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Synthesis, Characterization, and Biological Applications of New Series of Azo Triazole Organometallic Derivatives and Their Silver Nanoparticle Forms

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SOME metal salts can react with 3-amino-1,2,4-triazole derivatives as Ni(II) acetate, Co(II) acetate and Cu(II) acetate afforded metallated products **Ia-d**, **IIa-d,** and **IIIa-e** in the form of mono-metal, dimetal, and metal bis products. Elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral elucidated the structures of the newly synthesized compounds.

Some of the azo metal compounds were treated with silver nanoparticles. Transmission electron microscopy is used to determine the morphology and particles size via TEM image of nanoparticles and nano form of compounds. The antimicrobial activity of the recently synthesized compounds and its nano form has been measured against gram-positive and gram-negative bacteria and fungi.

Keywords: Organometallic, Azo-triazole, Copper acetate, Nickel acetate and cobalt acetate, Silver nano particles, Antimicrobial activity.

Introduction

The most important compounds of synthetic organic dye are azo dyes which are widely used as colorants in the textile industries,[1-2] also they have several other applications[3-4] as, for example, colorants for advanced printing and photography[5]; medication, food and cosmetic applications. Some dyes have been used in the biomedical field.[6] Dyes based on heterocyclic amines have been studied widely due to their good thermal,[7-8] optical[9] and medicinal properties, such as antibacterial,[10] antiviral,[11] antifungal[12] and antioxidant activities.[13] Many kinds of azo dyes have been synthesized but azo triazole derivatives are relatively rare.[14]

Organometallic compounds also have great attention for research due to the presence of carbon-metal bond which can either be sigma bond by the direct carbon-metal bond or pi–bond and lone pair by a metal complex bond. An organometallic compound, as a rule, contains both metals, transition metals, Semimetals, Lanthanides, and Actinides. Their chemical bonding reactivity and stabilities are predicted by using 18-electron rule. Some are normal covalent bonds, in which sets of electrons are shared between atoms, others are multicentre covalent bonds in which the bonding includes multiple molecules[15] what's more, the third sort is ionic bonds, in which the holding electron combine is given by just a single atom,[16] in donor-acceptor bonds, the metal particle is associated with hydrocarbons with various bonds between carbon atoms.

When metal atoms form covalent bonds with carbon atoms, the electrons are typically shared unequally. Therefore, the bond is polarized; numerous organometallic compounds have large

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activities towards chemical synthesis.[Kirsch, 2013 #72][17-19] The organo-magnesium halides (Grignard reagents), for instance, are used broadly in synthetic organic chemistry, like organo-lithium and organo-boron compounds. Alkyl aluminum compounds and titanium salts are likewise, utilized as catalysts in the polymerization of unsaturated hydrocarbons, such as ethylene and propylene.[18, 20][Kulinkovich, 2000 #74]

Organometallic compounds containing lead, tin, and mercury are on the whole industrially critical. A large number of organo compounds, for instance, are utilized as pharmaceuticals, pesticides, stabilizers.[19] Organometallic compounds have recently been found to be promising anticancer drug candidates.[21-22] Likewise, metallation of imines 2-(4-(dimethyl amino) benzylidene amino) benzene thiazol and 2-(benzylidene amino)-benzene thiazol with Hg(II), Ni(II) acetate, and palladium bromide gave organometallic compounds have Antimicrobial and Anticancer Activity.[23-25] Also, metallation of Schiff bases and their nanoparticle forms showed high antimicrobial and anticancer activity. [23, 26]

In the present work, some dyes derivatives of the 3-amino-1,2 ,5 -triazole ring have been synthesized and metallated by different metals acetate and then treated with silver nanoparticles. And study the expected biological effects of the new compounds.

Experimental

Reagents and materials

All the chemicals were obtained from Aldrich Chemical Company Wisconsin, USA. Precoated aluminum sheets (silica gel 60 F254, Merck, Darmstadt, Germany were used for thin-layer chromatography (TLC) and spots were seen under UV light. Melting points were measured on Stuart SMP10 melting point apparatus. IR spectra were recorded on Perkin–Elmer model 1600 FT-IR RX1 spectrophotometer as KBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX-400 FT NMR spectrometer using DMSO as solvent against tetramethylsilane (TMS) (δ=0.00). Multiplicities of the signals were designated as follows: s, singlet; d, doublet; m, multiplet. Chemical shift values are given in δ. The mass spectra were measured on a MICROMASS.

General procedure for preparation of the heterocyclic azo compounds (I), (II) & (III)

Azo dyes compounds were synthesized according to the literature method[27] using diazotized 3-amino-1,2,4-triazole followed by coupling with different phenols β-naphthol, α-naphthol and resorcinol into appropriate reaction condition to produce compounds **(I), (II) and (III)** respectively.

Compound (**I**) brown crystals, yield 86%; mp = 92-94^{o}C. FT-IR (KBr, cm^{-1}): ν = 3498 (O-H), 3255 (N-H), 3050 (C-H, ar.), 1680 (C=N), 1599 (N=N). ^{1}H NMR(400 MHz, DMSO-*d6*) δ: 9.51 (s,1H,NH), 8.78 (s, 1H, N= CH), 7.23-8.54 (m, 6H, aromatic), 3.7 (s,OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 109.09, 119.08, 123.07, 123.35, 126.43, 126.55, 127.99, 128.17, 129.73, 135.06, 139.80, 155.77. The MS spectrum showed the molecular ion peak at *m/z* 239. Anal. Calcd (%) for $C_{12}H_9N_5O$: C, 60.25; H, 3.79; N, 29.27. Found: C, 60.32; H, 3.82; N, 29.18%.

Compound (**II**) reddish brown crystals, yield 84%; mp = 85-87^{o}C. FT-IR (KBr, cm^{-1}): ν = 3498 (O-H), 3288 (N-H), 3010 (C-H, ar.), 1661 (C=N), 1596 (N=N). ^{1}H NMR(400 MHz, DMSO-*d6*) δ: 10.10 (s, 1H,NH), 8.14 (s, 1H, N= CH), 7.06-8.12 (m, 6H, aromatic), 4.13 (s,OH); ^{13}C NMR (100 MHz, $CDCl_3$ δ: 108.48,118.77, 122.42, 12.71, 124.98, 125.01, 126.53, 126.87, 127.82, 133.66, 134.86, 153. 61. The MS spectrum showed the molecular ion peak at *m/z* 239. Anal. Calcd for $C_{12}H_9N_5O$: C, 60.25; H, 3.79; N, 29.27. Found: C, 60.27; H, 3.84; N, 29.20%.

Compound (**III**) violet crystals, yield 81%; mp = 160-162^{o}C. FT-IR (KBr, cm^{-1}): ν = 3519(O-H), 3402 (O-H), 3169 (N-H), 3010 (C-H, ar.), 1695 (C=N), 1595 (N=N); ^{1}H NMR(*400 MHz, DMSO-d6*) δ: 8.91 (s,1H,NH), 8.27 (s, 1H, N= CH), 6.29-7. 92 (m, 3H, aromatic), 5.91 (s, OH), 6.13 (s, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 80.62, 109.65, 129.57, 129.98, 141.05, 147.18, 151.02, and 151.10. The MS spectrum showed the molecular ion peak at *m/z* 205. Anal. Calcd for $C_8H_7N_5O_2$: C, 46.83; H, 3.44; N, 34.13. Found: C, 46.62; H, 3.52; N, 34.18%.

Metallation of compounds I, II and III by different metals acetate

General procedure

A mixture of metal acetate (1mmol) and compounds (**I**, **II** and **III** (1mmol) in 30 ml methanol was allowed to react under stirring for three hours, and after that left overnight. A

distinct change in color was observed, whereby a precipitate was separated out, filtered off, dried and recrystallized from the suitable solvent. The filtrate was concentrated to precipitate crystals which were recrystallized from (acetic acid –ether).

