

Transition Metal Complexes of 2-methoxybenzaldehyde-N-(5-chloro-2-oxo-1, 2-dihydro-3H-indol-3-ylidene) hydrazone as New Tyrosinase Inhibitors: Design, Structural Description & In-Vitro Assessment

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ABSTRACT

The copper-containing tyrosinase is a trans-membrane glycoprotein responsible for catalyzing the rate limiting steps in mammalian melanogenesis, resulting in darker skin color and some dermatological disorders, e.g. melasma, age spots and sites of actinic damage. There are limited studies available on this enzyme in general and limited potent inhibitors are reported in particular. This led us to search for some new potent inhibitors for this enzyme. In this study a new series of coordination compounds from Co (II), Ni (II), Cu (II) and Zn (II) have been synthesized using an isatin derived hydrazone ligand i.e. 2-methoxybenzaldehyde-N-(5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazone. Structural characterization of synthesized complexes was carried out by means of employing standard methods e.g. elemental analysis, magnetic susceptibility measurements, molar conductance measurements, IR and UV-Vis. spectroscopy. The characterization suggested that complexes of Co (II), Ni (II) and Zn (II) acquired octahedral geometry with the bi-dentate ligand, while square plane geometry was deduced for Cu (II) complex. A comparative study for screening tyrosinase inhibitory potential was done for all compounds and it was observed that coordination complexes were more effective inhibitors of tyrosinase when compared to the free ligand.

Keywords: tyrosinase, melanogenesis, hydrazone, spectroscopy.

1. INTRODUCTION

Tyrosinase is a copper-containing trans-membrane glycoprotein that is responsible for catalyzing the rate limiting steps in mammalian melanogenesis and determining their skin and hair color¹. Hyper pigmentation in epidermal tissues is reported to be the cause of many dermatological disorders, for example melasma, age spots and sites of actinic damage². Tyrosinase is also found responsible for negative enzymatic browning in vegetables and fruits during post-harvest treatment, which decreases the nutritional quality of food products³. An extensive survey of the literature revealed that the subject tyrosinase inhibition hasn't been adequately studied⁴ and there is a great room for new and better tyrosinase inhibitors that can add up to available therapeutics and it all urged us to search for new potent synthetic inhibitors.

There are several starting organic precursors as candidate compound, we chose to start with one of hydrazone derived from isatin for its broad spectrum of biological potentials⁵⁻⁹. Hydrazone derivatives form an important class of starting compounds to be used in various scenarios of biological significance. The presence of donor atoms such as nitrogen, sulfur or oxygen not only renders them as good prospect inhibitors, but also provides effective coordination capabilities towards complexation¹⁰.

In this present work, we aimed to synthesize four complexes of 2-methoxybenzaldehyde-N-(5-chloro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)hydrazone with Co(II), Ni(II), Cu(II) and Zn(II). These newly synthesized metal complexes were fully characterized by standard physical and spectroscopic methods including IR spectroscopy, UV-visible spectroscopy and elemental analysis. Tyrosinase inhibitory potential of metal complexes along with free ligand were evaluated in-vitro and compared with the standard i.e. kojic acid.

2. MATERIAL & METHODS

All the reagents were obtained from Sigma-Aldrich and BDH and of analytical grade and used directly. All the solvents were distilled prior to use.

2.1 Physical measurements

CHN of ligand and its complexes, were measured by Perkin Elmer 2400 series II CHNS/O analyzer. Perkin Elmer Series 200 Atomic Absorption Spectrometer was used to determine the metal contents of the synthesized complexes. Shimadzu IR-prestige-21 was used to record the infrared spectrum of all compounds. JENWAY 4701 conductivity meter was used to measure the molar conductance at room temperature in DMSO. UV/Vis spectra were obtained by Perkin-Elmer Lambda-5 UV/Vis spectrometer while measurements of magnetic susceptibility were recorded by Sherwood MSB Mk1. Tyrosinase inhibition assay was performed on Spectramax-340 micro plate reader (Molecular Device USA).

