Preparation, Biological Activity and Characterisation of Novel Macroacyclic (N₂O₄ and N₂O₂) Schiff Base Ligands and Their Zn(II), Cd(II) and Hg(II) Complexes

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New macroacyclic N₂O₄(L₁) and N₂O₂(L₂) Schiff base ligands have been synthesized from 1,4-di-(4-fluoro-2-aminophenoxy)butane (DFAB) with salicylaldehyde and 1,4-di-(4-fluoro-2-aminophenoxy)butane (DFAB) with anthracene-9-carbaldehyde, respectively. Zn(II), Cd(II) and Hg(II) complexes of the Schiff base ligands have also been prepared and all compounds have been characterised by IR, ¹H NMR, ¹³C NMR spectroscopy and mass spectrometry. We are also especially interested in the antibacterial activity of these new complexes. The in vitro antibacterial activity of the metal ions, free ligands and their complexes were tested against the gram-positive bacteria and gram-negative bacteria by paper disc diffusion and minimum inhibitory concentration (MIC) methods. It is apparent the metal complexes have good antibacterial activity but related free ligands and metal ions have not antibacterial activity.

Key words: Schiff base, Macroacyclic, Antibacterial activity.

Schiff bases and their complexes, a typically of chelators are capable of forming coordinate bonds with many metal ions through azomethine group and phenolic group or via its azomethine or phenolic groups. The chemistry of Schiff base ligands and their metal complexes have attracted increasing interest owing to their role in the understanding of molecular processes occurring in biochemistry, antifungal, antibacterial, anticancer, catalytic fields and as encapsulating ligands for radiopharmaceuticals. Therefore, The chemistry of Schiff base ligands and their metal complexes have attracted a lot of interest due to their facile synthesis and wide range of applications including pigments, intermediates in organic synthesis and as polymer stabilizers.

MATERIALS AND METHODS

4-fluorophenol is commercially available from Merck and is used without any changes. All other solvents and materials were of reagent grade and used without further purification. IR spectra were recorded (KBr) on a Bruker VERTEX 70 spectrometer. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker- AV400MHz. Mass spectra were recorded on a 5973 Tecnology Agilent (HP) spectrometer (EI = 70 eV).

RESULTS

Synthesis

In this work, a new diamine 1,4-di-(4-fluoro-2-aminophenoxy)butane has been synthesized by modified previous procedure. Then, macroacyclic (N₂O₄ and N₂O₂) Schiff base ligands and their complexes have been synthesized and characterized by IR, ¹H-NMR and ¹³C-NMR spectroscopy and mass spectrometry.
Synthesis of 1,4-di-(4-fluoro-2-nitrophenoxy) butane (DFNB)

To a solution of 0.16 g 4-fluoro-2-nitrophenol (1 mmol) in 50 cm³ ethanol was added 0.04 g NaOH (1 mmol) in 5 cm³ water under stirring at room temperature. After the color of solution changed from yellow to red, a solution of 0.1 g 1,4-dibromo butane (0.5 mmol) in 10 cm³ ethanol was added dropwise and was refluxed for 48 h according to scheme 1. After completion of the reaction, the cream solid crude was filtered and washed with ethanol. mp: 121 °C. IR (KBr, ì, cm⁻¹): 1518 ìas(N–O), 1342 ìs(N–O), 1194 ì(C–O), 1020 ì(C-F). m/z: 368 [M+].

1H NMR (ä, DMSO-d₆, MHz): 1.63 (4H; Ha), 3.15 (4H; Hb), 7.15-7.32 (6H; Hd, He, Hg). 13C NMR (ä, DMSO-d₆, MHz): 23.22 (2C; Ca), 65.43 (2C; Cb), 117.53-132.71 (12C; Cc, Cd, Ce, Cf, Cg, Ch).

