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New Metronidazole Derivative and Some of Its Complexes with Antibiofilm study

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Abstract

Synthesis of a new ligand, namely [bis(2-(2-methyl-5-nitro-1*H-imidazol*-1-yl)ethyl) hydrogen borate] (BIB), utilizing the reaction of metronidazole with boric acid in a (2:1) mole ratio The metal complexes were synthesized utilizing the reaction of ($NiCl_2.6H_2O$ and $CuCl_2 .2H_2O$) with (BIB) ligand in a 2:1 (L:M) mole ratio. All synthesized compounds were characterized utilizing spectroscopic techniques such as infrared (FTIR), nuclear magnetic resonance of protons(^{1}H NMR), ultra violet and visible radiation (UV-Vis), thermal analysis (TG), atomic absorption (A.A.S.), micro elemental analysis (C.H.N.S.), melting point (m.p.), magnetic susceptibility, molar conductivity, and chloride content measurements. All complexes were paramagnetic and electrolyte, and the suggested geometries were the tetrahedral of nickel and the distorted octahedral of copper complexes. Against the Gram-negative bacterium *Pseudomonas auroginosa* (G-), all synthesized compounds were evaluated as anti-biofilm agents. Strikingly, the copper (II) complexes tested exhibit significant activity against biofilms and were better at removing biofilms than metronidazole (an antibiotic that is currently used to treat infections), ligand (BIB), and nickel (II) complexes.

Keywords: Metronidazole, Boric acid, FT-IR, Anti-biofilm.

1.Introduction

Nitroimidazole chemical compounds have active nitro groups on either the 2' or 5' positions of the imidazole ring. While 2'-nitroimidazoles exhibit pharmacological properties that are anti-ischemic and anti-inflammatory, 5-nitroimidazoles exhibit pharmacological characteristics that are anti-parasitic [1]. The 5-nitroimidazole compound, namely [metronidazole (MTN)], is the prototype and most commonly utilized drug in this class. It's one of the most versatile antibiotics in clinical use, effective against a wide range of anaerobic

microorganisms ranging from protozoa to bacteria. The World Health Organization has classified it as an essential medication [2].

Metronidazole (MTN) is a crystalline powder that is slightly soluble in water [3]. Metronidazole exhibits a wide range of pharmacological effects, with an emphasis on its antiviral, antibacterial, anti-proliferative, and antifungal activities in particular [4]. Metronidazole's initial clinical trials revealed that it was effective in treating amoebic liver abscess and invasive amoebic dysentery [5].

Boric acid B(OH)3 is a weak inorganic acid that is odorless and soluble in water. It is utilized as an efficient acid catalyst in organic synthesis for various selective transformations of simple and complex molecules [6]. B(OH)3 is utilized as an antiseptic in mouthwashes, talcum powder, protective ointments, and eyewashes. Also, boric acid is used in industrial applications such as optical and sealing glasses, textile fiber glass, heat-resistant borosilicate glass, porcelain enamels, and ceramic glazes [7].

In this study, a new metronidazole derivative was synthesized using boric acid as a BIB ligand (**Figure 1**). In addition, this (BIB) ligand is being mixed with [Ni (II) and Cu (II)] metal ions to create metal complexes (**Figure 2**). To prove the proposed structure, all synthesized compounds are characterized using physicochemical and spectral studies. The biological activity of synthesized compounds was evaluated.

2.Experimental Design

Material and Instruments

Without additional purification, all chemicals were used as supplied (utilizing Hayman, Fieser, BDH, HiMedia, Aarti Drugs Ltd., Fluka-Garantie, Merck, Sigma, Ajenta Pharm, Intron Biotech, and PSI).

Synthesis of bis(2-(2-methyl-5-nitro-*1H-imidazol*-1-yl)ethyl) hydrogen borate (BIB):

The mixture of metronidazole (0.1 g, 0.5842 mmol) in 8 mL of distilled water and boric acid (0.0180 g, 0.2921 mmol) was heated under reflux for 8 hours with stirring. The solution was tested by TLC technique, and the eluents were toluene, chloroform, and methanol (3:2:0.6, v/v/v), respectively. A part of the solution was evaporated, and he white product was obtained by cooling in an ice bath, scratching, washing with cold distilled water, and drying in an oven at 80 °C.

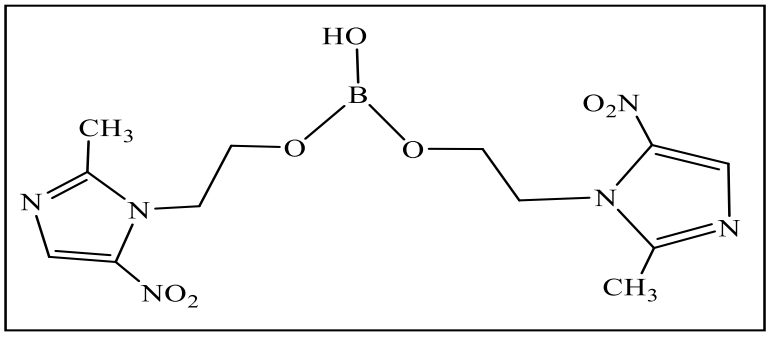

Figure 1: Structure of the ligand (BIB).

Synthesis of BIB complexes with (Ni (II) and Cu (II)) metal ions (C_1 and C_2) :

A warm solution of the BIB (0.1 gm, 0.2718 mmol) in 5 ml distilled water was added to a solution of metal salt (0.0323 gm, 0.0231 gm, 0.1355 mmol) in 2 ml of distilled water for $NiCl_2.6H_2O$ and $CuCl_2.2H_2O$, respectively. The mixtures were heated for 5 hours under reflux with stirring. The products were collected by the partially evaporated solvent in the presence of an ice bath and crashing, and they were then washed with cold distilled water and dried in an oven at 80 ^{0}C.

