal*	*B. bronc.*
F1	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
F2	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
F3	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
F4	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
F5	**Nil**	**Nil**	**10.9±0.7**	**Nil**	**Nil**	**Nil**
F6	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
C1	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
C2	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
C3	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
A1	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
A2	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
A3	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
A4	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
A5	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
A6	**11.4±0.8**	**11.5±0.1**	**Nil**	**11.4±0.8**	**11.0±0.2**	**Nil**
CO1	**15.9±2.4 MIC (0.4)mg/ml**	**12.9±1.3 MIC (0.1)mg/ml**	**11.3±0.7**	**11.6±0.4**	**18.3± 0.2 MIC (0.05)mg/ml**	**10.8±0.1**
CO2	**11.5±0.05**	**Nil**	**11.1±0.05**	**12.1±0.9 MIC (0.06)mg/ml**	**11±1.2**	**11.4±0.3**
CO3	**10.2±0**	**Nil**	**Nil**	**10.6±0.6**	**Nil**	**Nil**
DMSO	**Solvent**	**Solvent**	**Solvent**	**Solvent**	**Solvent**	**Solvent**
Roxithromycin	**12.2±0.8**	**11.9±0.7**	**17.1±1.8**	**14.6±0.17**	**13.9±2.0**	**16.2±4.4**
Cefixime	**24.4±2.3**	**27.7±2.8**	**23.2±1.3**	**22.9±4.3**	**29.5±2.4**	**24.7±0.6**

M. leuteus = Micrococcus leuteus, *B. sub = Bacillus subtillis, S. aureus = Staphylococcus aureus, E. coli = Escherichia coli, S. setubal = Salmonella setubal, B. bronch = Bordetella bronchiseptica*

Table-2: Antifungal activity of Schiff bases and their complexes.

Sample code	Percentage growth inhibition					
	A. niger	*A. fumigatus*	*A. flavus*	*F. moniliformes*	*F. solani*	*Mucor sp.*
F1	1.75	-13.55	22.6	16.6	47.2	1.54
F2	24.56	11.8	46.8	4.16	33.3	11.3
F3	-12.2	-25.4	16.4	13.5	1.85	5.15
F4	2.63	4.2	28.1	16.6	46.2	3.09
F5	2.63	25.4	47.6	3.125	57.4	-3.6
F6	18.4	-11	25	-9.3	53.7	14.9
C1	20.17	11.8	14.06	-6.2	53.14	-3.6
C2	-2.63	-15.2	11.7	25	24.8	9.79
C3	-8.77	16.9	1.56	22.9	42	-0.51
A1	-8.77	Zero	13.2	13.5	17.9	-7.2
A2	zero	7.62	7.03	42.7	-9.25	-1.5
A3	-0.87	29.6	28.9	33.3	zero	9.2
A4	13.15	23.7	1.5	29.1	24	2.06
A5	14.03	38.12	8.5	16.6	63.5	4.12
A6	-1.75	4.2	0.78	12.5	27.2	0.51
CO1	-5.26	6.77	50.7	21.8	48.1	3.6
CO2	19.2	22.08	35.1	14.5	7.4	31.3
CO3	25.4	3.38	13.2	19.7	25.3	0.51
Linear growth in negative control(mm)	57	59	64	48	54	97

A.niger= Aspergillus niger, A.fumigatus= Aspergillus fumigatus, A.flavus= Aspergillus flavus)
F.moniliformes= Fusarium moniliformes , F.solani=Fusarium solani

$$\% \text{ age inhibition of fungal growth} = \frac{100 - \text{linear growth in test (mm)}}{\text{Linear growth in neg. control (mm)}} \times 100$$

Criteria for significance:
Below 40% inhibition=low activity
40-60% inhibition= moderate activity
60-70% inhibition= Good activity
70% and above =Significant activity

Table-3: Cytotoxicity data of Schiff bases and their complexes.

