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PREPARATION AND STUDY OF TWO NEW SCHIFF BASES AND THE STUDY OF BIOLOGICAL EFFECTIVENESS ON INFECTED MICE BY THE PARASITE ENTAMOEBAHISTOLYTICA

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ABSTRACT

In the present work two new Schiff bases are synthesized, the first from reaction of 2-hydroxy-1-naphthaldehyde with sulphamethoxazole, and the second from reaction of 4-Acetmidobenzaldehyde with 3,4-Diamino toluene. These Schiff bases were verified by some spectral data (^{1}H-NMR , IR, and mass spectra).

Stool samples were collected from people infected with maebiasis to infect laboratory mice. It has been determined LD_{50} for the two ligands - after dissolved dimethyl sulfoxide DMSO - and account for them safe therapeutic dose, reaching 0.14 g / kg and 0.24 g / kg, respectively. Reached the less impact to the second ligand rate about 2.9., the highest inhibiting effect of metronidazole was at a rate of 0, while in the seventh day of them , the first ligand was a compromise in effect, amounting to at a rate of 1.1.

KEYWARDS: Biological Effectiveness

INTRODUCTION

Schiff bases are compounds which contain imine or azomethine (-C=N-) group. It products condensation primary amines with carbonyl compounds, and attended for the first time by the chemist Hugo Schiff in 1864[1, 2].

Schiff bases have gained importance in medical fields and medicines as a result of a wide range of biological activities such as anti-inflammatory[3-5], analgesic[4-6], antimicrobial[7, 8], anticonvulsant[9], antitubercular[10], anticancer[11, 12], antioxidant[13], As for the parasites it is biological activities to schiff bases are antiamoebic[14], antigiardial[15], antitrypanosomal[16], anti-malarial [17], anti-*Echinococcusgranulosus*[18], anthielmentes[19], and so forth.Azomethinenitrogen atom in the group could possibly be involved in the formation of a hydrogen bond with the active centers of cellular components and interfere with the natural processes of the cell [20, 21].

Several studies [22-24] showed that the presence of a lone pair of electrons in an sp2 hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, schiff bases are generally excellent chelating agents, [24, 25] especially when a functional group like –OH or –SH is present close to the azomethine group so as to form a five or six membered ring with the metal ion. Versatility of schiff base ligands and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable.

Amebiasis, also known as amebic dysentery or intestinal amebiasis, is an acute or chronic protozoal infection caused by *Entamoebahistolytica*. This infection produces varying degrees of illness, from no symptoms at all or mild diarrhea to fulminant dysentery. Extraintestinalamebiasis can induce hepatic abscess and infections of the lungs, pleural cavity, pericardium, peritoneum and, rarely, the brain. Ninety percent of people with amebiasis do not have symptoms. The prognosis is generally good, although complications can increase mortality. Brain abscess, a rare complication, is usually fatal [26].

MATERIAL AND METHODS

All chemicals are purchased from BDH, and used without further purifications. FT-IR spectra are recorded in KBr, Shimadzu spectrophotometer in the range of 4000-200 cm^{-1} used KBr disc to the two ligands. Melting points are measured with an electro thermalsturat apparatus, model SMP30. ^{1}HNMR spectra are recorded on a Bruker DRX System AL500 (500 MHz) spectrometer in DMSO, chemical shift in ppm relative to internal TMS. The micro analysis (C, H, N) of ligands are carried out by using CHNS-O PerkinElmer model 2400-11-Mass spectra are recorded with Agilent technologies 5975 mass spectrometer.

Synthesis of Ligand L_1

2-hydroxy-1-naphthaldehyde (0.02 mol, 3.44 g) dissolved in 25 ml of absolute ethanol mixed with sulphamethoxazole (0.02 mol, 5.07 g) with a few drops of acetic acid. After the addition was completed the reaction mixture was refluxed for (3hrs). Recrystallized by ethanol.