Anti-Biofilm:

Determination of the minimum inhibitory concentration (MIC) of Ligand (BIB) and its Complexes:

Following a microdilution method, different concentrations of this compound (8 - 1024 mg/mL) were utilized to estimate the MIC.

Biofilm quantification protocol:

The biofilm formation was evaluated on a polystyrene 96-well microplate. Bacterial strains were cultivated overnight for 24 hours at 37 °C in brain heart infusion (BHI) broth culture with 0.2% glucose, both in the presence and absence of MIC concentrations of chemical compounds. After removing the medium, biofilm-containing wells were washed three times with normal saline before being fixed with 200 µl of 99% methanol. The microplate was washed three times with distilled water, dyed for 15 minutes with 200 µl of (0.1%) crystal violet, and dried at room temperature. After the dye that was adhered to the biofilm was solubilized in 200 µl of pure ethanol, The absorbance was measured at 630 nm [8]. The experiments were repeated three times, and the data were shown as absorption means.

Effect of Ligand (BIB) and Complexes at Sub- MIC on Biofilm:

The same approach was employed for the previously mentioned biofilm formation study (one isolate was chosen). On the other hand, (BHI) broth contains such compounds at sub-MIC concentrations. At 37ºC, the plates were incubated for 24 hours. Following that, all wells were rinsed, stained, and read at 630 nm. Positive controls were also performed by adding 200µl of a compound-free fresh bacterial strain (compatible with the 0.5 McFarland standards).

3.Results

The hypothesized structures of the investigated compounds were supported by the physical and analytical data **(Table 1)**.

Table 1. Data from the analysis as well as the physical properties of the (BIB) ligand and its metal complexes.

Comp	The Molecular Formula	Color	Yied %	m.p (°C)	M.wt (g/mol)	Theoretical% (Experimental) % C%	H%	N%	M %	Cl %
BIB	$C_{12}H_{17}BN_6O_7$	White	97%	(148-150)	367.8	39.15 (39.41)	4.65 (5.37)	22.83 (23.22)	___	_____
C_1 Ni(II)	$[C_{24}H_{36}B_2N_{12}O_{15}NiCl]Cl.H_2O$	light green	76%	(158-160)	901.29	31.95 (32.71)	4.21 (4.74)	18.63 (19.39)	6.51 (7.33)	7.87 (8.76)
C_2 Cu(II)	$[C_{24}H_{40}B_2N_{12}O_{17}CuCl].Cl$	green	82%	(156-158)	924.14	31.16 (32.08)	4.32 (5.18)	18.17 (17.21)	6.87 (7.41)	7.68 (6.75)

Table 2. The BIB ligand and its metal ion complexes' name and molecular formula.

Comp	The Molecular Formula	Name
BIB	$C_{12}H_{17}BN_6O_7$	bis(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl) hydrogen borate.
C_1 Ni(II)	$[(L_1)_2Ni(H_2O)Cl].Cl.H_2O$	[aqua chloro bis{ bis(2-(2-methyl-5-nitro-1*H*-imidazol-1-yl)ethyl) hydrogen borate} Nickel(II)]hydrate. Chloride.
C_2 Cu(II)	$[(L_1)_2Cu(H_2O)_3Cl].Cl$	[Tri aqua chloro bis{ bis(2-(2-methyl-5-nitro-1*H*- 277midazole-1-yl)ethyl) hydrogen borate} Copper(II)] Chloride.

FT-IR spectra:

The infrared spectra of (Nickel and Copper) complexes showed changes in profile and shifting in the frequency of (υ OH) **(Table 3)** as a result of coordination with metal ions [9]. At (1483-1487) cm^{-1}, a new band appears in the spectra of the ligand and its complexes; this band is attributed to the (υ B-O) group [10]. The stretching of (C=N) the imidazole ring has not shown any change in frequency or profile, and these are because of the lack of coordination with metal ions through (C=N)[11]. The spectra of the Ni(II) complex exhibit lattice water at (3437) and coordinated water at (3365) cm^{-1} as well as the lower frequency bands [(991) and (678)]cm^{-1}.The coordinate H_2O of the Cu(II) complex appeared at (3388)cm^{-1} and the lower frequency band [(767) and (680)]cm^{-1}. Low-frequency bands appeared in complex spectra due to υM-O, υM-Cl [12,13] as shown in **Figure 3 and 4**.

Table 3. Main band of FTIR for the ligand (BIB) and it complexes.

Compound	υ OH	H_2O lattice (coordinate)	υ C=N	υ B-O	υ M-O	υ M-Cl
MTZ	3222	_____	1535	_____	_____	_____
BIB	3415	_____	1535	1487	_____	____
(Ni) C_1	3384	3437 (3365) (991) (678)	1535	1487	457	347
(Cu) C_2	3406	(3388) (767) (680)	1535	1483	424	333