Compound (**Ia**) brown crystals, yield 76%; mp = 110°C; FT-IR (KBr, cm^{-1}): ν = 3498 (O-H), 3255 (N-H), 3050 (C-H, ar.), 1672 (C=O), 1628 (C=N), 1599 (N=N), 480(C-Co), 453(O-Co). ^{1}HNMR (400 MHz, DMSO-*d6*) δ: 9.85 (s, 1H, NH), 9.16 (s, 1H, N= CH), 7.17-8.44 (m, 5H, aromatic), 4.23 (s, OH), 1.34 (s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.76, 109.09, 119.08, 123.07, 123.35, 126.43, 126.55, 127.99, 128.17, 129.73, 135.06, 139.80, 155.77, 189.78. EI-MS showed the molecular ion M^+ at 356. Anal. Calcd for $C_{14}H_{11}N_5O_3Co$: C, 47.21; H, 3.11; N, 19.66; Co, 16.54. Found: C, 47.43; H, 3.21; N, 19.61; Co, 16.47 %.

Compound (**Ib**) reddish brown crystals, yield 48%; mp = 280-282°C. FT-IR (KBr, cm^{-1}): ν = 3426 (O-H), 3289 (N-H), 3095 (C-H, ar.), 1629 (C=N), 1598 (N=N), 520 (C-Ni) and 448 (O-Ni); ^{1}H NMR(400 MHz, DMSO-d6) δ: 9.50 (s,1H,NH), 8.78 (s, 1H, N= CH), 7.93-8.75 (m, 5H, aromatic), 4.21(s, OH), 3.20 (s, Ni-OH); ^{13}C NMR (100 MHz, $CDCl_3$) 108.19, 118.28, 124.47, 126.42, 126.50, 128.15, 129.69, 129.98, 136.13, 148.06, 148.80, 154.01. EI-MS showed the molecular ion M^+ +1 at 315.8. Anal. Calcd for $C_{12}H_9N_5O_2Ni$: C, 45.91; H, 2.89; N, 22.31; Ni, 18.70. Found: C, 45.82; H, 2.94; N, 22.44; Ni, 18.82 %.

Compound (**Ic**) red crystals, yield 41%; m.p.118°C; FT-IR (KBr, cm^{-1}): ν = 3472 (O-H), 3249 (N-H), 3052 (C-H, ar.), 1629 (C=O), 1598 (C=N), 1580 (N=N), 552 (C-Ni), 439 (O-Ni). ^{1}HNMR (400 MHz, DMSO-*d6*) δ: 10.05 (s, 1H, NH), 9.38 (s, 1H, N= CH), 7.72-8.94 (m, 5H, aromatic), 4.40 (s, OH), 1.52 (s, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 16.76, 110.19, 118.08, 122.18, 133.38, 129.55, 136.95, 137.09, 138.77, 139.13, 141.08, 142.83, 159.17, 188.98. EI-MS showed the molecular ion M^+ at 355.9. Anal. Calcd for $C_{14}H_{11}N_5O_3Ni$: C, 47.24; H, 3.11; N, 19.67; Ni, 16.49. Found: C, 47.32; H, 2.99; N, 19.41; Ni, 16.63%.

Compound (**Id**) reddish brown crystals, yield 86% ; m.p.300>°C; FT-IR (KBr, cm^{-1}): ν = 3440 (O-H), 3290 (N-H), 3077 (C-H, ar.), 1670 (C=O), 1616 (C=N), 1568 (N=N), 587(C-Cu), 559(O-Cu); ^{1}H NMR(400 MHz, DMSO-d6): δ: 9.48 (s,1H,NH), 9.09 (s, 1H, N= CH), 6.94-8.52 (m, 5H, aromatic), 4.26 (s, OH) 1.42(s,CH_3) ; ^{13}C NMR (100 MHz, $CDCl_3$) 20.53, 108.59, 118.038, 123.47, 123.65, 127.13, 127.15, 127.99, 128.57, 129.78, 135.34, 139.60, 154.74 , 185.78 .EI-MS showed the molecular ion M^+ at 360.94. Anal. Calcd for $C_{14}H_{11}N_5O_3Cu$: C, 46.60; H, 3.07; N, 19.41; Cu, 17.61. Found: C, 46.49; H, 2.98; N, 19.33; Cu, 17.66 %.

Compound (**IIa**) reddish brown crystals, yield 52%; m.p. 270-272°C; FT-IR (KBr, cm^{-1}): ν = 3470 (O-H), 3275 (N-H), 3100 (C-H, ar.), 1609 (C=N), 1593 (N=N) , 554 (C-Co) , 487 (O-Co); ^{1}HNMR(400 MHz, DMSO-d6) δ: 8.65 (s, 1H,NH), 8.20 (s, 1H, N= CH), 6.89-7.97 (m, 5H, aromatic), 4.40 (s, OH), 3.58 (s, Co-OH); ^{13}C NMR (100 MHz, $CDCl_3$)109.08, 120.07, 123.51, 123.76, 124.78, 124.97, 127.43, 127.87, 129.17, 131.96, 134.74, 154. 76. EI-MS showed the molecular ion M^+ +2 at 334. Anal. Calcd for $C_{12}H_{11}N_5O_3$ Co: C, 43.39; H, 3.34; N, 21.08; Co, 17.74. Found: C, 43.43; H, 3.29; N, 21.13; Co, 17.67%.

Compound (**IIb**) brown crystals, yield 44%; m.p.d. 110-112°C; FT-IR (KBr, cm^{-1}): ν = 3459 (O-H), 3290 (N-H), 3124 (C-H, ar.), 1629 (C=N), 1594 (N=N), 534 (C-Co). ^{1}H NMR (400 MHz, DMSO-d6) δ: 9.03(s, 2H, NH), 8.30 (s, 2H, N= CH), 8.10-6.90 (m, 10H, aromatic), 4.42 (s, 2OH); ^{13}C NMR (100 MHz, CDCl3) δ: 109.98, 119.77, 122.12, 123.18, 125.08, 126.31, 127.41, 128.77, 129.11, 133.19, 136.18, 157. 61. EI-MS showed the molecular ion M^+ at 535. Anal. Calcd for $C_{24}H_{16}N_{10}O_2$ Co: C, 53.84; H, 3.01; N, 26.16; Co, 11.01. Found: C, 53.65; H, 3.12; N, 26.18; Co, 11.17 %.

Compound (**IIc**) reddish brown crystals, yield 74%; m.p.<350°C; FT-IR (KBr, cm^{-1}): ν = 3396 (O-H), 3198 (N-H), 3130 (C-H, ar.), 1632 (C=N), 1591 (N=N), 483 (C-Ni). ^{1}HNMR (400 MHz, DMSO-d6): δ: 9.64 (s, 2H, NH), 9.02 (s, 2H, N= CH), 6.61-8.11 (m, 10H, aromatic), 4.39 (s, 2OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 109.98, 121.18, 124.19, 125.76, 125.78, 124.17, 127.43, 127.87, 129.71, 134.76, 139.24, 159. 16. EI-MS showed the molecular ion M^+ at 535. Anal. Calcd for $C_{24}H_{16}N_{10}O_2$ Ni: C, 53.87; H, 3.01; N, 26.17; Ni, 10.97. Found: C, 53.76; H, 3.21; N, 26.25; Ni, 10.85 %.

Compound (**IId**) dark brown crystals, yield 68%; m.p.<350°C; FT-IR (KBr, cm^{-1}): ν = 3455

(O-H), 3281 (N-H), 3100 (C-H, ar.), 1631 (C=N), 1590 (N=N), 540 (C-Cu), 463 (O-Cu); ^{1}H NMR(400 MHz, DMSO-d6) δ :8.43(s, 1H,NH), 8.00 (s, 1H, N= CH), 7.09-7.90 (m, 5H, aromatic), 4.31 (s, OH), 3.29 (s, Cu-OH); ^{13}C NMR (100 MHz, $CDCl_3$)107.98,119.07, 123.12, 123.81, 124.88, 125.91, 127.43, 127.77, 127.97, 132.66, 135.66, 155. 61. EI-MS showed the molecular ion M^+ +2 at 338. Anal. Calcd for $C_{12}H_9N_5O_2Cu\,H_2O$: C, 42.79; H, 3.29; N, 20.79; Cu, 18.87. Found: C, 42.87; H, 3.26; N, 20.66; Cu, 18.81%.

