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Synthesis, characterization and antidiabetic studies of Co(ii) glimepiride complex

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Abstract

Cobalt complex of glimepiride was synthesized by reaction of glimepiride with cobalt (II) chloride hexahydrate. The metal complex was characterized based on elemental analysis, UV, IR, and ^{13}C NMR spectroscopy. The elemental analysis result where in agreement with the calculated values, the electronic spectrum of the ligand showed intra ligand charge transfer (ILCT) which was assigned to the chromophores present in the ligand. The electronic spectrum of the complex suggested intra ligand charge transfer (ILCT), ligand to metal charge transfer (LMCT), and d-d transition. IR spectrum of cobalt complex showed the involvement of two carbonyls in coordination to the metal complex. This shows that glimepiride acted as a tridentate ligand in the cobalt complex. ^{13}C NMR spectrum of the cobalt complex showed the involvement of the pyrrole ring in coordination to the metal ion. The structure of cobalt complex was assigned as octahedral in which the lig and molecules lies horizontally joining the central metal atom. The metal complex showed remarkable hypoglycemic activity as compared with the parent drug ligand in alloxan induced albino rat. After 8 hours of experimental time, cobalt metal complex showed significant reduction in blood sugar level more than glimepiride (Co glimepiride complex 217.50±21.93 to 134.25±10.90), and (glimepiride drug 258.25±25.38 to 187.75±18.71). The metal complex had eligibility to lower blood glucose for more duration of time even after 21 days of experimental time than glimepiride drug (Co glimepiride complex 121.25±1.50 to 93.00±3.46), and (glimepiride drug 122.50±2.51 to 100.25±2.87), and hence showed more hypoglycemic activity.

Keywords: Glimepiride, synthesis, infrared, ^{13}C NMR, hypoglycemic activities

1. Introduction

The introduction of metal ions into a biological system for the treatment or diagnosis of diseases is just one of the many subdivisions within the field of bioinorganic chemistry. While the field of bioinorganic chemistry is considered by many to be a relatively new field, this is contrary to historical fact, which has shown that metals were used as far back as ancient civilizations of Mesopotamia, Egypt, India, and China [1]. Many of the inorganic compounds utilized in these times were used in an empirical way with little attempt to design the compounds, and with little to no understanding of the molecular basis of their mechanism of action. Modern trends in the area of coordination chemistry involve studies on synthesis, structure and applications. The role of metal ions in living systems has been well established in recent years. The reasons for the persistent interest in the complexes are many, but the most important among them must be their role in various biochemical, pharmaceutical, industrial and chemical processes [1]. Certain coordination compounds, which occur in nature, are of biological importance. The participation of metallo-proteins in respiratory, photosynthetic, nitrogen fixation, biosynthetic and metabolic processes is essential to the foundations of life. Coordination complexes have a long history of use as chemotherapeutic agents. The successful application of inorganic complexes as drugs involves the recognition of their bioinorganic modes of action coupled with the traditional pharmacokinetic parameters of uptake, distribution and excretion. Further, the rational approaches to chemotherapy and particularly the notion of selective toxicity must be placed in an inorganic context [2]. The use of transition metal complexes as medicinal