Synthesis of 1,4-di-(4-fluoro-2-aminophenoxy) butane (DFAB)

0.37 g 1,4-di-(4-fluoro-2-nitrophenoxy)butane (1 mmol) was dissolved in 50 cm³ HCl (5 N) at temperature of 40-50°C. Then 2.3 g SnCl₂.2H₂O (10 mmol) was gradually added to the mixture of reaction. After the addition of SnCl₂.2H₂O, a turbid solution was formed. The mixture of reaction was refluxed till the solution become colorless and transparent (24 h) according to scheme 2. After completion of the reaction, the white solid crude was filtered and washed with cold ethanol. Yield: mp: 132-133 °C. IR (KBr, ì, cm⁻¹): 1022 ì(C-F), 3505 ìs(N–H), 1223 ì(C–O). m/z: 395 [M+].

1H NMR (ä, DMSO-d₆, MHz): 1.93 (4H; Ha), 4.14 (4H; Hb), 6.94-7.59 (14H; Hd, He, Hg, Hk, Hl, Hm, Hn), 8.99 (2H; Hi), 13.7 (2H; Hphenolic). 13CNMR (ä, DMSO-d₆, MHz): 25.88 (2C; Ca), 68.85 (2C; Cb), 106.42-155.76 (24C; Cc, Cd, Ce, Cf, Cg, Ch, Cj, Ck, Cl, Cm, Cn, Co), 167.15 (2C; Ci).

Template synthesis of metal complexes of L₁ with M²⁺ (M = Zn, Cd and Hg)

To a solution of 0.12 g salicylaldehyde (1 mmol) in 20 cm³ ethanol was added appropriate amount of M²⁺ (1 mmol) and it was stirred at room temperature for 2 h. After that dropwise a solution of 0.2 g diamine salt (0.5 mmol) in 10 cm³ water and 30 cm³ ethanol over 30 minutes. Then the solution of NaOH (5M) was added drop by drop until pH of the mixture of reaction reached 7. The reaction was stirred at room temperature for 24 h to be completed according to scheme 3. After completion of the reaction, the whitly precipitate was filtered and washed with acetonitrile and cold methanol. mp 150-151 °C. IR (KBr, ì, cm⁻¹): 3470i(C–Hamine), 1618i(C=N), 1115i(C–O), 1021i(C-F). m/z: 395 [M+].

OH
F
NO₂
SnCl₂·H₂O
HCl

Scheme 1. Outcome of 1,4-di-(4-fluoro-2-nitrophenoxy)butane condensation between 4-fluoro-2-nitrophenol and 1,4-dibromo butane

OCH₂CH₂CH₂CH₂O
SnCl₂·H₂O

Scheme 2. Outcome of 1,4-di-(4-fluoro-2-aminophenoxy)butane condensation between 1,4-di-(4-fluoro-2-nitrophenoxy)butane and SnCl₂·2H₂O

Synthesis of macrocyclic Schiff base ligand (L₁)

To a solution of 0.12 g salicylaldehyde (1 mmol) in 20 cm³ ethanol was added dropwise a solution of 0.2 g diamine salt (0.5 mmol) in 10 cm³ water and 30 cm³ ethanol over 30 minutes. Then the solution of NaOH (5M) was added drop by drop until pH of the mixture of reaction reached 7. The reaction was stirred at room temperature for 24 h to be completed according to scheme 3. After completion of the reaction, the whitly precipitate was filtered and washed with acetonitrile and cold methanol.

OH
F
NO₂
NaOH

Scheme 1. Outcome of 1,4-di-(4-fluoro-2-nitrophenoxy)butane condensation between 4-fluoro-2-nitrophenol and 1,4-dibromo butane

