Synthesis, Characterization, and Antimicrobial Activity Studies of New Schiff Base Tetridentate Ligands and Their Manganese (II) Complexes

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Authors’ contributions

This work was carried out in collaboration by all authors. Author MHAD designed, managed the study, wrote the introduction and experimental sections in the manuscript. Author EABS wrote the chemistry in the results and discussion section in the manuscript. Author DSAN managed the chemistry and microbial experimental parts of the study in the manuscript. Authors WMAH and KSS managed and wrote the microbial experimental part of the study in the manuscript. Authors EABS and MHAD wrote the first draft. All authors read and approved the final manuscript.

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ABSTRACT

Two Schiff base tetridentate ligands 4 and 5 were synthesized by condensation reactions of 4-hydroxy-3-methoxy-5-((2-nitrophenyl)diazenyl) benzaldehyde 1 with 1,2-diaminoethane 2 and 1,2-diaminobenzene 3, respectively. Treatment of synthesized ligands with the manganese (II) chloride MnCl₂ 6 gave two complexes 7 and 8 of general formula ML. The synthesized ligands and their complexes were characterized by FTIR, UV-Vis, Mass spectroscopy, and elemental analysis. The biological activity of the synthesized compounds were tested. It was found that ligand 5 and the complexes 7 and 8 possess antibacterial activity against the gram –ve Proteus mirabilis.

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1. INTRODUCTION

In September 1862, Hugo Joseph Schiff was discovered the new double bond between carbon atom and nitrogen atom as C=N linkage imine functional group from the condensation reaction between aldehydes or ketones with primary amines or amino acids in the presence of an acid catalyst [1,2]. Organic compounds containing the substituted imine are called azomethines, aldmines, anils, benzanils, ketimines or Schiff’s bases [1,3,4]. The condensation of two equivalent aldehydes or ketones with a diamine produced the bis-Schiff base compounds, which have symmetric or unsymmetric/asymmetric structures [5,6].

Both unsymmetrical and symmetrical bis-Schiff bases can be coordinated as ligands with the central transition metal ion to form complexes. The complexes of unsymmetrical bis-Schiff base ligands are used as irregular binding model with peptides [7], while the intercalation of some symmetrical bis-Schiff bases with DNA and UV-Vis spectroscopy has been studied [8,9]. However, some symmetrical bis-Schiff base structures are studied and characterized by 1D and 2D NMR spectroscopy and X-Ray Crystallography analysis [10-12].

Recently, we have been reported the synthesize and characterize of the diazo dye 1 [13]. The aim of this paper is to synthesize and characterize the new bis-Schiff base tetradentate ligands 4 and 5 derived from condensation reactions of compound 1 with 1,2-diaminobenzene 2 and 1,2-diaminobenzene 3, respectively. The Mn(II) complexes of these ligands 7 and 8 have also been studied. All the synthesized compounds were characterized by melting points, FTIR, UV–Vis, Mass spectroscopy, and elemental analysis. Additionally, the microbial inhibitory of these derivatives 4, 5, 7 and 8 against some pathogenic bacteria such as Staphylococcus aureus as gram +ve, Escherichia coli and Proteus mirabilis as gram –ve were evaluated.

2. EXPERIMENTAL DETAILS

2.1 General

The melting points were determined with Electrothermal Melting Point Apparatus. FTIR spectra were recorded with JASCO FTIR 4600, Single beam, Path Laser, by KBr disk technique in the frequency range of 4000–400 cm⁻¹. UV–Vis spectra were recorded with JASCO V–750 Spectrophotometer in ethanol at the wavelength range of 900–200 nm. All experiments were done at PCB Research Laboratory, Faculty of Science, Hadhramout University, Mukalla, Hadhramout, Yemen. Mass spectra were carried out on Direct Inlet part to mass analyzer in Thermo Scientific GCMS model ISQ, and Elemental analyses were carried out using FLASH 2000 CHNS/O analyzer, Thermo Scientific at the Regional Center for Mycology and Biotechnology RCMB, Al-Azhar University, Nasr City, Cairo, Egypt. All microbial experiments were done at Alsalam Medical Center, Mukalla, Hadhramout, Yemen.

2.2 Synthesis

2.2.1 Preparation of 4-Hydroxy-3-methoxy-5-((2-nitrophenyl)diazenyl)benzaldehyde 1

Compound 4-hydroxy-3-methoxy-5-((2-nitrophenyl)diazenyl)benzaldehyde 1 was prepared as described previously [13].