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compounds has become more and more prominent. These complexes offer a great diversity in their action; they do not only have anti-cancer properties but have also been used as anti-inflammatory, anti-infective and anti-diabetic compounds [3]. Development of transition metal complexes as drugs is not an easy task; considerable effort is required to get a compound of interest. Beside all these limitations and side effects, transition metal complexes are still the most widely used chemotherapeutic agents and make a large contribution to medicinal therapeutics in a way that is unimaginable; a few years back [4]. Transition metal complexes are vital in the catalysis, materials synthesis, photochemistry, and biological systems [5]. They display diverse chemical, optical and magnetic properties. The continued interest and quest in coordination chemistry of metal complexes of bioactive ligands are enhanced mainly due to their use as models for more intricate naturally occurring primary bioinorganic system. The fundamental chemical properties utilized by the metals ions in biological systems would be far simpler to study through the less complicated synthetic analogs. Model studies in search of mechanism of the biological reaction, mediated by metal ions led to the synthesis of a large number of bioactive ligands and their complexes [5]. The synthesis of new bioactive ligands and complexes would provide new vehicles in the enhancement of drugs. Recently, the benefits of these properties in medicinal chemistry have begun to emerge. The misconception that organometallic complexes lack stability under conditions in which living organisms thrive, has been one reason why they have not been considered as suitable candidates for pharmaceuticals until quite recently. However, in the last decade, there has been a flurry of activities with regard to the development of organometallics in a biological context. Organometallic complexes which are stable in physiological environments are now being developed as, for example, anticancer agents, and radiopharmaceuticals for diagnosis and therapy, and biosensor probes [6]. Cobalt is a vital trace metal found both in animals and humans with therapeutic use in pharmacological fields. In the form of vitamin B_{12} (cobalamin), this metal plays a number of crucial roles in many biological functions. Vitamin B_{12} alone contains water-soluble vitamin that is stored in the liver and must come from the diet. Cobalamin is required for DNA synthesis, red blood cells formation, maintenance of the nervous system, growth and development of children. Cobalt is used in the management of anaemia with pregnant women, because it stimulates the production of red blood cells. Cobalt was found to boost the effects of insulin and its action [7]. Treatment with cobalt chloride decreases the glycemia of diabetic rats which may be mediated by gene expression of glucose transporter 1 **(**GLUT-1) gene expression [8]. Treatment with cobalt chloride showed significant decline in blood glucose in streptozotocin (STZ) induced diabetic rats. The glucose lowering effect of glucosaminic acid-cobalt chelate, has been reported to be effective against diabetes [9]. Cobalt is the most important contributor to metal ion toxicity in patients both in single and pure form. Various forms of complexes of cobalt have been described to lower the possible toxicity of cobalt without altering its therapeutic effect [10]. Cobalt therapy may prove effective in improving the impaired antioxidant status during the early state of diabetes and ascorbic acid supplementation at this dose potentiates the effectiveness of cobalt action [11].

Glimepiride is a third generation sulfonylurea drug used for the treatment of type 2 diabetes which lowers blood sugar level by stimulating the release of insulin through pancreatic beta cells and by inducing increased activity of intracellular insulin receptors [12, 13]. Complexation of sulphonylureas with transition and inner transition metal has been studied in detail [14]. The structure of glimepiride is shown in Figure 1.

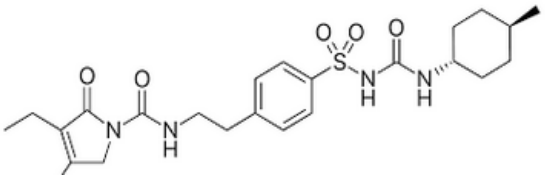

Figure 1: Structure of Glimepiride

2. Materials and methods

2.1 Chemicals and Solvents

All chemicals and reagent used in the experimental work were of analytical grade. Pure glimepiride drug, $CuCl_2.2H_2O$, $CoCl_2.6H_2O$,$Y(NO_3)_3.6H_2O$, Sc_2O_3 and alloxan-monohydrate were imported from E. Merck Co Germany.

2.2 Physical Measurement

Melting points of the complex was determined using MPA160 melting point apparatus. The molar conductance of the complex was determined using MRC conductivity meter with a cell constant of 1.05 in 50 ml of distilled water. Atomic absorption spectroscopy was carried out on Buck-2010 spectrometer (Buck instrumental company). Five (5ml) of nitric acid was mixed together with 2 ml of perchloric acid, 0.5 g of sample was digested with the mixed acid for 3 hr at temperature of 150-250 °C. The digest was allowed to cool, filtered and the filtrate made up to 50 ml with distilled water. The elemental analysis for C, H, N, O, and S was carried out. Infrared spectrum was collected on Perkin Elmer Paragon 1000 FT-IR spectrophotometer (spectrum BX) equipped with cesium iodide window (4000-350 cm^{-1}) in KBr pellets. The UV-Visible spectrum was obtained on Perkin Elmer (lambda 25) spectrometer (200 -800 nm) using distilled water as solvent. The^{13}C Nuclear Magnetic Resonance (NMR) spectrum was obtained using Varian 400 MHz Unity INOVA, using DMSO as solvent.