OCH₂CH₂CH₂CH₂O
SnCl₂·H₂O

Scheme 2. Outcome of 1,4-di-(4-fluoro-2-aminophenoxy)butane condensation between 1,4-di-(4-fluoro-2-nitrophenoxy)butane and SnCl₂·2H₂O
Complex (1)  
\[ \text{[Zn(L1)](NO}_3\text{)}_2; \text{Yield 91 \%; m.p> 200 \degree C;} \]
FAB MS (positive FAB in nitrobenzyl alcohol): m/z 578 [C\textsubscript{30}H\textsubscript{24}F\textsubscript{2}ZnN\textsubscript{2}O\textsubscript{4}]+. IR (KBr, ì, cm\textsuperscript{-1}): 2954 (C–H aromatic), 2933 (C–CH\textsubscript{2} aliphatic), 2875 (C–CH\textsubscript{2} iminic), 1619 (C=N), 1217 (C–O). 1H NMR (\ä, DMSO-d\textsubscript{6}, MHz): 1.89 (4H; Ha), 3.96 (4H; Hb), 5.00 (4H; He, Hg), 6.21-6.74 (10H; Hd, He, Hf, Hg, Hn), 8.99 (2H; Hi). 13C NMR (\ä, DMSO-d\textsubscript{6}, MHz): 26.09 (2C; Ca), 68.58 (2C; Cb), 100.57-156.36 (24C; Cc, Cd, Ce, Cf, Cg, Ch, Cj, Ck, Cl, Cm, Cn, Co), 167.4 (2C; C).  

Complex (2)  
\[ \text{[Cd(L1)](NO}_3\text{)}_2; \text{Yield 75 \%; m.p=190-192 \degree C;} \]
FAB MS (positive FAB in nitrobenzyl alcohol): m/z 627 [C\textsubscript{30}H\textsubscript{24}F\textsubscript{2}CdN\textsubscript{2}O\textsubscript{4}]+. IR (KBr, ì, cm\textsuperscript{-1}): 2956 (C–H aromatic), 2932 (C–CH\textsubscript{2} aliphatic), 2879 (C–CH\textsubscript{2} iminic), 1618 (C=N), 1217 (C–O). 1H NMR (\ä, DMSO-d\textsubscript{6}, MHz): 1.92 (4H; Ha), 4.09 (4H; Hb), 6.94-7.59 (14H; Hd, He, Hf, Hg, Hk, Hl, Hm, Hn), 8.99 (2H; Hi). 13C NMR (\ä, DMSO-d\textsubscript{6}, MHz): 25.88 (2C; Ca), 68.86 (2C; Cb), 106.33-156.36 (24C; Cc, Cd, Ce, Cf, Cg, Ch, Cj, Ck, Cl, Cm, Cn, Co), 163.86 (2C; C).  

Complex (3)  
\[ \text{[Hg(L1)]Cl}_2; \text{Yield:90 \%; m.p > 200 \degree C;} \]
FAB MS (positive FAB in nitrobenzyl alcohol): m/z 715 [C\textsubscript{30}H\textsubscript{24}F\textsubscript{2}HgN\textsubscript{2}O\textsubscript{4}]. IR (KBr, ì, cm\textsuperscript{-1}): 3501 (C–H iminic), 1622 (C=N), 1213 (C–O), 1017 (C–F). m/z: 683 [M\textsuperscript{+}]. 1H NMR (\ä, DMSO-d\textsubscript{6}, MHz): 1.73 (4H; Ha), 3.73 (4H; Hb), 6.72-7.03 (24H; Hd, He, Hf, Hg, Hk, Hl, Hm, Hn, Ho, Hq, Hs, Ht, Hu, Hv), 8.87 (2H; Hi). 13CNMR (\ä, DMSO-d\textsubscript{6}, MHz): 163.86 (2C; C).  

Synthesis of metal complexes of L\textsubscript{2} with M\textsuperscript{2+} (M = Zn, Cd and Hg)  
To a solution of 0.2 g anthracene-9-carbaldehyde (1 mmol) in 20 cm\textsuperscript{3} ethanol was added appropriate amount of M\textsuperscript{2+} (1 mmol) and it was stirred at room temperature for 24 h to be completed according to scheme 4. After completion of the reaction, the white precipitate was filtered and washed with acetonitrile and cold methanol. Yield: 91%. mp 142-143 ÚC. IR (KBr, ì, cm\textsuperscript{-1}): 3501 (C–H iminic), 1622 (C=N), 1213 (C–O), 1017 (C–F). m/z: 683 [M\textsuperscript{+}]. 1H NMR (\ä, DMSO-d\textsubscript{6}, MHz): 1.73 (4H; Ha), 3.73 (4H; Hb), 6.72-7.03 (24H; Hd, He, Hf, Hg, Hk, Hl, Hm, Hn, Ho, Hq, Hs, Ht, Hu, Hv), 8.87 (2H; Hi). 13CNMR (\ä, DMSO-d\textsubscript{6}, MHz): 163.86 (2C; C).  