2.2 Synthesis of Hydrazone Ligand

Hydrazone ligand (code BH-5) was prepared by refluxing 10mL solution of 0.5M 5-Chloro isatin with 10mL hydrazine hydrate in 100mL methanol for 2-3 hour at 70°C. Filter off the resulting yellow color crystals of monohydrazone then these crystals were further reflux with 10mL solution of 0.5M 2-methoxybenzaldehyde; for 3-4 hour in methanol. On cooling, orange colored ppt were filtered and then washed with CCl₄, recrystallization was done by using methanol and dried in vacuum to get the required hydrazone¹¹.

2.3 Synthesis of Metal (II) complexes

Metal complexes of the hydrazone were synthesized by refluxing 25mL solution of 0.2M metal chloride and 30mL of 0.4M solution of BH-5 in methanol for 2-3 hours. The resulting solution was concentrated, filtered and washed with CCl_4 and then recrystallized by slow evaporation from methanol solution^{12, 13}.

2.4 Procedure for Tyrosinase Inhibition Activity

Tyrosinase inhibitory activity of the hydrazone ligands and its metal complexes was performed as per reported method by Kubo & Kinoshita, 1998 with slight modifications. Testing compounds i.e. ligand and complexes were dissolved in dimethyl sulfoxide (DMSO) at desired concentrations.

40 μL solution (1.5mM) of substrate i.e. L-3,4-dihydroxyphenylalanine (L-DOPA) was dissolved in 40 μL of 0.1M phosphate buffer (pH 6.8). 40 μL of sample was then added into the buffer-substrate solution and incubate it for 10min at room temperature. After incubation, 40 μL of the aqueous solution of tyrosinase was added in the mixture. Absorbance of the solution was recorded at 475nm. Triplicate trials were done for each tested sample. Kojic acid was used as standard inhibitor for tyrosinase¹⁴.

IC_{50} values i.e. inhibitory concentration at which 50% of enzyme activity was inhibited were calculated to assess the tyrosinase inhibitory potential of the hydrazone ligand and its complexes. The percentage inhibition was estimated by given equation:

$$\% \text{ Inhibitory} = \frac{(A_0 - A_s)}{A_0} \times 100$$

Where, A_0 is the absorbance of the control (without samples) and A_s is the absorbance of the samples.

3. RESULTS & DISCUSSION

3.1 Synthesis of Complexes

The results of elemental analysis of synthesis of complexes have been presented in Table-1, and the corresponding calculated values of molar conductivity and magnetic moment are listed in Table-2. With the help of the data obtained, we suggest 1:2 metal-to-ligand molar ratios between the neutral bidentate hydrazone ligand and metal ions. Moreover, octahedral geometry have been suggested for Co(II) and Ni(II) complexes while square planar and tetrahedral geometries were proposed for Cu(II) and Zn(II) complexes¹².

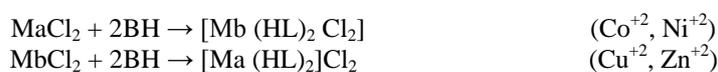
Table-1. Elemental Analysis of Metal Complexes of BH-5

| COMPOUNDS | | [Co(BH-5) ₂ Cl ₂] | [Ni(BH-5) ₂ Cl ₂] | [Cu(BH-5) ₂ Cl ₂] | [Zn(BH-5) ₂ Cl ₂] |
|--------------|-------|--|--|--|--|
| Formula mass | | 754.99 | 753.99 | 758.99 | 759.99 |
| Color | | Orange | Spring green | Navy blue | White |
| % Yield | | 69 | 67 | 62 | 49 |
| M% | Found | 7.72 | 7.78 | 8.41 | 8.47 |
| | Cal | 7.78 | 7.75 | 8.34 | 8.56 |
| C% | Found | 50.66 | 50.72 | 50.69 | 50.22 |
| | Cal | 50.75 | 50.77 | 50.44 | 50.32 |
| H% | Found | 3.03 | 3.14 | 3.04 | 3.21 |
| | Cal | 3.19 | 3.2 | 3.17 | 3.17 |
| N% | Found | 11.29 | 10.96 | 10.88 | 10.79 |
| | Cal | 11.1 | 11.1 | 11.03 | 11.01 |
| Outer Cl% | Found | --- | --- | 9.71 | 9.84 |
| | Cal | --- | --- | 9.35 | 9.34 |

