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Synthesis, Spectral Structural Studies and 5α-Reductase Inhibitory Activities of Co^{II}, Ni^{II}, Cu^{II}, Zn^{II} Mixed Ligand Complexes

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Abstract : The mixed ligand complexes of Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} with catechol (L_1) and 2,3-Diamino-5-bromopyridine (L_2) were synthesized. The bonding nature and shape of complexes were characterized through spectral instrument (IR, NMR, UV-vis), magnetic susceptibility, molar conductivity and elemental analysis. The ligands act as bidentate ligands and all complexes have octahedral geometry with non-electrolytes nature. The ligands and complexes have been screened against 5α-reductase inhibitor *in vivo,* and all tested compounds showed a good inhibition activity towards 5α-reductase enzyme. Zn^{II} complex is found to be the most potent 5α-reductase inhibitor and the lowest toxicity compared to the other tested compounds and standard drug anastrozole.
Key words : Mixed ligand-Catechol-2,3-Diamino-5-bromopyridine-5α-reductase–Anastrozole.

Introduction

The discovery of metal-based drugs is one of the fastest growing and challenging areas of pharmaceutical research. This field gained momentum after the discovery of the antitumor activity of cisplatin which is one of the most widely used anticancer drugs[1].The most important factor in the designing of metal based chemotherapeutic agents is the selecting of metal ions[2-4], since using essential metals (as cobalt, copper, nickel and zinc) may be less toxic than non-essential metals such as platinum. Whereas these metal ions could modify the pharmacological activity of the ligands due to the changes in their charge density distribution, redox potentials, size andshape[5].

On the other hand 5α-Reductase is an NADPH-dependent enzyme that increases the conversion of testosterone to the more potent androgen dihydrotestosterone (DHT), which binds with higher affinity to the androgen receptor (AR) [6]. Androgen-dependent diseases were always associated with the increase of DHT concentration, where these diseases including prostatic hyperplasia, male pattern baldness, hirsutism, acne and prostate cancer[7-10]. The reduction of DHT concentration is useful in treatment of these androgen-dependent diseases, though the search for potent inhibitors of this enzyme appears to be a promising therapeutic target for many researches.

In view of the interesting potential applications of metal complexes in pharmacology and as part of our previous works in bioinorganic chemistryto design and synthesize new potent chemotherapeutic agents[11-14], we described here the synthesis and characterization of Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} mixed ligand complexes derived

from catechol and 2,3-diamino-5-bromopyridine. All the prepared complexes were screened against 5 α-reductase inhibitor activity *in vivo*.

Experimental

Materials

2,3 diamino- 5- bromopyridin obtained from Acros, catechol and metal chlorides were Merck. Organic solvents were reagent grades.

Physical measurements

All melting points were measured on a Gallenkamp melting point apparatus and were uncorrected. Elemental analyses were determined at the micro analytical center, Cairo University. IR spectra were recorded in the 4000-400 cm^{-1} on a (Jasco FTIR- 6100 Japan) spectrometer, using KBr pellets. ^{1}H and ^{13}C NMR were recorded on a Bruker DPX 300, δ values relative to the deuterated DMSO. Magnetic susceptibilities were measured at 20°C by the Gouy method at the Faculty of Science, Cairo University. The molar conductance measurements were measured in solution of the metal complexes in DMF (10^{-3}) using Metrohem 660 conductivity meter. Electronic absorptions were recorded on a (PG Instruments ltd., +80+ UV-Vis) automatic spectrophotometer in DMSO.

Synthesis of mixed ligand complexes

To a solution of metal chlorides (1.0 mol) in ethanol, 2,3 diamino-5- bromopyridine was added (1.0 mmol). The mixture was stirred at 70°C for 1 hour and then (1.0 mmol) of catechol in ethanol was added. The resulting mixture was stirred for 2 hours at the same temperature. After cooling, the resulting precipitate was filtered and washed with diethyl ether then dried.