Template synthesis of metal complexes of L\textsubscript{2} with M\textsuperscript{2+} (M = Zn, Cd and Hg)  
To a solution of 0.2 g anthracene-9-carbaldehyde (1 mmol) in 20 cm\textsuperscript{3} ethanol was added appropriate amount of M\textsuperscript{2+} (1 mmol) and it was stirred at room temperature for 24 h to be completed according to scheme 4. After completion of the reaction, the white precipitate was filtered and washed with acetonitrile and cold methanol. Yield: 91%. mp 142-143 ÚC. IR (KBr, ì, cm\textsuperscript{-1}): 3501 (C–H iminic), 1622 (C=N), 1213 (C–O), 1017 (C–F). m/z: 683 [M\textsuperscript{+}]. 1H NMR (\ä, DMSO-d\textsubscript{6}, MHz): 1.73 (4H; Ha), 3.73 (4H; Hb), 6.72-7.03 (24H; Hd, He, Hf, Hg, Hk, Hl, Hm, Hn, Ho, Hq, Hs, Ht, Hu, Hv), 8.87 (2H; Hi). 13CNMR (\ä, DMSO-d\textsubscript{6}, MHz): 163.86 (2C; C).  

Scheme 3. Outcome of of macroacyclic Schiff base ligand (L\textsubscript{1}) condensation between 1,4-di-(4-fluoro-2-aminophenoxy)butane and salicylaldehyde  

Scheme 4. Outcome of of macroacyclic Schiff base ligand (L\textsubscript{2}) condensation between 1,4-di-(4-fluoro-2-aminophenoxy)butane and anthracene-9-carbaldehyde
stirred at room temperature for 2 h. After that dropwise a solution of 0.2 g diamine salt (0.5mmol) in 10 cm$^3$ water and 30 cm$^3$ ethanol over 30 minutes. Then the solution of NaOH (5M) was added drop by drop until pH of the mixture of reaction reached 7. The mixture of reaction was refluxed for 36 h. After completion of the reaction, the white precipitate was filtered and washed with acetonitrile and cold methanol.

**Complex (4)**

\[
[Zn(L_2)](NO_3)_2; \text{Yield 91 \%; m.p> 200 ^\circ C; FAB MS (positive FAB in nitrobenzyl alcohol): m/z 747 [C_{46}H_{33}F_{2}ZnN_2O_2]^+}. \text{IR (KBr, } \bar{\nu}, \text{ cm}^{-1}): 2943 \bar{\nu}(\text{C–Haromatic}), 2922 \bar{\nu}(\text{C–Haliphatic}), 2864 \bar{s}(\text{C–Himinic}), 1620 \bar{\nu}(\text{C=N}), 1214 \bar{\nu}(\text{C-O}), 1016 \bar{\nu}(\text{C-F}). 1H NMR (\delta, \text{ DMSO-d}_6, \text{MHz}): 1.84 (4H; Ha), 3.86 (4H; Hb), 6.81-7.51 (24H; Hd, He, Hg, Hl, Hm, Hn, Ho, Hq, Hs, Ht, Hu, Hv), 8.87 (2H; Hi). 13CNMR (\delta, \text{ DMSO-d}_6, \text{MHz}): 23.92 (2C; Ca), 60.12 (2C; Cb), 104.18-170.66 (40C; Cc, Cd, Ce, Cf, Cg, Ch, Cj, Ck, Cl, Cm, Cn, Co, Cp, Cq, Cr, Cs, Ct, Cu, Cv, Cw), 178.13 (2C; Ci).