Table-2: Magnetic Moment & Conductometric Data of the Metal Complexes of BH-5

| Complexes | μ_{eff} (BM) | Conductance (μS) |
|--|-------------------------|-------------------------------|
| [Co(BH-5) ₂ Cl ₂] | 4.76 | 14 |
| [Ni(BH-5) ₂ Cl ₂] | 3.21 | 15 |
| [Cu(BH-5) ₂ Cl ₂] | 1.85 | 154 |
| [Zn(BH-5) ₂ Cl ₂] | D | 152 |

The fundamental chemical equations for the synthesis of metal complexes can be written as:



*BH = hydrazone ligand

Complexes were solid and non-hygroscopic at room temperature and showed no sign of decomposition or volatility. Complexes were highly soluble in DMSO and DMF and showed low solubility in other organic solvents while insoluble in water. The results shown in Table-1 is in good agreement with the expected complexes composition. The proposed geometry for the metal complexes have been given in Fig-1.

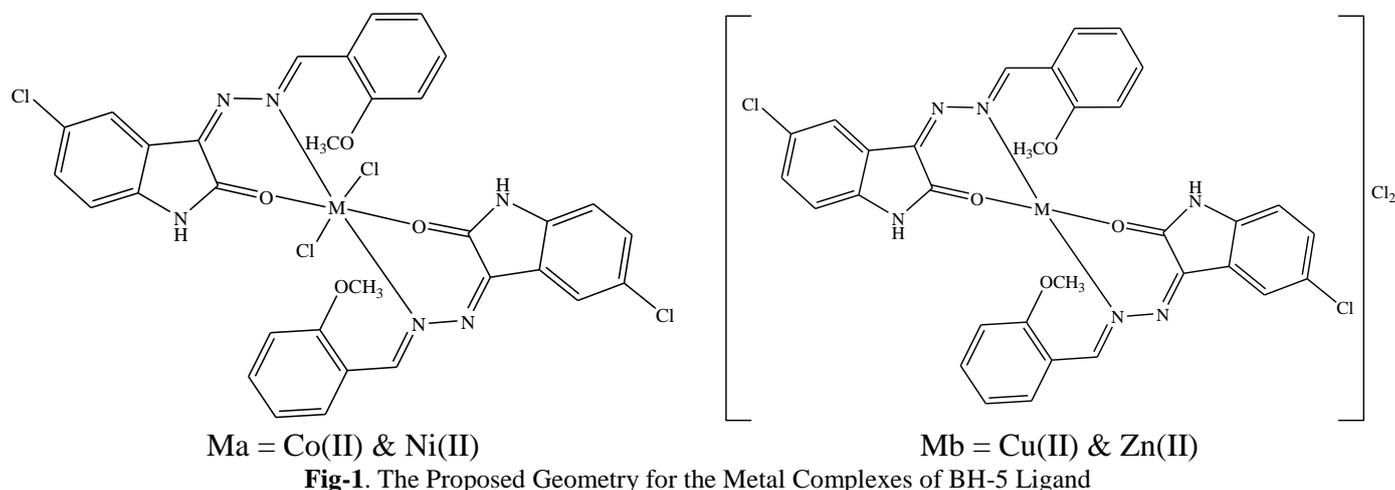


Fig-1. The Proposed Geometry for the Metal Complexes of BH-5 Ligand

3.2 Electronic Spectra and Magnetic Studies

The electronic spectrum of hydrazone ligand show two broad peaks between 280-360nm, which were assigned to $n-\pi^*$ transitions of the aldimine and ketimine moieties, respectively.

The electronic spectra of Co(II) complex showed three absorption bands with the maximum absorbance at 11601cm^{-1} , 19194cm^{-1} and 25707cm^{-1} , may assigned to the ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$, ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$ transitions respectively. Magnetic moment for Co (II) complexes were found to be 4.76BM, which along with electronic data, adequately confirm high spin octahedral Co (II) complexes¹².