Spectral characterization of Co^{II} complex

Yield (65%) m.p. above 300; Anal.calcd. for ($C_{11}H_{14}BrCoN_3O_4$) (M.wt., 391.09): C, 33.78; H , 3.61 ; N, 10.74 ; O, 16.36 %, Found C,33.76; H, 3.59; N,10.68; O, 16.32%. IR (KBr, Cm^{-1}): 3401 ν(H_2O), 3209, 3170 ν(NH_2), 1515 δ(NH_2), 1214 ν(C-O), 836, 745 γ(H_2O), 590 ν(M-N), 436 ν(M-O); UV-vis (1 x 10^{-4} M, DMF), λ_{max}/nm= 320, 380, 512; Molar conductance :Λm($\Omega^{-1}cm^2mol^1$) = 11.3;μ_{eff} = 4.75B.M.

Spectral characterization of Ni^{II} complex

Yield 77°C, m.p. above 300°C; Anal.calcd. for ($C_{11}H_{14}BrN_3NiO_4$) (M.wt.390.85): C, 33.80; H, 3.61; N, 10.75;O, 16.37 % Found C, 33.87; H, 3.65; N, 10.72;O, 16.40 %.IR (KBr, Cm^{-1}): 3373 ν(H_2O), 3220, 3192 ν(NH_2), 1515 δ(NH_2), 1214 ν(C-O), 860 , 759 γ(H_2O), 578 ν(M-N), 470 ν(M-O); UV-vis (1 x 10^{-4} M, DMF), λ_{max}/nm =329, 405, 520, 590; Λm ($\Omega^{-1}cm^2mol^{-1}$) =17.4; μ_{eff} = 3.12 B.M.

Spectral characterization of Cu^{II}complex

Yield 71 %,m.p. above 300°C; anal. calcd. for ($C_{11}H_{14}BrCuN_3O_4$) (M.wt. 395.70): C, 33.39; H, 3.57; N, 10.62; O, 16.17bv % , Found C, 33.42; H,3.61; N, 10.59;O, 16.22 %.IR (KBr, Cm^{-1}): 3402 ν(H_2O), 3320, 3181 ν(NH_2), 1518 δ(NH_2) , 1208 ν(C-O), 838, 777 γ(H_2O), 582 ν(M-N), 453 ν(M-O);UV-vis (1 x 10^{-4} M, DMF), λ_{max}/nm = broad band centered at 489nm; Λm ($\Omega^{-1}cm^2mol^{-1}$) =14.2;μ_{eff} = 1.91B.M.

Spectral characterization of Zn^{II}complex

Yield 59°C,m.p. above 300°C; Anal.calcd. for ($C_{11}H_{14}BrN_3O_4Zn$) (M. wt. 397.53): C, 33.24; H, 3.55; N, 10.57; O, 16.10 % . Found C, 3.28; H, 3.59; N, 10.52; O, 16.14 %.IR (KBr, cm^{-1}) :3373 ν(H_2O), 3220, 3192 ν(NH_2), 1515 δ(NH_2), 1214 ν(C-O), 860 , 759 γ(H_2O), 578 ν(M-N), 470 ν(M-O); ^{1}H-NMR (DMSO-d_6): 4.53(Coordinated water);5.12 (br, s, 2H, NH_2), 5.49 (br, s, 2H, NH_2), 6.67-6.89 (m, 4H, Ph , s, 2H,Py);^{13}C-NMR (DMSO-d_6): 106.2, 116.21,118.7, 119.1,130.9, 134.2,142.5, 144.7(Carbons of phenyl and pyridine rings); UV-vis (1 x 10^{-4} M, DMF), λ_{max}/nm =335; Λm ($\Omega^{-1}cm^2mol^{-1}$) =17.4; μ_{eff} = Dia.

Biological assay

Treatment of animals

Animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and were kept on a constant standard diet. Twenty-three groups, each of 12 male Sprague- Dawley rats in the postnatal third days, were treated subcutaneously with the 5α-reductase inhibitor (tested compound or reference standard). The tested compounds were dissolved in 5% Tween 80 in water. The solvent was used for both standard and negative control group, beginning on the postnatal third day until the age of seven weeks. Twenty-one groups were used to test the activities, of which one was used as the positive control for anastrozole and another served as the negative control group. After, scarifying blood was withdrawn for testosterone and dihydrotestosterone determination[15,16].

