Research Article

Synthesis of Complexes of Azole Derivatives with Ethylenediamine and Biological Evaluation

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Abstract

Starting from lauric acid two novel metal complexes of Cu (II) and Co (II), containing azoles derivative nucleus was synthesized by involving multiple-step procedure. This procedure involves four reactions. The first one relates to the esterification of lauric acid using ethanol in the presence of sulphuric acid. As for the second reaction, it is the addition of hydrazine. The third one consists on the cyclization in basic medium, then finally, the coordination with the éthanediamine. In the first part, the resultant compound was characterized by IR and NMR spectroscopy. Its bactericidal activity was evaluated, in the second part, by determining minimum inhibitory concentration (MIC) values and inhibitory zone diameter against gram positive bacteria (Bacillus subtilis (BS) and Listeria monocitgenes (LM)) and gram negative bacteria such as Klebsiella pneumonia (KP) and Pseudomonasaeruginosa (PA). The antifungal activities were tested against four phytopathogenic ungal strains namely Fusariumgraminearum, Aspergillusochraceus, Aspergillusparasiticus and Penicillium expansum. Some of the tested compounds displayed promising antibacterial and antifungal activities.

Keywords: azoles derivative, complexes, antifungal activities,lauric acid, antimicrobial acrivites

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Introduction

The Carbon-nitrogen bonds are ubiquitous in natural medicines [1]. and organic materials such as azoles [2]. The formation of bonds of this type is among the most important chemical transformations in heterocycle synthesis and medicinal chemistry. Heterocycles are a structural pattern that interning in a multitude of bioactive important natural products [3], pharmaceutical [4], given their wide field of application, as analgesic [5], antidiabetic [6], antimicrobial [7], antiallergic [8-9], anticonvulsant [10], antifungal [11], and finally antidepressants [12], that is why we are interested in the synthesis of azole derivatives, by developing a method of forming carbon-nitrogen bonds, by exploiting the reactivity of the acid function, and who has us among syntheses a derivative from fatty acids by involving reactions.

Esterification, addition of hydrazine and finally cyclization in basic medium. The synthesized azole derivatives have been characterized by different spectroscopic methods, IR, 1H and 13C NMR. The latter are merged by coordinate with the éthanediamine [13], leading to schiff-base ligands; the nitrogen site has been used to study their complexation with metallic cations such as Cu2+ and Co2+. The complexes obtained, after spectroscopic characterization by different methods, have been biologically tested in relation to certain molds belonging to the genera: Aspergillus, Alternaria, Penicillium and Rhizopus, and on bacteria of the genus: Staphylococcus, listeria, Pseudomonas, Bacillus and Eschirichia, as well as yeasts of the genus Candida.

Materials and Reagents

All reactions were monitored by thin layer chromatography (TLC) using silica gel F254 supplied by MERCK, using mixture of different polar and nonpolar solvents in varying proportions and spots were observed using iodine as visualizing agent.

All Melting points were determined in open capillary tubes on a BÜCHI 540 melting point apparatus and are uncorrected.

The Infrared spectra of reactants and product in the range of 4000-400 cm-1 were recorded as potassium bromide discs on a Shimadzu FTIR-8300 Fourier Transform infrared spectrophotometer.

The1H and13C NMR Spectra were measured in Chloroform-d (CDCl3) on Bruker AM 300 MHZ Spectrometer (University of Oran, Essenia), relative to the internal standard tetramethylsilane (TMS), and chemical shift values are expressed in parts per million (δ, ppm).

General procedure for the preparation of ethyl laurate B

This ester was prepared following the standard procedure reported in the literature [14]. Lauric acid (5g, 0.025mol) was dissolved in excess of ethanol (200 mL) with 5 mL of concentrated sulfuric acid and the mixture was refluxed at 80°C in an oil bath for 6-7 h, the progress of the reaction was monitored by TLC. The excess of acid was neutralized with sodium bicarbonate then the solvent was evaporated and the product was collected.

General procedure for the preparation of N'-dodecanoylmethanedihydrazide C

To a solution of lauric ethyl ester B (4.47 g, 0.019mol) in ethanol (100 mL), Nalco (1.76g, 0.019mol) was added and heated for 10 h on oil bath. The progress of the reaction was monitored by TLC.