Compound (**IIIa**) dark red crystals , yield 39%; m.p.<350°C; FT-IR (KBr, cm^{-1}): ν = 3502-3335 (O-H), 3221 (N-H), 3050 (C-H, ar.), 1623(C=O), 1587 (C=N), 1570 (N=N), 559(C-Co), 471(O-Co); ^{1}HNMR(400 MHz, DMSO-d6) δ: 8.99 (s,1H,NH), 8.35 (s, 1H, N= CH), 6.30-7. 94 (s, 2H, aromatic), 6.25 (s,OH), 6.23 (s,OH), 1.90 (s,CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 20.34, 90.12, 109.65, 128.78, 129.86, 142.80, 146.31, 151.62, 152.34, 186.34. EI-MS showed the molecular ion M^+ at 322 . Anal. Calcd for $C_8H_7N_5O_3Co.H_2O$: C, 37.28; H, 2.82; N, 21.74; Co, 18.29. Found: C, 37.45; H, 2.90; N, 21.66; Co, 18.17%.

Compound (**IIIb**) violet crystals, yield 37%; mp = 260°C; FT-IR (KBr, cm^{-1}): ν = 3509-3336 (O-H), 3240 (N-H), 3052 (C-H, ar.), 1589 (C=N), 1576 (N=N), 557(C-Co).). ^{1}HNMR (400 MHz, DMSO-d6): δ: 9.44 (s, 2H, NH), 8.92 (s, 2H, N= CH), 6.98-8.21 (m, 4H, aromatic), 4.28 (s, 4OH); ^{13}C NMR (100 MHz, $CDCl_3$)δ: 111.26, 122.98, 124.33, 125.16, 127.08, 129.17, 129.87, 130.79, 134.12, 139.22, 159. 16. EI-MS showed the molecular ion M^+ at 467. Anal. Calcd. for $C_{16}H_{12}N_{10}O_4Co$: C, 41.13; H, 2.59; N, 29.98; Co, 12.61. Found: C, 41.18; H, 2.68; N, 29.79; Co, 12.53%

Compound (**IIIc**) reddish brown crystals, yield 78%; mp 350>°C; FT-IR (KBr, cm^{-1}): ν = 3414-3345 (O-H), 3290 (N-H), 3110 (C-H, ar.), 1588 (C=N), 1567 (N=N), 551(C-Ni), 467(O-Ni).^{1}HNMR(400 MHz, DMSO-d6): δ: 9.85 (s, 1H, NH), 8.22 (s, 1H, N= CH), 6.98 (s, 1H, aromatic), 4.92 (s, 2 OH), 3.07 (s, 2 Ni-OH); ^{13}C NMR (100 MHz, $CDCl_3$)δ: 104.08, 119.98, 131.12, 123, 152.66, 153.88, 157.12. EI-MS showed the molecular ion M^+ at 373 Anal. Calcd for $C_8H_7N_5O_4Ni_2.H_2O$: C, 25.79; H, 2.43; N, 18.80; Ni, 31.51 Found: C, 25.59; H, 2.51; N, 18.93; Ni, 31.66%.

Compound (**IIId**) green crystals, yield 48%; m.p.<350°C; FT-IR (KBr, cm^{-1}): ν = 3455-3340 (O-H), 3325 (N-H), 3092 (C-H, ar.), 1693(C=O), 1591 (C=N), 1581 (N=N), 559 (C-Cu), 473 (O-Cu); ^{1}HNMR(400 MHz,DMSO-d6) δ: 8.35 (s,1H,NH) , 8.17(s, 1H, N= CH), 6.30-7.94 (s, 2H, aromatic), 6.24(s, OH), 5.83 (s, OH), 1.88(s,CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 19.54, 98.12, 110.15, 127.65, 129.54, 141.91, 144.97, 152.54, 152.87, 181.05. EI-MS showed the molecular ion M^+ at 327. Anal. Calcd for $C_{10}H_9N_5O_4Cu$: C, 36.76; H, 2.78; N, 21.43; Cu, 19.45. Found: C, 36.65; H, 2.82; N, 21.35; Cu, 19.42%.

Compound (**IIIe**) pal yellow crystals, yield 37%; m.p.d. 110°C; FT-IR (KBr, cm^{-1}): ν = 3441-3327 (O-H), 3327 (N-H), 3100 (C-H, ar.), 1699 (C=O), 1695 (C=O), 1635 (C=N), 1583 (N=N), 547(C-Cu), 470 (O-Cu). ^{1}HNMR (400 MHz,DMSO-d6) δ: 8.33 (s, 1H, NH) , 8.23(s, 1H, N= CH), 6.54 (s, 1H, aromatic), 5.33 (s, OH), 1.79(s, CH_3) 1.77(s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) 19.13, 99.32, 110.51, 129.35, 130.54, 144.11, 149.79, 159.50, 158.67, 179.15. EI-MS showed the molecular ion M^+ at 466.7. Anal. Calcd for $C_{12}H_{11}N_5O_6Cu_2.H_2O$: C, 30.91; H, 2.81; N, 15.02; Cu, 27.25. Found: C, 30.82; H, 2.89; N, 15.23; Cu, 27.14%.

Result and Discussions

The aim of the work is synthesize of azo dyes compounds by diazotization reaction followed by metallation and evaluation of the new compounds and their nanoparticle forms as antimicrobial**.**

Some azo dyes were synthesized using diazotized 3-amino-1,2,4-triazole followed by coupling with various phenols β-naphthol, α-naphthol and resorcinol into appropriate reaction condition to produce compounds (**I**), (**II**) and (**III**) respectively. The structure of azo compounds was confirmed by using different spectral data, the FT-IR spectra of compounds (**I**), (**II**) and (**III**) showed the presence of (N=N) absorbance at 1599cm^{-1}, 1596 cm^{-1}, 1595 cm^{-1} respectively. In addition OH group in 3519-3498 cm^{-1} range, NH group in 3255-3169 cm^{-1}range, and C-H, aromatic ring in 3010 -3050 cm^{-1} range, for the compound (**I**) ^{1}H NMR spectrum showed δ 9.51(s,1H,NH); 8.78(s, 1H, N= CH); 7.23-8.54 (m, 6H, aromatic); 3.7(s, OH); and in ^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**I**) 109.09, 119.08, 123.07, 123.35, 126.43, 126.55, 127.99, 128.17, 129.73, 135.06, 139.80, 155.77. El-MS spectrum demonstrated the molecular ion peak at *m/z* 239.

Also for compound (**II**) the ^{1}H NMR spectrum showed δ 10.01 (s, 1H, NH), 8.14 (s, 1H, N= CH), 7.06-8.12 (m, 6H, aromatic), 4.13 (s, OH). In ^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**II**), 108.48,118.77, 122.42, 12.71, 124.98, 125.01, 126.53, 126.87, 127.82, 133.66, 134.86, 153. 61, El-MS spectrum demonstrated the molecular ion peak at *m/z* 239.

For compound (**III**) the ^{1}H NMR spectrum showed δ 8.91(s, 1H, NH), 8.27(s, 1H, N= CH), 6.29-7. 92 (m, 3H, aromatic), 5.91(s, OH), 6.13(s, OH); In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**III**) 80.62, 109.65, 129.57, 129.98, 141.05, 147.18, 151.02, 151.10. El-MS spectrum demonstrated the molecular ion peak at *m/z* at 205. All data obtained confirm the success of synthesis of the azo compounds.

The prepared azo dyes were metallated by different metal acetate Co (II), Ni (II) and Cu (II) acetate to give newly metallated compounds (c*f.* Scheme 1-3). FT-IR, ^{1}H NMR, ^{13}CNMR, Mass spectrum, and elemental analysis are used to confirm the structure of the newly synthesized compounds.