**Complex (5)**

\[
[Cd(L_2)](NO_3)_2; \text{Yield 89 \%; m.p> 200 ^\circ C; FAB MS (positive FAB in nitrobenzyl alcohol): m/z 797 [C_{46}H_{33}F_{2}CdN_2O_2]^+}. \text{IR (KBr, } \bar{\nu}, \text{ cm}^{-1}): 2942 \bar{\nu}(\text{C–Haromatic}), 2920 \bar{\nu}(\text{C–Haliphatic}), 2870 \bar{s}(\text{C–Himinic}), 1620 \bar{\nu}(\text{C=N}), 1217 \bar{\nu}(\text{C-O}), 1015 \bar{\nu}(\text{C-F}). 1H NMR (\delta, \text{ DMSO-d}_6, \text{MHz}): 1.85 (4H; Ha), 3.90 (4H; Hb), 6.81-8.12 (24H; Hd, He, Hg, Hl, Hm, Hn, Ho, Hq, Hs, Ht, Hu, Hv), 8.87 (2H; Hi). 13CNMR (\delta, \text{ DMSO-d}_6, \text{MHz}): 23.86 (2C; Cc), 60.23 (2C; Cb), 101.58-171.66 (40C; Cc, Cd, Ce, Cf, Cg, Ch, Cj, Ck, Cl, Cm, Cn, Co, Cp, Cq, Cr, Cs, Ct, Cu, Cv, Cw), 180.14 (2C; Ci).

**Complex (6)**

\[
[Hg(L_2)]Cl_2; \text{Yield: 78 \%; m.p>; 200 ^\circ C, FAB MS (positive FAB in nitrobenzyl alcohol): m/z 885 [C_{46}H_{33}F_{2}HgN_2O_2]^+}. \text{IR (KBr, } \bar{\nu}, \text{ cm}^{-1}): 2965 \bar{\nu}(\text{C–Haromatic}), 2941 \bar{\nu}(\text{C–Haliphatic}), 2878 \bar{s}(\text{C–Himinic}), 1621 \bar{\nu}(\text{C=N}), 1214 \bar{\nu}(\text{C-O}), 1016 \bar{\nu}(\text{C-F}). 1H NMR (\delta, \text{ DMSO-d}_6, \text{MHz}): 1.91 (4H; Ha), 3.92 (4H; Hb), 6.82-8.21 (24H; Hd, He, Hg, Hl, Hm, Hn, Ho, Hq, Hs, Ht, Hu, Hv), 8.92 (2H; Hi). 13CNMR (\delta, \text{ DMSO-d}_6, \text{MHz}): 24.13 (2C; Ca), 60.23 (2C; Cb), 103.54-171.27 (40C; Cc, Cd, Ce, Cf, Cg, Ch, Cj, Ck, Cl, Cm, Cn, Co, Cp, Cq, Cr, Cs, Ct, Cu, Cv, Cw), 178.17 (2C; Ci).

**Antibacterial activity**

The in vitro antibacterial activity of the metal ions, free ligands and their complexes were tested against the gram-positive bacteria; Bacillus anthracis (RTCC 1036), Staphylococcus epidermidis (PTCC 1114), Staphylococcus aureus (RTCC 1885), Enterococcus faecalis (RTCC 2121), and Gentamycin (GE) and Tetracycline (TE) as standard compounds.

