Biochemical Characterization and Antimicrobial Activity against Some Human or Phyto-Pathogens of New Diazonium Heterocyclic Metal Complexes

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String of vanadium (IV), zirconium (IV), palladium (II), platinum (IV) and uranium (VI) chelates of 2-cyano-2-[(2-nitrophenyl)hydrazono]thioacetamide (Cnphta) were prepared and characterized by physicochemical, spectroscopic and thermal analyses. The formulae of the isolated solid complexes were assigned as [VO-(Cnphta)2(H2O)]SO4·5H2O (1), [ZrO(Cnphta)2(H2O)]Cl2·4H2O (2), [Pd(Cnphta)2]Cl2 (3), [Pt(Cnphta)2Cl2]Cl2 (4) and [UO2(Cnphta)2](NO3)2·5H2O (5). The infrared assignments clearly showed that Cnphta ligand coordinated as a bidentate feature through the hydrazono nitrogen and the thioacetamide nitrogen for V(IV), Zr(IV) and U(VI) but displayed different behavior for Pd(II) and Pt(IV). Results of the molar conductivities measurements showed that the metal complexes were electrolytes in contrast with Cnphta ligand. The interpretation, mathematical analysis and evaluation of kinetic parameters were also carried out. In addition, the studied ligand and its new chelates were tested for their antimicrobial activity against some human or phytopathogenic microorganisms. The new metal complexes explicated promising antibacterial activity against all tested bacteria especially Staphylococcus aureus and Bacillus subtilis. Regarding the antifungal activity, all metal complexes were able to inhibit the mycelium growth of both tested pathogenic fungi. In particular Zr(IV) and Pt(IV) complexes showed the highest significant fungicidal effect against A. fumigatus similar to positive control.

Keywords: Cnphta, metal complexes, spectroscopic, human and phytopathogens, antimicrobial activity.

Introduction

Recently, there is a high interest in programs targeted at manufacturing of functionally substituted heterocyclic compounds from low-cost raw materials to evaluate them as biodegradable agrochemicals and environmentally friendly and safe pesticides.[1–3] The pharmaceutical importance of organic compounds containing azo group have been found to exhibit a wide range of biological activities like antibacterial, antitumor and as potent local anesthetics.[4,5] Aryl diazonium salts represent a well-known and important group to synthesize heterocyclic compounds that possess many diverse biological activities such as bactericidal, pesticidal, anticonvulsant, anti-inflammatory and antithyroid diseases and tuberculostatic treatment.[6–8] During the last two decades, considerable attention has been paid to the chemistry of the metal complexes of organic heterocyclic compounds containing nitrogen and other donors due to their stability and promising biological activities.[9–15]

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nitrophenyl]hydrazono]thioacetamide (Cnphta). The objective of this research is to document the synthesis, structural characterization and biological activity of new prepared chalcone metal complexes with V(IV), Zr(IV), Pd(II), Pt(IV) and U(VI) using hetero organic molecule Cnphta. In the present research, Cnphta was prepared via the coupling 2-nitrobenzidiazonium chloride with 2-cyano-ethane-thioamide in presence of sodium acetate in ethanol. The chemical formulas and structures of Cnphta (Figure 1) and its metal complexes were established via magnetic conductance measurements, elemental analysis, UV/VIS, mass (MS), IR and 1H-NMR spectroscopy as well as thermogravimetric analyses (TG/DTG) and kinetic parameters. The antibacterial activity of the studied Cnphta and the new prepared complexes were evaluated against some human or phyto- pathogenic bacteria Staphylococcus aureus, Bacillus subtilis (Gram-positive, G+=ve) and Escherichia coli and Pseudomonas aeruginosa (Gram-negative, G-ve). Whereas the antifungal activity were carried out against Aspergillus flavus and A. fumigatus.