Synthesis of Ligand L_2

4-Acetmidobenzaldehyde (0.02 mol, 3.26 g) dissolved in 25 ml of absolute ethanol mixed with 3,4-Diamino toluene (0.01mol, 1.22 g) with a few drops of acetic acid. After the addition was completed the reaction mixture was refluxed for (4hrs). Recrystallized by ethanol. their physical properties and analytical data are recorded in Table 1.

Table 1: Physical Properties and Analytical Data for the Synthesized Ligands (L_1, L_2)

Compound	Molecular formula	Molecular weight	Melting point (C°)	Color	Yield%	PH
L_1	$C_{21}H_{17}N_3O_4S$	407.44	243 – 244	Yellow	70	7.6
L_2	$C_{25}H_{24}N_4O_2$	412.48	290 – 291	Yellowish orange	69	6.3

Determination of lethal dose (LD50)

Males of the *M. musculus*Balb/C strain were dosed orally to determine the LD50 using a stomach tube with two ligands (L_1, L_2),after dissolved by Dimethyl sulfoxide (DMSO). The animals were monitored for 72 h and weakness, unstable walking, loss of balance and death was checked during this period. Injection started with low dose then continued to high dosages based on Litchfield and Wilcoxon (1949) equation[27]:

$$LD_{50} = \text{highest dosage} - \frac{\sum ab}{n}$$

Where, LD50 is the lethal dose 50, highest dosage is the dose with 100% mortality of mice, a is the value of

difference between the previous and next dose, b is the summation of dead animal for each dose (previous dose + next dose / 2) and n is the number of animals used for each dose.

Parasite Study

Feces Samples

Entamoebahistolytica parasites collection with the two stages trophozoite and cyst of feces of some infected patients with amoebiasis,in Bint Al-Huda hospital in Thi-Qarcity. Samples are placed in a sterile plastic bottles and brought to the laboratory directly, note that these samples were severe cases only.

Stool Examination

Examination of feces samples with method direct wet film preparation using the normal saline and Lugol's iodine solution[28].

Parasite Purification

Method of Snyderand Meleney 1941 [29]. Has been used to purficate the parasite (cyst) , to final concentration of 10^4 cysts / 1ml .

Infection of Laboratory Animals

Male mice of *M. musculus*Balb/c albino strain aged 11 to 12 weeks old(30∓2 gm) were used in this study.Animals were also screened for any protozoal infection via stool examination for three consecutive days. Mice free from parasitic infections were used.Animals were divided mainly into (5) groups, each containing (8)mice. Four of these groups have been administered orally1 ml of normal saline containing 10^4cyst/ml of *Entamoebahistolytica* using sterile stomach tube capacity 1 ml, Feces were examined daily for 10 days to check for infection (Figure 1).

A positive control group was infected without treatment and the negative control group was left without infection and treatment (Table 2

Table 2: Experimental Albino Mice Model

Treatment	Number	Mice Group
Treated with first ligand	8	L_1group
Treated with second ligand	8	L_2group
Treated with metronidazole	8	Metronidazole group
Infected without treatment	8	Positive control group
Without infection and treatment	8	Negative control group

Figure 1: Showed the Presence of Blood and Mucus with the Faeces of Infected Animals

Treatment of the Infected Mice

Treatment of mice infected with thecyst stage parasite of amoebic dysentery after 10 days of injections, where the two groups were injected with first and second ligandswith a dose of 0.14 and 0.24 g / kg, respectively, and the third group was given 10 mg / kg of metronidazole[30]for 7 days after dissolved all dimethyl sulfoxide DMSO.