Synthesis of compounds (Ia), (Ib), (Ic) and (Id).

Activation of C-H bonds is a standout amongst the most essential academic and industrial topics in modern chemistry.[28] One part of this work that has aroused widespread interest concerns ortho-metallation of aromatic rings.[29] The starting points of this work date back to the pioneering work of Wittig [Gilman, 1934 #95] [30] and Gilman [31] who showed that aromatic rings substituted with certain heteroatom groups (often N- or O-donor substituent) undergo specific lithiation in the ortho position. The reaction of compound (**I**) with cobalt acetate in methanol at room temperature gave rise to mono cobalated product (**Ia**) the reaction may proceeds via coordination of cobalt acetate to azo nitrogen followed by electrophilic substitution (of aromatic moiety) at ortho position the suggested mechanism of the reaction may take place as in (c*f.* Scheme 4). The structure of the compound (**Ia**) was confirmed by elemental analyses, FT-IR, ^{1}H NMR, ^{13}C NMR and Mass spectra. The FT-IR spectrum showed new absorption bands at 1672 cm^{-1}, 480 cm^{-1} and 453 cm^{-1} due to ν C=O, C-Co and O-Co respectively. The ^{1}H NMR shows δ 9.85 (s, 1H, NH), 9.16 (s, 1H, N=CH), 7.17-8.44 (m, 6H, aromatic), 4.23 (s,OH), 1.34 (s,CH_3). In ^{13}C NMR spectrum, the following signals are characteristic of the structure of the compound (**Ia**) 19.76, 109.09, 119.08, 123.07, 123.35, 126.43, 126.55, 127.99, 128.17, 129.73, 135.06, 139.80, 155.77, 189.78 and the MS spectrum shows the molecular ion peak M^+ at 356. When compound (**I**) reacted with nickel acetate in methanol at room temperature mono nickelated product (**Ib**) was obtained as a precipitate and another product (**Ic**) was produced via evaporation of the filtrate. The suggested mechanism of the reaction may take place as in (c*f.* Scheme 5). The structure of compound (**Ib**) was consistent with the data obtained from elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR, and MS spectra. The FT-IR spectrum showed new absorption bands at 520cm^{-1} and 448cm^{-1} due to ν C-Ni and O-Ni respectively. The ^{1}H NMR spectrum shows δ 9.50 (s, 1H, NH), 8.78 (s, 1H, N= CH), 7.93-8.75 (m, 6H, aromatic), 4.21(s, OH), 3.20 (s, Ni-OH). In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**Ib**) 108.19, 118.28, 124.47, 126.42, 126.50, 128.15, 129.69, 129.98, 136.13, 148.06, 148.80, 154.01and the MS spectrum showed the molecular ion (M^+ +1) at 315.8. Compound (**Ic**) was confirmed by FT-IR spectrum which showed new absorption bands at 1629 cm^{-1}, 552 cm^{-1} and 439cm^{-1} due to ν C=O , C-Ni and O-Ni respectively and the MS spectrum showed the molecular ion M^+ at 355.9.

Compound (**Id**) was obtained from the reaction of compound (**I**) and copper acetate in methanol at room temperature. The structure of compound **(Id)** was inferred based on elemental analyze, FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. The FT-IR spectrum showed new absorption bands at 1670 cm^{-1}, 587 cm^{-1} and 559 cm^{-1}due to ν C=O, ν C-Cu and ν O-Cu respectively. The ^{1}H NMR spectrum shows δ 9.48 (s, 1H, NH), 9.09 (s, 1H, N= CH), 6.94-8.52 (m, 5H, aromatic), 4.26 (s, OH) 1.42 (s, CH_3). In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (Id) 20.53, 108.59, 118.038, 123.47, 123.65, 127.13, 127.15, 127.99, 128.57, 129.78, 135.34, 139.60, 154.74 and 185.78.The MS spectrum showed the presence of the molecular ion peak at *m/z* at 360.9.

Synthesis of compounds (IIa), (IIb), (IIc) and (IId).

The reaction of compound (**II**) with cobalt acetate in methanol at room temperature gave rise to mono cobalated product (**IIa**), which obtained

as a precipitate and another product cobalt bis-compound (**IIb**) produced via evaporation of the filtrate, spectral data and elemental analysis confirmed the structures, where, the FT-IR spectrum for compound (**IIa**) showed new absorption bands at 554 cm^{-1} and 487 cm^{-1} due to ν C-Co and O-Co respectively. The ^{1}H NMR spectrum showed δ: 9.03(s, 2H, NH), 8.30 (s, 2H, N= CH), 8.106.90- (m, 10H, aromatic), 4.42 (s, 2OH); In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (IIb)δ: 109.98, 119.77, 122.12, 123.18, 125.08, 126.31, 127.41, 128.77, 129.11, 133.19, 136.18, 157. 61.The MS spectrum showed the molecular ion peak at *m/z* at 535.

The reaction of compound (**II**) with nickel acetate in methanol at room temperature produce nickel bis-compound (**IIc**) the structure was confirmed on the bases of its elemental analysis, FT-IR, and MS spectra. The FT-IR spectrum showed a new absorption band at 483cm^{-1} due to ν C-Ni. The MS spectrum showed the presence of the molecular ion peak at *m/z* at 535.

A mono copper product (**IId**) was obtained by reaction of compound (II) with copper acetate in methanol at room temperature. Elucidation of the structure was based on elemental analysis, IR and spectral data. The FT-IR spectrum showed new absorption bands at 540 cm^{-1} and 463 cm^{-1}due to ν C-Cu and ν O-Cu respectively. The ^{1}H NMR spectrum showed δ 8.43 (s, 1H, NH), 8.00 (s, 1H, N= CH), 7.09-7.90 (s, 2H, aromatic), 4.31 (s, OH), 3.29 (s, Cu-OH). In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**IId**) 107.98, 119.07, 123.12, 123.81, 124.88, 125.91, 127.43, 127.77, 127.97, 132.66, 135.66, 155. 61. The MS spectrum showed the molecular ion M^{+} +2 at 338.

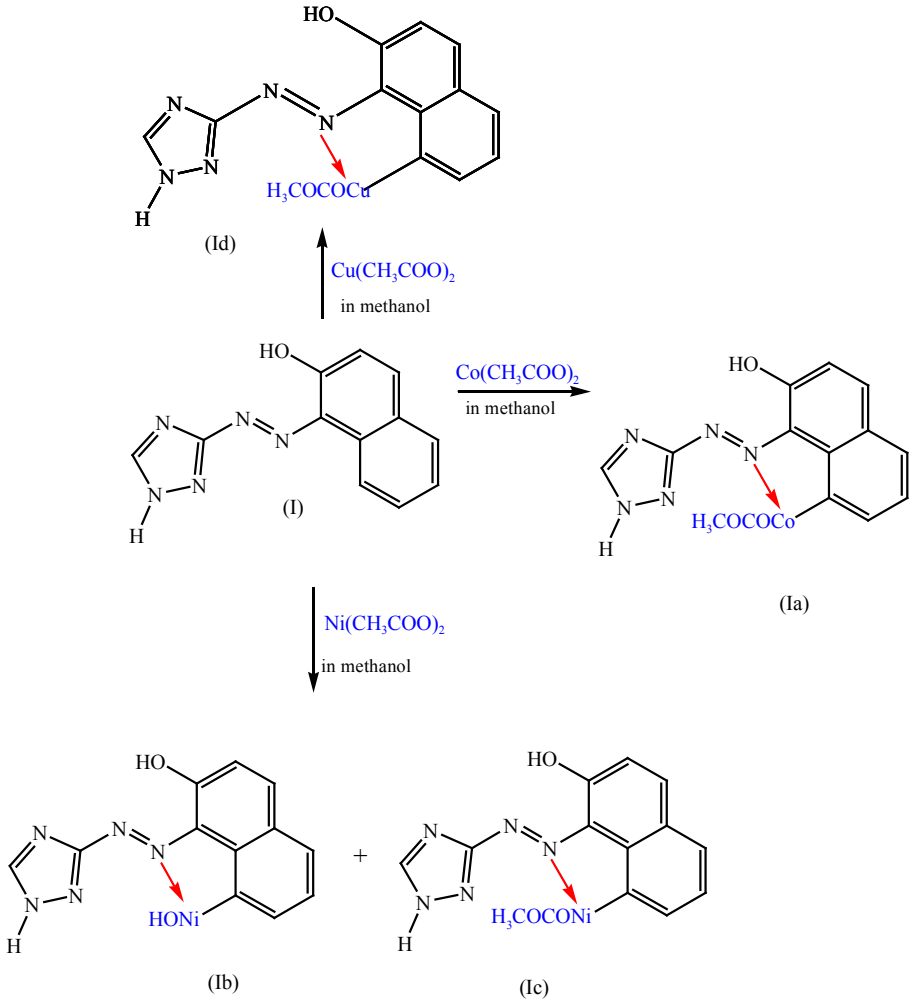
Synthesis of compounds (IIIa), (IIIb), (IIIc), (IIId) and (IIIe).