Results and Discussion

The new synthesized chelates of (1) V(IV), (2) Zr(IV), (3) Pd(II), (4) Pt(IV) and (5) U(VI) with Cnphta are stable in air and insoluble in most of the organic solvents, colored and non-hygroscopic in nature. All the complexes were solvable in dimethyl formamide and dimethyl sulfoxide. The exquisite physical properties and distinctive data of the Cnphta ligand and its chelates were evaluated (Table 1). In order to decide whether SO4$^{2-}$, Cl$^{-}$ and NO$_3$$^{-}$ anions are coordinated or outside the coordination sphere, the conductance of 1.0 × 10$^{-3}$ DMSO solutions of the complexes were measured at room temperature (Table 1). The observed value for the complex (1) is 95.40 S cm$^{-1}$ mol$^{-1}$ shows 1:1 electrolyte, while (2), (3), (4) and (5) complexes with values 150.45, 144.81, 141.17 and 156.36 S cm$^{-2}$ mol$^{-1}$, respectively, are 1:2 electrolytes. Comparison of the analyses for both the calculated and found data indicates that the compositions of the isolated complexes are coincided with the proposed formulae. The magnetic moment value for the complex (1) found at 1.65 B.M reveals the presence of one unpaired electron per V(IV) ion.

IR Absorption Spectra

The IR spectra of Cnphta ligand and its metal complexes are shown in Figure S1 and listed in Table 2. The IR spectra of the complexes were used with free ligand for the determination of coordinating sites that may be involved in complexation. All Cnphta complex showed abroad band in the 3422 – 3436 cm$^{-1}$ zone, the presence of these bands confirms the presence of water molecules.$^{[17-19]}$

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**Table 1.** Elemental analysis and physico-analytical data for Cnphta and its metal complexes.

| Compounds                        | Yield % | M.p./°C | Color          | Found (calc.) (%) | \(\Lambda\) (S cm$^{-2}$ mol$^{-1}$) |
|----------------------------------|---------|---------|----------------|-------------------|------------------------------------|
| Cnphta                           |         |         | Yellowish      | 43.26             | C 43.36 H 2.81 N 28.01 Cl – M –   |
| 249H.96, C$_6$H$_7$N$_3$O$_5$S   | 73      | 228     | Dark Brown     | 28.00             | C 28.08 H 3.38 N 18.20 Cl – – M – |
| [VO(C$_3$H$_7$N$_2$O$_2$S)$_2$(H$_2$O)]SO$_4$ . 5H$_2$O | 769.13 | 240     | Yellowish-White| 28.10             | C 28.18 H 3.13 N 18.12 Cl – – M – |
| [ZrO(C$_3$H$_7$N$_2$O$_2$S)$_2$(H$_2$O)]Cl$_2$ . 4H$_2$O | 766.35 | 247     | Black          | 31.89             | C 31.97 H 2.07 N 20.57 Cl – – M – |
| [Pd(C$_3$H$_7$N$_2$O$_2$S)$_2$]Cl$_2$ | 675.55 | 90      | Orange-Brown   | 25.76             | C 25.86 H 1.60 N 16.67 Cl – – M – |
| [Pt(C$_3$H$_7$N$_2$O$_2$S)$_2$]Cl$_2$ | 835.21 | 85      | Yellowish-Green| 21.88             | C 21.99 H 2.44 N 17.10 Cl – M –   |
| [UO$_2$(C$_3$H$_7$N$_2$O$_2$S)$_2$](NO$_3$)$_2$ . SH$_2$O | 982.16 | 235     |                |                   | C 23.34 H 2.81 N 28.01 Cl – M –   |

