SYNTHESIS OF NEW BORON COMPOUNDS WITH AMOXICILLIN AND SOME OF ITS METAL COMPLEXES WITH USE THEM IN ANTIBACTERIAL, ASSESSMENT OF HEPATOPROTTECTIVE AND KIDNEY ACTIVITY, ANTICANCER AND ANTIOXIDANT APPLICATIONS

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ABSTRACT

Objective: New ligand (7-[2-amino-2-(4-hydroxyphenyl)-acetamido-3,3-dimethyl-6-oxo-2-thia-5-aza bicyclo[3,2,0]heptane-4-carboxylic boric anhydride]) with its Co (II), Ni (II) and Cu (II) complexes. And new mixed ligand copper complex was synthesized.

Methods: The ligand was synthesized by the reaction of boric acid with amoxicillin (1:1) and the mixed ligand complex has been synthesized by the reaction of the ligand, 4-aminoantipyrine and Cu (II) ion (1:1:1).

Results: All studied compounds were characterized by the spectral method: Fourier transform infrared, ultraviolet-visible, thermal analysis (TG and DTG), flame atomic absorption and nuclear magnetic resonance. Also CHNS, melting point, magnetic susceptibility and molar conductivity.

Conclusion: According to the obtained data, all complexes were non electrolyte and the geometry was octahedral for all complexes. All synthesized compounds were tested as antibacterial agents against Escherichia Coli, Pseudomonas aeruginosa as Gram-negative bacteria (G-) and Staphylococcus aureus as Gram-positive bacteria (G+). The results showed that copper complexes were more active in (10-2M) than the other compounds.

The medicinal applications (hepatoprotective and kidney in serum of mice, histopathological of liver and kidney, anticancer and antioxidant in human cell were studied of the synthesized compounds and gave a good results in all tested.

Keywords: Boron compounds, Boric acid, Amoxicillin, Anticancer, Antioxidant.

INTRODUCTION

Boric acid and borates are exists in water, food and soil. They are used as least toxic pesticides to kill insects, mites, fungi, algae, fleas and wood decay fungi [1]. The mechanism of killing contains working to poison the stomach and absorbing the waves which protect insects. Also boric acid used as antiseptic in talcum powder, mouth-washes, eyewashes and protective ointments. Another application includes reducing the flammability of cellulosic materials used in production of leather [2]. Amoxicillin trihydrate (2-Amino-2,3,3-dimethyl-6-oxo-2-thia-5-aza bicyclo[3,2,0]heptane-4-carboxylic acid) is one of aromatic compounds which has a special properties and used as antipyretic, anti-inflammatory, analgesic, antimicrobial and anticancer [6].

In the present work we are synthesis a new derivative of amoxicillin with boric acid, also the metal complexes of this derivative (ligand) with Co (II), Ni (II) and Cu (II) ions were synthesized. We are synthesis a mixed ligand complex from the reaction of amoxicillin and 4-aminoantipyrine with Cu (II) ion. The medicinal applications and the biological activity were studied for all synthesized compounds.

EXPERIMENTAL PART

Chemicals

All chemicals were used as received without further purification (supplied by BDH, Spain, Aldrich, Merck, Fluka AG, Buchs SG, LOBA Chime, Hayman, Fluka-Garantie and fieser).

METHODS

Synthesis of 7-[2-amino-2-(4-hydroxyphenyl)-acetamido-3,3-dimethyl-6-oxo-2-thia-5-aza bicyclo[3,2,0]heptane-4-carboxylic boric anhydride (L) (Fig. 1)

The mixture of amoxicillin trihydrate (0.1 g, 0.238 mmol) in 10 mL H2O and boric acid (0.0147 g, 0.238 mmol) in 1 mL H2O was heated under reflux for 17 h with stirring. The resulting solution was heated to evaporate a part of solvent and then diethyl ether was added in presence of ice-bath and crushing to precipitate the yellow product, washed with hot water and dried in oven.

Synthesis of metal complexes with Co (II), Ni (II) and Cu (II) (C1-C3)

The mixture of L (0.1 g, 0.238 mmol) in 5 mL methanol and metal salt (0.244 mmol, 0.058, 0.057 and 0.041 g) of CoCl2·6H2O, NiCl2·6H2O and CuCl2·2H2O respectively in 1 mL methanol was heated under reflux for (5) h with stirring. The part of solvent was evaporated and diethyl ether was added in presence of ice-bath and crushing to increase the quantity of product, washed with methanol and dried in oven.