Compounds	No of shrimps	No of shrimps killed			$LD_{50\ Value}$
	used at each dose level	1000ppm	100ppm	10ppm	(ppm) or (µg/ml)
F1	30	20	19	16	23.25
F2	30	16	7	4	909
F3	30	30	21	Zero	66.7
F4	30	23	6	1	327.1
F5	30	7	2	1	>1000ppm
F6	30	2	zero	Zero	>1000ppm
C1	30	4	2	Zero	>1000ppm
C2	30	6	2	1	>1000ppm
C3	30	8	1	Zero	>1000ppm
A1	30	26	17	13	26.5
A2	30	30	22	4	43.8
A3	30	30	20	9	31.8
A4	30	30	28	8	20.19
A5	30	24	15	10	62.33
A6	30	30	26	3	35.78
CO1	30	28	23	19	2.95
CO2	30	29	26	16	7.54
CO3	30	27	20	10	31.961

Table-4: Antitumor activity of Schiff bases and their complexes.

Sample Code	Average number of tumors per disc	%age of tumor inhibition
F1	0.8	88.50%
F2	0.9	87.10%
F3	3.8	45.70%
F4	2.8	60%
F5	2.1	70%
F6	0.9	87.10%
C1	1.7	75.70%
C2	1.1	84.20%
C3	4	42.80%
A1	1.1	84.20%
A2	2.3	67.10%
A3	0.2	97.10%
A4	2	71.40%
A5	1.6	77.10%
A6	2.2	68.50%
CO1	4.4	37%
CO2	3.7	47%
CO3	3.2	57%
Control	7	

DNA Damaging Analysis

These synthesized Schiff bases were subjected to DNA damaging analysis. The results showed that only two compounds N-(2-hydroxy benzylidene) 4-ferrocenylaniline (F2) and Nickel (II) complex of organic Schiff bases (CO3) had DNA damaging activity at 20mg/ml concentration. DNA damaging activity of F2 at 20mg/ml concentration is shown in Fig. 1. All other compounds showed no observable DNA damaging activity at any concentration studied.

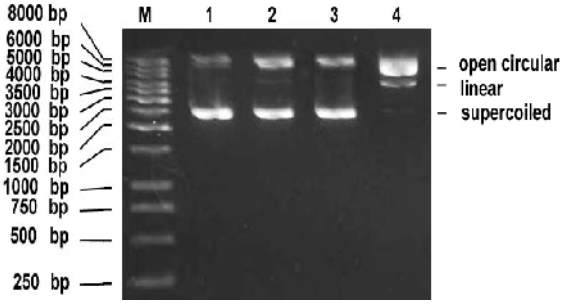


Fig. 1: 1% agarose gel presenting DNA damaging activity of F2 [N-(2-hydroxy benzylidene) 4-ferrocenylaniline].

Lane 1 1Kb DNA ladder (M)
Lane 2 Negative control untreated Plasmid DNA untreated
Lane 3 Plasmid DNA treated with 1mg/ml of F2 compound
Lane 4 Plasmid DNA treated with 10 mg/ml of F2 compound
Lane 5 Plasmid DNA treated with 20mg/ml of F2 compound

Conclusions

Twelve Schiff bases and their metal complexes were synthesizes and characterized by their physical properties, NMR and IR spectroscopic methods. It can be deduced from the biological evaluation of these Schiff bases that the difference in response of the compounds arises because of their composition and structural differences. It has been suggested that the presence of ferrocene and transition metals such as Co, Cu, Ni may impart biological activity to Schiff bases. Our results show that among these compounds, organic Schiff bases showed better antifungal activity while their complexes showed better antibacterial activity. However, ferrocene did not show any synergistic effect on antifungal and antibacterial activities. On the other hand, the ferrocenyl Schiff bases and its complexes had significant antitumor activity. This is a direct evidence that ferrocene enhances the antitumor activity of organic compounds. Furthermore most of the compounds did not show DNA damaging activity at any concentration. Only two compounds N-(2-hydroxy benzylidene) 4-ferrocenylaniline (F2) and Nickel (II) complex (CO3) of organic Schiff base had DNA damaging activity at 20mg/ml concentration. We conclude that some of these compounds can be used as new generation antibacterial and antifungal agents, and particularly, ferrocenyl Schiff bases as drugs against cancer.

Acknowledgement

We wish to express our appreciation for the financial support from Luleå University of Technology Sweden and the University Research Fund (URF) Quaid-I-Azam University Islamabad Pakistan.

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