2.3 Synthesis of glimepiride cobalt complex

The complex was prepared following reported procedure by [15]. Co (II) salt solution was prepared by dissolving 5.9 g (0.025 mol) $CoCl_2.6H_2O$, in 25 ml ethanol. The solution of the metal salt was added slowly with stirring in a separate 20 ml of ethanol solution of 12.25 g of glimepiride (0.025 mol) at room temperature maintaining the $_PH$ between 6.0 - 6.5 by adding dilute solution of NaOH. After refluxing the mixture for 3 hours and cooling, the complex separated out [16, 17]. The complex formed was washed with ethanol, recrystallized, filtered, dried in vacuum and weighed. The yield was recorded.

2.4 Hypoglycemic activities

Sixteen (16) healthy albino rats weighing between 105-140 g were obtained from the Animal Breeding Units Department of Veterinary Pharmacology, University of Nigeria, Nsukka. The animals were housed in clean cages with filter tops in specific pathogen free environment with 12 h light/dark cycle and were provided standard feed (Vital feed Nig Ltd.) tap water, and were allowed to acclimatize for 2 weeks prior to the commencement of the experiment under tropical humid condition in the animal house of the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

3. Experimental Design

The animals were randomly grouped into 4 groups of 4 animals each after 2 weeks of acclimatization; the groups were labelled as shown below.
Group 1 – Normal control group; this group of animals were not induced with diabetes but were fed with normal diet for 21 days.
Group 2 – Diabetic control group; this group of animals were induced with diabetes but were not administered any anti-diabetic agent for 21 days
Group 3 – [Co(GMP)] group; this group were made diabetic and were treated with $[Co(C_{24}H_{34}N_4O_5S).(H_2O)_2]$ (glimepiride cobalt complex) for 21 days.
Group 4 - GMP group; this group of diabetic animals received oral administration of $C_{24}H_{34}N_4O_5S$ (pure glimepiride) for 21 days.

3.1 Sample collection

On the 7th, 14th, and 21st day, blood samples of the animals were collected respectively through the tail, and blood glucose level checked using glucose meter.
Blood Glucose Level, Hematological and Lipid Profile Studies. The animals were distributed randomly into 4 groups with 4 animals in each group. The first group served as positive control (normal control), the second group served as negative control (diabetic control), the third group served as glimepiride Co complex, while the fourth grouped served as pure glimepiride (5 mg/kg) drug. All treatment was done through oral routes for 21 days using gavage.

3.2 Diabetes induction

The rats were made diabetic by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan-monohydrate (160 mg/kg). Eight days later, rats with blood glucose concentrations above 190 mg/dL were considered diabetic and such rats were used for the study.

4. Dosage of cobalt complex administered

The dosage of the complex (5 mg/kg) was prepared and administered orally using gavage.

4.1 Acute and Sub-acute test

The acute test was carried out on the first day of treatment. Blood sample was collected from the tail of each rat in all groups before and at 2, 4, 6, and 8 hr following treatment and glucose levels determined using Glucosemeter (ACCUCHEK, Roche Diagnostics) with code number of 527. For the sub-acute studies, the test was repeated on day 7, 14 and 21.

4.2 Statistical analysis

Results were expressed as mean ±SM, mean were analyzed for difference and analysis of variance (one way ANOVA) using SPSS 20.0 software.