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**Figure 1.** 2-Cyano-2-[(2-nitrophenyl)hydrazono]thioacetamide (Cnphta).
The Cnphta ligand showed stretching vibration bands attributed to NH, NH$_2$, CN, C=N and C=S at 3322, 3230, 2206, 1604 and 1219 cm$^{-1}$, respectively. The shift of NH (~3311 cm$^{-1}$) and NH$_2$ (~3221 cm$^{-1}$) to lower frequency values with different intensities for the complexes spectra of (1), (2) and (5) proved that Cnphta chelated through the N$_{hydrazono}$ and N$_{thioacetamide}$ entities with V(IV), Zr(IV) and U(VI). The spectra of (3) and (4) chelates signaling the change of ν(C≡N) (~2215 cm$^{-1}$) and ν(C=N) (1611 cm$^{-1}$) to higher frequency specified the participation of the C≡N and C=N units in interaction with metal ions. New bands matched to ν(M=N) vibration supporting the pointed out the mode of coordination (Figure 2) at 628 and 574 cm$^{-1}$ for (1), at 632 and 574 cm$^{-1}$ for (2), at 644 and 590 cm$^{-1}$ for (3), at 655 and 578 cm$^{-1}$ for (4) and at 655 and 628 cm$^{-1}$ for (5) (Table 2) which are absent in the spectrum of Cnphta. The infrared spectra of the synthesized complexes display changes in the aromatic ring vibrations in comparison to the corresponding absorption bands for free ligand. According to the proposed structure for the complexes under investigation (Figure 2), the four nitrogen atoms of Cnphta ligand positioned in the equatorial regions around the metal ions generate a plane of six membered rings with C$_2v$ symmetry. The C$_{2v}$ complex, [UO$_2$(Cnphta)$_2$]$^{2+}$ was suggested to demonstrate 147 vibrational fundamentals, which all are monodegenerate$^{[19-22]}$. These are distributed between A$_1$, A$_2$, B$_1$ and B$_2$ motions; all are IR and Raman active, except for the A$_2$ modes which are only Raman active. The uranyl moiety has two stretching vibration peaks (asymmetric and symmetrical) at 949 and 864 cm$^{-1}$$^{[20-23]}$. The calculated bond length and force constant values are 1.71 Å and 281.96 Nm$^{-1}$ according to the known method$^{[24,25]}$. The results are very consistent with the data found for other di-oxo-uranium(VI) chelates.

**Electronic Spectroscopy of the Complexes**

Figure S2 depicts the electronic solid reflection spectra of Cnphta and its chelates in the wavelength scale from 200 to 800 nm. It can be seen that free Cnphta...
ligand reflected at 231 and 249 nm may be attributed to \( \pi-\pi^* \) transition and the second band observed at 380 nm is assigned to \( n-\pi^* \) transitions (Table 3).\(^{[26]} \) The complexes reflection bands were shifted to higher (bathochromic shift) and lower (hypsochromic shift) positions, as well as the absence of the band at 231 nm and the inclusion of new bands from 400 nm to 517 nm that can be allocated to the ligand to metal charge-transfer suggested the creation of their chelates.\(^{[27,28]} \) Furthermore, the chelates (1), (3) and (4) provided new bands corresponding to the d-d transitions which showed from 524 to 578 nm.\(^{[29]} \)

\(^{1}\text{H-NMR Spectra}\)

\(^{1}\text{H-NMR} \) spectra of Cnphta, (2), (4) and (5) compounds were recorded in (\( \text{D}_6 \))DMSO (Figure S3 and Table 4). The protons of \(-\text{NH}\) and \(-\text{NH}_2\) signals of (2), (4) and (5) chelates were marginally differentiated in the Cnphta spectrum, and it was observed at: 11.55 – 11.12 ppm (s, 2H, \( \text{D}_2\text{O} \)) and at: 9.77 – 10.08 ppm (s, 4H, \(-\text{NH}_2, \text{D}_2\text{O}\)), implying that the Cnphta ligand was chelated in (2) and (5) complexes through the two nitrogen atoms of \(-\text{NH}\) and \(-\text{NH}_2\). These protons peaks in the (4) complex, which were found at 11.12 and 10.04 ppm, showed no measurable difference with Cnphta, indicating that the two groups are not coordinated. Furthermore, owing to the existence of water molecules in the complexes, the \(^{1}\text{H-NMR} \) spectra for (2), (4) and (5) chelates indicate a novel peak in the range 3.00 – 4.15 ppm. Matching the Cnphta’s key peaks to their complexes, the intensity of \(-\text{CH} \) aromatic protons signals was increased and reported in the range \( \delta: 6.89 – 8.40 \) ppm (m, 8H, \( \text{Ar-H} \)).\(^{[30]} \)