Synthesis of mixed ligand complex with Cu (II) ion (Mix.)

The mixture of L (0.115 g, 0.281 mmol) in 3 mL methanol, 4-aminoantipyrine (0.057 g, 0.281 mmol) in 1 mL methanol and CuCl2·2H2O (0.048 g, 0.281 mmol) in 1 mL methanol was heated under reflux for 5 h. Part of solvent was evaporated and diethyl ether was added in presence of ice-bath and crushing to increase the product. The black product washed several time with methanol and dried in oven.

Antibacterial activity

Antibacterial activity of the ligand and its metal complexes was studied against Pseudomonas aeruginosa and Escherichia Coli as Gram-negative (G-) and S. aureus as Gram-positive (G+) by the agar well-diffusion
RESULTS AND DISCUSSION

Physical properties and elemental analysis

The physical properties, elemental and analytical data are illustrated in Table 1.

FT-IR Spectroscopy

The FT-IR spectrum of the ligand showed bands at 3456, 3379 cm\(^{-1}\) which refer to stretching vibration of NH\(_2\) [16]. The band of v N-H (amide) appeared at 3205 cm\(^{-1}\) [16]. The spectrum of exhibited absence of carboxylic OH band (3300–2621 cm\(^{-1}\) in amoxicillin) and appear new band at 1342 cm\(^{-1}\), which due to O=O [17, 18]. The spectrum showed band at 3525 cm\(^{-1}\) which was assigned to phenolic OH group [16]. The band of v C=O (carboxylic and β-lactam) was appeared at 1770 cm\(^{-1}\) [16, 18]. While the band of v C=O of amide appeared at 1683 cm\(^{-1}\) [16]. The data can be shown in Table 2. The spectra of complexes C\(_2\)C\(_2\) Mix showed shifting in some band positions. The bands of v NH shifted to higher frequency of asymmetry bands and to lower frequency of symmetric bands (Table 2) and this refer to coordination with metal ions through the nitrogen atom of NH\(_2\) [4]. The band of v NH (amide) was shifted to higher frequency in all complexes and this is attributed to coordination with metal ions. The spectrum of mixed ligand showed a new band at 1560 cm\(^{-1}\) due to imine (υ C=N) [19]. All spectra of complexes exhibited new bands at lower frequency which refer too M-O, v M-N and v M=Cl [20]. The data can be shown in Table 2.

NMR Spectroscopy

\(^1\)H-NMR and \(^{13}\)CNMR were used to characterized the ligand (L) and its metal complexes using d\(^6\)-DMSO as solvent.

\(^1\)H-NMR Spectroscopy

The spectra of all compounds showed peak at 8.25 ppm which refer to chemical shift of DMSO as a solvent. The spectra of L exhibited absence the proton peak of carboxylic OH (about δ 10 ppm in amoxicillin) [21] and appear new proton peak of B-OH at δ 8.74 pp [22] (Table 3). The spectra of Co (II), Ni (II) and Cu (II) complexes (C\(_2\)-C\(_2\)) were showed shifting to higher value in proton peak position of N-H amide and NH\(_2\) groups (Tables 4-6) in a comparison with the spectrum of ligand (L) [21]. The spectrum of (Mix.) exhibited further proton peaks which attributed to 4-aminoantipyrine [19]. The spectrum of (Mix.) also showed shifting in position of chemical shift of N-H amide and NH\(_2\) group in the ligand (Table 7) because the complexation with metal ions through nitrogen atoms of N-H amide and NH\(_2\). The spectrum of (Mix.) also showed shifting to higher values in position peaks of N-CH and this is because formation of imin group in neighboring atom [21]. The spectra of the ligand and C\(_2\) can be shown in Figs. 2-4.