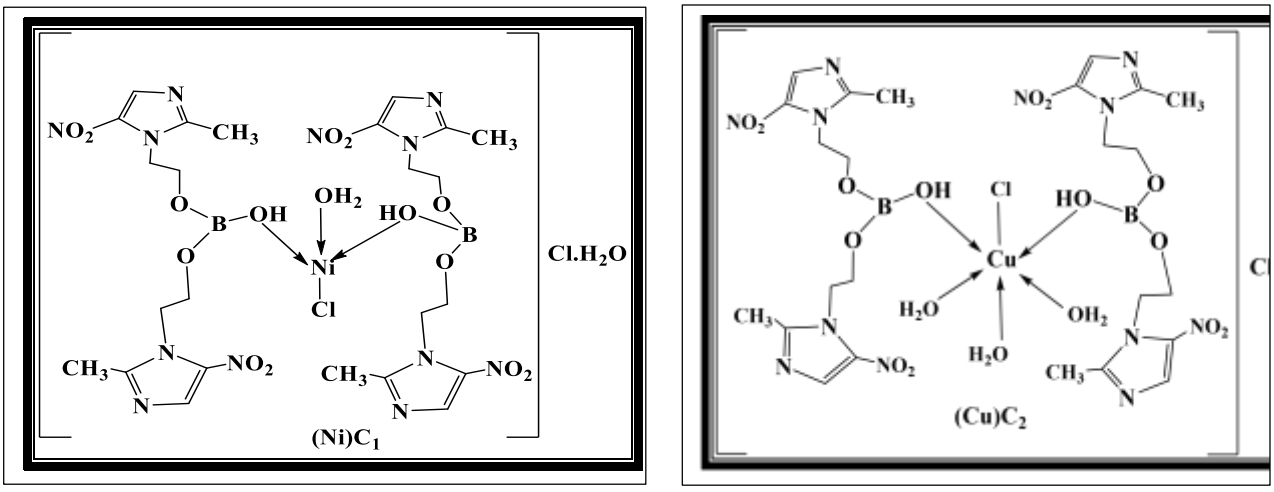

Figure 2. The suggested structures of synthesized complexes.

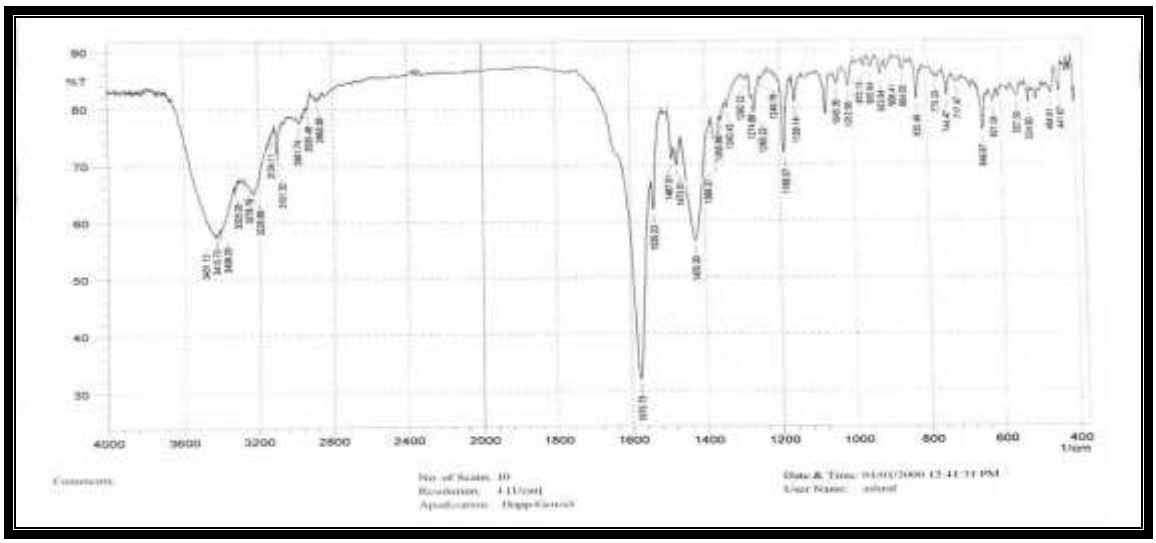

Figure 3.FT-IR spectrum of the (BIB) ligand.

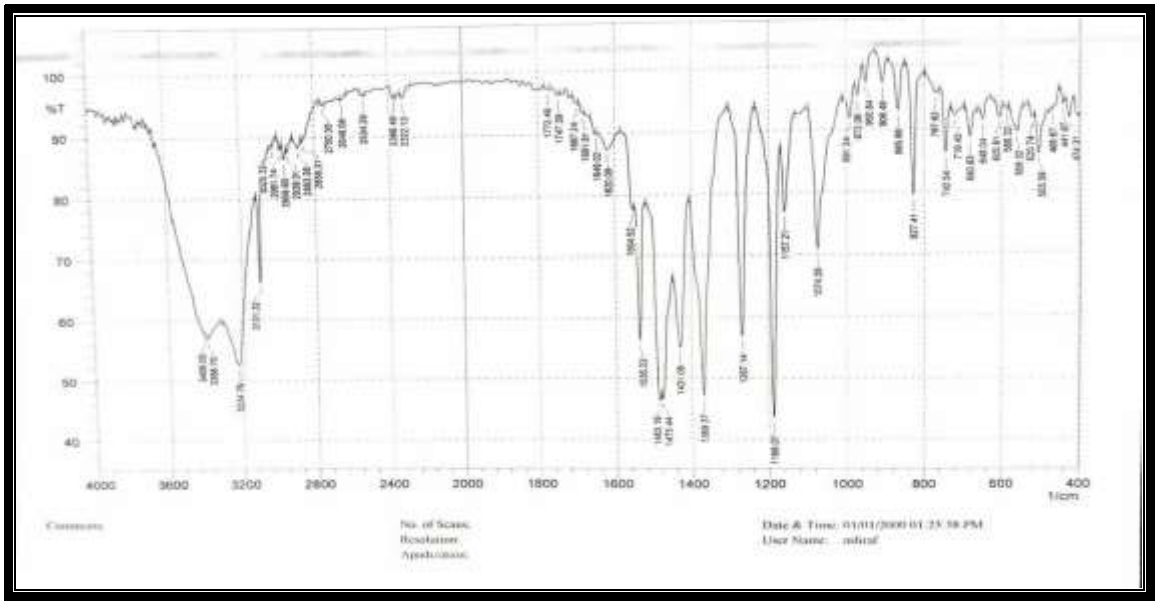

Figure 4. FT-IR spectrum of the Cu(II) complex C_2.