\(^{1}\text{H-NMR Spectra}\)

Mass spectral analyses of representative chelates were performed to validate the formula weights suggested on the basis of elemental analysis. The mass spectra of Cnphta, (1), (2), (3), (4) and (5) compounds depicted in (Figure S4), showed the parent peaks at \( m/z \) (%): 249 (57.93%), 769 (30.47%), 765 (35.50%), 674 (70.63%), 833 (44.80%) and 982 (66.66%), respectively. \( \text{Scheme 1} \) demonstrates Cnphta’s fragmentation mode. Where, at \( m/z = 249 \) (57.93%) allocated to the molecular ion.
peak [a] losses NO\textsubscript{2} to produce [b] at m/z = 203 (14.86\%), also it losses CH\textsubscript{2}NS to give fragment [c] at m/z = 189 (11.00\%). The molecular ion peak [a] losses C\textsubscript{2}H\textsubscript{2}N\textsubscript{2}S to produce [d] at m/z = 122 (30.00\%) and it also losses C\textsubscript{2}H\textsubscript{2}N\textsubscript{2}S to produce fragment [e] at m/z = 151 (21.00\%). It losses C\textsubscript{3}H\textsubscript{2}N\textsubscript{2}O\textsubscript{2} to produce [f] at m/z = 72 (16.49\%) and losses C\textsubscript{2}H\textsubscript{2}N\textsubscript{2}S to produce fragment [g] at m/z = 137 (10.00\%). The molecular ion peak [a] losses NH\textsubscript{2} to produce fragment [h] at m/z = 233 (42.93\%). Scheme 2 manifest the fragmentation manner of (5) complex as representative case. Where at m/z = 982 (66.66\%) allocated to the molecular ion peak [a] losses N\textsubscript{2}O\textsubscript{4} to produce [b] at m/z = 890 (50.00\%) and it losses C\textsubscript{12}H\textsubscript{2}N\textsubscript{2}O\textsubscript{4} to produce [c] at m/z = 738 (23.55\%). The molecular ion peak [a] losses C\textsubscript{2}H\textsubscript{2}NO\textsubscript{2} to produce [d] at m/z = 860 (28.77\%) and it losses C\textsubscript{2}N\textsubscript{2} to produce [e] at m/z = 930 (19.00\%). The molecular ion peak [a] losses C\textsubscript{2}N\textsubscript{2}O\textsubscript{4} to produce [f] at m/z = 838 (17.89\%) and it losses NO\textsubscript{2} to produce [g] at m/z = 936 (13.20\%).