\(^{13}\)CNMR Spectroscopy

The \(^{13}\)CNMR data are listed in (Tables 8-12). The chemical shift of DMSO as a solvent appeared at δ 40 ppm. The \(^{13}\)CNMR spectrum of the ligand (L) showed shifted to higher values of carboxylic C=O group

| Comp. | The molecular formula | Color | m.p (°C) | Yield% | M.wt | Micro elementa analysis found (Calc.) | Metal content% | Chloride content% |
|-------|-----------------------|-------|----------|--------|------|--------------------------------------|----------------|------------------|
|       |                       |       |          |        |      | C% | H% | N% | S% |                      |                |                 |
| L     | C\(_2\)H\(_6\)N\(_2\)O\(_2\)SB | Yellow | 182–184 | 51.54  | 468.82 | 46.39 (46.96) | 3.2 (4.89) | 10.04 (10.27) | 7.13 (7.82) | --            |                |
| C1    | C\(_2\)H\(_6\)N\(_2\)O\(_2\)SBCo. 2Cl 2H\(_2\)O | Green | 210–212 | 42.85  | 574.73 | 33.21 (33.41) | 4.43 (4.18) | 7.21 (7.31) | 5.48 (5.56) | 10.13 (10.25) | 12.12 (12.35) |
| C2    | C\(_2\)H\(_6\)N\(_2\)O\(_2\)SBNi. 2Cl 2H\(_2\)O | Brown | 240–242 | 50.00  | 574.49 | 34.33 (33.42) | 4.13 (4.20) | 7.18 (7.31) | 5.64 (5.57) | 9.93 (10.21) | 12.24 (12.35) |
| C3    | C\(_2\)H\(_6\)N\(_2\)O\(_2\)SBCu. 2Cl 2H\(_2\)O | Black | 140 Dec | 57.14  | 579.366 | 34.01 (33.14) | 4.22 (4.14) | 7.44 (7.25) | 5.01 (5.52) | 10.70 (10.97) | 12.05 (12.25) |
| Mix.  | C\(_2\)H\(_6\)N\(_2\)O\(_2\)SBCu. 2Cl 2H\(_2\)O | Black | 228–230 | 52.08  | 764.36 | 42.39 (43.28) | 4.32 (4.57) | 10.62 (10.98) | 4.22 (4.18) | 8.12 (8.31) | 8.99 (9.28) |

Dec: Decompose, M.wt: Molecular weight
comparison with parent drug [23] and this is because the binding with B(OH)₂. The spectra of metalcomplexes (C₁–C₃) exhibited shifted to higher values in the chemical shift of C=O (β-lactam), C=O amide and CH-NH₂ and this is attributed to complexation with metal ions [23].

In (Mix.) spectrum, the carboxylic C=O band absent and appeared a new bands at δ 159.36, which assigned to δ C=N (imino group) [19]. As well as the C=O (β-lactam), C=O (amide) and CH-NH₂ bands were shifted to higher values and this is because coordination with metal ions.

### Table 2: Characteristic infrared absorption bands of the ligand and its metal complexes

| Comp | δNH₂ | δNH amide | δOH carboxylic Acid | δOH phenolic | δC=O β-lactam and carboxylic Acid | δC=O amide | δB-O | δC=N | δM-O | δM-N | δM-Cl |
|------|------|-----------|---------------------|--------------|----------------------------------|------------|------|------|------|------|------|
| L    | 3456 | 3205      | ----                | 3525         | 1770                             | 1683       | 1342 | ---- | ---- | ---- | ---- |
| C1   | 3471 | 3232      | ----                | 3527         | 1730                             | 1645       | 1345 | ---- | ---- | 435  | 568  | 389  |
| Co   | 3350 | 3235      | ----                | 3533         | 1750                             | 1652       | 1346 | ---- | ---- | 438  | 565  | 362  |
| C2   | 3482 | 3235      | ----                | 3533         | 1750                             | 1652       | 1346 | ---- | ---- | 438  | 565  | 362  |
| Ni   | 3355 | 3251      | ----                | 3529         | 1733                             | 1654       | 1340 | ---- | ---- | 489  | 568  | 360  |
| Cu   | 3483 | 3225      | ----                | 3523         | 1750                             | 1652       | 1334 | 1560 | 536  | 449  | 366  |

### Table 3: 1HNMR data for the ligand

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| OH Phenolic | 9.33 (1H), s |
| B-OH | 8.74 (2H), s |
| N-H Amide | 8.64 (1H), s |
| C-H aromatic | 7.37-7.23 (4H), m |
| CHNH (β-lactam) | 5.62 (1H), d |
| CHS (β-lactam) | 5.32 (1H), d |
| NH₂ | 4.98 (1H), s |
| CH-NH₂ | 4.98 (1H), s |
| N-CH | 3.99 (1H), s |
| 2CH₃ | 1.49 (3H), s, 1.40 (3H), s |