5. Result and Discussion

Physical properties, elemental analysis, selected Infra-red spectral data, electronic spectral data, mass spectral data, ^{13}C-NMR spectral data, mean (±SD) hypoglycemic activities (acute test) and mean (±SD) hypoglycemic activities (7 – 21) days of glimepiride and its Co(II) complex are shown in Tables 1- 8 respectively.
The colour of glimepiride Co(II) complex (Table 1) suggests d-d transition. Melting points of the complex as compared to glimepiride suggests that new products were formed. The molar conductance of the metal complex was found to be less than 30 $Ohm^{-1}cm^2mole^{-1}$, this shows that they are non-electrolyte [18]. The % compositions of the C,H,N O, S and the metal in the complex also suggest the formation of new product (Table 2). The elemental analysis of glimepiride and its complex shows that the experimental values are in agreement with the calculated values. It also suggests that the mole ratio of the ligand and metal is 2:1.
The IR spectra of the ligand and the complex were recorded on Perkin Elmer Paragon 1000 FT-IR spectrophotometer, range 4000-350 cm^{-1}, and the assignments given in Table 3. The proposed structure was proposed based on IR absorption bands. A prominent band at 3468.57 cm^{-1} in the ligand, have been assigned ν(NH) stretch. In the Co complex, the NH stretch appeared at 3494.53 cm^{-1}. This shift suggests coordination through the NH to cobalt. The C=O stretching frequency for the ligand appeared at 1640.41 cm^{-1}. This band shifted to 1651.73 cm^{-1} in Co glimepiride complex [19]. This

shift suggests the involvement of the C=O group in the complexation to the metal ion. The C-O stretch was absent in the ligand, but was present in the complex. The appearance of the C-O stretch suggests conversion of the C=O to C-O during complexation. S=O of sulfone was not involved in coordination because there was no shift noticed in its vibration frequency.

The electronic absorption bands of the ligand and the cobalt complex have been summarized in Table 4 above. The absorption band for the ligand at 200 nm has been assigned n-π^* transition. The chromophores in the ligand that exhibit this type of transition are C=O, S=O, C=C respectively. This transition is also called intra-ligand charge transfer (ILCT). For the Co complex, the absorption band at 250 nm, 450 nm, and 600 nm have been assigned π-π^* (ILCT), LMCT, and d-d transition respectively.

The mass spectral data of glimepiride and its cobalt complex are present in Table 5. For glimepiride, the molecular ion peak was observed at m/z 491, due to $[C_{24}H_{34}N_4O_5S]^{+\cdot}$,which is in agreement with the structure of the ligand. The peak at m/z 352, corresponds to $[C_{16}H_{20}N_3O_4S]^{+\cdot}$ Due to loss of $C_8H_{14}NO^{\cdot}$ m/z 140. Mass spectrum of Co complex suggests molecular ion peak at m/z 1039 $[C_{48}H_{67}CoN_8O_{10}S_2]^{+\cdot}$which corresponds to the molecular weight of the complex with base peak at m/z 591 $[C_{24}H_{30}CoN_5O_7S]^{+\cdot}$.Other peaks of appreciable intensity were observed at m/z 1021, 963, 845, 486, 815, 421, 351, 300, 220, 175, 149, 112 and 60.

The ^{13}C-NMR spectral data of glimepiride and its cobalt complex are present in Table 6. In glimepiride ligand, C=O (pyrrole ring) resonated at 174 ppm, but this band shifted downfield (187 ppm) in the Co complex. This shift suggest complexation of the ligand to the metal ion through the C=O group. In glimepiride ligand, the signal due to presence of C=O (RCONH) was observed at 153 ppm, this signal shifted downfield in Co complex (164 ppm) as a result of coordination of the ligand to the metal center through the C=O group. Peaks observed between 154-159 ppm and between 134-137 ppm indicates C=C carbons of the pyrrole ring.

Based on the elemental analysis, electronic, IR and ^{13}C NMR spectra, the following structure (Figures 2) have been proposed for the metal complex.