The reaction of compound (**III**) and cobalt acetate in methanol at room temperature gave rise to compounds (**IIIa**) and (**IIIb**) The structure of compounds) were inferred based on elemental analyses, FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectra. The FT-IR for compound (**IIIa**) showed new absorption bands at 1693 cm^{-1}, 559 cm^{-1}, 471 cm^{-1} due to C=O, C-Co and O-Co respectively. The ^{1}H NMR spectrum shows δ 8.99 (s, 1H, NH), 8.35 (s, 1H, N= CH), 6.30-7.9 (m, 6H, aromatic), 6.25(s, OH), 6.23(s, OH), 1.90(s, CH_3). In ^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**IIIa**) 20.34, 90.12, 109.65, 128.78, 129.86, 142.80, 146.31, 151.62, 152.34, 186.34. The MS spectrum showed the molecular ion M^{+} at 322.

For compound (**IIIb**) the The ^{1}H NMR spectrum shows δ: 9.44 (s, 2H, NH), 8.92 (s, 2H, N= CH), 6.98-8.21 (m, 4H, aromatic), 4.28 (s, 4OH); In ^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (IIIb) 111.26, 122.98, 124.33, 125.16, 127.08, 129.17, 129.87, 130.79, 134.12, 139.22, 159. 16. and the MS spectrum showed the presence of the molecular ion peak at *m/z* 467.

Metallation of compound (**III**) using nickel acetate in methanol at room temperature afforded new compound (**IIIc**) spectral data and elemental analysis confirmed its structure, where, FT-IR for compound (**IIIc**) showed new absorption bands at 551 cm^{-1},467 cm^{-1} due to ν C-Ni and ν O-Ni respectively. The ^{1}H NMR spectrum shows δ: 9.85 (s, 1H, NH), 8.22 (s, 1H, N= CH), 6.98 (s, 1H, aromatic), 4.92 (s, 2 OH), 3.07 (s, 2 Ni-OH); In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (IIIc)δ: 104.08, 119.98, 123,124.22, 131.12, 152.66, 153.88, 157.12. The MS spectrum showed the presence of the molecular ion peak at *m/z* 373.

The reaction of compound (**III**) and copper acetate in methanol at room temperature gave the mono copperated product (**IIId**) and the diCopperated product (**IIIe**). The structures were consistent with the data obtained from elemental analysis, FT-IR, and MS spectra. For compound (**IIId**) the FT-IR spectrum showed new absorption bands at 1693 cm^{-1}, 559 cm^{-1} and 473 cm^{-1} due to, ν C=O, ν C-Cu and ν O- Cu respectively. The ^{1}H NMR spectrum shows δ 8.35 (s, 1H, NH) , 8.17 (s, 1H, N= CH), 6.30-7.94 (s, 2H, aromatic), 6.24 (s, OH), 5.83 (s, OH), 1.88 (s,CH_3). In^{13}C NMR spectrum the following signals are characteristic of the structure of the compound (**IIId**) 19.54, 98.12, 110.15, 127.65, 129.54, 141.91, 144.97, 152.54, 152.87, 181.05. The MS spectrum showed the presence of the molecular ion peak at *m/z* 327. For compound (**IIIe**), the FT-IR spectrum showed new absorption bands at 1699 cm^{-1}, 1695 cm^{-1}, 547 cm^{-1} and 470cm^{-1} due to ν C=O, ν C=O, ν C-Cu, and ν O-Cu respectively and EI-MS showed the molecular ion peak at *m/z* 466.7.



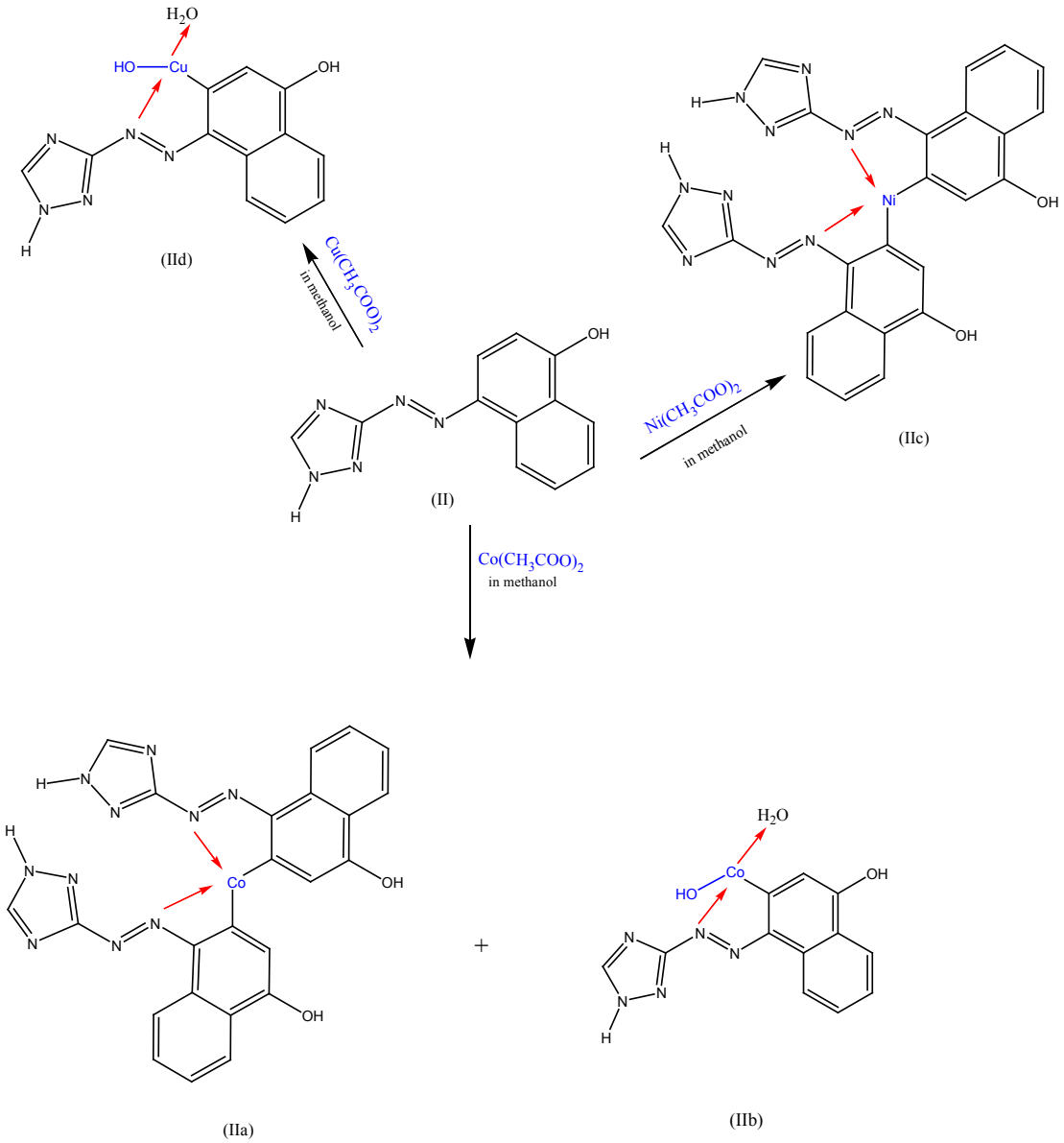
(Scheme 1)