DMSO: Dimethyl sulfoxide

### Table 4: 1HNMR data for C1

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| OH Phenolic | 9.20 (1H), s |
| N-H Amide | 9.09 (1H), s |
| B-OH | 8.83 (2H), s |
| C-H aromatic | 8.03-6.50 (4H), m |
| CHNH (β-lactam) | 5.69 (1H), d |
| CHS (β-lactam) | 5.42 (1H), d |
| NH₂ | 5.29 (2H), s |
| CH-NH₂ | 5.29 (1H), s |
| N-CH | 3.96 (1H), s |
| H₂O | 3.57 (4H), s |
| 2CH₃ | 1.29 (3H), s, 1.16 (3H), s |

DMSO: Dimethyl sulfoxide

### Table 5: 1HNMR data for C2

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| OH Phenolic | 9.26 (1H), s |
| N-H Amide | 8.99 (1H), s |
| B-OH | 8.85 (2H), s |
| C-H aromatic | 8.02 – 6.50 (4H), m |
| CHNH (β-lactam) | 5.70 (1H), d |
| CHS (β-lactam) | 5.47 (1H), d |
| NH₂ | 5.32 (2H), s |
| CH-NH₂ | 5.32 (1H), s |
| N-CH | 3.99 (1H), s |
| H₂O | 3.43 (4H), s |
| 2CH₃ | 1.67 (3H), s, 1.38 (3H), s |

DMSO: Dimethyl sulfoxide

As well as the C=O (β-lactam), C=O (amide) and CH-NH₂ bands were shifted to higher values and this is because coordination with metal ions.
Electronic spectra
The electronic spectra of synthesized compounds were recorded in methanol (10−4 M) at room temperature. The spectral data were listed in Table 13. The electronic spectrum of ligand exhibited high intensity bands in 356 nm (28089 cm−1), which due to π→π* transition [24]. In addition the spectrum of ligand showed low intensity bands at 373 nm (26809 cm−1), which assigned to nπ*→ π* transition [24]. The data were listed in Table 13. The spectrum of C1 complex showed change in position of π→π* transition (Table 14). The spectrum of C1 complex exhibited two bands at 585 nm (17049 cm−1) and 805 nm (12422 cm−1) which assigned to T1g→T2g (p) and T1g↔T2g respectively [25]. The magnetic moment value of C1 complex were found (Meff= 4.55 B.M) and this value is agreement with octahedral geometry [25]. The data were listed in Table 14. The spectrum of Ni (II) complex showed two bands at 610 nm (16393 cm−1) and 951 nm (10515 cm−1) which due to 3A1g→3T1g(F) and 3A1g→3T2g transition of octahedral Ni (II) complexes [25, 26]. The Meff of nickel C1 were 3.42 B.M. This value agreement with octahedral geometry [25-27]. The data were listed in Table 13. The spectra of copper complexes (C2 and Mix.) complexes showed change in nπ*→π* transition (Table 14). The spectrum of C2 complex showed three bands at 541 nm (18484 cm−1), 651 nm (15360 cm−1) and 761 nm (13140 cm−1) which due to B1g↔Eg, B2g↔B2g and B2g↔A1g transitions (Table 14) of distorted octahedral copper complexes [25] (Fig. 5). The spectrum of mixed ligand complex (Mix.) (Fig. 6) exhibited one

Table 6: 1H NMR data for C3

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| OH Phenolic            | 9.35 (1H), s             |
| N-H Amide              | 9.00 (1H), s             |
| B-OH                   | 8.75 (1H), s             |
| C-H aromatic           | 8.03–6.74 (4H), m        |
| CHNH (β-lactam)        | 5.76 (1H), d             |
| CHS (β-lactam)         | 3.55 (2H), s             |
| NH2                    | 5.20 (2H), s             |
| CH-NH2                 | 3.94 (1H), s             |
| H2O                    | 3.36 (4H), s             |
| 2CH3                   | 1.55 (3H), s 1.40 (3H), s|

DMSO: Dimethyl sulfoxide

Table 7: 1H NMR data for mix

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| OH Phenolic            | 9.41 (1H), s             |
| N-H Amide              | 9.04 (1H), s             |
| B-OH                   | 8.88 (2H), s             |
| C-H aromatic           | 7.87–6.55 (9H), m        |
| CHNH (β-lactam)        | 5.76 (1H), d             |
| CHS (β-lactam)         | 5.36 (1H), d             |
| NH2                    | 5.29 (2H), s             |
| CH-NH2                 | 5.29 (1H), s             |
| N-CH                   | 4.32 (1H), s             |
| H2O                    | 3.40 (4H), s             |
| N-CH3                  | 3.40 (3H), s             |
| C-CH3                  | 2.50 (3H), s             |
| 2CH3                   | 1.63 (3H), s 1.51 (3H), s|