2.2.2 Synthesis of bis-Schiff bases 4 and 5

Both bis-Schiff bases 4 and 5 were synthesized from 1 by the method described earlier, with some modification [14-16]. In 100 mL dried round bottom flask equipped with a reflux condenser, a 6.03 g of compound 1 (0.02 mol) was dissolved in 15 mL of absolute ethanol containing a drop of glacial acetic acid and refluxed for about 10 min on an oil bath. The solution was mixed dropwise for 10 min with 1,2-diaminobenzene 2 (0.60 g, 0.01 mol) in 15 mL hot absolute ethanol or 1,2-diaminobenzene 3 (1.08 g, 0.01 mol) in 15 mL hot absolute ethanol. The resulting mixture was stirred for 5 hr, and then amount of crashed ice was added with stirring for 30 min. The separated solid was filtered by vacuum filtration, washed with cooled water and then with distilled water, and finally recrystallized from ethanol to yield colored crystals of bis-Schiff base 4 or 5.

2.2.3 Synthesis of complexes 7 and 8

The complexes 7 and 8 were synthesized from 4 and 5, respectively, as the method reported previously, with some modification [17]. In 100 mL dried round bottom flask equipped with a reflux condenser, a 6.27 g of 4 (0.01 mol) or 6.75
g of 5 (0.01 mol) was added to a solution of the manganese(II) chloride 6 MnCl₂.4H₂O (1.98 g, 0.01 mol) in 20 mL of absolute ethanol. The solution was refluxed for 3 hr on an oil bath. The obtained mixture was cooled with stirring by adding a crashed ice for 30 min. The resulting solid was filtered by vacuum filtration, washed with cooled water and then with distilled water, and recrystallized from ethanol to yield colored crystals of the complex 7 or 8.

2.3 Methodology of Antimicrobial Activity

2.3.1 Bacterial strains

The bacteria strains that used in this study are most commonly involved in causing gastroenteritis, wound and burn exudates, urinary tract infection and ear secretions. Three bacteria strains *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis* were isolated from different patients attending Alsalam Medical Center laboratory in Mukalla, Hadhramout – Yemen, and used throughout this study. The isolated bacteria were subcultured on Nutrient agar Difco and incubated aerobically at 37°C for 24 hr. Organisms were maintained in the laboratory on nutrient agar slopes at 4°C [18].

2.3.2 Determination the minimal inhibitory concentration of 4, 5, 7 and 8

The minimum inhibitory concentration MIC of 4, 5, 7 and 8 was determined by using a different dilution for that compounds, which diluted with sterile ethanol 75%. It was weighed amount of 4, 5, 7 and 8, separately, and dissolved in ethanol 75%. The following concentration was prepared 1:2, 1:4, 1:8, 1:16 and 1:32 from the stock of each solutions [18,19].

2.3.3 Antimicrobial activity

The antibiotic susceptibility tests were carried out by the Kirby–Bauer disk diffusion technique according to Clinical Laboratory Standard Institute guidelines [20,21]. About 20 mL of the sterilized medium was poured into each sterile 9 cm diameter of Petri-dish and allowed to solidify. Bacterial suspension for each strain tested was adjusted at 3 x 10⁸ CFU/mL by McFarland scale which prepared by mixing 0.1 mL of 1.0% dehydrate barium chloride with 9.9 mL of 1.0% sulfuric acid H₂SO₄ as described by [22]. About 0.1 mL of the prepared bacterial suspension was spread evenly onto the agar surface using a cotton swab and kept in a refrigerator for 2 hr. The compound diffuses into the agar resulting in a concentration gradient that is inversely proportional to the distance from the disc. The test medium was Mueller–Hinton agar [23]. Thereafter, all prepared plates were incubated at 37°C for 24 hr [24]. After that, the size of the inhibition zone around the disc is a measure in mm of the degree of inhibition [20,21]. Seven of different antibiotics disks were used as a reference for evaluation of antibacterial: Ciprofloxacin, Amikacin, Clarithromycin, Cefaclor, Cefepime, Azithromycin and Co-trimoxazole [25]. The antibiotics were compared to the effect of synthesized compounds to antimicrobial activity of these pathogenic bacteria.