For Ni(II) complex, UV-Vis, spectrum showed three sharp peaks at 11696cm^{-1} , 15674cm^{-1} and 25641cm^{-1} which have been assigned to the ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}$; ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$ and ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$ transitions respectively. Magnetic moment for Ni (II) complex were found to be 3.21 that was also consistent with octahedral structure¹⁵.

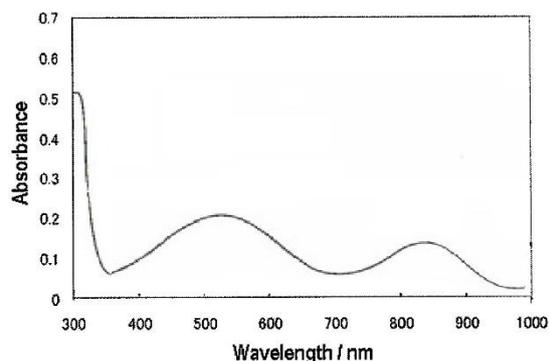
UV-Vis spectrums of Cu (II) complex showed two prominent peaks, one low intensity band at 13908cm^{-1} and other broad band at 26247cm^{-1} correspond to ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ and ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_{1g}$ transitions, predicting square-planar Cu (II) complex. Magnetic moment values of 1.85BM, also confirming square planner geometry and the absence of metal-metal interaction in the complex¹³.

Zn (II) complex exhibited single absorption maxima at 28490cm^{-1} , arises possibly due to intra-ligand transitions. Normally, Zn (II) ion forms tetrahedral complexes and its d^{10} electronic configuration confirm its diamagnetic nature¹².

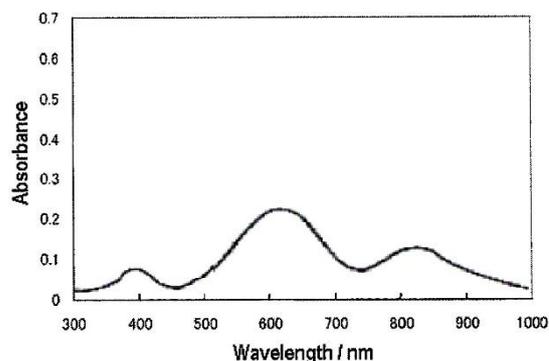
Table-3 presents electronic data of complexes, while UV-visible spectra of complexes have been presented in Fig-2.

Table-3 Electronic Spectroscopic Data of the Metal Complexes of BH-5 Ligand

| Complexes | Bands maxima $\nu \text{ cm}^{-1}$ ($\lambda \text{ nm}$) |
|--|--|
| [Co(BH-5) ₂ Cl ₂] | 25707 (289), 19194 (521), 11601 (862) |
| [Ni(BH-5) ₂ Cl ₂] | 25641 (390), 15674 (638), 11696 (855) |
| [Cu(BH-5) ₂ Cl ₂] | 26247 (381), 13908 (719) |
| [Zn(BH-5) ₂ Cl ₂] | 28490 (351) |



(a)



(b)