General procedure for the preparation of 4-amino-5-undecyl-2,4-dihydro-3H-1,2,4-triazol-3-one D

A mixture of N'-dodecanoylmethanedihydrazide C, potassium hydroxide (KOH) was refluxed on a oil-bath for 5-7 h. The progress of the reaction was monitored by TLC. After cooling to room temperature, the resulting precipitate was washed with a mixture of water and dichloromethane, and then dried.

General procedure for the preparation of ligand E

A mixture of compound (D) (3.82 g, 0.015 mol), ethane-1, 2-diamine (0.45 g, 0.015mol) and ethanol (10 ml) was refluxed on a oil-bath for 6-8h. The progress of the reaction was monitored by TLC. After cooling in an ice bath, the resulting precipitate was filtered, washed with water and dried.

General procedure for the preparation complexed ligand Co (F)

To a methanolic solution (1.3g, 0.0024 mol in 20 mL) of the required metal was added a methanolic solution (0.43g, 0.0024mol in 20 mL) of ligand HL in a 1:1 (metal: ligand) molar ratio for Co(II) metal, Then the mixture was gently heated under reflux for 30 minutes and a crystalline colored precipitate was formed at room temperature. The precipitates were filtered out, washed with cold diethyl ether and dried in desiccators.

General procedure for the preparation complexed ligand Cu (G)

To a methanolic solution (1.6g, 0.003mol in 20 mL) of the required metal was added a methanolic solution (0.56g, 0.003mol in 20 mL) of ligand HL in a 1:1 (metal: ligand) molar ratio for Cu(II) metal, Then the mixture was gently heated under reflux for 30 minutes and a crystalline colored precipitate was formed at room temperature. The precipitates were filtered out, washed with cold diethyl ether and dried in desiccators

Biology

Determination of minimum inhibitory concentration (MIC)

A disk diffusion assay according to the standard protocols (CLSI, 2006) [15]. The solutions of each tested compounds were prepared in DMSO to get a concentration of 100 mg/mL. From this stock solution, serial dilutions of the compounds (100, 50, 25, 12.5, 6.25, 3.125, 1.560, 0.781, 0.390, and 0.195 mg/mL) were prepared. The MIC was recorded in each case as the minimum concentration of the compound, which inhibited the visible growth of the tested microorganism, and DMSO was used as a negative control.

Antibacterial activity

In order to evaluate the antibacterial activity four bacteria have been used: Klebsiella pneumonia (KP) and Pseudomonasaeruginosa (PA) ATCC 70603 and ATCC 27853, as gram negative bacteria and Bacillus subtilis (BS) and Listeria monocitgenes (LM) as gram positive bacteria.

All microorganisms were obtained from Microbiology Laboratory, Institute of Biology, University of Dr. Moulay Tahar, Saïda. The bacterial strains were maintained on Muller-Hinton agar and the tested compounds were dissolved

in DMSO to make a stock solution of 100 mg/mL and the other concentrations were prepared by dilution. A suspension of the organisms was introduced onto the surface of sterile agar plates, and then incubated at 37 °C for 24 hrs. After incubation, MIC was determined.

Antifungal activity

The antifungal activities of the synthesized compounds D, E, F and G were studied in different concentrations (1.25, 2.5, 5 and 7.5 µg/ml) against four phytopathogenic fungal strains namely Fusariumgraminearum, Aspergillusochraceus, Aspergillusparasiticus, Penicilliumexpansum, The antifungal activity was determined by the Fandohan method [16].