^{1}H-NMR Spectroscopy:

The data on ^{1}HNMR for BIB ligand are listed in **Table 4,** which is supported by **Figure 5,** and the ^{1}HNMR spectrum in DMSO-d6 is shown in **Figure 6**. The spectrum of the ligand exhibits a chemical shift at (δ3.99 ppm) because of the (B-OH) proton [14, 15]. The multiple peaks noticed in the range (δ4.35-4.40ppm) and (δ3.66ppm) referred to N-CH_2 and O-CH2, respectively [16, 17]. Chemical shifts of the methyl group CH_3 and residual DMSO at (δ2.44ppm) [16] The peak observed at (δ8.04ppm) attributed to imidazole protons [18].

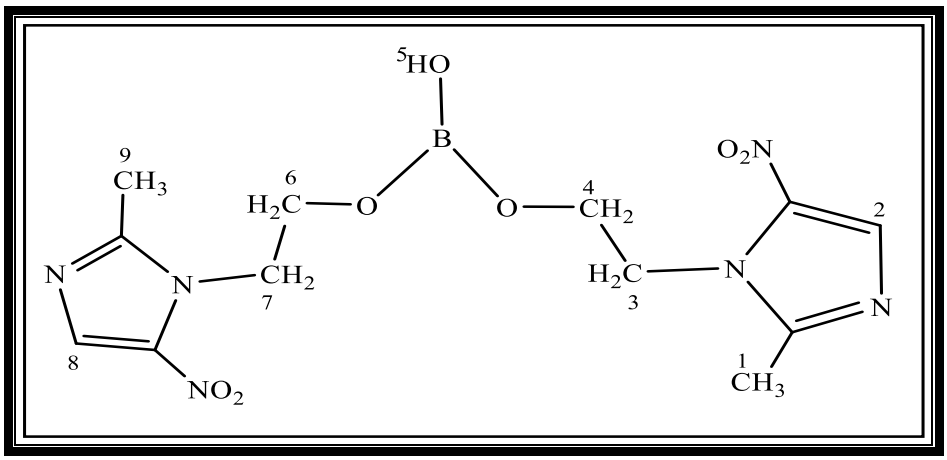

Figure 5. Proton positions in (BIB) structure.

Table 4. Chemical shifts for ^{1}HNMR of BIB**.**

Assignments in D^6-DMSO	Mark	Chemical shifts δ (ppm)
CH_3	1,9	(2.44),6H,s
CH(imidazole)	2,8	(8.04),2H,s
N-CH_2	3,7	(4.35-4.40),4H,m
O-CH_2	4,6	(3.66),4H,m
B-OH proton	5	(3.99)1H,s

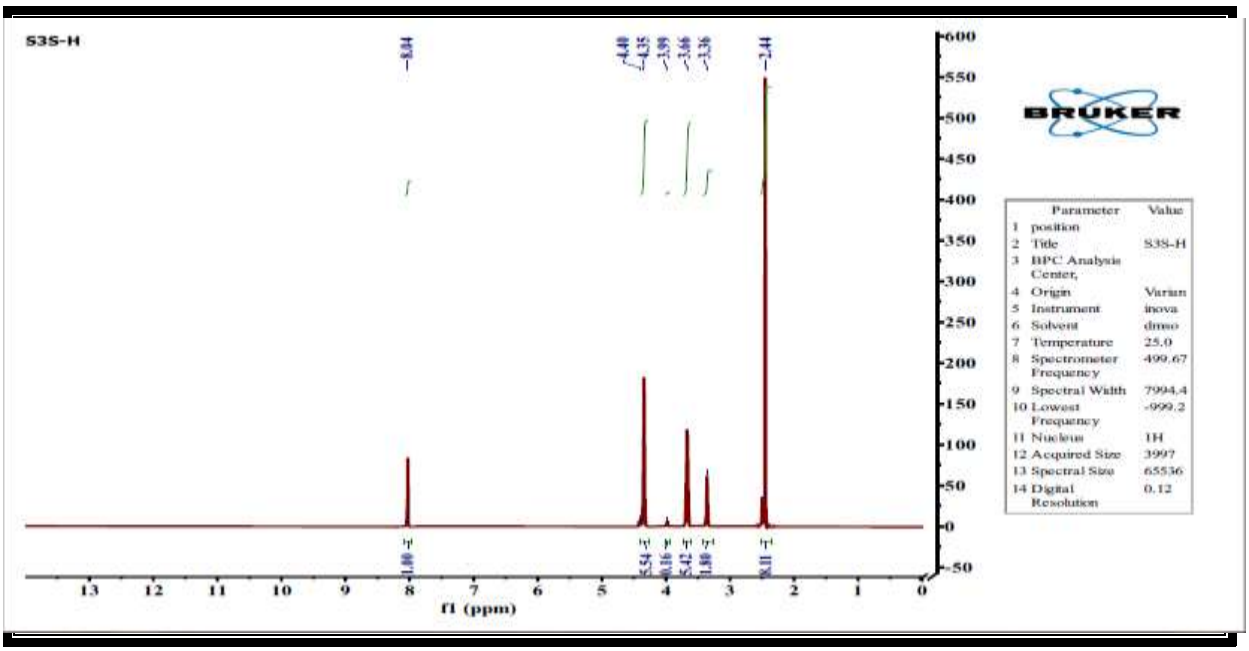

Figure 6.^{1}H-NMR Spectrum of BIB.