3. RESULTS AND DISCUSSION

3.1 Ligands and Complexes

4-Hydroxy-3-methoxy-5-((2-nitrophenyl) diazenyl) benzaldehyde 1 was synthesized and characterized previously [13]. Ligand 4 was obtained as a reddish orange solid by condensation reaction of compound 1 with 1,2-diaminoethane 2. It has a melting point about 124°C. The interaction of 4 with a manganese dichloride MnCl₂ 6 gave a complex 7 which is a reddish brown solid, Scheme 1. It has a melting point > 400°C. It is soluble in ethanol, stable toward air and moisture. Table 1 described the physical properties and elemental analyses of synthesized compounds.

The FTIR spectrum of 4 exhibited absorption frequency at 1631 cm⁻¹ which was a characteristic of the stretching vibration of imine functional group C=N. Two bands at 1170 cm⁻¹ and 1283 cm⁻¹ indicated the stretching vibrations of the methoxy functional group –OCH₃. The FTIR spectrum of 4 also showed a broad band at 3349 cm⁻¹ due to the ν –OH of phenol, two absorption bands around 3173 and 3109 cm⁻¹ due to the ν C-H unsaturated, and two bands at 1429 cm⁻¹ and 1469 cm⁻¹ for the symmetric and asymmetric stretching vibrations of the azo functional group –N=N– [13], respectively. Additional peaks around 1345 cm⁻¹ and 1484 cm⁻¹ were observed that were assigned to the symmetric and asymmetric stretching vibrations of the nitro functional group –NO₂, respectively. On the other hand, the C-H aldehydic and C=O bands disappeared in the FTIR spectrum of 4 as compared to the FTIR spectrum of compound 1.
Scheme 1. Synthetic route towards ligand 4 and complex 7

Table 1. Physical properties and elemental analysis of ligands and their complexes

| Cpd. | Mol. Formula | Mol. Wt. | m.p. °C | Yield % | Elemental Analysis |
|------|--------------|----------|---------|---------|-------------------|
|      |              |          |         |         | Calc. (Found) %   |
|      |              |          |         |         | C    | H    | N    | C    | H    | N    |
| 4    | C_{30}H_{26}N_{8}O_{8} | 626.594 | 124     | 86      | 57.51 (57.78) | 4.18 (4.31) | 17.88 (17.62) |
| 5    | C_{34}H_{26}N_{8}O_{8} | 674.638 | 199     | 70      | 60.53 (60.41) | 3.89 (4.06) | 16.61 (16.89) |
| 7    | C_{30}H_{26}N_{8}O_{8}MnCl_{2} | 752.438 | > 400   | 60      | 47.89 (48.07) | 3.48 (3.71) | 14.89 (14.97) |
| 8    | C_{34}H_{26}N_{8}O_{8}MnCl_{2} | 800.482 | 220     | 85      | 51.02 (51.26) | 3.27 (3.58) | 14.00 (13.86) |

A comparison of the FTIR spectra of complex 7 and 4 displayed a significant changes in the intensities and positions of some bands. The absorption band of the C=N group in a complex 7 was shifted to a lower frequency 1598 cm\(^{-1}\), indicating the involvement of this group in coordination with a manganese ion [17,26]. This was supported by the appearance of a new band
around 449-480 cm\(^{-1}\) which was a characteristic absorption frequency of manganese nitrogen bond Mn-N [17,26]. Also, the band of methoxy functional group –OCH\(_3\) changed in shape, and also there was a new band around 504-518 cm\(^{-1}\) that was assigned to the stretching vibration of manganese oxygen bond Mn-O [17,26], indicating that coordination also take place through the oxygen of methoxy functional group. However, the absorption bands of both azo – N=N– and phenolic hydroxyl –OH functional groups were not significantly affected, suggesting these groups were not participating in coordination, Fig. 1.

The UV-Vis spectrum of 4 in ethanol exhibited three bands, the first and second bands were observed at 274.4 nm and 321.6-335.2 nm which were indicated the \(\pi\rightarrow\pi^*\) transitions of the aromatic rings and imine functional group, respectively [14-16,27]. The third band was located at 445.6-444.8 nm which was assigned to the intramolecular charge-transfer and resulted from \(\pi\rightarrow\pi^*\) transition between benzene rings through the azo functional group –N=N– as a bridged [13]. While the UV-Vis spectrum of an ethanolic solution of the complex 7 referred to the bathochromic shift of the \(\lambda_{\text{max}}\) of the imine functional group C=N as compared to its value in UV-Vis of 4. It is observed at 342-344 nm suggesting the participation in the bond formation with manganese ion in complex 7 [26,28,29]. The spectrum also showed the absorption at 273.2 nm due to the \(\pi\rightarrow\pi^*\) transitions of the aromatic rings, and the absorption band of the azo functional group remained unaffected, suggesting the nonparticipation of this functional group in the coordination with manganese ion.