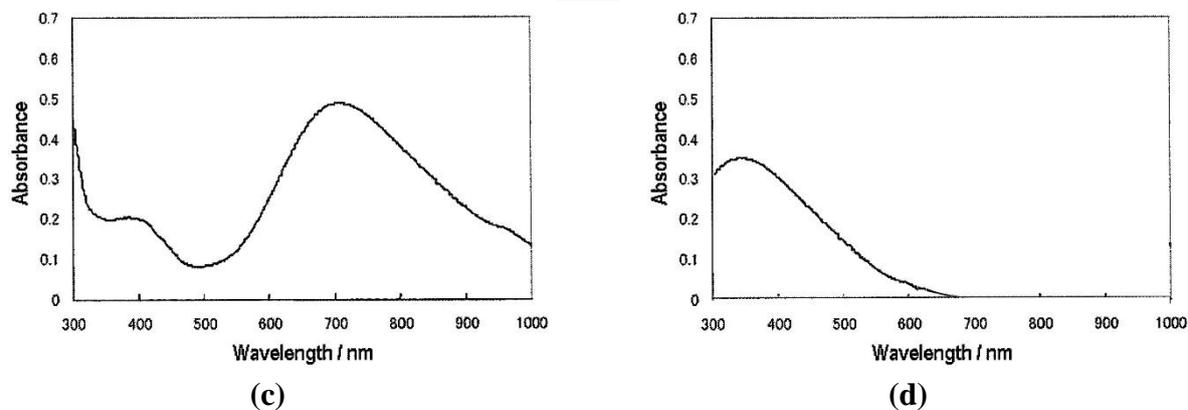


Fig-2 UV-visible spectrum of (a) Co(II) complex, (b) Ni(II) complex, (c) Cu(II) complex and (d) Zn(II) complex with hydrazone ligand i.e. BH-5

3.3 I.R Spectral Studies

Hydrazone ligand showed a strong band at 3273cm^{-1} that was assigned to indol ring (N—H) while lactonyl carbon (C=O) exhibited its characteristic band at 1737cm^{-1} . Strong band with a peak at 1608cm^{-1} , corresponding to aldimine group i.e. HC=N was also observed. Infra-red spectrum of ligand also showed some other strong bands e.g. at 1670cm^{-1} , 1296cm^{-1} , 1024cm^{-1} and 835cm^{-1} which were attributed to ketimine (C=N), phenolic (C—O), hydrazinic (N—N) and C—Cl groups.

Infrared spectra of the synthesized complexes exhibited the band of stretching frequency of aldimine HC=N group in between $1568\text{--}1579\text{cm}^{-1}$. When compared with free hydrazone, these significant decreases in the frequencies i.e. $29\text{--}40\text{cm}^{-1}$ after complexation, designates the coordination of aldimine nitrogen atom with the metal ions.

IR spectra of the metal complexes showed another prominent $32\text{--}42\text{cm}^{-1}$ shift in the frequencies of carbonyl group confirming the binding of metal ions through carbonyl oxygen, present at the indole end in the hydrazone ligand.

The location of bands in the IR spectrum of the metal complexes due to stretching frequency of N—H (indol ring) and C=N (ketimine) did not show any considerable shift as compared to the frequencies of the ligands, therefore indicated the absence of any sign of coordination from these groups.

In the spectrum of Co (II) and Ni (II) complexes, some additional bands were also observed in the region of $518\text{--}526\text{cm}^{-1}$, $411\text{--}458\text{cm}^{-1}$ and $361\text{--}364\text{cm}^{-1}$, attributed to the stretching frequencies of M—O, M—N and M—Cl bonds respectively. Interestingly, these bands were absent in the spectrum of Cu (II) and Zn (II) complexes, indicating the absence of inner sphere chloride ion in their coordination sphere^{12, 13, 16}. The details of the infrared absorption bands of ligand and its metal complexes have been presented in Table 4.

Table-4: Significant IR Spectral Bands of the BH-5 Hydrazone and its Metal Complexes

| Tentative Assignments | BH-5 | [Co(BH-5) ₂ Cl ₂] | [Ni(BH-5) ₂ Cl ₂] | [Cu(BH-5) ₂ Cl ₂] | [Zn(BH-5) ₂ Cl ₂] |
|-----------------------|------|--|--|--|--|
| ν (OH) | --- | --- | --- | --- | --- |
| ν (NH) | 3273 | 3266 | 3267 | 3268 | 3272 |
| ν (C=O) | 1737 | 1705 | 1699 | 1702 | 1695 |
| ν (C=N) | 1670 | 1661 | 1665 | 1664 | 1666 |
| ν (HC=N) | 1608 | 1573 | 1572 | 1579 | 1568 |
| ν (C—O) | 1296 | 1286 | 1291 | 1291 | 1297 |
| ν (N—N) | 1024 | 1018 | 1014 | 1027 | 1023 |
| ν (C—Cl) | 835 | 822 | 841 | 827 | 832 |
| ν (M—N) | --- | 458 | 456 | 411 | 442 |
| ν (M—O) | --- | 519 | 525 | 526 | 518 |
| ν (M—Cl) | --- | 364 | 361 | --- | --- |

Infrared spectral results provided substantial evidences for the complexation of hydrazone ligands with the metal ions in the bidentate mode from its aldimine nitrogen and carbonyl oxygen. These results also predicted the presence of chloride ion in the coordination sphere, bounded with the metal ions except in Cu (II) and Zn (II) complexes.