Pharmacological studies

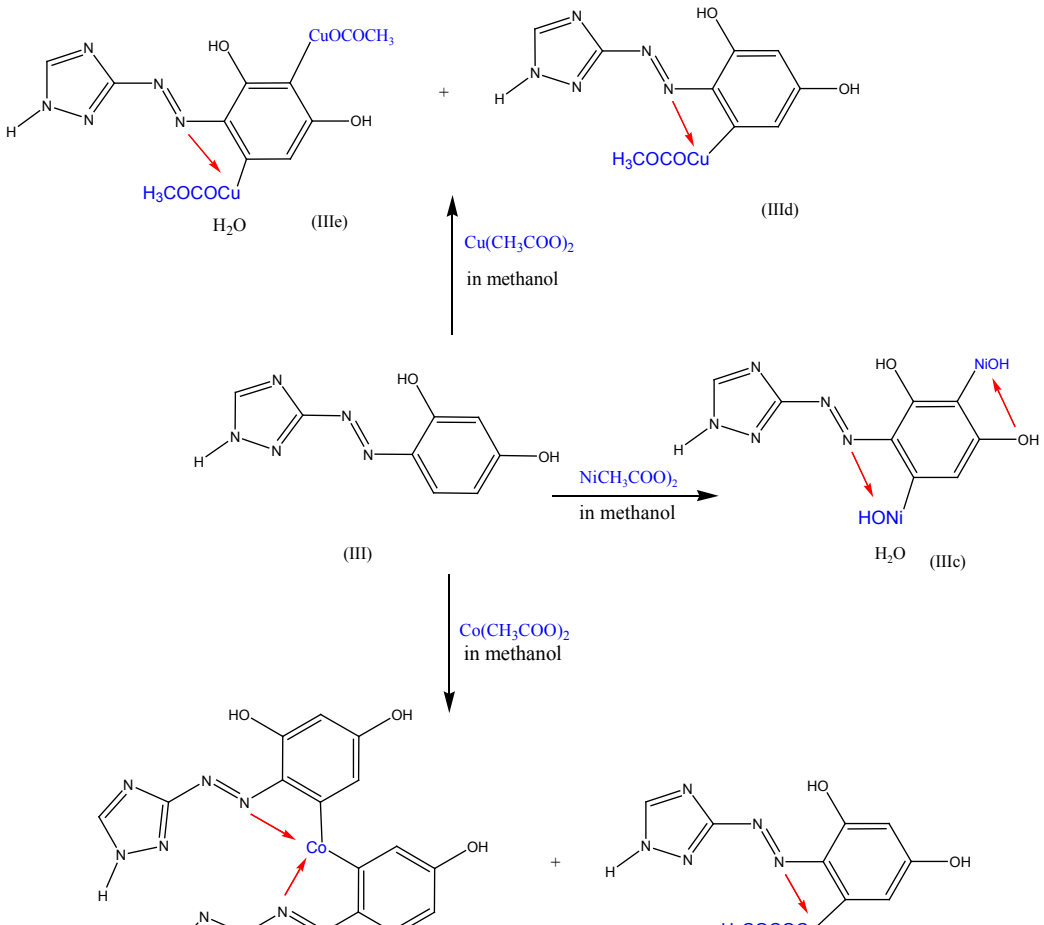
New synthesized organomettallic compounds were evaluated for antimicrobial activity against one strain Gram-positive bacteria ***Staphylococcus aureus*** and ***Escherichia coli*** Gram-negative bacteria as well as two pathogenic fungi, as ***Aspergillus flavus*** and ***Candida albicans***. The results of the biological studies are shown in **figures 5**. The data are compared with standard antibiotics, **Ampicillin** as Gram-negative and Gram-positive bacteria. **Amphotericin** was used as an antifungal standard reference. The in vitro antibacterial and antifungal activities demonstrated that most compounds have higher antimicrobial activity in comparison with that of the standard.

In **figure 1,** compounds **Ia, IIa, IIc, IId, IIIc,** and **IIId** showed significant results in inhibiting ***Staphylococcus aureus*** growth with 25, 24, 23, 27, and 28 mm zone of inhibition respectively when compared to the other compounds and **Ampicillin** reference. Also **figure 2** showed the effects of Compounds **IIa** and **IIIc** against ***Escherichia coli*** produced 26 and 28 mm zone of inhibition respectively; this was compared to the effect of **Ampicillin** reference, while the rest of tested compounds showed moderate effects.

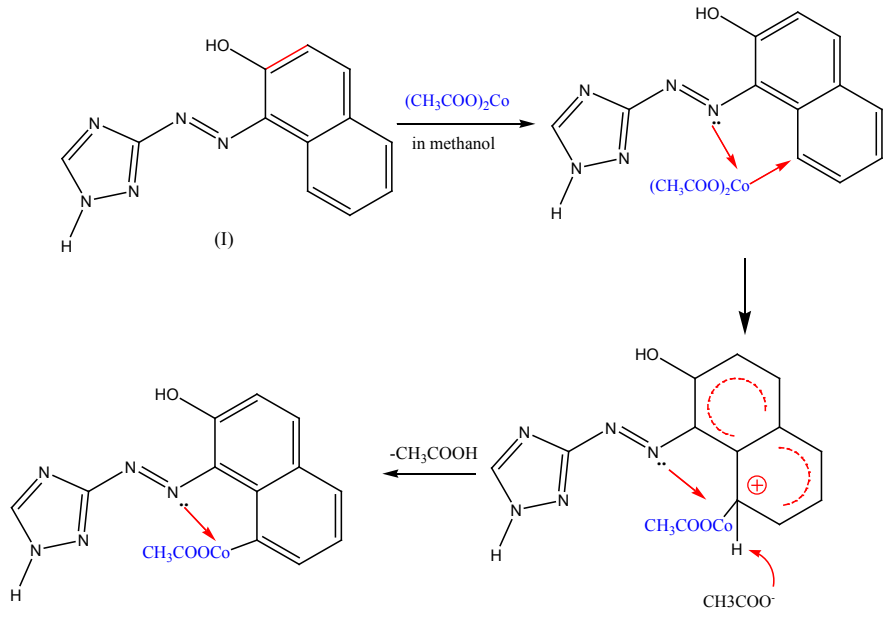
The promoted bactericidal activities of copper containing compounds can be explained on the basis of Overtone's concept [32] and Tweedy's chelation theory,[33] which attributed the high strength of organometallic compounds against pathogens to rise liposolubility due to the low polarity of metal ion through the carbon-metal bond, where the polarity of the metal was reduced due to the partial sharing of its cationic bond with the donor sites. This enhanced lipophilicity facilitates penetration and diffusion of the


H2O
HO
Cu
OH
(IId)
Cu(CH3COO)2
in methanol
(II)
Ni(CH3COO)2
in methanol
Ni
(IIc)
Co(CH3COO)2
in methanol
Co
(IIa)
+
(IIb)