The number of cysts was counted based on the following formula to calculate the effective dose:

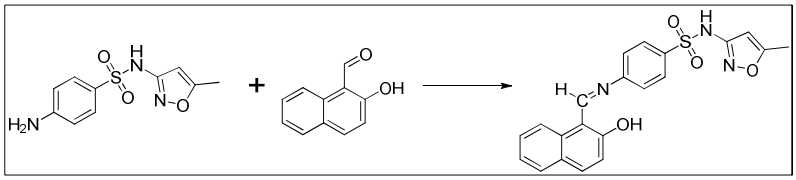
$$\text{Effective dose group} = \frac{\text{Number of cysts in positive control group} - \text{Number of cysts in treated}}{\text{Number of cysts in positive control group}}$$

STATISTICAL ANALYSIS

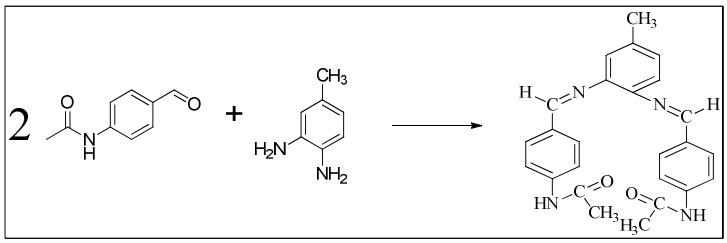
The use of statistical software (SPSS) for statistical analysis as the results were analyzed using the least significant difference (LSD) test and scale analysis of variance (ANOVA Table) under the 0.05 level

RESULTS AND DISCUSSION

The L_1, L_2 are synthesized in a good yield as follows Scheme1, 2.



Scheme 1: Synthesis of L_1



Scheme 2: Synthesis of L_2

^{1}H-NMRspectra for the L_1, L_2

The ^{1}H-NMR spectrum of the Schiff base L_1 which exhibited a singles at 3.3ppm is assigned to protons of CH_3(3H),6.1 due to heterocyclic ring CH (1H) and the multi singlet's in the 8.03 – 7.3ppm are assigned of the benzene ring

(10H), 8.9 due to CH=N- (1H), 9.3 due to N-H (1H) and at 9.8ppm due to OH (1H) protons (Figure 1).

The ^{1}H-NMR spectrum of the Schiff base L_2 which exhibited a singles at 3.2ppm is assigned to protons of CH_3(3H), 3.4 due to CH_3 related toCONH- and the multi singlet's in the 8.03 – 7.33ppm are assigned of the benzene ring (11H), 8.9 due to CH=N- (1H) and 9.2 due to N-H (2H) protons (Figure 2).

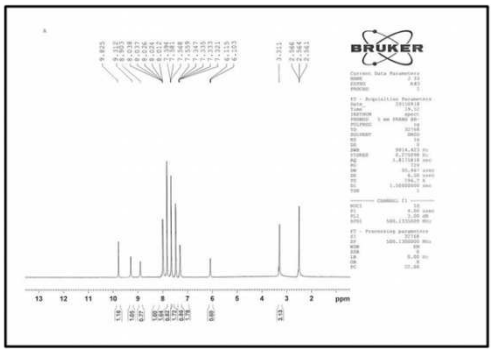


Figure 1: ^{1}H-NMR Spectra of L_1

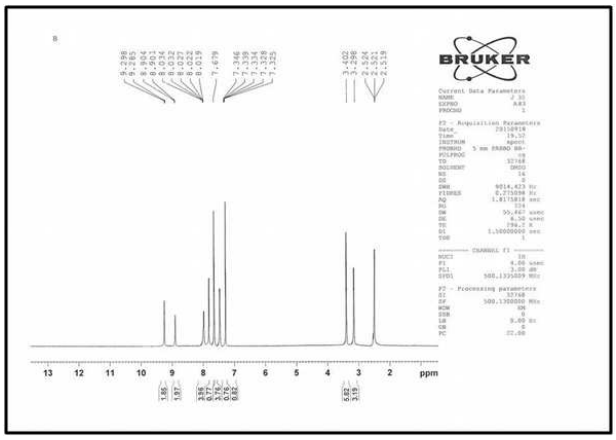


Figure 2: ^{1}H-NMR spectra of L_2

IR Spectra

The IR spectra of the Schiff bases under study (Figs. 3 and 4) are recorded in the solid state using the KBr disc technique. Selected bands of diagnostic importance arecollected in Table 3.