Radioimmuno assay for testosterone and dihydrotestosterone

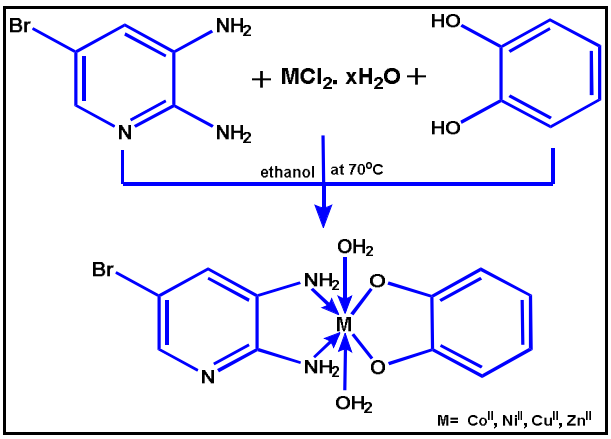
Testosterone and dihydrotestosterone were measured by radioimmuno assay in serum extracts using specific antisera without prior chromatography[16]. Serum samples of 0.5 mL were extractedwith 2 mL of freshly purified peroxide-free diethyl ether by shaking for 60 sec on a Vortex mixer. The aqueous phase was frozen at – 70 °C, the ether phase containing steroids was transferred to a conical test tube and evaporated in BSA/ phosphate buffer (pH = 7.4) containing (1,2,6,7-3H)- testosterone or (1,2,6,7-3H)-dihydrotestosterone and then specific antisera were added and incubated over a period of 24 h at 4 °C under non-equilibrium conditions. Bound hormone and free hormone were separated by adsorption on dextran-coated charcoal. The activity of each sample was determined in a Beckman-counter (USA) using a commercially available scintillation cocktail (Mini-RIA, Zinsser, Spain).The hormone level in the sample was calculated from a standard curve by means of a computer program (KIA-Calc, LKB, Canada), using appropriate control sera. Steroid levels of rats treated with different doses of 5-reductase inhibitors were compared with vehicle-treated controls. The relative potency was calculated by dividing the ED_{50} (dose that causes 50% of pharmacological response in the test) of anastrozole by that of a tested compound.

Determination of acute toxicity

LD_{90} was determined by using 108 adult male albino rats and injecting them with different increasing doses of agents. Dose that killed 90% of the tested animals was calculated according to Austen et al[17].

Results and discussions

The mixed ligand complexes were prepared in a good yield by heating equal molar ratio ethanolic solution of metal chlorides, catechol (L_1) and 2,3-diamino-5- bromo-pyridine (L_2) Scheme 1.These complexes dissolved only in DMSO and DMF, The proposed structure is in a good agreement with results of the micro analytical data (Figure 1).