DMSO: Dimethyl sulfoxide

Table 8: 13C NMR data for the Ligand

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| COOB                   | 173.36                   |
| C=O (β-lactam)         | 170.16                   |
| C=O (amide)            | 160.15                   |
| C (heterocyclic ring)  | 139.92                   |
| Aromatic carbon        | 130.30–112.80            |
| CHNH (β-lactam)        | 61.17                    |
| CHS (β-lactam)         | 58.96                    |
| CH-NH2                 | 57.84                    |
| C-S                    | 26.51                    |
| 2CH3                   | 20.30, 18.15             |

Table 9: 13C NMR data for C1

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| C=O (β-lactam)         | 177.33                   |
| C=O (amide)            | 175.50                   |
| COOB                   | 173.70                   |
| C (heterocyclic ring)  | 140.40                   |
| Aromatic carbon        | 130.24–113.38            |
| CH-NH2                 | 64.39                    |
| CHNH (β-lactam)        | 61.53                    |
| CHS (β-lactam)         | 59.39                    |
| C-S                    | 25.51                    |
| 2CH3                   | 17.58, 16.35             |

Table 10: 13C NMR data for C2

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| C=O (β-lactam)         | 176.63                   |
| C=O (amide)            | 175.43                   |
| COOB                   | 173.18                   |
| C (heterocyclic ring)  | 137.54                   |
| Aromatic carbon        | 133.55–115.18            |
| CH-NH2                 | 63.35                    |
| CHNH (β-lactam)        | 62.79                    |
| CHS (β-lactam)         | 58.53                    |
| C-S                    | 26.88                    |
| 2CH3                   | 18.48, 15.83             |

Table 11: 13C NMR data for C3

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| C=O (β-lactam)         | 176.22                   |
| C=O (amide)            | 174.52                   |
| COOB                   | 173.18                   |
| C (heterocyclic ring)  | 138.54                   |
| Aromatic carbon        | 132.84–112.13            |
| CH-NH2                 | 63.35                    |
| CHNH (β-lactam)        | 62.79                    |
| CHS (β-lactam)         | 58.53                    |
| C-S                    | 26.88                    |
| 2CH3                   | 20.87, 18.18             |

Table 12: 13C NMR data for Mix

| Assignments in d6-DMSO | Chemical shifts δ (ppm) |
|------------------------|-------------------------|
| C=O (β-lactam)         | 176.71                   |
| C=O (amide)            | 175.35                   |
| C=N C=O (4-aminoantipyrine) | 159.36                 |
| C (heterocyclic ring)  | 140.43                   |
| C (4-aminoantipyrine)  | 134.54                   |
| Aromatic carbon        | 134.54–110.27            |
| C=O (4-aminoantipyrine) | 115.72                 |
| CH-NH2                 | 66.31                    |
| C (β-lactam)           | 63.53                    |
| C (β-lactam)           | 57.94                    |
| N-CH3                  | 39.08                    |
| C-S                    | 23.57                    |
| 2CH3                   | 21.69, 18.37             |
| CH3 (4-aminoantipyrine) | 18.37                  |
Table 13: Electronic transitions, molar conductivity, spectra, magnetic susceptibility and suggested geometry of the ligand and its metal complexes

| Comp | Band positions nm (cm⁻¹) | Assignment | Molar conductivity (S.cm².mol⁻¹) in Methanol | µeff. (B.M) | Suggested geometry |
|------|--------------------------|------------|---------------------------------------------|-------------|--------------------|
| L    | 356 (28089) 370 (27027) | (π→π*)     | -----                                       | -----       | -----              |
| C1   | 363 (27548) 585 (17094) 805 (12422) | (π→π*) | 25                                           | 4.55        | Octahedral         |
|      | 351 (28490) 610 (16393) 951 (10515) | (T₂g→T₁g (P)) |                                           |             |                    |
|      | 363 (27548) 541 (18484) 651 (15360) 761 (13140) | (π→π*) | 30                                           | 1.96        | distorted octahedral |
| C3   | 363 (27548) 541 (18484) 651 (15360) 761 (13140) | (π→π*) | 42                                           | 1.89        | distorted octahedral |
| Misc. | 363 (27548) 758 (13192) | (π→π*)     | -----                                       | -----       |                    |