Table 3: Selected Bands of Diagnostic Importance from the IR Spectra of Schiff Bases L_1, L_2

رمز المركب	Compound	N–H Str.	R–H Aromatic Str.	C–H Aliphatic Str.	C=N Str.	C=O Str.	C=C Str.	C–N
L_1	$C_{21}H_{17}N_3O_4S$	—	3051 w	2970 w	1631 s	—	1546 s	1408 m
L_2	$C_{25}H_{24}N_4O_2$	3259 w	3062 w	2816 w	1674 s	1600 s	1543 s	1411 m
Str. = Stretching , m= medium , s=strong , w=weak								

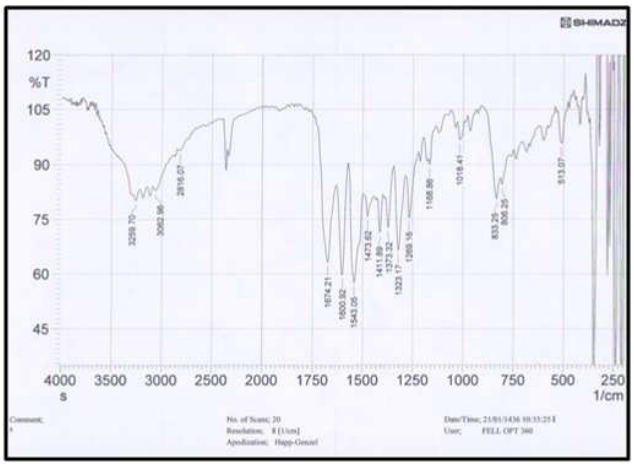


Figure 3: IR Spectra of Schiff Bases L_1

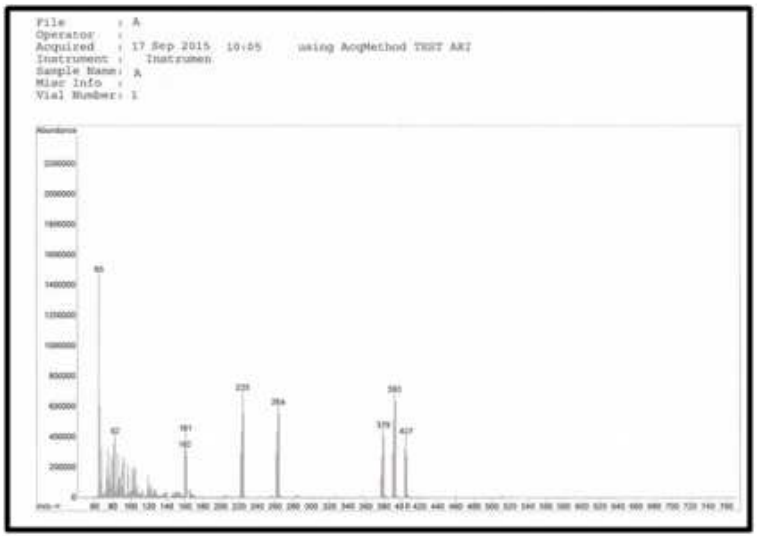


Figure 4: IR Spectra of Schiff Bases L_2

Mass Spectra

The mass spectra of first ligand show the molecular ion peak at m/z 407, spectrum is also characterized by the appearance of other bands when(390, 379, 264, 225, 161, 160, 82, 65) m/z due to $[C_{21}H_{16}N_3O_3S]^{+\cdot}$, $[C_{20}H_{17}N_3O_3S\ ]^{+\cdot}$, $[C_{11}H_{10}N_3O_3S\ ]^{+\cdot}$, $[C_9H_9N_2O_3S\ ]^{+\cdot}$, $[C_4H_5N_2O_3S\ ]^{+\cdot}$, $[C_4H_4N_2O_3S\ ]^{+\cdot}$, $[C_4H_4NO\ ]^{+\cdot}$, $[C_5H_5]^{+\cdot}$ respectively . The second ligand show the molecular ion peak at m/z 412, spectrum is also characterized by the appearance of other bands when (251, 161, 134, 119, 91, 65) m/z due to $[C_{16}H_{15}N_2O]^{+\cdot}$, $[C_9H_9N_2O]^{+\cdot}$, $[C_8H_8NO]^{+\cdot}$, $[C_7H_5NO]^{+\cdot}$, $[C_7H_7]^{+\cdot}$, $[C_5H_5]^{+\cdot}$ respectively (Figure 5, 6).