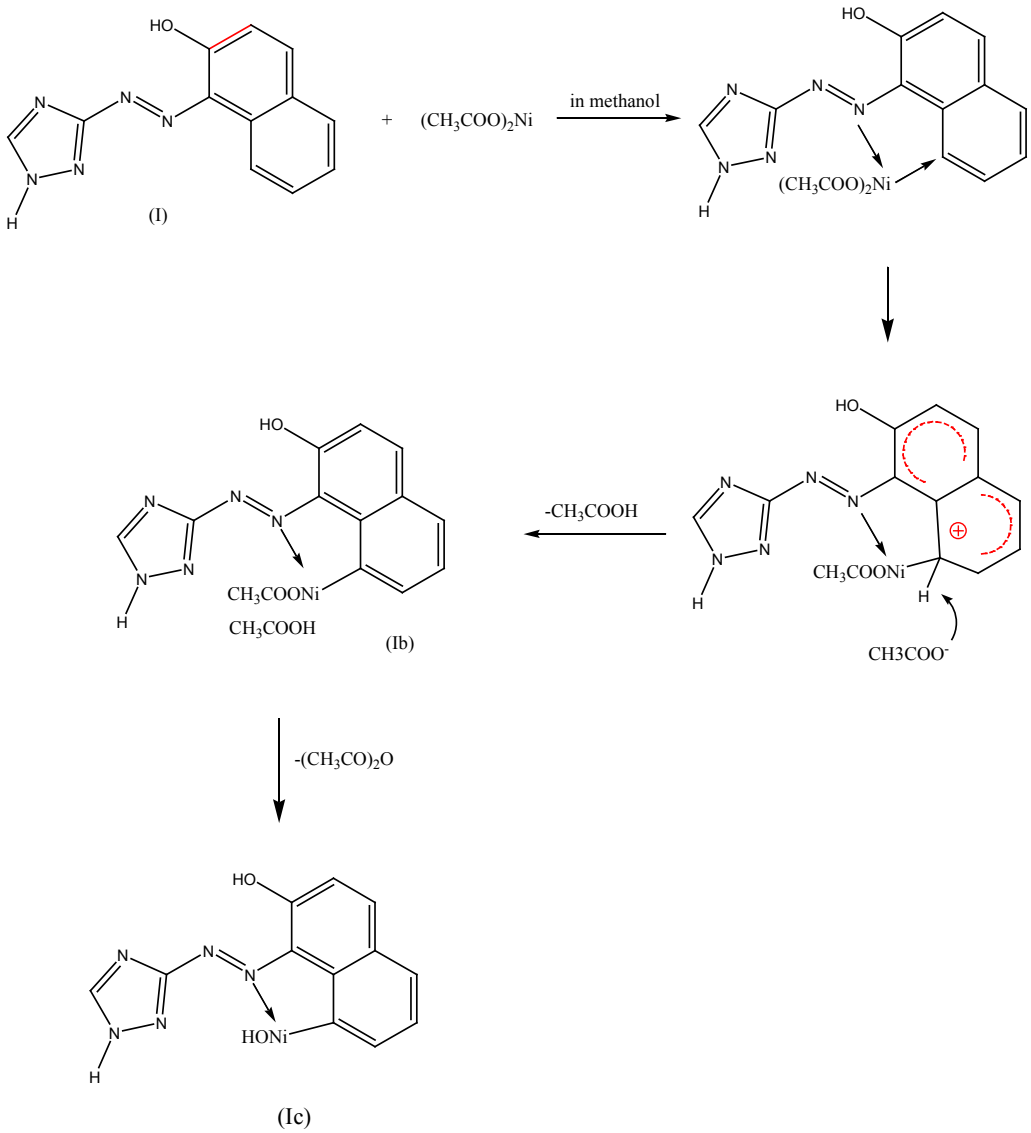
(Scheme 2)



(Scheme 3)



(Scheme 4)


HO
N
N
N
N
H
(I)
+
(CH3COO)2Ni
in methanol
(CH3COO)2Ni
CH3COONi
H
CH3COO-
-CH3COOH
CH3COONi
CH3COOH
(Ib)
-(CH3CO)2O
HONi
(Ic)

(Scheme 5)

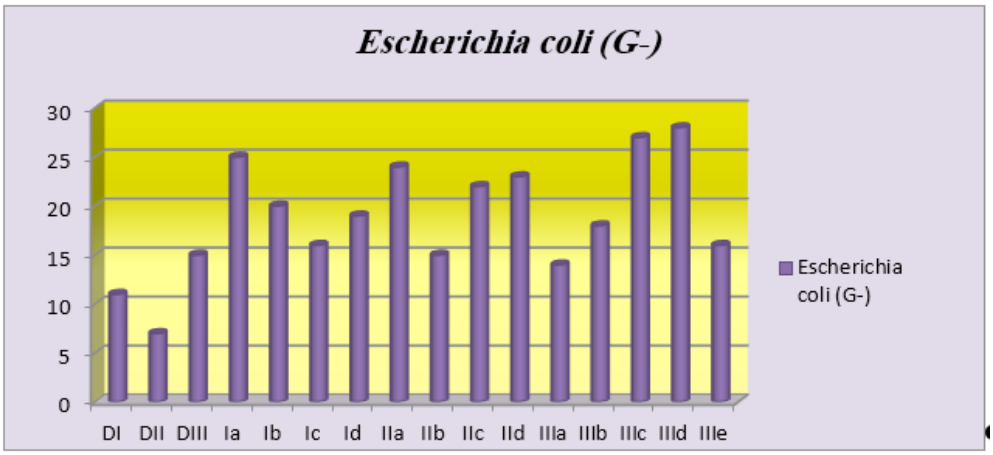


Fig. 1: Graph of the zone of inhibition (ZOI, mm) new organometallic compounds against (G-) bacterial cells *Escherichia coli*

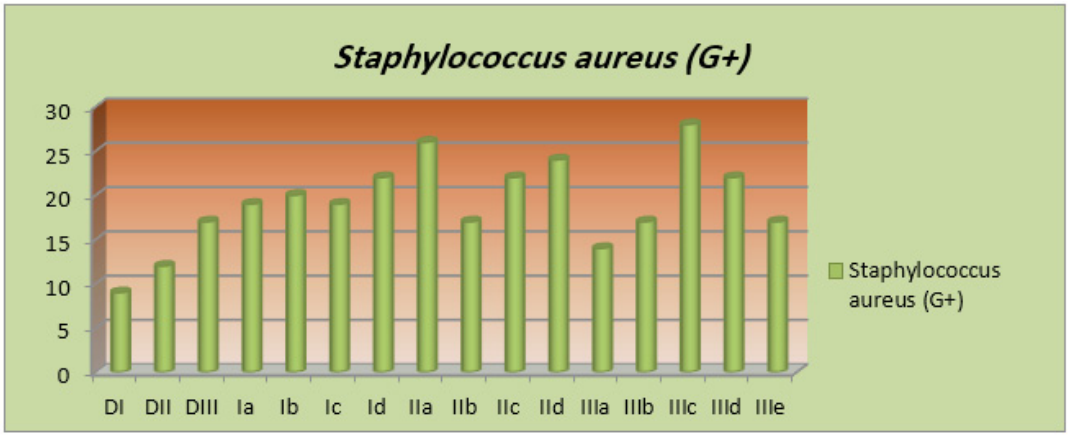


Fig. 2: Graph of the zone of inhibition (ZOI, mm) new organometallic compounds against (G+) bacterial cells *Staphylococcus aureus*

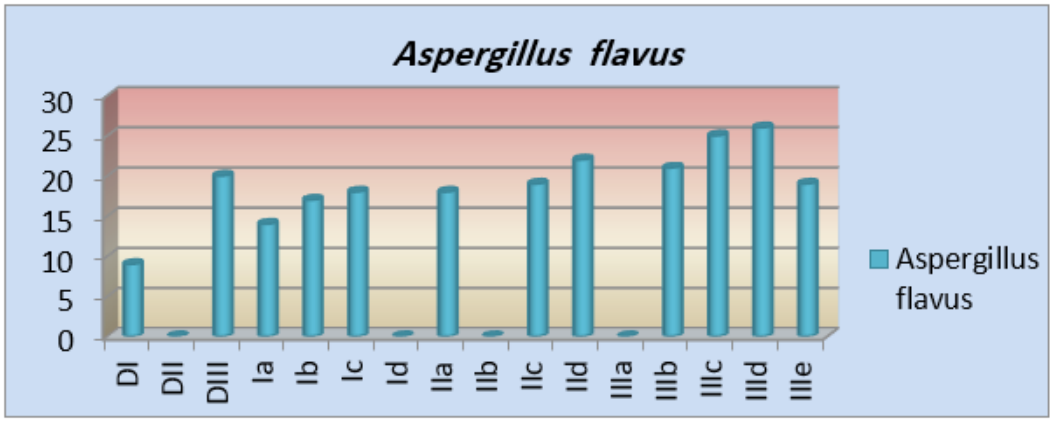


Fig. 3: Graph of the zone of inhibition (ZOI, mm) new organometallic compounds against *Aspergillus flavus* fungal strains