Table 14: Thermal decomposition data of the ligand (L) and its metal complexes

| Molecular formula and Molecular weight | Step | Temp. rang of the Decomposition °C | DTG Temp °C | Suggested | Mass loss% |
|---------------------------------------|------|------------------------------------|-------------|-----------|-----------|
|                                       |      |                                    |             |           |           |
| C₃H₆N₂O₂SB                           | 1    | 35–313.95                          | 101, 225    | H₂BO₂, 2CH₂, OH | 26.36    |
|                                       | 2    | 313.95–644.86                      | 330, 375, 410, 517, 625 | NH₃, SClHClD | 24.7     |
|                                       | 408.82 |                                    |             | HO(C₂H₅)CHCONH(C₂H₅)NO | 48.92 |
| C₃H₆N₂O₂SCo.                          | 1    | 40–184.70                          | 55, 100     | 2H₂O, OHH | 9.22     |
| 2Cl₂ZH₂O                              | 2    | 184.70–272.07                      | 235         | 2Cl₂OH, CH₂ | 17.92 |
| 574.73                                | 3    | 272.07–642.59                      | 300, 330, 450, 595, 625 | (HO)C₂H₅CH(NH₂)COCH₂ | 28.71    |
|                                       |      |                                    |             | NHCH₂(CO)CH(N)SCCH₂OBCo | 44.14 |
|                                       |      |                                    |             | H₂O       | 3.13     |
| Ni₂Cl₂ZH₂O                            | 2    | 189.13–259.07                      | 227         | H₂O, 2Cl₂ | 15.49 |
| 574.49                                | 3    | 259.07–644.69                      | 275, 318, 400, 475, 558, 583, 633 | SC₁₂(H₂O)₂COOB(OH)₂ | 30.6 |
|                                       |      |                                    |             |           | 30.8     |
|                                       |      |                                    |             |           |          |
| C₃H₆N₂O₂SBCu                          | 1    | 25–258.56                          | 45, 85, 100 | HO(C₂H₅)CH(NH₂) | 50.77 |
| 2Cl₂ZH₂O                              | 2    | 125.56–384.19                      | 230, 280    | CONH₂(C₂H₅)NO | 13.94 |
| 579.36                                | 3    | 384.19–644.61                      | 440, 465, 520, 590, 635 | 2Cl₂NH₂CO₂ | 22.6 |
|                                       |      |                                    |             |           | 22.59    |
| C₃H₆N₂O₂SBCo.                         | 1    | 25–161.83                          | 40, 75, 100, 120 | 2Cl₂OH, CH₂ | 12.42 |
| Cu₂ZH₂O                               | 2    | 161.83–230.82                      | 187         | Cu₂O        | 51.01 |
| 764.36                                | 3    | 230.82–360.37                      | 285, 345    | C₂(C₂H₅)N(CH₂)N(C₂H₅)CO₂SC₂OH | 34.66 |
|                                       |      |                                    |             |           | 34.97    |
|                                       |      |                                    |             |           |          |
| Table 15: The biological activity for compounds in 10⁻² and 10⁻³ M

| Compound | P. aureginosa Inhibition zone diameter (mm) | S. aureus Inhibition zone diameter (mm) | E. coli Inhibition zone diameter (mm) | Suggested |
|----------|---------------------------------------------|----------------------------------------|--------------------------------------|-----------|
|          | 10⁻² | 10⁻³ | 10⁻² | 10⁻³ | 10⁻² | 10⁻³ |
| DMSO     |      |      |      |      |      |      |
| Amoxicillin |      |      |      |      |      |      |
| Boric acid |      |      |      |      |      |      |
| L        |      |      |      |      |      |      |
| C1       |      |      |      |      |      |      |
| C2       |      |      |      |      |      |      |
| C3       |      |      |      |      |      |      |
| Mix.     |      |      |      |      |      |      |

Fig. 5: Structure of the complex (C₃, C₂ and C₁)

band at 758 nm (13192 cm⁻¹), this band due to B₂g→A₁g transition of distorted octahedral Cu (II) complex [25] (Fig. 7). The magnetic moment of copper complexes were 1.96 and 1.89 B.M of C₂ and Mix. respectively, these values of Meff agreement with distorted octahedral geometry [25]. All complexes exhibited a nonelectrolyte behavior [28] (Table 13).
Thermal analysis of the ligand and its metal complexes
The TG and DTG analysis were performed under nitrogen gas in the range heating 16–650°C and the heating rate (10°C/min). This technique was used to study the thermal stability of synthesized compounds as well as to characterize the suggested structures. The thermal decomposition data were listed in (Table 14) and the thermographs of the ligand (L) and C₁ complex were shown in (Figs. 2 and 8). The results showed that the stability of the ligand and its complexes was increase as the following order Mix. < C₁ < L₁ < C₂ < C₃. The results of degradation exhibited good agreement in percentage of calculate and found mass loss and this confirm the suggested structures of synthesized compounds [29].