**Thermal Studies**

Thermogravimetric analyses for prepared compounds were carried out under N\textsubscript{2} flow to support the proposed formulae and structures (Figure S5). A survey of the literature reveals that the order of decomposition by pyrolysis of the constituents of solid complexes is water, anion, ligand and final residue, corresponding to either metal oxide or free metal. The thermograms of Cnphta showed a thermal stability up to 150 °C then decomposed in one step in the range 150–650 °C with mass loss 99.90\% correspond to the loss of HSCN + 3C\textsubscript{2}H\textsubscript{2} + 2CO + 2N\textsubscript{2} (Table 5). The data of the thermogram of [VO(Cnphta)\textsubscript{2}(H\textsubscript{2}O)SO\textsubscript{4}·5H\textsubscript{2}O complex (1) shows that the complex was decomposed with two steps. The first step occurs in the range 50–150 °C with mass loss 11.67\% (calc. = 11.70\%) corresponds to removal of 5H\textsubscript{2}O molecules. The last step at two maxima at 256 and 503 °C with weight loss 69.23\% (calc. = 69.18\%) correspond to elimination of 8 C\textsubscript{2}H\textsubscript{2} + 2CO + 2SO\textsubscript{2} + 5N\textsubscript{2} giving VSO\textsubscript{4} as a final product. The TG and DTG data of complex (2) proved that the complex completely decomposed in to stages. The first one at 88 °C with weight loss 9.33\% (calc. = 9.39\%) correspond to loss of lattice water molecules. The final step occurs at 176–618 °C with mass loss 62.05\% (calc. = 62.00\%) corresponding to the loss of 8 C\textsubscript{2}H\textsubscript{2} + 2CO + 2SO\textsubscript{2} + 5N\textsubscript{2} and ZrO\textsubscript{2} + 8C are the final product. The Pd(II) complex decomposed in one step ranged at 25–550 °C with weight loss 84.93\% (calc. = 85.04\%) correspond to loss of 6C\textsubscript{2}H\textsubscript{2} + 2HSCN + 4CO + Cl\textsubscript{2} + 4N\textsubscript{2} giving Pd as a final product. The TG of Pt(IV) complex proceeds approximately at one step which found with two maxima 295, 527 °C with weight loss 61.67\% (calc. = 61.31\%) corresponding to the loss of 5C\textsubscript{2}H\textsubscript{2} + 2H\textsubscript{2}S + 4N\textsubscript{2} + 2NO + 2Cl\textsubscript{2}, giving PtO\textsubscript{2} + 8C as a final residue. The thermal decomposition of complex (5) proceeds via two main degradation steps. The first step occurs at two temperatures maxima 55 and 127 °C, with weight loss 9.10\% (calc. = 9.16\%) attributed to the loss of 5H\textsubscript{2}O. The second step of decomposition occurs at three temperatures maxima 240, 348 and 496 °C with weight loss 63.40\% (calc. = 63.34\%) corresponding to loss 7C\textsubscript{2}H\textsubscript{2} + 4CO + 2SO\textsubscript{2} + 2NO + 5N\textsubscript{2} (Table 5).

**Table 3. UV/VIS spectral data of Cnphta and its metal complexes.**

| Assignments (nm) | Cnphta | Cnphta complexes with |
|------------------|--------|-----------------------|
| π-π* transitions | 231, 249 | 240, 244, 251, 262, 227 |
| n-π* transitions | 380 | 379, 391, 281, 370, 374, 392, 281, 374, 281, 374, 272 |
| Ligand-metal charge transfer | – | 400, 517, 426, 429, 469, 487, 449, 494, 419 |
| d-d transitions | – | 530, 549, 578, – | 543, 572, 577, 524, 543, 577, – |

**Table 4. Selected 1H-NMR data of Cnphta and its metal complexes 2, 4 and 5.**

| Compounds | δH\textsubscript{H\textsubscript{2}O} | δH\textsubscript{–CH aromatic} | δH\textsubscript{–NH amine} | δH\textsubscript{–NH hydrazid} |
|-----------|------------------|-----------------|-----------------|------------------|
| Cnphta | 6.89–8.01 | 7.29–8.42 | 9.78, 10.08 | 11.12 |
| 2 | 3.00, 3.33 | 6.92–8.35 | 10.04 | 11.12 |
| 4 | 3.14, 4.15 | 7.28–8.40 | 9.77, 10.07 | 11.55 |
| 5 | 3.01, 3.42 | 7.28–8.40 | – | – |
| 2 in D\textsubscript{2}O | 3.01, 3.42 | 7.28–8.40 | – | – |
Scheme 2. Fragmentation pattern of complex (5).