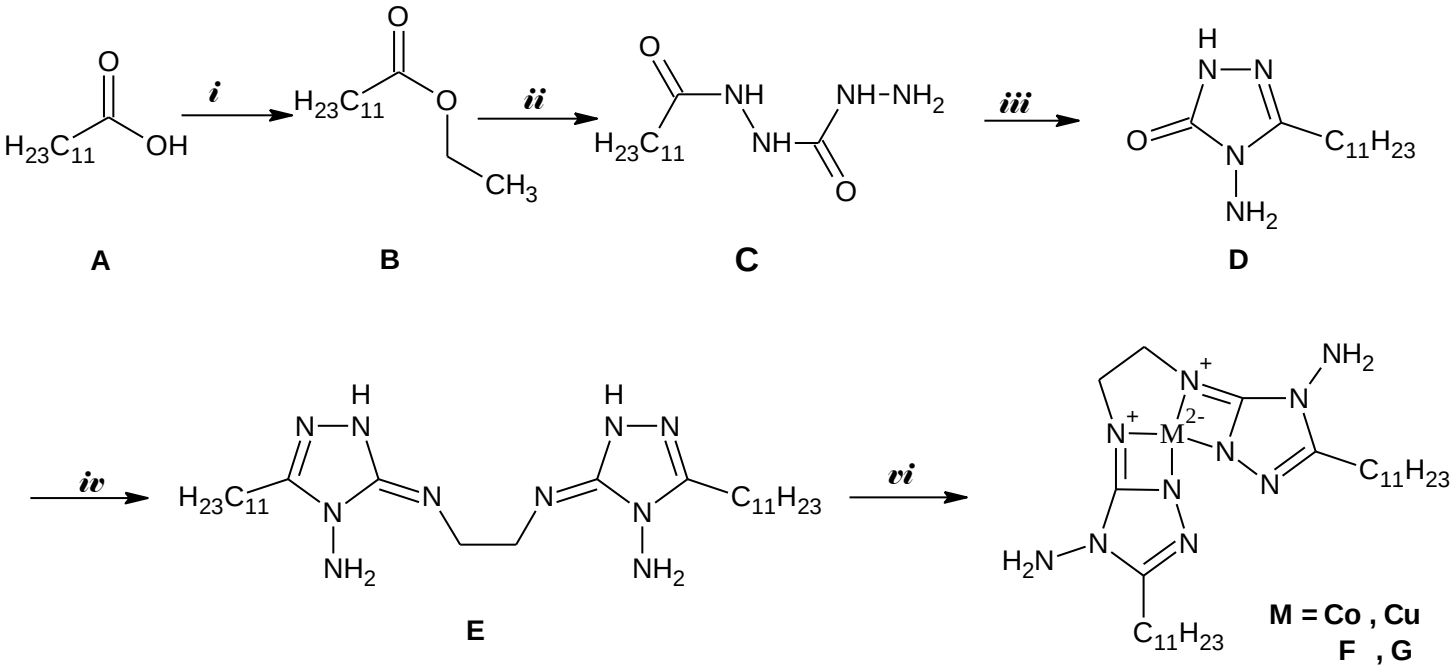
In vitro Screening of Antifungal Activity of synthesized compounds was determined on the potato dextrose agar (PDA) as the growth medium for the tested fungi, and PDA was prepared by dissolving potato extract (200 g), D-glucose (20 g) and agar (15 g) in distilled water (1000 ml).Finally, the medium was transferred to a flask, sealed, sterilized by autoclaving at 121 C for 30 min and cooled down. The compounds were tested at various concentrations and have been incorporated into the PDA culture medium maintained molten at a temperature of 40 to 45°C. After the mixture flow and solidification; Mycelial implants of 6 mm diameter on the pathogen fungi are deposited in the center of the Petri dish containing PDA medium with 04 concentrations for each synthesized compounds to be studied. The Petri-dishes were incubated at 28°C for 3-5 days. Taking into account the mycelial growth of the control [17]. After the completion of incubation period, the Relative inhibition rate of the circle mycelium compared to blank assay was calculated via the following equation:

$$\text{Antifungal index}\ (\%) = [(1 - Da)/Db] \times 100\% \qquad (1)$$

Where: *Da*is the diameter of the test growth area, *Db*is the diameter of the growing area of the control.

Results and Discussion

The target compounds F and G were synthesized by a multiple-step procedure as shown in **Scheme 1**. The synthetic route started from esterfication of lauric acid 1 using ethanol in the presence of sulphuric acid. This ester reacted with nalco in ethanol to give compound C. 4-amino-5-undecyl-2, 4-dihydro-3H-1,2,4-triazol-3-one (D) was obtained by ring closing reaction upon treating compound C with potassium hydroxide in ethanol. This compound was converted to give the ligand LH by ring-alkylation reactions using 4-amino-5-undecyl-2,4-dihydro-3H-1,2,4-triazol-3-one (D) with ethylenediamine (2:1). Finally, the products F and G were obtained by refluxing compound E with the required metal in methanol.