| Bacterial     | Complex (1) | Complex (2) | Complex(3) | L1 | Zn(NO3)2 | Cd(NO3)2 | HgCl2 | TE | GM |
|---------------|-------------|-------------|------------|----|---------|---------|-------|----|----|
| B. anthracis  | 30          | 25          | 98         | -  | -       | -       | -     | 20 | 25 |
| S. epidermidis| 40          | 44          | 20         | -  | -       | -       | -     | 25 | 25 |
| S. aureus     | 40          | 40          | 86         | -  | -       | -       | -     | 25 | 30 |
| E. faecalis   | 20          | -           | 80         | -  | -       | -       | -     | 30 | 15 |
| B. subtilis   | 30          | 35          | 25         | -  | -       | -       | -     | 20 | 20 |
| P. aeruginosa | 20          | -           | 25         | -  | -       | -       | -     | -  | 15 |
| K. pneumonia  | 50          | 25          | 100        | -  | -       | -       | -     | 20 | 25 |
| E. aerogenes  | 30          | 25          | 74         | -  | -       | -       | -     | 20 | 15 |
| E. coli       | 30          | 25          | 73         | -  | -       | -       | -     | 18 | 10 |

Minimum inhibitory concentration or MIC (mg/ml)

| B. anthracis  | 6.25        | 12.51       | 82.51      | -  | -       | -       | -     | -  | - |
| S. epidermidis| 1.56        | 1.56        | 25         | -  | -       | -       | -     | -  | - |
| S. aureus     | 1.56        | 1.56        | 25         | -  | -       | -       | -     | -  | - |
| E. faecalis   | 25          | -           | 100        | -  | -       | -       | -     | -  | - |
| B. subtilis   | 6.25        | 3.12        | 84         | -  | -       | -       | -     | -  | - |
| P. aeruginosa | 25          | 12.5        | 16         | -  | -       | -       | -     | -  | - |
| K. pneumonia  | 0.75        | -           | 97         | -  | -       | -       | -     | -  | - |
| E. aerogenes  | 6.25        | 12.5        | 25         | -  | -       | -       | -     | -  | - |
| E. coli       | 6.25        | 12.5        | 84.5       | -  | -       | -       | -     | -  | - |
Bacillus subtilis (PTCC 1715) and gram-negative bacteria; Pseudomonas aeruginosa (RTCC 1547), Klebsiella pneumonia (RTCC 1247), Enterobacter aerogenes (PTCC 1221), Escherichia coli (RTCC1330) by paper disc diffusion and minimum inhibitory concentration (MIC) methods. Bacteria cultures were obtained from Tehran islamicazad university hospital, microbiology department. Microbial strains were cultured overnight at 310 °K in Nutrient Broth. During the survey, these stock cultures were stored in the dark at 277 °K. Tetracycline and Gentamycine were used as standard compounds to determine the sensitivity of one strain/isolate in each microbial species tested. Antibacterial activity in the disc diffusion assay was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice. For investigation of antimicrobial activity of as prepared metal ions, free ligands and their complexes. The inhibitory effect of complexes on the growth of microbes were studied. The results can be seen in Table 1 and Table 2.

The results showed that antibacterial activity of the complexes exceeded the Tetracycline and Gentamycine used as standard compounds.

**DISCUSSION**

**Structural analysis**

A potentially macrocyclic Schiff base ligand \( \text{L}_1 \) was prepared by condensation reaction of 1,4-di-(4-fluoro-2-aminophenoxy)butane (DFAB) and salicylaldehyde in ethanol. A potentially macrocyclic Schiff base ligand \( \text{L}_2 \) was prepared by condensation reaction of 1,4-di-(4-fluoro-2-aminophenoxy)butane (DFAB) and anthracene-9-carbaldehyde in ethanol. DFAB was also synthesized by nucleophilic substitution reaction \( \text{SN}_2 \) of 4-fluoro-2-nitrophenol (DFNB) and 1,4-dibromo butane. The complexation of \( \text{L}_1 \) and \( \text{L}_2 \) were carried out toward \( \text{M}^{2+} \) (M = Zn, Cd and Hg) using one-pot template reactions. The resulted complexes were investigated by IR, \(^1\)HNMR, \(^{13}\)CNMR spectroscopy and mass spectrometry.

IR, NMR and mass data gives useful information on the structure of DFAB, DFNB, Schiff base ligands \( \text{L}_1 \) and \( \text{L}_2 \) and its metal complexes. The spectrum of the free ligands were compared with the spectrum of the metal complexes. The structurally significant IR, NMR and mass spectral data of free ligands and its metal complexes have been reported in the experimental section.