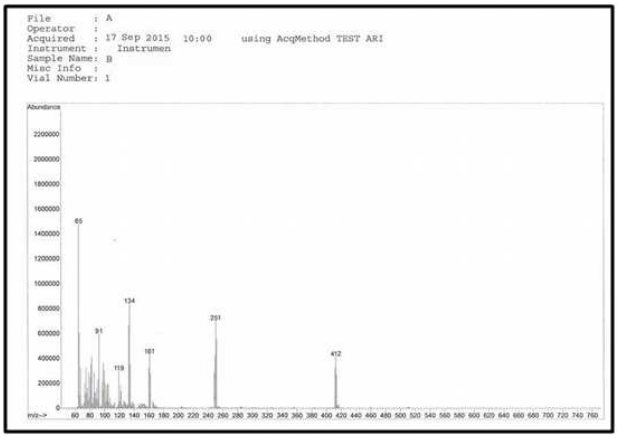


Figure 5: Mass spectra of Schiff bases L_1

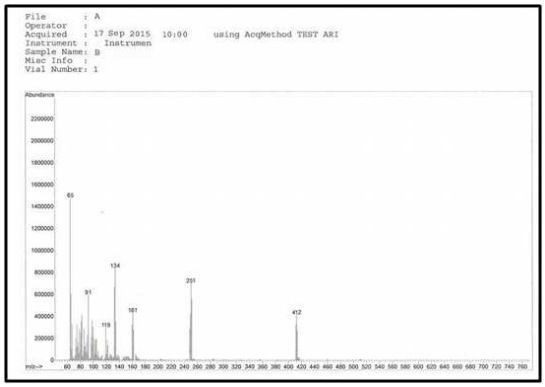


Figure 6: Mass Spectra of Schiff Bases L_2

The Effect of Two Schiff Bases and Metronidazole on *Entamoebahistolytica in Vivo*

At the level of probability ($p < 0.05$), the results of statistical analysis show a significant variation, remarkable inhibitory effect of the growth of cysts that reached the less impact to the second ligand rate about 2.9(effective dose 63%). in the seventh day of the injury and treatment. In the fifth day of the injury and treatment, the highest inhibiting effect of metronidazole was at a rate of 0(effective dose 85%), while in the seventh day of them , the first ligand was a compromise in effect, amounting to at a rate of 1.1(effective dose 56%) Table 4.

Table 4: The Effect of Two Schiff Bases and Metronidazole on Cyst Stage of *entamoebahistolytica in Vivo*

Treatment Time	Treated With Normal Saline	Treated With First Ligand (0.14 Gm/Kg)	Treated With Second Ligand (0.24 Gm/Kg)	Treated With Metronidazole (10ml/Gm)
First day	10	10	8.8	6.8
Second day	11.2	8	8	4.5
Third day	13.3	6.2	7.1	2.7
Fourth day	14	5	6.7	1
Fifth Day	15.1	4.4	5.2	0
Sixth day	17	2.3	4.1	0
Seventh Day	18.2	1.1	2.9	0

L.S.D. for days = 0.97 L.S.D. for treatment = 0.95

May be a inhibiting effect for two ligands result of its ability to form coordination bonds with some elements within the *Entamoebahistolytica* such elements within the synthesis of metallic enzymes like superoxide dismutases and iron hydrogenases and thus inhibiting the synthesis of these anti-oxidant enzymes which affects resistance to reactive oxygen species, and then the death of these parasites.

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