Scheme 1 Synthetic route of the final compounds A-G

Reagents and conditions

(*i*) C_2H_5OH, and conc. H_2SO_4, reflux for 8 h; (*ii*) $NH_2NHCO(NHNH_2)$ and C_2H_5OH, reflux for 5 h; (*iii*) KOH and C_2H_5OH, Reflux (*iv*) ethylenediamine and C_2H_5OH, (*vi*) metal (Co, Cu) and CH_3OH.

Both the free ligand and its metal complexes formations were detected by thin layer chromatography (TLC) via their Rf which were different from starting materials. The obtained complexes are of various colours varied from brick red to violet colour. It is to note that these colours are different from the ligand colour, wich indicate that the colours formed depend on the metal ions. The melting points of the complexes are different (higher) than that of the ligand an evidence for complexation. The physical and analytical data of all the compounds studied has been summarized in **Table 1**.

Table 1 Physical properties and analytical data of the synthesized compounds

Compound	Colour	Yield, %	Melting point, °C	Rf value	Solvent system $CHCl_3/CH_3OH$
B		81.5	liquid	0.65	8/2
C		81	Liquid	0.75	8/2
D		92.75	Liquid	0.9	8/2
E	White	60.15	120	0.83	8/2
F	Red	78.5	171-172	0.85	8/2
G	Violet	73.03	208	0.78	8/2

The IR spectra in the (4000–400 cm-1) region provide information regarding the coordination mode in the complexes were analyzed by comparison with the data for the free ligand. The IR data of the ligand and complexes are shown in **Table 2**.

Table 2 The characteristic infrared absorptions in ῡ cm-1 of the synthesized compounds B-G

Comp.	-C-H	C=O	NH_2 / NH	C-O-C	C=N	C-N	M-N
B	2976.0	1739.7	-	1016.4	-	-	-
C	2975.0	1737.7	3483.2	-	-	-	-
D	2925.8	1739.7	3460.1	-	-	1109.0	-
E	2923.9	-	3342.4	-	1566.1	1109.0	-
F	2923.9	-	3134.1-3219.0	-	1585.4	1058.8	1375.2
G	2954.7	-	3163.0-3276.8	-	1606.6	1041.5	1373.2

^{1}H NMR (300MHz, $CDCl_3$), δ (ppm) of B: 0.903 (3H, C1), 4.116 (2H, C2), 2.292 (2H, C4), 1.618 (2H, C5), 1.257 (2H, C6, C7, C8, C9, C10, C11, C12, C13), 0.88 (3H, C14). **^{13}C NMR (300MHz, $CDCl_3$), δ (ppm):** 14.229 (C1, C14), 59.796 (C2), 174.068 (C3), 34.396 (C4), 24.987 (C5), 29.612 (C6, C7, C8, C9, C10, C11), 31.918 (C12), 22.695 (C13).

^{1}H NMR (300MHz, $CDCl_3$), δ (ppm) of C: 5.026 (H, NH 2, 1, 16), 4.135 (2H, NH_2 17), 2.285 (2H, C4), 1.253 (2H, C6, C7, C8, C9, C10, C11, C12, C13), 1.624 (2H, C5), 0.854 (3H, C14). **^{13}C NMR (300MHz, $CDCl_3$), δ (ppm):** 14.249 (C14), 173.703 (C3), 34.394 (C4), 24.730 (C5), 29.453 (C6, C7, C8, C9, C10, C11), 31.605(C12), 22.464 (C13), 151.767 (C15).

^{1}H NMR (300MHz, $CDCl_3$), δ (ppm) of D: 5.075 (H, NH 1), 4.015 (2H, NH_2 17), 2.191 (2H, C4), 1.044 (2H, C6, C7, C8, C9, C10, C11, C12, C13), 1.522 (2H, C5), 0.786 (3H, C14). **^{13}C NMR (300MHz, $CDCl_3$), δ (ppm):**14.171 (C14), 34.394 (C4), 17.341 (C5), 29.358 (C6, C7, C8, C9, C10, C11), 31.814 (C12), 22.748 (C13).