Table 5. Thermogravimetric data of Cnphta and its metal complexes.

| Compounds | TG range (°C) | DTG<sub>max</sub> (°C) | % Estimated (calculated) | Assignment | Lost species |
|-----------|---------------|-------------------------|--------------------------|------------|--------------|
|           | Mass loss | Total mass loss |                     |            |              |
| Cnphta    | First step 260, 561 | 99.90 (100.00) | 99.90 (100.00) | HSCN + 3C₂H₂ + 2CO + 2N₂ |
| 1         | First step 103 | 11.67 (11.70) | 69.23 (69.18) | 8C₅H₈ + 2CO + 2SO₂ + 5N₂ |
|           | Second step 256, 503 | 19.10 (19.11) | 81.10 (80.88) | VSO₄ |
|           | Residue     | 11.67 (11.70) | 69.23 (69.18) | 8C₅H₈ + 2CO + 2SO₂ + 5N₂ |
| 2         | First step 88 | 9.33 (9.39) | 71.38 (71.39) | 5C₅H₈ + NH₃ + 2HCl + 2SO₂ + 0.5H₂ + 4.5N₂ |
|           | Second step 269, 352, 565 | 28.62 (28.60) | 84.33 (84.24) | ZrO₂ + 8C |
|           | Residue     | 15.67 (15.75) | 71.38 (71.39) | 5C₅H₈ + NH₃ + 2HCl + 2SO₂ + 0.5H₂ + 4.5N₂ |
| 3         | First step 383 | 84.33 (84.24) | 84.33 (84.24) | 6C₅H₈ + 2HSCN + 4CO + Cl₂ + 4N₂ |
|           | Residue     | 15.67 (15.75) | 71.38 (71.39) | 5C₅H₈ + NH₃ + 2HCl + 2SO₂ + 0.5H₂ + 4.5N₂ |
| 4         | First step 295, 527 | 61.67 (61.31) | 61.67 (61.31) | Pd |
|           | Residue     | 38.33 (38.68) | 72.50 (72.50) | 5C₅H₈ + 2H₂S + 4N₂ + 2NO + 2Cl₂ |
| 5         | First step 55, 127 | 9.10 (9.16) | 9.10 (9.16) | PtO₂ + 8C |
|           | Second step 240, 348, 496 | 27.50 (27.49) | 72.50 (72.50) | 7C₅H₈ + 4CO + 2SO₂ + 2NO + 5N₂ |
|           | Residue     | 27.50 (27.49) | 72.50 (72.50) | 7C₅H₈ + 4CO + 2SO₂ + 2NO + 5N₂ |

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Thermodynamic Parameters

The kinetic parameters $\Delta H^*$, $\Delta E^*$, $\Delta G^*$ and $\Delta S^*$ (Table 6) were tested using Coats-Redfern and Horowitz-Metzger models equations (Figure S6). Decomposition steps of activation energies were found in the range 48.36–153.11 kJ mol\(^{-1}\). The positive sign of $\Delta G^*$ for complexes demonstrated that the free energy of the final residue’s was higher than the initial compounds, implying all decomposition steps non-spontaneous process. For the subsequent decomposition stages of a given complex, the values of the activation, $\Delta G^*$, increased significantly. This explained that substantially rising the values of $T\Delta S^*$ from one stage to the next overrides the values of $\Delta H^*$. Negative $\Delta S^*$ values for the degradation process of metal complexes suggested the activated fragments have more ordered structure than reactants or decomposition reactions are slow.\(^{[5,33,34]}\)

\[
\ln \left[ \frac{1 - (1 - \infty)^{1-n}}{1-n} \right] = \ln \left( \frac{AR}{\beta E} \right) - \frac{E_a}{RT} \quad \text{for } n \neq 1 \tag{1}
\]

\[
\ln \left[ \frac{-\ln(1-\infty)}{T^2} \right] = \ln \left( \frac{AR}{\beta E} \right) - \frac{E_a}{RT} \quad \text{for } n = 1 \tag{2}
\]