**Table 2.** Antibacterial activity of \( \text{M}^{2+} \) complexes, \( \text{N}_2\text{O}_2 \) (\( \text{L}_2 \)), metal ions, Gentamycine (GE) and Tetracycline (TE) as standard compounds

| Bacterial          | Complex (4) | Complex (5) | Complex (6) | \( \text{L}_2 \) | \( \text{Zn(NO}_3\text{)}_2 \) | \( \text{Cd(NO}_3\text{)}_2 \) | \( \text{HgCl}_2 \) | TE | GM |
|--------------------|-------------|-------------|-------------|-----------------|-----------------|-----------------|-----------------|----|----|
| B. anthracis       | 50          | 47          | 70          | -               | -               | -               | -               | 20 | 25 |
| S. epidermidis     | 66          | 40          | 80          | -               | -               | -               | -               | 25 | 25 |
| S. aureus          | 12          | 90          | 40          | -               | -               | -               | -               | 25 | 30 |
| E. faecalis        | 45          | 93          | 42          | -               | -               | -               | -               | 30 | 15 |
| B. subtilis        | 100         | 97          | 45          | -               | -               | -               | -               | 20 | 20 |
| P. aeruginosa      | -           | 75          | 68          | -               | -               | -               | -               | 15 |    |
| K. pneumonia       | 75          | 100         | 68          | -               | -               | -               | -               | 20 | 25 |
| E. aerogenes       | 46          | 72          | 97          | -               | -               | -               | -               | 20 | 15 |
| E. coli            | 73          | 89          | 92          | -               | -               | -               | -               | 18 | 10 |

Minimum inhibitory concentration or MIC (mg/ml)

| B.anthracis       | 43          | 57          | 76          | -               | -               | -               | -               | -  |
| S.epidermidis     | 100         | 14          | 46          | -               | -               | -               | -               | -  |
| S.aureus          | 100         | 90          | 47          | -               | -               | -               | -               | -  |
| E. faecalis       | 75          | 46          | 73          | -               | -               | -               | -               | -  |
| B. subtilis       | 42          | 85          | 95          | -               | -               | -               | -               | -  |
| Paeruginosa       | 98          | 85          | 91          | -               | -               | -               | -               | -  |
| K.pneumonia       | 65          | 37          | 83          | -               | -               | -               | -               | -  |
| E.aerogenes       | 17          | 74          | 56          | -               | -               | -               | -               | -  |
| E. coli           | 75          | 19          | 27          | -               | -               | -               | -               | -  |
The vibration bands that appeared in the IR spectrum of DFNB at 1342 cm\(^{-1}\) and 1518 cm\(^{-1}\) are assigned to symmetric and asymmetric stretching vibrations of the NO\(_2\) groups in the molecule, respectively. The disappearance of vibration band at 3500 cm\(^{-1}\) related to stretching vibration of phenolic OH groups confirmed the formation of DFNB. The band at 3505 cm\(^{-1}\) in the IR spectrum of DFAB is ascribed to the N-H stretching vibration. The vibration band at 1223 cm\(^{-1}\) indicates that etheric bond cleavage has not occurred through reduction of NO\(_2\) groups. The vibration band related to stretching vibrations of the NO\(_2\) groups is absent showing the reduction of NO\(_2\) groups has been completed. The strong absorption bands at approximately 1618 cm\(^{-1}\) and 1622 cm\(^{-1}\)in the IR spectrums of L\(_1\) and L\(_2\) are ascribed to the stretching vibration of iminic C=N bond, respectively. The \(^1\)H NMR spectrum of the ligand L\(_1\) showed signals at 1.93 (4H); 4.14 (4H), 6.94-7.59 (14H), 8.99 (2H), 13.7 (2H) ppm which are attributed to methylene \(\text{H}_a\); methylene \(\text{H}_b\), iminic and phenolic hydrogens, respectively. The \(^1\)H NMR spectrum of the ligand L\(_2\) showed signals at 1.73 (4H); 3.73 (4H), 6.72-7.43 (24H), 8.87 (2H), 13.7 (2H) ppm which are attributed to methylene \(\text{H}_a\); methylene \(\text{H}_b\), iminic and phenolic hydrogens, respectively. The \(^1\)H NMR spectrum of L\(_1\) showed signals at 1.73 (4H); 3.73 (4H), 6.72-7.43 (24H), 8.87 (2H), 13.7 (2H) ppm which are attributed to methylene \(\text{H}_a\); methylene \(\text{H}_b\), iminic and phenolic hydrogens, respectively. The \(^13\)C NMR spectrum of L\(_1\) showed signals at 25.88 (2C); 68.85 (2C), 106.42-155.76 (24C) and 167.15 (2C) ppm which are attributed to methylene \(\text{C}_a\); methylene \(\text{C}_b\), aromatic and iminic carbons, respectively. The \(^13\)C NMR spectrum of L\(_2\) showed signals at 23.75 (2C); 59.94 (2C), 103.13-172.56 (40C) and 178.17 (2C) ppm which are attributed to methylene \(\text{C}_a\); methylene \(\text{C}_b\), aromatic and iminic carbons, respectively.