From both FTIR and UV-Vis studies, it can be suggested that 4 binds to the manganese ion through the imine functional group and oxygen of the methoxy functional group. Elemental analysis CHN of 4 was in a good agreement with the calculated value, Table 1. Also, the mass spectrum of 4 exhibited a molecular ion peak at m/z 626.86, which was equivalent to its molecular weight, Fig. 2. This confirmed the structure of 4. Elemental analysis of complex 7 indicated that the metal:ligand ratio is 1:1 in the complex 7, Table 1. Moreover, the mass spectrum of complex 7 showed a molecular ion peak at m/z 752.34 which was the same as the molecular weight of the complex 7, Fig. 2. This confirmed the proposed ratio of M:L is 1:1. So, according to the results obtained from FTIR, UV-Vis, Mass spectra and CHN analysis, it can be concluded that the 4 interacts with manganese ion and a tetradentate structure can be suggested for the complex 7, as shown in Fig. 3.

![Fig. 1. FTIR Spectra of synthesized ligand 4 and complex 7](image-url)
Ligand 5 was obtained as a reddish brown solid by condensation reaction between compound 1 and 1,2-diaminobenzene 3. It has a melting point about 199°C. Complex 8 has been synthesized by the interaction of 5 and manganese dichloride MnCl₂, Scheme 2. It is a reddish brown solid, soluble in ethanol. It is thermally stable and has a melting point about 220°C. Physical properties and elemental analysis were tabulated in Table 1.

The FTIR spectrum of 5 showed a band at 1628 cm⁻¹ due to the ν C=N stretching vibration frequency. Two bands at 1139 and 1283 cm⁻¹ were assigned to ν =C-OCH₃ of the methoxy functional group. However, the FTIR spectrum of 5 also showed a broad band centered at 3349 cm⁻¹ indicated ν –OH of phenol, two bands around 3173 and 3109 cm⁻¹ due to the ν C-H unsaturated, and other two bands appeared at 1414 cm⁻¹ and 1573 cm⁻¹ that assigned to the symmetric and asymmetric stretching vibrations of the azo functional group –N=N– of the 5, respectively [13]. Both bands observed around 1345 cm⁻¹ and 1484 cm⁻¹ were attributed to the symmetric and asymmetric stretching vibration
frequencies of the nitro functional group –NO$_2$, respectively. On the other hand, there were no the characteristic absorption frequencies of the aldehydric functional group in the FTIR spectrum of 5 as compared to the FTIR spectrum of compound 1.

A comparative study of the FTIR spectra of 5 and complex 8 exhibited that the band of $\nu$ C=N in the complex 8 observed to be lowered at 1598 cm$^{-1}$ due to the participation of imine functional group in coordination with the manganese ion [17,26]. This confirmed by the appearing of new band around 503-517 cm$^{-1}$ which was attributed to the manganese nitrogen bond Mn-N [17,26]. There was a change in the shape of methoxy band, in addition of a new band appeared around 540 cm$^{-1}$ which was a characteristic band of manganese oxygen bond Mn-O [17,26], indicating the participation of methoxy functional group in the coordination with the manganese ion. The absorption bands of both the azo functional group $\nu$ –N=N– and phenolic functional group $\nu$ –OH were unaffected in the complex 8 indicating that these groups remain uncoordinated, as shown in Fig. 4.

**Scheme 2. Synthetic route towards ligand 5 and complex 8**
The UV-Vis spectrum of 5 displayed three bands in an ethanolic solution. The first band observed at 273.2 nm due to the $\pi \rightarrow \pi^*$ transition of benzene rings. The second band located at 321.6-337.2 nm for $\pi \rightarrow \pi^*$ transition of imine functional group C=N [14-16,27], while the third band observed at 480.4-506 nm that was attributed to azo functional group –N=N– [13]. Additionally, the UV-Vis spectrum of the complex 8 in ethanol solvent showed a band at 273.2 nm for $\pi \rightarrow \pi^*$ transition of benzene rings. The second band is observed at 340.4-345-6 nm due to the $\pi \rightarrow \pi^*$ transition of imine functional group C=N. This band showed a red shift due to the coordination with a manganese ion in the complex 8 [26,28-29]. However, the spectrum exhibited that the absorption of the azo functional group remained unchanged, indicated this group was not involved in coordination in complex 8.