3.4 Tyrosinase Inhibition Activity

It was observed that the parent hydrazone ligand was weak tyrosinase inhibitor. It is generally observed that after complexation with metal ions, the bioactivity of organic molecules gets altered^{17, 18}. Similar behavior was observed for BH-5 (weak tyrosinase inhibitor) but its metal complexes were found to be good tyrosinase inhibitors.

These noticeable increases in the inhibitory activity suggested that the resultant metal complexes irreversibly interact with the enzyme and limit its efficiency. BH-5 after coordination with metal ion, redistribute the electrostatic charges and resultant complex have a more stabilized structure, this stability and charge distribution i.e. polarity, are mainly responsible for specific interaction with the enzyme. During this interaction, enzyme may change its confirmation and loses its efficiency.

Results obtained from the tyrosinase inhibition activity are given in Table-5, and the graphical illustration of % tyrosinase inhibition has been represented in Fig-3.

Table-5: IC₅₀ values for Tyrosinase Inhibition Activity of BH-5 Ligand and its Metal Complexes

| COMPOUNDS | IC ₅₀ (μM) Mean ± SEM | % Inhibition (100μM) Mean ± SEM |
|--|-------------------------------------|------------------------------------|
| BH-5 | > 500 | 8.5 ± 0.39 |
| [Co(BH-5) ₂ Cl ₂] | > 200 | 20.6 ± 0.69 |
| [Ni(BH-5) ₂ Cl ₂] | NA | NA |
| [Cu(BH-5) ₂ Cl ₂] | > 200 | 33.5 ± 0.15 |
| [Zn(BH-5) ₂ Cl ₂] | > 200 | 11.2 ± 0.21 |
| * Kojic acid | 43.7 ± 0.04 | 89.01 ± 0.03 |

SEM standard error of mean

NA (not active)

*Standard for Tyrosinase Inhibition Activity

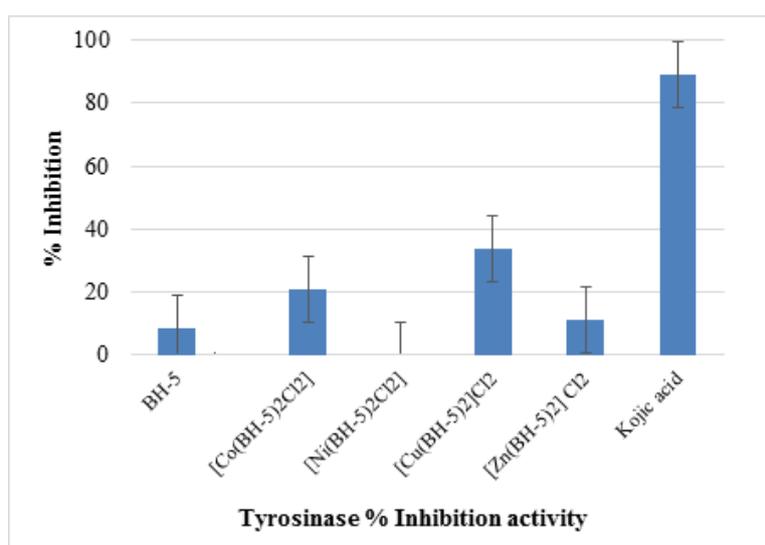


Fig-3 Tyrosinase Inhibition activity of Metal Complexes of BH-5 at 100μM

4. CONCLUSION

Four new metal complexes were synthesized from 2-methoxybenzaldehyde-N-(5-chloro-2-oxo-1, 2-dihydro-3H-indol-3-ylidene)hydrazone. Complexes were characterized using standard physical and analytical methods. Ligand was found to be bound to the central atoms in 1:2 metal-to-ligand molar ratios. Octahedral geometry was suggested for Co(II) and Ni(II) complexes while square planar and tetrahedral geometries were proposed for Cu(II) and Zn(II) complexes. The results of tyrosinase inhibition activities showed that the complexes were more potent enzyme inhibitors than the parent ligand.

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