Table1. Physical properties of glimepiride and its cobalt complex

Ligand/metal complex	Color	Yield (%)	Melting point (°C)	Molar conductance ($Ohm^{-1}cm^2mole^{-1}$)
GMP	White	-	207	25.7
[Co(GMP)]		86.48	260	27.71

Table 2. Elemental analysis of glimepiride and its cobalt complex

Ligand/ metal complex	% C Found (Calc.)	% H Found (Calc.)	% N Found (Calc.)	% O Found (Calc.)	% S Found (Calc.)	% Co Found (Calc.)
GMP	58.77	6.95	11.92	16.10	5.22	
[Co(GMP)]	55.20 (55.48)	6.90 (6.50)	10.42 (10.78)	15.12 (15.40)	6.05 (6.17)	5.55 (5.67)

Table 3. Selected Infra-red spectral data of glimepiride and its cobalt complex (cm^{-1})

Ligand/ complex	ν(NH) (RCONH)	ν(C=O)	ν(S=O) (sulfone)	ν(C-O)	ν(C-H) (aromatic)	ν(C-H) (aliphatic)
GMP	3468.57	1640.41	1316	-	3062.91	2937.14
[Co(GMP)]	3494.53	1651.73	1321.34	1199.62	3080	2931.42

Table 4. Electronic spectral data of glimepiride and its cobalt complex.

Ligand/complex	λmax (nm)	Assignment
GMP	200	n-π^* (ILCT)
[Co(GMP)]	250 450 600	n-π^* (ILCT) LMCT d-d transition

Table 5. Mass spectral data of glimepiride and its cobalt complex

Ligand/complex	Ms (m/z) ratio	Assignment
GMP	491 $[C_{24}H_{34}N_4O_5S]^{+\cdot}$ 352 $[C_{16}H_{20}N_3O_4S]^{+\cdot}$	Molecular ion peak Base peak
[Co(GMP)]	1039 $[C_{48}H_{67}CoN_8O_{10}S_2]^{+\cdot}$ 1021 $[C_{47}H_{61}CoN_8O_{10}S_2]^{+\cdot}$ 963 $[C_{43}H_{52}CoN_8O_{10}S_2]^{+\cdot}$ 591 $[C_{24}H_{30}CoN_5O_7S]^{+\cdot}$ 845 $[C_{36}H_{48}CoN_7O_9S_2]^{+\cdot}$	Molecular ion peak Due to loss of a methyl group Due to loss of C_4H_{10} Base peak Due to the loss of $C_{11}H_{15}N_2$

Table 6. ^{13}C-NMR spectral data of glimepiride and its metal complex (δ ppm)

Ligand/complex	C=O (pyrrole ring)	C=O (RCONH)	C=O (NHCONH)	4C (aromatic)	C-CH_3 (pyrrole ring)	C-CH_2CH_3 (pyrrole ring)
GMP	174	153	152	127	154	134
[Co(GMP)]	185	162	152	127	157	137

Table 7. Mean (±SD) hypoglycemic activities of glimepiride and its complex (acute test)

Mean values of fasting blood sugar levels of albino rats (mg/dl)					
Group	0hr	2hrs	4hrs	6hrs	8hrs
N Control	91.50^{b}±4.51	93.50^{e}±5.26	87.50^{f}±4.43	87.50^{d}±4.79	84.50^{e}±4.43
D Control	368.50^{a}±1.03	379.75^{a}±6.84	390.50^{a}±5.57	415.50^{a}±29.37	467.50^{a}±20.20
[Co(GMP)]	337.25^{a}±45.47	325.75^{b}±36.22	217.50^{e}±21.93	145.00^{c}±12.90	134.25^{d}±10.90
GMP	365.25^{a}±47.99	61.25^{c}±29.55	258.25^{d}±25.38	213.75^{b}±11.09	187.75^{b}±18.71

Where GMP=Glimepiride

Means with different superscripts in the same column are significantly (P<0.05) different. N control represents Normal control, D control represents Diabetic Control while GMP represents Glimepiride.