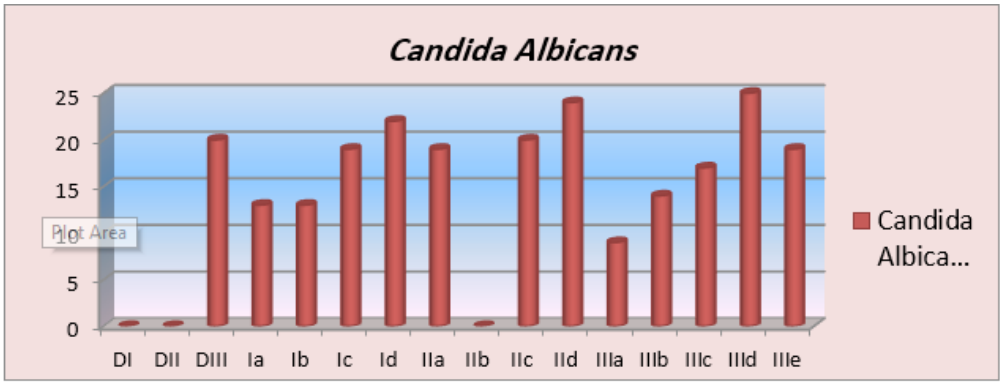


Fig. 4: Graph of the zone of inhibition (ZOI, mm) new organometallic compounds against *Candida Albicans* fungal strains

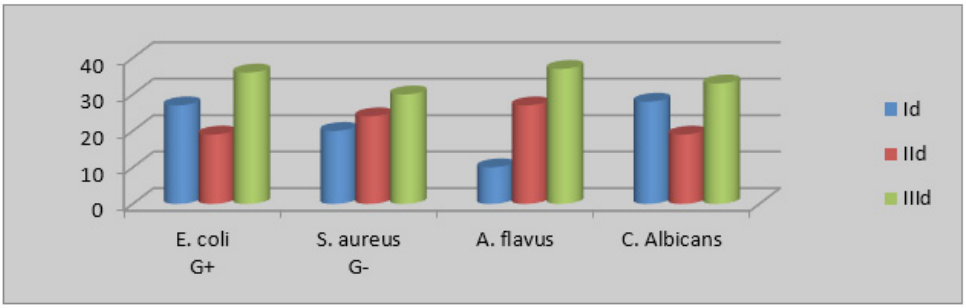


Fig. 5: Graph of the zone of inhibition (ZOI, mm) of nano-forms for compounds Id, IId and IIId against bacterial cells and fungal strains

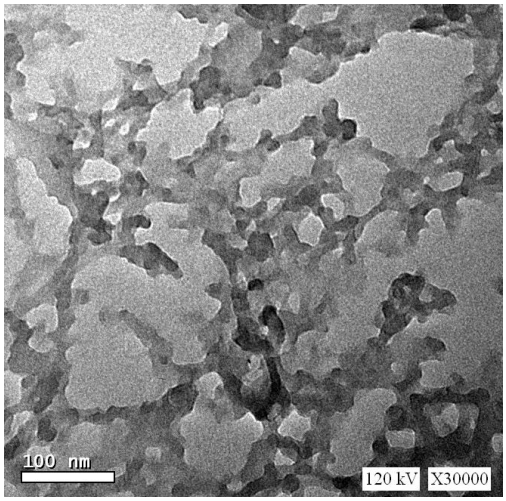


Fig. 6: TEM of compound Id after addition of Ag-NPs solution

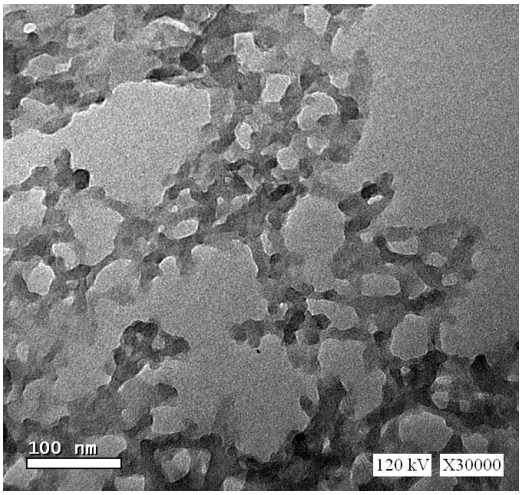


Fig. 7: TEM of compound IId after addition of Ag-NPs solution

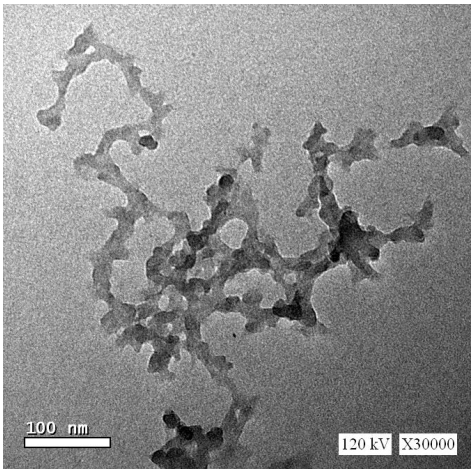


Fig. 8: TEM of compound IIId after addition of Ag-NPs solution

target compound into the lipid membrane of the pathogen along with blocking metal active enzymatic binding sites in the microorganisms. On the other hand, **figure 3** and **figure 4** showed significant inhibition of compound **IId**, **IIIc**, and **IIId** against ***Aspergillus flavus*** and ***Candida albicans*** with 22, 25 and 26 mm zone of inhibition when compared to other compounds. Evaluation of antimicrobial activity revealed that all the synthesized organomettallic compounds were effective in inhibiting the bacterial and fungal growth but with some exceptions.

The antibacterial and antifungal activities of the silver nano forms (Ag-NPs) of the compounds **Id**, **IId**, and **III**d were screened to match their impact with relevancy the parent compounds. The nanoform for compound **IIId** showed high activity, whereas the nanoforms of **Id** and **IId** compounds showed sturdy activities towards bacterium and fungi. The results obtained are shown in **figure 5.**

Synthesis of silver-nanoparticles

Silver Nanoparticles were prepared by chemical reduction methods.[32] In our work date seed is used as reducing agent,[34] where, 10 ml date seed extraction was added to 0.01gm silver nitrate and the solution completed to 100 ml by adding 90 ml distilled water, this mixture was put in the ultrasonic bath for 30 min until the solution turned to pale yellow solution. The appearance of pale yellow color in the colorless solution has been taken as indicative of AgNPs synthesis by almost all the researchers.[35] UV/ Visible spectrum analysis of the synthesized AgNPs showed λ_{max} at 446 nm in agreeing with the significant range for AgNPs.[36]

AgNPs was added to selected azo triazole cupperated compounds as **Id**, **IId**, and **IIId**, Transmission electron microscopy is used to determine the morphology and particle size via TEM of nanoforms of compounds **Id, IId,** and **IIId** are shown in **figures 6, 7** and **8** respectively.

Conclusion

Our research work involves synthesis diazo derivatives of the 3-amino-1,2,4-triazole ring then metallated by different metal acetate and then treated with silver nanoparticles. The newly synthesized azo metal compounds are characterized by different modern analytical techniques and purity is checked by TLC. Most of the organometallic compounds possess good antimicrobial activities against one strain Gram-positive bacteria Staphylococcus aureus and Escherichia coli Gram-negative bacteria as well as two pathogenic fungi, as Aspergillus flavus and Candida albicans. Especially organocopper compounds have high biological activity.

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Author Contributions

All authors are equally contributed to this article.

Conflicts of Interest

The authors declare no conflict of interest.

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