From FTIR and UV-Vis observation, it can be suggested that the 5 coordinates with the manganese ion through the nitrogen of imine functional group and oxygen of methoxy functional group. Elemental analysis CHN of 5 revealed a good agreement with the calculated value, Table 1. Moreover, the mass spectrum of 5 exhibited a molecular ion peak at m/z 674.45, which was equivalent to its molecular weight, Fig. 5. These analyses confirmed the structure of ligand 5.

Elemental analysis of complex 8 indicated that general composition a M:L ratio of 1:1, Table 1. This supported by the mass spectrum of complex 8 in that a molecular ion peak located at m/z 800.15 which was the same as the molecular weight of the complex 8, Fig. 5. Both elemental analysis and mass spectrum were confirmed the proposed composition a M:L ratio of 1:1. According to the FTIR, UV-Vis, Mass spectra, and CHN analysis studies, the tetradentate structure has been suggested of the complex 8, as shown in Fig. 6.
3.2 Microbiology Activity

The ligands 4, 5 and their Schiff base complexes 7 and 8 were screened against *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. The results are tabulated in Table 2. It was found that ligand 5, complex 7 and complex 8 have antibacterial activities against the gram –ve *Proteus mirabilis*, and they have not antibiological activity against both *Staphylococcus aureus* and *Escherichia coli* at room temperature. The concentration 0.125 mM of complex 8 showed inactive of all pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*. By the way, the ligand 5 has a high anti-bacterial effect against *Proteus mirabilis* at the concentrations 0.125 and 0.25 mM with [++=IZ 13-19 mm] as compared to the complexes 7 and 8. However, ethanol 75% was tested of all pathogenic bacteria used in this study, and the obtained results were without inhibition zone.
Table 2. Inhibitory growth activity of both ligands 4, 5 and their complexes 7 and 8 against pathogenic bacteria (diameter of inhibition zone = IZ)

| Compound | Conc. mM | Mean diameter of inhibition zone IZ (mm) |
|----------|---------|----------------------------------------|
|          |         | gram +ve | E. coli | gram –ve | P. mirabilis |
| 4        | 2.0     | –        | –       | –        | –           |
|          | 1.0     | –        | –       | –        | –           |
|          | 0.5     | –        | –       | –        | –           |
|          | 0.25    | –        | –       | –        | –           |
|          | 0.125   | –        | –       | –        | –           |
|          | 0.0625  | –        | –       | –        | –           |
| 5        | 2.0     | –        | –       | +        | –           |
|          | 1.0     | –        | –       | +        | –           |
|          | 0.5     | –        | –       | +        | –           |
|          | 0.25    | –        | –       | ++       | –           |
|          | 0.125   | –        | –       | ++       | –           |
|          | 0.0625  | –        | –       | +        | –           |
| 7        | 2.0     | –        | –       | +        | –           |
|          | 1.0     | –        | –       | +        | –           |
|          | 0.5     | –        | –       | +        | –           |
|          | 0.25    | –        | –       | +        | –           |
|          | 0.125   | –        | –       | +        | –           |
|          | 0.0625  | –        | –       | +        | –           |
| 8        | 2.0     | –        | –       | +        | –           |
|          | 1.0     | –        | –       | +        | –           |
|          | 0.5     | –        | –       | +        | –           |
|          | 0.25    | –        | –       | +        | –           |
|          | 0.125   | –        | –       | –        | –           |
|          | 0.0625  | –        | –       | +        | –           |
| ethanol 75% | –    | –        | –       | –        | –           |
| Cf      | 5*      | +++      | –       | –        | –           |
| Am      | 30*     | +++      | –       | –        | –           |
| Ct      | 15*     | +++      | –       | –        | –           |
| Ca      | 30*     | –        | ++      | +++      | –           |
| Ce      | 30*     | –        | ++      | ++       | –           |
| Az      | 15*     | –        | ++      | ++       | –           |
| Cm      | 25*     | –        | +++     | +++      | –           |