Table 8. Mean (±SD) hypoglycemic activities of glimepiride and its Co(II) complex (sub-acute test)

Mean values of fasting blood sugar levels of albino rats (mg/dl)			
Group	7 days	14 days	21 days
N Control	96.25^{d}±4.66	94.50^{f}±4.7	91.50^{d}±6.45
D Control	319.25^{a}±4.99	333.00^{a}±9.76	331.00^{a}±10.65
[Co(GMP)]	121.25^{c}±1.50	99.75^{ef}±1.26	93.00^{cd}±3.46
GMP	122.50^{c}±2.51	107.25^{cd}±1.50	100.25^{bc}±2.87

Means with different superscripts in the same column are significantly (P<0.05) different. N control represents Normal control, D control represents Diabetic Control while GMP represents Glimepiride.

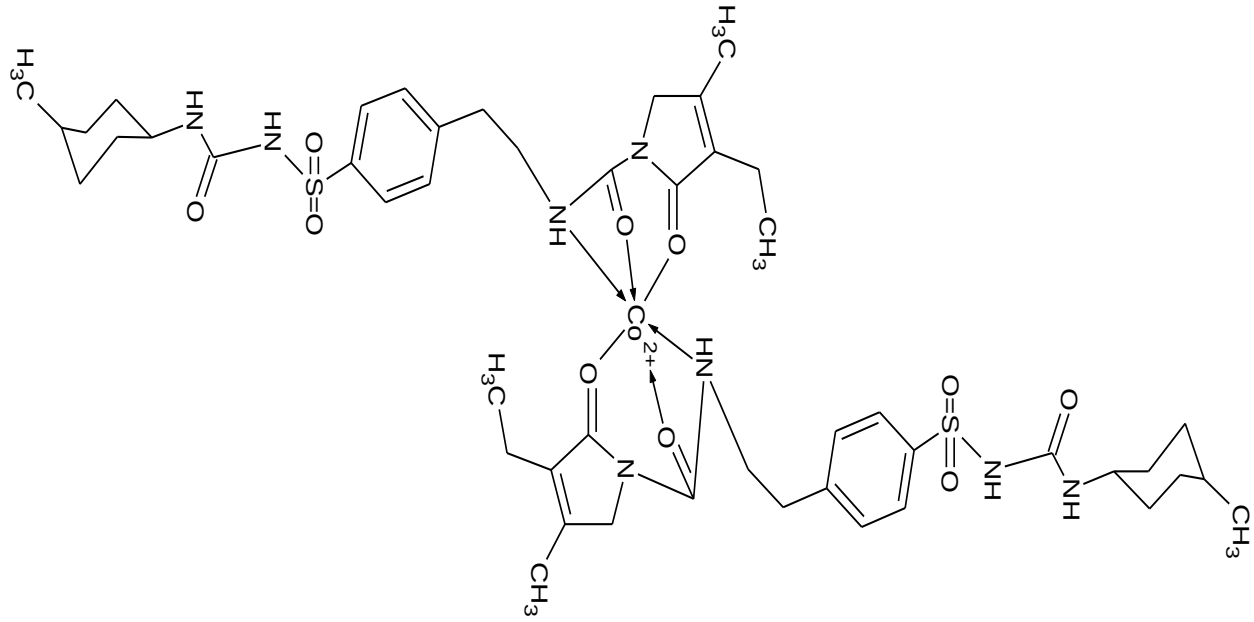

Figure 2: Proposed structure of cobalt (II) glimepiride complex

6. Conclusion

Complex of Co(II) with glimepiride was successfully synthesized. The melting point, conductivity, color, IR spectrum, UV spectrum, mass spectrum, ^{13}C NMR spectrum and elemental analysis all suggested that new product was formed. The glimepiride complex however, showed enhanced hypoglycemic activity and kept the blood sugar level lower compared with the parent glimepiride drug even after experimental period.

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