Biological activity
The antibacterial activity of the ligand (L) and its metal complexes have been evaluated against (P. aeruginosa, E. coli (G-) and Staphylococcus aureus (G+)). The bacterial activity was test with two concentration (10⁻² and 10⁻³ M) of the primary materials and all synthesized compounds. The C₁ and Mix. in 10⁻² M were the most effective against the studied microorganisms. The ligand (L) exhibited small activity with S. aureus comparition with its complexes. All synthesized compounds in 10⁻³ M were more active from the parint drug against S. aureus. The antibacterial data were listed in (Table 15). DMSO solvent was used as control [30].

Hepatoprotective and kidney evaluation
Hepatoprotective evaluations included assessment of liver function enzyme (GOT, GPT and ALP) and renal function test (Urea, Creatinine, TSP and Alb) in serum. The results were listed in (Tables 16 and 17). The obtained results showed the positive effect of all studied compound on GOT, GPT, ALP, Urea, Creatinine, TSP and Alb.

Histopathological evaluation
The results of histopathological evaluation of liver and kidney tissue which treated with C₁ and Mix. showed the positive effect...
of studied compounds and no negative effect were on tissue observed.

**Anticancer activity (cytotoxicity assays)**

Study of anticancer activity for amoxicillin, L, C<sub>3</sub>, and Mix was carried out on different cancer cell lines (AMJM, SK-7 and CMF-3). The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

\[
\text{Inhibition rate} = \frac{A - B}{A} \times 100
\]

Where A and B are the optical density of control and the optical density of test.

The study of anticancer activity for the ligand exhibited high percentage of cytotoxicity comparison with amoxicillin, this is due to presence of boron element and this is agreement with literature [31]. The increase in percentage of cell inhibition due to presence of β-lactam ring in all studied compounds as well as presence of aromatic ring in Mix (4-Aminobenztpyrine) and this is enhanced the anticancer activity [32]. Also found that the Schiff base ligand improve the anticancer activity [33]. The Schiff base linkage (–C=N) is an essential structural requirement for antitumor activities [34, 35]. Cytotoxicity assays of ligand and its complexes are shown in Tables 18-20 and Figs. 9-11 for L and C<sub>3</sub>.

**DPPH radical scavenging activity (RSA) (antioxidant activity)**

2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used in RSA for the evaluation of antioxidant and this is the rapid technique for screening the RSA of specific compounds or extracts [35]. DPPH is a stable free radical that can accept an electron or hydrogen radical and get converted to a stable, diamagnetic molecule. The results of this study showed that the ligand exhibited a negligible DPPH activity. In

### Table 16: Effect of the Ligand (L) and its metal complexes on liver function enzymes (GOT, GPT and ALP) in sera of albino male mice

| Groups | Dose (mg/Kg) | GOT (Mean±Standard error of mean) | GPT (Mean±Standard error of mean) | ALP (Mean±Standard error of mean) |
|--------|--------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Control   | 0.625        | 38.50±0.50                        | 50.50±2.50                        | 64.00±4.00                        |
| L         | 0.625        | 21.00±1.00                        | 35.50±1.50                        | 49.00±3.00                        |
| C<sub>3</sub> | 0.625        | 27.50±0.50                        | 50.00±2.50                        | 84.50±4.50                        |
| Mix.      | 0.625        | 42.00±1.50                        | 58.90±4.00                        | 90.50±3.50                        |

GOT: Glutamic oxaloacetic transaminase, GPT: Glutamate pyruvate transaminase, ALP: Alkaline phosphatase