Thermogravimetric analysis (TGA):

The ligand's thermal degradation (BIB) and its complexes were investigated by (TGA) and from (25-800) ^{o} C in argon. Using this technique, the suggested structures were characterized, as was the thermal stability of the synthesized compounds studied. In the following order, the BIB ligand and its complexes thermal stability increased: (C_1 > BIB > C_2) **(Table 5) [3].**Thermal decomposition was utilized to confirm the structures where the results of degradation exhibit high agreement with the found mass loss (practical 100% and theoretical 99.9%), which confirms the

proposed structures of the synthesized compounds. The thermogram of the ligand (BIB) and nickel complex C_1 is shown in **Figure 7.8**.

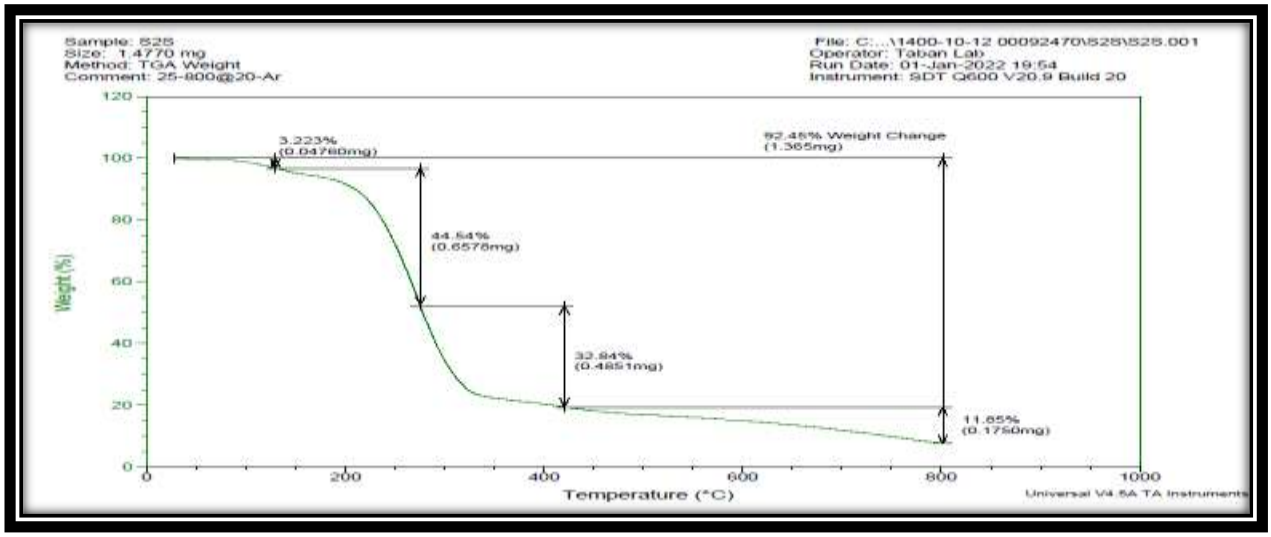

Figure 7. The thermo-gram of the (BIB) ligand.

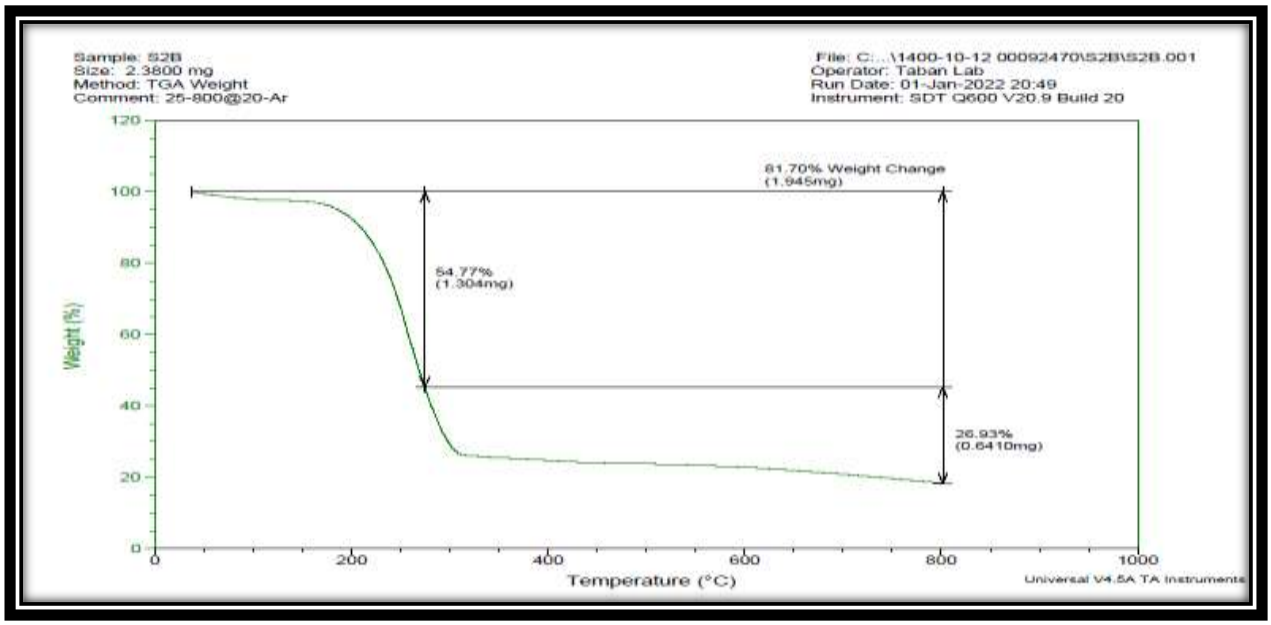

Figure 8. The thermo-gram of Nickel complex C_1**.**

Table 5. TGA of ligand (MBIB) and their complexes:

Comp.	Compounds (M.wt)(gm/mol)	Step	Temp. rang of the Decomposition C°	Suggested Assignment	Mass loss%	
					Cal.	Found
BIB	$C_{12}H_{17}BN_6O_7$ 367.8	1	25-135	CH_3	4.078	3.223
		2	135-275	$N_3C_7O_2H_9$	45.40	44.54
		3	275-420	$N_3C_2O_3H_5$	32.35	32.84
		4	420-800	2C+O	10.87	11.85
		Residue	>800	B+O	7.28	7.55
Ni C_1	$[C_{24}H_{36}B_2N_{12}O_{15}NiCl]Cl .H_2O$ 901.29	1	25-278	$2H_2O+2Cl+N_6O_7C_{13}H_{20}+B$	54.34	54.77
		2	278-800	$N_6O_4C_8H_7$	27.84	26.93
		Residue	>800	$O_3H_7C_3B$ Ni	17.80	18.30
Cu C_2	$[C_{24}H_{40}B_2N_{12}O_{17}CuCl].Cl$ 924.14	1	25-262	$3H_2O+2Cl+2(N_3O_2C_6H_8)+O$	48.58	48.94
		2	262-800	$N_6O_9C_{12}H_{18}B$ +Cu	50.24	50.26
		Residue	>800	B	1.16	0.81

UV-Vis Spectral Studies:

The UV-Vis spectra of BIB and its metal complexes in distilled water are listed in **Table 6.** The spectrum of the ligand **(Figure 9)** exhibits the band at 313nm (31948 cm-1) due to the π→ π* transition [19]. This band shifted to a lower frequency in nickel and copper complexes, and this is a result of the coordination with metal ions. The Ni (II) complex **(Figure 10)** appears to have two bands at [962 nm (10395 cm^{-1}) and 785 nm (12738 cm^{-1})] which were attributed to[$^3T_{1(F)} \rightarrow {}^3A_{2(F)}$ and $^3T_{1(F)} \rightarrow {}^3T_{1(P)}$] transitions of the tetrahedral Ni (II) complex [20]. The *M*eff of Ni(II)C_1 was 2.78 B.M. This value agrees with the tetrahedral geometry of nickel complexes [20, 21]. In the spectrum of the copper (C_2) complex, there were two absorption bands at [960 nm (10416 cm^{-1}) and 739 nm (13531 cm^{-1})] that were assigned to the $^2B_1g \rightarrow {}^2A_1g$ and $^2B_1g \rightarrow {}^2B_2g$ transitions, respectively [20]. The magnetic moment of the copper complex was 2.23 B.M., and these values of *M*eff agree with the copper complex's (C2) distorted octahedral geometry [21, 22]. The molar conductance in distilled water for each synthesized complex was measured using (10^{-3} M). The complexes ($C_1$and C_2) are (1:1) exhibit electrolyte behavior [23, 24].

Table 6. Electronic transitions of the BIB and its complexes, proposed geometry, molar conductivity, and magnetic susceptibility.

Comp	ג nm (cm^{-1})	Assignment	Molar conductivity ($S.cm^2 .mol^{-1}$) in H_2O	M_{eff} (B.M)	Geometry
BIB	313(31948)	(π -π*)	_____	_____	_____
	315(31746)	(π -π*)			
C_1(Ni)	785(12738)	$^3T_{1(F)} \rightarrow {}^3T_{1(P)}$ (υ_2)	131	2.78	Tetrahedral
	962(10395)	$^3T_{1(F)} \rightarrow {}^3A_{2(F)}$ (υ_1)			
	357(28011)	(π -π*)			
C_2(Cu)	739(13531)	$^2B_1g \rightarrow {}^2B_2g$ (υ_2)	169	2.23	Distorted
	960(10416)	$^2B_1g \rightarrow {}^2A_1g$ (υ_1)			Octahedral