\[
\ln[-\ln(1-\infty)] = \frac{E_a \theta}{RT^2} \quad \text{for } n = 1 \tag{3}
\]

\[
\ln \left[ \frac{1 - (1 - \infty)^{1-n}}{1-n} \right] = \ln \left( \frac{AR}{\beta E} \right) - \frac{E_a}{RT} + \frac{E_a \theta}{RT^2} \quad \text{for } n \neq 1 \tag{4}
\]

\[
\Delta H^* = E_a - RT \tag{5}
\]

\[
\Delta S^* = R \ln hA \tag{6}
\]

\[
\Delta G^* = \Delta H^* - T\Delta S^* \tag{7}
\]

Antimicrobial Activity

Cnphta and its complexes were verified for bactericidal effect against two G+ve (S. aureus K1 and B. subtilis K22) and two G-ve (E. coli K32 and P. aeruginosa SW1) compared to the positive controls Ampicillin, Amoxycillin and Cefaloxin (Table S1). Whereas the fungicidal activity of the studied compounds were verified against two serious pathogenic fungi (A. flavus and A. fumigatus) compared to the positive control Ketoconazole (Table S1). The results of antibacterial and antifungal assay were represented in Figures 3 and 4 where all tested treatments showed remarkable bactericidal and fungicidal activities against all tested pathogens. The biological activity of the above mentioned complexes can be explained through the Overtone’s concept. The antimicrobial activity of the parent ligand can be enhanced by the chelation with the studied
metal ions. In particular, the chelation process enhances the delocalization of the electrons increasing the lipophilicity of the central ions and ease the penetration through the microbial cell membrane.\[^{35-37}\]

Furthermore, the increase in lipophilicity aid the tested compounds to penetrate deeper into the microorganisms cells which blocks the metal binding sides of microbial enzymes.\[^{36,38-42}\] The metal complexes can also disturb the respiration process of the microorganism and block the synthesis of proteins inhibiting the growth of the organism. The antibacterial activity of metal complexes can be ordered as following: \(3 > 4 > 5 > 2 > 1\), whereas the antifungal can be ordered as following: \(2 > 4 > 3 \geq 5 > 1\). In all cases the bioactivity of prepared complexes were higher than the parent ligand.

**Conclusions**

A new hetero organic compound 2-cyano-2-[(2-nitrophenyl)hydrazono]thioacetamide was synthesized and reacted with some metal ions (V(IV), Zr(IV), Pd(II), Pt(IV) and U(VI)) to form the corresponding complexes. The suggested chemical formulae and the probable structures of Cnhta and its metal complexes were supported by elemental analyses, molar conductivity, magnetic, \(^1\)H-NMR, MS, IR, UV/VIS and TG measurements. The infrared data indicated that Cnhta chelated with metal ions through two N atoms leading to the formation of octahedral geometries for all complexes except Pd(II) complex which tends to be square planar structure. The data of thermal studies for the compounds supporting the chelation, nature and the number of water in the complexes. Results showed that the tested compounds have noteworthy antimicrobial effect against all tested pathogens especially against *S. aureus, B. subtilis* and *A. fumigatus* compared to the positive controls. The chemical structure of the free ligand Cnhta is important factor related to the promising antimicrobial activity of some trace elements. In addition, the metal complexes demonstrated highly significant activities as compared to the single Cnhta ligand. The outcomes of this research indicate the possible use of these new prepared compounds against the new resistant pathogenic strains to the commonly used antibiotics or fungicides.