### Table 17: Effect of the Ligand (L) and its Metal Complexes on Renal Function Test (Urea, Creatinine, Alb and TSP) in Sera of Albino Male Mice

| Groups | Dose (mg/Kg) | Urea (Mean±Standard error of mean) | Creatinine (Mean±Standard error of mean) | Alb (Mean±Standard error of mean) | Total protein (Mean±Standard error of mean) |
|--------|--------------|-----------------------------------|------------------------------------------|-----------------------------------|---------------------------------------------|
| Control | 0.625        | 31.55±1.45                        | 0.55±0.045                               | 3.05±0.15                         | 7.65±0.35                                   |
| L       | 0.625        | 42.70±2.70                        | 0.31±0.01                                | 2.80±0.10                         | 7.95±0.35                                   |
| C<sub>3</sub> | 0.625        | 32.70±0.10                        | 0.085±0.005                              | 3.00±0.10                         | 8.05±0.05                                   |
| Mix.    | 0.625        | 33.95±1.25                        | 0.065±0.005                              | 2.95±0.05                         | 8.40±0.05                                   |

Alb: Albumin, TSP: Total serum protein

### Table 18: Cytotoxicity Assays (AMJM) Cell of the Ligand and its Metal Complexes

| Comp. | Conc. (6.125 µg/mL) | Conc. (12.5 µg/L) | Conc. (25 µg/mL) | Conc. (50 µg/m) | Conc. (100 µg/L) |
|-------|---------------------|-------------------|------------------|-----------------|-----------------|
| Amoxicillin | 2.78                | 4.16              | 4.16             | 4.16            | 2.78            |
| L     | 6.67                | 8.33              | 13.33            | 18.33           | 23.33           |
| C<sub>3</sub> | 16.18              | 20.59             | 32.35            | 44.12           | 61.77           |
| Mix.  | 16.07               | 32.04             | 48.08            | 49.84           | 65.86           |

### Table 19: Cytotoxicity Assays (SKOV-3) Cell of the Ligand and its Metal Complexes

| Comp. | Conc. (6.125 µg/L) | Conc. (12.5 µg/L) | Conc. (25 µg/mL) | Conc. (50 µg/mL) | Conc. (100 µg/mL) |
|-------|-------------------|-------------------|------------------|-----------------|------------------|
| Amoxicillin | 2.70                | 2.09              | 4.16             | 2.71            | 5.55             |
| L     | 6.67                | 8.33              | 13.33            | 18.33           | 23.33           |
| C<sub>3</sub> | 7.69               | 17.30             | 29.34            | 32.69           | 42.30           |
| Mix.  | 7.81               | 9.33              | 15.92            | 26.56           | 32.81           |

### Table 20: Cytotoxicity assays (MCF-7) cell of the ligand and its metal complexes

| Comp. | Conc. (6.125 µg/L) | Conc. (12.5 µg/L) | Conc. (25 µg/mL) | Conc. (50 µg/mL) | Conc. (100 µg/mL) |
|-------|-------------------|-------------------|------------------|-----------------|------------------|
| Amoxicillin | 3.12                | 2.34              | 4.68             | 3.12            | 5.46             |
| L     | 2.63                | 3.94              | 6.57             | 6.57            | 8.54             |
| C<sub>3</sub> | 16.70              | 21.71             | 25.05            | 25.05           | 25.05           |
| Mix.  | 20.00              | 34.15             | 42.25            | 53.44           | 61.79           |
Table 21: DPPH RSA for the ligand and its metal complexes with comparison ascorbic acid

| Comp. | Conc. (25 µg/mL) | Conc. (50 µg/mL) | Ascorbic acid |
|-------|-----------------|-----------------|---------------|
|       | % Scavenging activity |                  |               |
| L     | 30              | 46              | 78            |
| C1    | 30              | 56.25           | 85            |
| C2    | 33.33           | 40              | 73.33         |
| C3    | 40              | 71.11           | 91.10         |
| Mix   | 37.78           | 6.22            | 77.78         |

RSA: Radical scavenging activity

complexes, the results showed that the scavenging activity % of C3 and Mix. (Cu (II) complexes) were more than the free ligand and this is due to the presence of Cu (II) ion [36]. while the other complexes exhibited different scavenging activity. (Table 21)

CONCLUSION
New compound was synthesized by the insertion of boron on amoxicillin, also some metal complexes of this ligand were synthesized as well as we are synthesized the mixed ligand (copper complex with ligand and 4-aminoantipyrine) was synthesized. All compounds were characterized by different techniques. The antibacterial activities for all compounds were evaluated. The results showed that copper complexes were more active in 

authors' contribution
All authors have contributed equally.

CONFLICTS OF INTEREST
Authors have no conflicts of interest.

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