Scheme 1. Synthesis of Co^{II}, Ni^{II}, Cu^{II}, Zn^{II} mixed ligand complexes

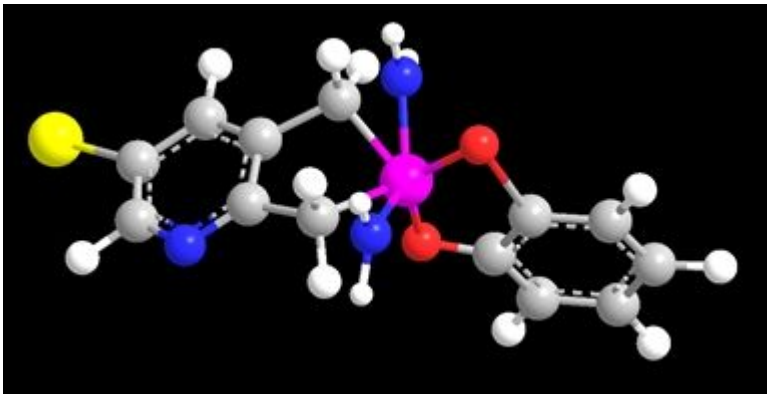


Figure 1. Stereochemistry of the metal complex

Infrared spectra

Significant frequencies have been selected by comparing the IR spectra of ligands and their complexes. The disappearance of ν (OH) 3448 cm^{-1} band of catechol (L_1) and the shift of phenolic (C-O) stretching frequency to lower energy regions in all mixed ligand complexes indicate the deprotonating of OH group and coordinates to metal ion.[18,19]The NH_2 bands at 3373 $\nu_{as}(NH_2)$, 3220 $\nu_s(NH_2)$ and 1570 cm^{-1} (NH_2 deformation) in the ligand (L_2) are shifted to lower frequency in the mixed ligand complexes suggesting that coordination of NH_2 group to the metal ion.[20] The appearance of non-ligand bands at 563-590 cm^{-1} and 436-470 cm^{-1} may be assigned to the ν (M-N) and ν(M-O) respectively.[21,22]All complexes show a broad band around 3450 cm^{-1} and some new bands in region, 743-777 cm^{-1} and 836-865 cm^{-1} which are attributed to ν(OH) of stretching wagging and rocking mode of the coordinated water molecule.[23,24]

NMR Spectra

^{1}H NMR spectra data of the ligands (L_1, L_2) and zinc complex are measured in DMSO solvent.The shifts (δ) of the different types of protons of the ligands and zinc complex are tabulated in Table 1.In comparison with ^{1}H NMR spectrum of ligands (L_1, L_2) zinc complex confirm that OH proton (9.62 ppm. in L_1) is absent in the complex, suggesting the deprotonating of OH proton with metal ion upon chelation.[25] The signals due to NH_2 protons are shifted to downfield suggesting the involvement of the NH_2 groups in coordination. Other signals due to aromatic protons of L_1 and L_2 are slightly shifted to downfield in the spectrum of zinc complex upon chelation. The signal at 4.53ppm may be due to the coordinated water.[26]

^{13}C NMR spectrum of zinc complex is similar to that of the ligands (L_1, L_2) but with down field shiftin the two carbons attached to OH groups in ligand (L_1) from 145.7 ppm to 142.5 ppm. It is found also that the two carbons attached to NH_2 groups in the ligand (L_2) was down field shifted from 132.4, 147.92 to 130.94, 144.7 ppm in zinc complex. Other carbons signals of L_1 and L_2 are slightly shifted to downfield in the spectrum of zinc complex upon chelation.

Table1. NMR spectral data of ligands and Zn- complex

Compound	^{1}H NMR δ (ppm)	Assignment	^{13}C NMR δ(ppm)	Assignment
L1	9.62 6.81- 7.12	(s,2H, two OH group) (m, 4H, phenyl ring)	116.24, 119.89, 145.7	Carbons of phenyl ring
L2	5.37 5.67 6.74 6.82	(br, s, 2H, NH_2 group) (br, s, 2H, NH_2 group) (s, 1H, pyridine ring) (s, 1H, pyridine ring)	106.9, 119.1, 132.43, 134.8, 147.9	Carbons of pyridine ring
Zn^{II}complex	5.12 5.49 6.67- 6.89 4.53	(br, s, 2H, NH_2 group) (br, s, 2H, NH_2 group) (m, 4H, Ph , s, 2H, Py) (coordinated water)	106.2, 116.21 118.7, 119.1 130.9, 134.2 142.5, 144.7	Carbons of phenyl and pyridine rings

Molar conductance

The molar conductivity of the mixed ligand complexes is measured in DMF (10^{-3} solution) at room temperature. The observed molar conductivity values of the measured complexes were in the range 10.6-17.2$\Omega^{-1}cm^2mol^{-1}$, suggesting that all complexes are non-electrolytes and there are no any chloride ions outside their coordination sphere.[27]

Electronic spectra and magnetics momentums

Cu^{II} complex shows magnetic moment value at 1.91 B.M corresponding to one unpaired electron consistent to an octahedral geometry.[28]this compound exhibit a broad low intensity shoulder band centered at 498 nm assigned to the $^2E_g \rightarrow {}^2T_{2g}$ transition in accord with an octahedral geometry.[29,30]