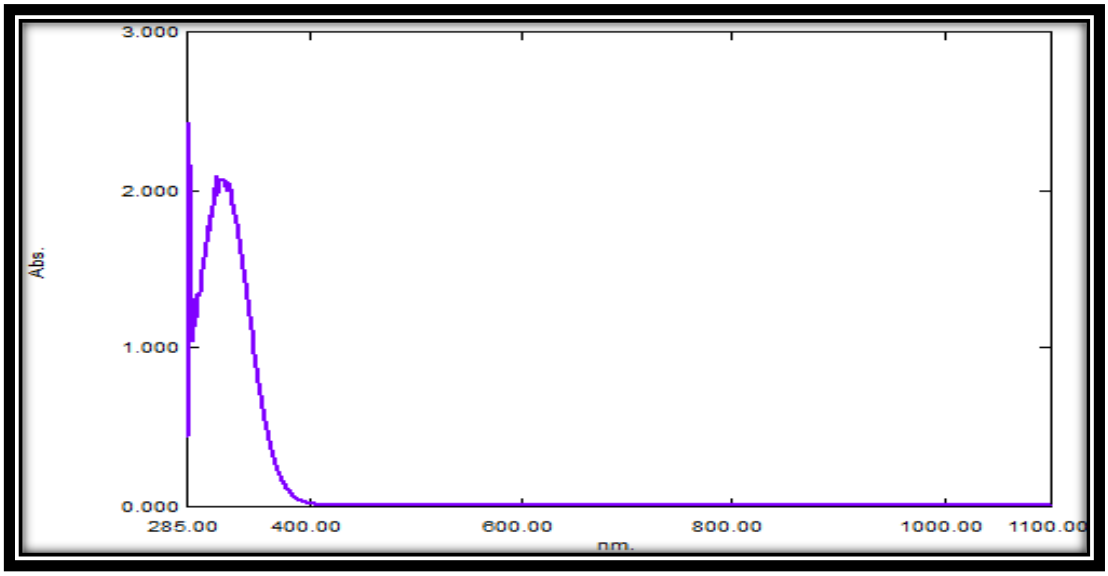

Figure 9. UV-Vis spectrum of BIB

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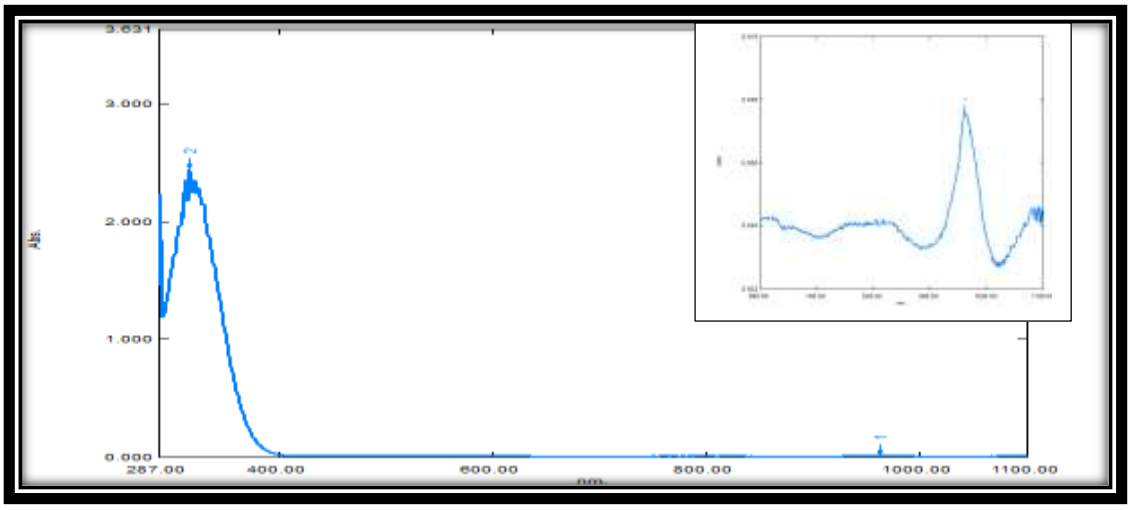

Figure 10. UV-Vis spectrum of C_1 complex.

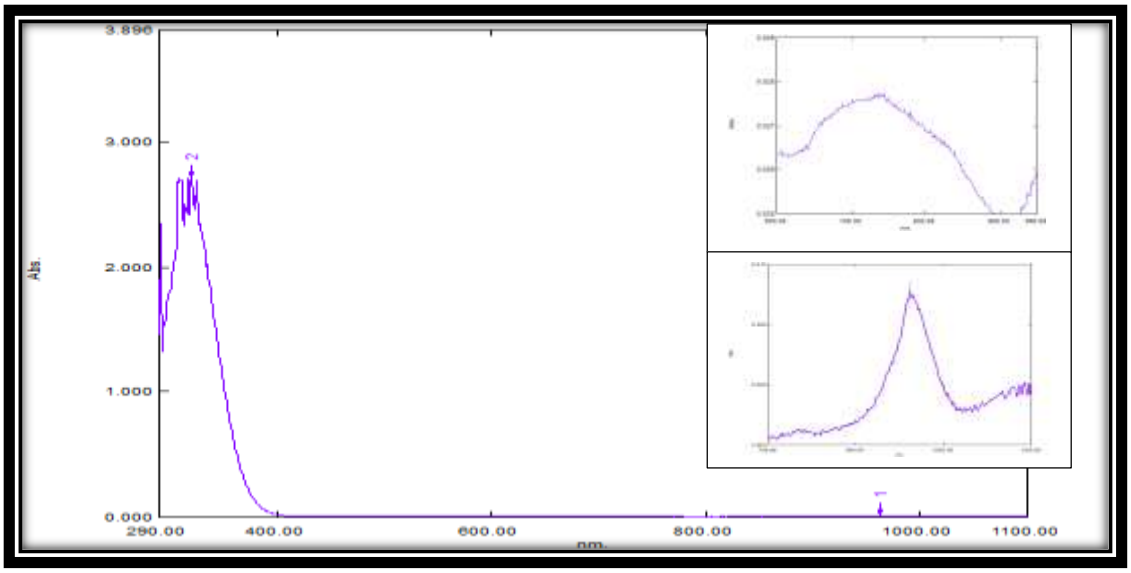

Figure 11.UV-Vis spectrum of C_2 complex.