**Experimental Section**

**Chemicals**

All chemicals used were of the analytical reagent grade (AR) and of highest purity and are commercially from different sources. VOSO\(_4\)-H\(_2\)O (99.9 %), ZrOCl\(_2\)-8H\(_2\)O (99.9 %), PdCl\(_2\) (99.9 %), PtCl\(_4\) (99.9 %) and
Synthesis of Cnphta

Cnphta was prepared by dissolving 5 mmol (0.5 g) of 2-cyanoethanethioamide (A) in 20 mL ethanol and 5 mmol (0.9275 g) of 2-nitrobenzene diazonium chloride (B) in 30 mL ethanol in presence of CH₃COONa in 1:1 molar ratio and cooled to 0°C on ice bath (Scheme 3). The resulting solid was filtered, dried and purified by recrystallization from ethanol to afford compound.

Synthesis of Metal Complexes

The dark-brown solid complex [VO-(Cnphta)₂(H₂O)]SO₄·5H₂O (1) was prepared by addition of VO(SO₄)·H₂O (0.5 mmol, 0.091 g) in 20 mL acetone to Cnphta (1 mmol, 0.25 g) in 20 mL acetone. The reaction mixture was stirred at room temperature for 24 h. The mixture was left for slow evaporation, then the formed precipitate as filtered off, washed several times using bidistilled water and dried over CaCl₂ in a desiccator. Whereas, yellowish-white [Zr₂(C₅H₅N₂O₂S)₂(H₂O)]Cl₂·4H₂O (2), black [Pd-(C₅H₅N₂O₂S)₂]Cl₂ (3), orange-brown [Pt-(C₅H₅N₂O₂S)₂]Cl₂ (4) and yellowish-green [UO₂(C₅H₅N₂O₂S)₂](NO₃)₂·5H₂O (5) solid complexes were synthesized as mentioned previously, utilizing acetone as a solvent and corresponding metal salts, respectively, in 1:2 molar ratios (M:Cnphta).

Antimicrobial Investigation

Tested Bacterial Strains

The tested bacterial strains, S. aureus K1, B. subtilis K22 (G+ve) and E. coli K32 and P. aeruginosa (G-ve) have been conserved as pure cultures in the collection of the School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Potenza, Italy.

Tested Fungal Strains

The tested phytopathogenic fungi, A. flavus and A. fumigatus, were stored as pure cultures at 4°C on potato dextrose agar (PDA) in the mycotheca of SAFE, University of Basilicata, Potenza, Italy. Both tested fungi were previously identified using morphological and molecular methods. The genomic DNA (gDNA) of each tested fungi was extracted using a Qiagen Genomic DNA Kit (Qiagen, Heidelberg, Germany). The extracted gDNA was amplified using TS4 and TS5 primers[43,44] and the obtained amplicons were sequenced and matched to those present in GenBank using Simple Local Alignment Search Tool program from 1990 (BLAST, USA).[45]
Microbicidal Assay

The antibacterial and antifungal activity of all tested compounds (1), (2), (3), (4) and (5) were examined following the method of Beecher and Wong process\(^{(46)}\) with minor modifications. Briefly, the Mueller-Hinton culture media was prepared and was inoculated with each tested pathogen. Five mm diameter holes were drilled with a sterile cork-borer on surface media. One hundred μL of each tested compounds, after completely dissolved in DMSO at 10\(^{-3}\) M, were injected into holes and then all plates were incubated at 37°C for 20 h for bacteria and 7 days at 30°C for fungi. The antimicrobial activity was estimated by measuring the diameter of inhibition zone (mm± SDs) compared to Ampicillin, Amoxycillin and Cefaloxin as positive controls for bactericidal assay and Ketoconazole for fungi.

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Author Contribution Statement

M.S. El-Attar: Formal analysis, Data curation, Investigation. Writing - review & editing. H.S. Elshafie: Investigation, Data curation, Writing - review & editing, Supervision. S.A. Sadeek: Data curation, Writing – review& editing, Supervision. A.F. El-Farargy: Data curation, Writing, review & editing, Supervision. S.I. El-Desoky: Methodology, Formal analysis, Data curation, Investigation. W.H. El-Shwiniy: Methodology, Formal analysis, Data curation, Investigation. I. Camele: Data curation, Writing - review & editing, Supervision.

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