The observed magnetic moment value (4.75B.M) of Co^{II} complex agrees with the value for octahedral cobalt complexes.[31] The UV-vis spectra of cobalt complex show only d-d transition band at 512 nm and the others expected two d-d transition bands cannot be observed. This may be attributed to the masking this band by the strong charge transfer band of the ligand.[32-34]

The magnetic moment of Ni^{II} complex was found to be 3.12 B.M, which is within the range of values corresponding to an octahedral geometry.[35] The spectra of this complex shows the appearance of two bands at ν_1 520nm assigned to$^3A_{2g} \rightarrow {}^3T_{2g}$ and $\nu_2$590 nm for$^3A_{2g} \rightarrow {}^3T_{1g}$(f); but the third peak for $^3A_{2g} \rightarrow {}^3T_{1g}$ (p) transition was not observed, which may be lost in the low energy tail of the charge transition.Theband appeared at 324 nm was assigned to the charge transfer.[34]

The Zn^{II} complex was found to be diamagnetic, the UV spectra of zinc complex exhibited only high intensity band at 335 nm assigned to a ligand–metal charge transfer.[36] Analysis the results of IR spectra, molar conductivity and NMR spectra are consistent to octahedral geometry zinc complex (Figure 1).

Pharmacological screening

Ligands and complexes were tested for their 5α-reductase inhibitor activity in vivo, testosterone and dihydrotestosterone hormone levels are measured by radioimmuno assays; the values of ED_{50}, $LD_{90}$and relative potency to the reference drug anastrozole given in Table 2 and represented in figures 2-4. All the tested compounds showed 5α-reductase inhibitor activities with ED_{50}(0.92-3.54 mg kg^{-1}) and low acute toxicity LD_{90}. The ligands (L_1, L_2) exhibit a low inhibitory activity for 5α-reductase enzyme with 31-43% activity of the reference drug anastrozole, nickel and cobalt complexes showed moderate inhibitory activities with 57-61% potency relative to anastrozole, whereas copper and zinc complexes showed good inhibitory activities with 92-120% potency relative to anastrozole. The potency of these compounds decreases in the following order; zinc complex > copper complex > nickel complex > cobalt complex > L_1> L_2.

It is clear that the chelation causes drastic change in pharmacological effect of the ligands.[5,37] Zn^{II}complex is found to be the most potent 5α-reductase inhibitor and the lowest toxicity compared to the other tested compounds and standard drug anastrozole, which may be due to the generally positive properties of zinc for prostatic tissue which effects on the intraprostatic concentrations of testosterone.

Table 2. Evaluation of ED_{50}, LD_{90} and 5α-reductase inhibitor activities relative to Anastrozole

Compound	ED_{50}[a] (μg kg^{-1})	LD_{90}[b] (μg kg^{-1})	Potency relative to Anastrazole®
L_1	2.54	577	0.43
L_2	3.54	654	0.31
Ni^{II} complex	1.91	768	0.57
Co^{II} complex	1.78	890	0.61
Cu^{II} complex	1.18	1098	0.92
Zn^{II} complex	0.91	1130	1.19
Anastrazole®	1.09	3.69	1.00

[a]ED_{50}: Dose cause 50% of pharmacological response in test.

[b]LD_{90}: Dose kill 90% of the tested animals.