4.Anti-biofilm Activity

Assessing biofilm formation of *P. aeruginosa* isolates

To quantify biofilm intensity, absorbance at 630nm was determined using a microplate reader.As a result, the absorbance values correlate to the degree of biofilm thickness formed utilizing the isolates in question. Based on the limits summarized the obtained results were categorized into four groups (strong, moderate, weak, and non-biofilm producer) **(Table 7)**. The present study indicated that out of 15 (*Pseudomonas aeruginosa*) isolates, 4 (26.6%) formed a weak biofilm, 5 (33.3%) formed a moderate biofilm, whereas 6 (40.0%) formed a strong biofilm [25-28].

Table 7. Biofilm intensity based on estimated cutoff value*of *P. aeruginosa* isolates.

ID Biofilm	intensity	OD630 Limits number of isolates	Number of isolates	percentage %
1	Non-biofilm producer	< 0.05	0	0
2	Weak	0.05 - 0.10	4	26.6 %
3	Moderate	0.10 - 0.20	5	33.3 %
4	Strong	≥ 0.20	6	40.0 %

cutoff value = 0.05 (defined as the Mean of control OD_{630} plus 3 Standard deviation).

Determination of the (Metronidazole, BIB and all complexes) MIC

MIC is the lowest antibiotic concentration (µg /mL) that inhibits the growth of a given strain of bacteria [29-32]. The susceptibility of the one higher biofilm producer isolate of *P.aeruginosa* (*P.aeruginosa* #5) towards metronidazole, BIB, and all complexes was tested. Variations in susceptibilities to metronidazole, BIB, and all complexes for isolate *(P.aeruginosa* no.5) were observed **(Table 8, Figure 12)**.

Table 8. The MIC and sub-MIC of MTN, BIB and all complexes against *P. aerougenosa* isolates.

Comp	compound code	*P. aerougenosa* isolates	MIC(µg/ml)	Sub MIC (µg/ml)
MTN	As	P_5	1024	512
BIB	As_1	P_5	512	256
C_1Ni	As_3	P_5	1024	512
C_2Cu	As_2	P_5	512	256

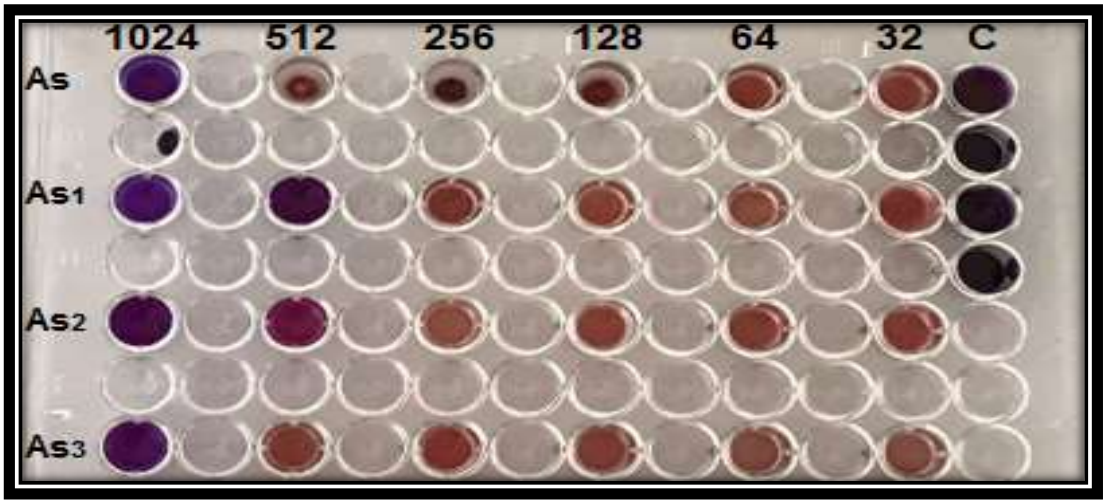

Figure 12. The MIC of MTN, BIB and All complexes against *P. aerougenosa.*

The Effect of Sub MIC of (MTN, BIB and all Complexes) on Bio-film Formation

As shown in **Table 9**, the study's results showed that metronidazole, its ligand (BIB), and all of its complexes at sub-MIC levels were very good at killing the biofilms of bacteria that were being tested. However, the effects of various compounds vary. Obviously, the biofilms were significantly ($P< 0.001$) reduced in isolates C_2Cu>MTZ >BIB > C_1Ni.

Table 9. Anti-biofilm activity of MTN, BIB, MBIB and all complexes sub-MIC.

Comp. code	Bacteria	OD	Before Treatment	After Treatment	*P*
MTN	5	Mean± SD	0.492±0.045	0.277±0.068	< 0.001
BIB	5	Mean± SD	0.492±0.045	0.281±0.086	< 0.001
C_1Ni	5	Mean± SD	0.492±0.045	0.306±0.038	< 0.001
C_2Cu	5	Mean± SD	0.492±0.045	0.222±0.032	< 0.001

Metals such as copper are used as antibacterial agents. The coordination of metal ions with aromatic ligands leads to increase antibacterial and cytotoxic activity [33, 34].

5. Conclusion

The reaction of metronidazole with boric acid generated a new ligand (BIB), and its metal complexes with Ni(II) and Cu(II) were generated in a 2:1 (BIB: M) mole ratio. Spectral and physicochemical techniques were used to characterize each compound that was synthesized. The

proposed structure of the Ni(II) complex was the tetrahedral geometry and the octahedral geometry of the Cu(II) complex, and the results showed that the complexes have electrolytic behavior. Against the Gram-negative bacterium *Pseudomonas auroginosa* (G-), all synthesized compounds were evaluated as anti-biofilm agents. The results showed that in comparison to other compounds, copper complexes were more active.

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