The IR spectra of Zn(II), Cd(II) and Hg(II) complexes exhibited vibration bands at 1620 cm\(^{-1}\), 1620 cm\(^{-1}\) and 1621 cm\(^{-1}\) respectively which can be assigned to the C=N stretching vibration of L\(_1\). The frequency of this vibration for Zn(II) complex is the same as Hg(II) complex. Also, vibration bands at 1214 cm\(^{-1}\), 1217 cm\(^{-1}\) and 1214 cm\(^{-1}\) confirmed the maintenance of C-O bonds of L\(_1\) for Zn(II), Cd(II) and Hg(II) complexes, respectively. The frequency of this vibration for Zn(II) complex is the same as Hg(II) complex. These results in addition to mass results clearly indicate the formation and coordination of macrocyclic ligand (L\(_1\)) to metal ions through one-pot template reaction.

Because single crystals of these complexes could not be isolated from any solvents, no definitive crystal structures could be assigned. However, on the basis of characterization results, the molecular ratio of the L\(_1\) and L\(_2\) to metal ions could be confirmed as 1:1.

**Antibacterial activity test**

The inhibition effect on bacteria growth was determined by disc diffusion method [17-20]. Each compound was dissolved in methanol as a solvent (1g/10 ml) and 50 ìl of each solution applied on the paper disc (the disc diameter was 6 mm). The impregnated discs with different solutions were left for complete evaporation of the solvent. Then disc papers were placed on the inoculated plates with the bacteria of interest. After incubation in the standard upside down position in 40 °C for 24 h, zones of growth inhibition around each of the discs were measured to the nearest millimetre. A blank, containing only methanol, showed no inhibition in a preliminary test. The macrodilutionbroth susceptibility assay was used for the evaluation of minimal inhibitory concentration (MIC). It is apparent the metal complexes have greater antibacterial activity but related free ligands and metal ions have not antibacterial activity. The results showed that in some cases the antibacterial activity of this complexes exceeded of other Schiff base complexes[21-24]. Tweedy’s chelation theory is a good clarification for this phenomenon.

**CONCLUSION**

A new macrocyclic Schiff base ligands derived from condensation of 1,4-di-(4-fluoro-2-
aminophenoxy)butane with salicylaldehyde and 1,4-di-(4-fluoro-2-aminophenoxy)butane with anthracene-9-carbaldehyde have been synthesized and its complexation capacity towards Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ has been studied by adopting one-pot template method. The structures of the complexes were confirmed by $^1$HNMR, $^{13}$CNMR, IR spectroscopy and mass spectrometry. We are also especially interested in the antibacterial activity of these new complexes, free ligands ($L_1$ and $L_2$) and metal ions. The results showed that in the metal complexes have greater antibacterial activity but related free ligands and metal ions have not antibacterial activity.

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