* Key to symbols: *: ug, Cf: Ciprofloxacin, Am: Amikacin, Ct: Clarithromycin, Ca: Cefaclor, Ce: Cefepime, Az: Azithromycin and Cm: Co-trimoxazole as antibacterial, Disc diameter = 6 mm. Highly active: +++ [IZ > 19 mm], Moderately active: ++ [IZ 13-19 mm], Slightly active: + [IZ 7-13 mm], Inactive: – [IZ < 7 mm]

4. CONCLUSION

In this study, two Schiff base ligands 4, 5 and their manganese complexes 7 and 8 have been synthesized. According to the UV-Vis and FTIR spectra, the ligands 4 and 5 coordinate to the manganese ion (II) through the imine nitrogen and the methoxy oxygen. The physico-chemical methods, FTIR, UV-Vis, Mass spectroscopy, and elemental analysis, suggest that the ligands 4 and 5 behave as the tetradentate and the metal:ligand ratio is 1:1. The microbiological activity study of these compounds shows that 5 and the complexes 7 and 8 have antibacterial activity against the gram –ve P. mirabilis, and 5 has a high antibacterial effect at the concentrations 0.125 and 0.25 mM with [++=IZ 13-19 mm] as compared to the complexes 7 and 8.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Layer RW. The chemistry of imines. Chem Rev. 1963;63(5):489-510. DOI: 10.1021/cr60225a003

2. Cozzi PG. Metal-salen Schiff base complexes in catalysis: Practical aspects. Chem Soc Rev. 2004;33:410-421. DOI: 10.1039/B307853C

3. El–Bayoumi MA, El–Aasser M, Abdel–Halim F. Electronic spectra and structures of Schiff's bases I. Benzanils. J Am Chem Soc. 1971;93(3): 586-590. DOI: 10.1021/ja00732a004

4. El–Bayoumi MA, El–Aasser M, Abdel–Halim F. Electronic spectra and structures of Schiff's bases II. N-Benzylins. J Am Chem Soc. 1971;93(3):590-592. DOI: 10.1021/ja00732a005

5. Lopez J, Liang S, Bu XR. Unsymmetric chiral salen Schiff bases: A new chiral ligand pool from bis-Schiff bases containing two different salicylaldehyde units. Tetrahedron Lett. 1998;39(24):4199-4202. DOI: 10.1016/S0040-4039(98)00784-9

6. Lopez J, Mintz EA, Hsu FL, Bu XR. Novel unsymmetric chiral Schiff bases possessing two different donor moieties: Unique tetradentate ligands from combination of salicylaldehyde and acetylacetone units. Tetrahedron Asymm. 1998;9(21):3741-3744. DOI: 10.1016/S0957-4166(98)00401-7

7. Atkins R, Brewer G, Kokot E, Mockler GM, Sinn E. Copper (II) and nickel (II) complexes of unsymmetrical tetradentate Schiff base ligands. Inorg Chem. 1985;24(2):127-134. DOI: 10.1021/ic00196a003

8. Parra MR, Garcia T, Lorenzo E, Pariente F. Comprehensive study of interactions between DNA and new electroactive Schiff base ligands application to the detection of singly mismatched Helicobacter pylori sequences. Biosens Bioelectron. 2007; 22(11):2675-2681. DOI: 10.1016/j.bios.2006.11.002

9. Helal MH, Al-Mudaris ZA, Al–Douh MH, Osman H, Wahab HA, Alnajjar BO, Abdallah HH, Majid AMSA. Diaminobenzene Schiff base, a novel class of DNA minor groove binder. Intern J Oncol. 2012;41(2):504-510. DOI: 10.3892/ijo.2012.1491

10. Al–Douh MH, Hamid SA, Osman H, Ng SL, Fun HK. 6,6'-Dimethoxy-2,2'-(6-phenylene bis(nitriломethylidyne)) diphenol. Acta Crystallogr E. 2007;63(8):o3570-o3571. DOI: 10.1107/S1600536807034812

11. Al–Douh MH, Osman H, Hamid SA, Kia R, Fun HK. 6,6'-Dimethoxy-2,2'-(6-phenylene bis(nitriломethylidyne)) diphenol chloroform disolvate. Acta Crystallogr E. 2009;65(4): o680-o681. DOI: 10.1107/S1600536809007302