^{1}H NMR (300MHz, D_2O), δ (ppm) of E: 2.077 (H, NH 1,23), 4.698 (2H, NH_2 17, 27), 2.846 (2H, C4, C28), 1.133 (2H, C6, C7, C8, C9, C10, C11, C12, C13, C37, C36, C35, C34, C33, C32, C31, C30), 1.762 (2H, C5, C29), 1.007 (3H, C14, C38), 2.846 (2H, C19, C20).

^{1}H NMR (300MHz, D_2O), δ (ppm) of F: 4.697 (2H, NH_2 17, 27), 2.626 (2H, C4, C38), 1.758 (2H, C6, C7, C8, C9, C10, C11, C12, C13, C37, C36, C35, C34, C33, C32, C31, C30), 2.011 (2H, C5, C29), 1.001 (3H, C14, C38), 2.013 (2H, C19, C20). **^{13}C NMR (300MHz, D_2O), δ (ppm):**15.842 (C14, C38), 16.043(C4, C28), 43.815 (C6, C7, C8, C9, C10, C11, C12, C13, C37, C36, C35, C34, C33, C32, C31, C30), 19.176 (C5, C29), 44.267 (C19, C20).

^{1}H NMR (300MHz, D_2O), δ (ppm) of G: 4.699 (2H, NH_2 17, 27), 1.910 (2H, C6, C7, C8, C9, C10, C11, C12, C13, C37, C36, C35, C34, C33, C32, C31, C30), 1.911 (2H, C5, C29), 1.026 (3H, C14, C38), 1.910 (2H, C19, C20).