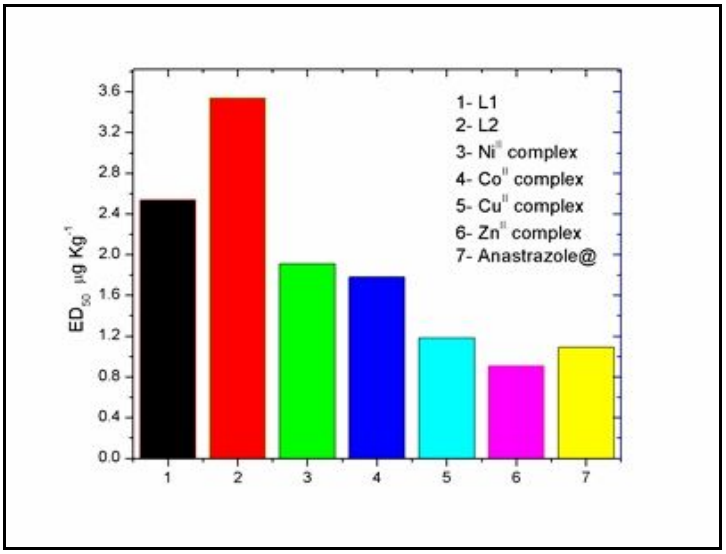


Figure 2. Evaluation of dose caused 50% of pharmacological response in the test

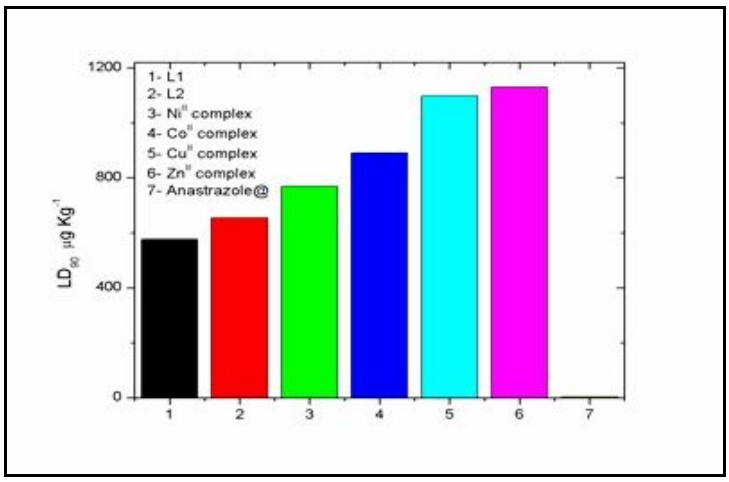


Figure 3. Evaluation of dose killed 90% of the tested animals

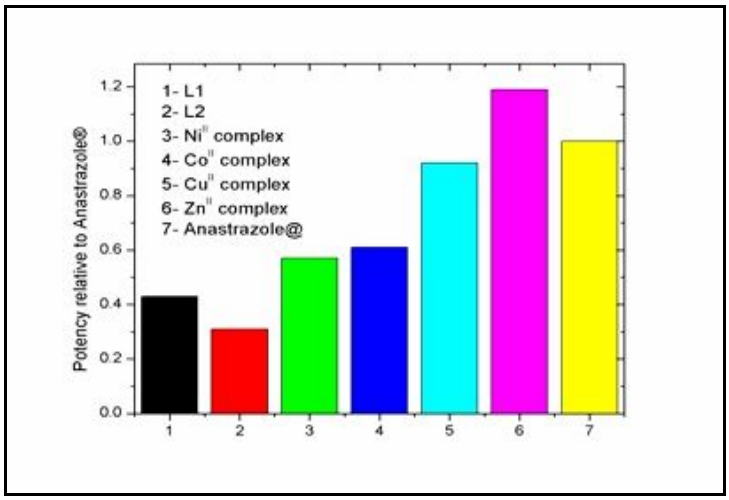


Figure 4. 5α-reductase inhibitor activity of tested compounds relative to anastrozole

Conclusion

The mixed ligand complexes of Co^{II}, Ni^{II}, Cu^{II} and Zn^{II} with catechol (L_1) and 2,3 diamino-5-bromopyridine (L_2) have been synthesized and their structure were confirmed by discussing their microanalytical and spectral data. All complexes are non-electrolytes. The two ligands (L_1, L_2) have potential binding sites towards metal ions; they act as bidentate ligands by coordinating through two phenolic oxygen atoms of L_1 and two nitrogen atoms of amino group in L_2. The remaining coordination positions being occupied by two water molecules to achieve an octahedral geometry. Ligands and complexes were tested for their 5α-reductase inhibitor activity in vivo, and the inhibitory potencies of these compounds ranged from moderate to potent

Zn^{II} complex is found to be the most potent 5α-reductase inhibitor and the lowest toxicity compared to the other tested compounds and standard drug anastrozole. These potent activities of copper and zinc complexes qualified them for further studies on pharmacological response of those metal complexes.

Conflict of interest

Authors declare no conflict of interest.

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