12. Al–Douh MH, Osman H, Hamid SA. Synthesis, spectroscopic and crystal structures studies of N,N'-bis(2-hydroxy-3-methoxybenzylidene) diaminebenzene derivatives. Univ Aden J Nat Appl Sci. 2010;14(2):325-347. [ISSN: 1606-8947]

13. Al–Douh MH, Selim EAB, Al–Haik WM, Mahram SMB, Al–Bakri AK, Al–Nohey DS, Sonbol KS. Synthesis and antimicrobial activity of some compounds derived from diazo dyes. Intern J Chem Stud. 2019; 3(4):6-12. [ISSN: 2581-348X]

14. Al–Douh MH, Al–Fatlawy AA, Abid OH. Synthesis and characterization of some 2-(N-benzoyl-N-pyrid-4-yl aminobenzyl)-aminobarbitalic acids via Schiff's bases. Hadh Stud Res. 2003;4(2):37-49. SBN–National Library Aden 18–2004

15. Al–Douh MH, Al–Fatlawy AA, Abid OH. Synthesis and characterization of 2-(N-benzoyl-N-pyrid-3-yl aminobenzyl)-aminobarbitalic acids via N-benzylidene pyridine-3-amines. Fac Sci Bull. 2003; 16(3):83-94. [ISSN: 1684–100X]

16. Al–Douh MH, Al–Fatlawy AA, Abid OH. Synthesis and characterization of some 2-(N-benzoyl-N-pyrid-2-yl aminobenzyl)-aminobarbitalic acids via N-benzylidene pyridine-2-amines. Univ Aden J Nat Appl Sci. 2004;8(1):181-194. [ISSN: 1606-8947]

17. Temel H, Cakir U, Otludil B, Ugras Hl. Synthesis, spectral and biological studies of Mn(II), Ni(II), Cu(II), and Zn(II)
complexes with a tetradentate Schiff base ligand. Complexation studies and the determination of stability constants (Ke). Synth React Inorg Metal-Org Chem. 2001; 31(8):1323-1337.

DOI: 10.1081/sim-100107201

18. Othman AS. Antibacterial activity of bee and Yemeni Sidr honey against some pathogenic bacterial species. Intern J Curr Microbiol Appl Sci. 2014;3(10):1015-1025. [ISSN: 2319-7706]

19. Andrews JM. Determination of minimum inhibitory concentrations. J Anitmicrob Chemother. 2001;48(S1):5-16. DOI: 10.1093/jac/48.suppl_1.5

20. Piddock LJ. Techniques used for the determination of antimicrobial resistance and sensitivity in bacteria. J Appl Bacteriol. 1990;68(4):307-318. DOI: 10.1111/j.1365-2672.1990.tb02880.x

21. CLSI M02-A10. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 10th Ed., Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA; 2010.

22. McFarland J. The Nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J Am Med Assoc. 1907;XLIX(14):1176-1178. DOI:10.1001/jama.1907.25320140022001f

23. Rajput A, Prajapati B, Chauhan B, Shah A, Trivedi T, Kadam M. Prevalence of metallo-betalactamases (MBL) producing Pseudomonas aeruginosa in a tertiary care hospital. Indian J Basic & Appl Med Res. 2012;1(4):304-308.

24. Joshi VK, Sharma S, Rana NS. Production, purification, stability and efficacy of bacteriocin from isolates of natural lactic acid fermented vegetables. Food Technol Biotechnol. 2006;44(3):435-439. [ISSN: 1330-9862 (FTB-1681)]

25. Lundstrom J. Simple Isoquinoline Alkaloids. In The Alkaloids: Chemistry and Pharmacology. Brossi, A., Ed., Academic Press, Inc., New York, USA, 1983;21:255-327. DOI: 10.1016/S0099-9598(08)60052-8

26. Kumar Y, Chandra S, Singh RP, Singh AK. Synthesis and characterization of Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) complexes of salicylaldehyde semicarbazone. Synth React Inorg Metal-Org Chem. 2001; 31(8):1323-1337. DOI: 10.1080/00945718408066654

27. Golchoubian H, Waltz WL, Quail JW. Synthesis, structure, and reactivity of macro cyclic heterobimetallic complexes of (Co, Cu), (Co, Ni), (Co, Zn), (Zn, Cu), (Zn, Ni) combinations. Can J Chem. 1999; 77(1):37-56. DOI: 10.1186/s11671-015-1179-6