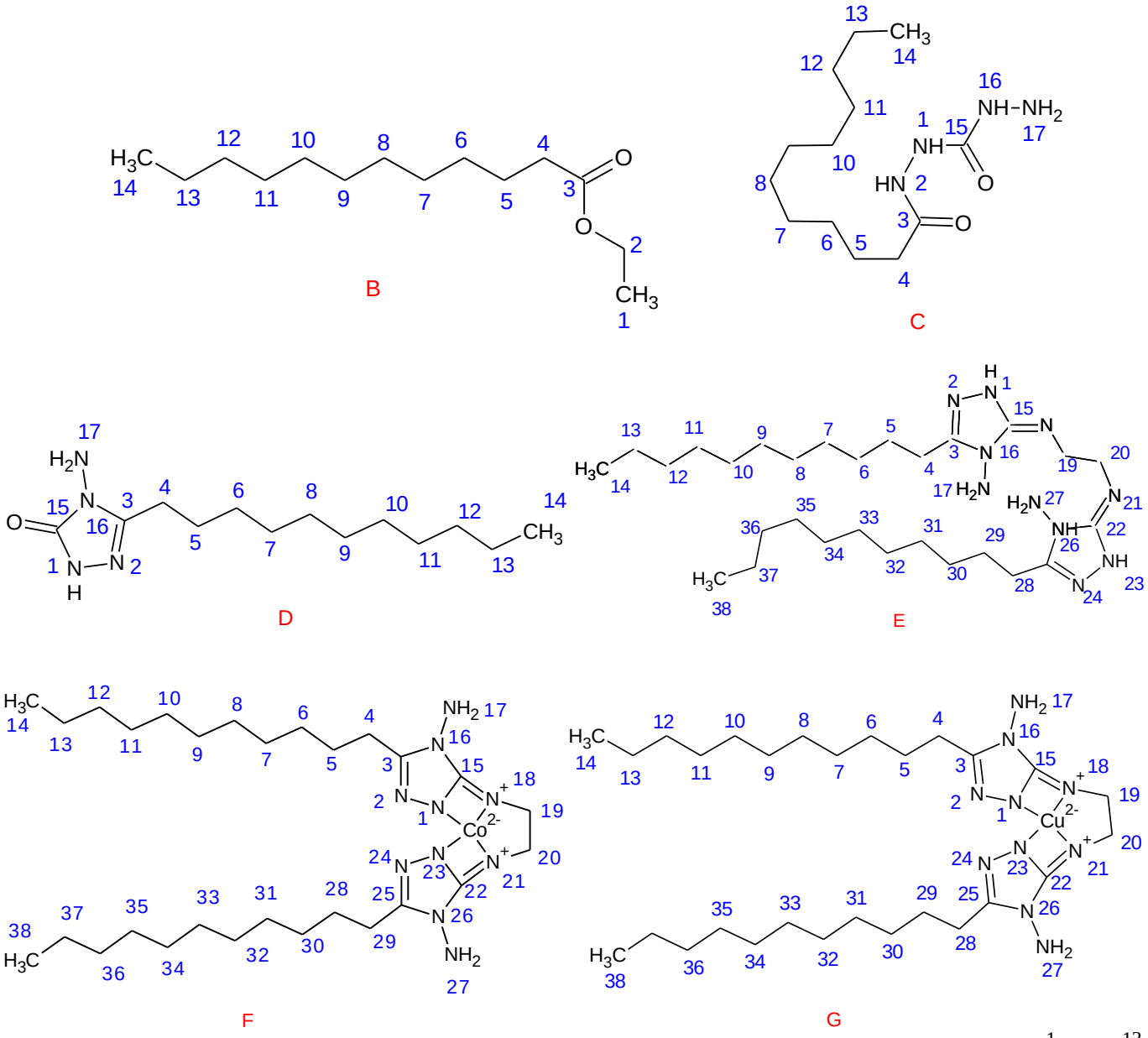


Figure 1 Schematic drawings developed according to ChemSketch of structures B, C, D, E, F and G for ^{1}H and ^{13}C NMR purposes

Antibacterial activities tests

The synthesized compounds have been tested in vitro for their antibacterial activity against four bacteria, such as *Klebsiella pneumonia (KP)* and *Pseudomonasaeruginosa (PA)* as gram negative bacteria and *Bacillus subtilis (BS)* and *Listeria monocitgenes (LM)* as gram positive bacteria. The obtained results are summarized in **Table 3**.

Table 3 Antibacterial activity of the synthesized compounds

Strains	**Compound and values of MIC in mg/mL**			
	G	**F**	**E**	**D**
PA	0.390	3.125	00.00	12.5
BS	25	50	00.00	25
LM	12.5	25	12.5	12.5
KP	12.5	25	00.00	50

As it is shown in Table 3, the results of evaluating the antibacterial properties suggest that all compounds have very good potential to act as antibacterial agents. The values of MIC ranged between: 0.195-100 mg/mL

Antifungal activities tests

The evaluation of antifungal activity revealed that all the synthesized compounds have a significant biological activity, the best activity was observed at a concentration of 7.5 µg/mL against all the tested fungi.The results of in vitro antifungal activities are presented in **Table 4**.

Table 4 Relative inhibition rate (%) of synthesized compounds against tested fungal strains

		Concentration [µg/ml] Espèces	**D**	**E**	**F**	**G**
Relative inhibition rate (%)	*F.graminearum*	1.25	53.33	23.33	53.33	28.57
		2.5	60	33.33	56.67	40.48
		5	63.33	40.00	60.00	52.38
		7.5	66.67	50.00	66.67	59.52
	A.parasiticus	1.25	9.20	29.09	26.36	52.73
		2.5	29	43.53	35.25	63.64
		5	35	58	50.00	65
		7.5	36.60	68.35	62.32	70.91
	A.ochracus	1.25	16.67	35.71	40.48	38.55
		2.5	50	52.38	45.24	55.47
		5	54.76	59.52	57.14	62.09
		7.5	71.43	76.19	59.52	79.22
	P.expansum	1.25	15.69	27.00	20.00	44
		2.5	35.08	39.12	33.00	61.22
		5	42.46	60.16	51.00	73.11
		7.5	64	80.43	71.20	83.44

Conclusion

In summary, we have described the synthesis of the ligand HL and his metal complexes, IR, NMR spectral techniques were used to confirm their formation. The newly synthesized compounds were evaluated for their antibacterial and antifungal activity. The results of biological tests and the in vitro antifungal activity indicated that most of the synthesized compounds exhibited promising results. These compounds can be considered as lead molecules for future investigations.

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