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SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIDIABETIC STUDY OF CU(II) AND FE(III) COMPLEXES OF GLIMEPIRIDE

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ABSTRACT
Pure glimepiride 1-(p-(2-(3-ethyle-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl)phenyl) sulfonyl)-3-(trans-4-methylcyclohexyl) urea is a third generation hypoglycemic sulfonylurea which is useful in the treatment of non insulin dependent diabetes mellitus (NIDDM) Glimepiride is a white crystalline powder, relatively insoluble in water but highly permeable (class-II) drugs the rate of oral absorption is often controlled by the dissolution rate in the gastrointestinal tract. Metal complex of glimepiride has been synthesized by reaction with Cu(II) and Fe(III) in the form of its chloride. The biological activity of many drugs has been shown to be enhanced on complexation with metal ions, hence promoting their use in pharmacology. For the structure elucidation of these complexes "monovariation method" has been used to ascertain the ligand –metal ratio in the complex. The molecular formula are $[Cu(C_{24}H_{34}N_4O_5S)_2 (H_2O)_2]$ and $[Fe(C_{24}H_{34}N_4O_5S)_2 (H_2O)_2]$. The ratio of complex formation with the above metal was further confirmed conductometrically using Job's method of continuous variation as modified by turner and Anderson.

KEYWORDS: Glimepiride, antidiabetic, glimepiride, spectroscopy, metal salts.

INTRODUCTION

Ayurveda and Unani system of medicines used several plants material and minerals including Gold, silver, Mercury, Bismuth, Copper, Iron and Arsenic as metals or as their salts have been used for several thousand years. Organo-metallic compounds emerged as important agents in the treatment of syphilis, tropical diseases etc.[1] According to Alfred Burger[2] medicinal chemistry is a science whose fundamental roots lie in the branches of chemistry and biology. It is a scientific discipline at the intersection of pharmacology and chemistry involved with designing, synthesizing and developing pharmaceutical drugs. It's involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. A survey of literature reveals that metal complexes of some drugs have been found to be more potent than the drug.

Pure glimepiride 1-(p-(2-(3-ethyle-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl) phenyl) sulfonyl)-3-(trans-4-methylcyclohexyl) urea trade name Amaryl is a third generation hypo- glycemic drug which is potent in the treatment of non-insulin dependent diabetes mellitus (NIDDM). Glimepiride exhibits slow gastrointestinal absorption rate and inter individual variations of its bioavailability.[3] The metal complexes from bidentate ligands have often been studied recently because of their applications in enhancement of drug action.[4,5] Transition metals are essential for normal functioning of living organisms and are, therefore, of great interest as potential drugs.[6] Our work on metal complexes of oral anti-diabetics agents of hypoglycemic activity, Present paper deals with the synthesis and structural studies of Glimepiride-Cu and Fe complexes.

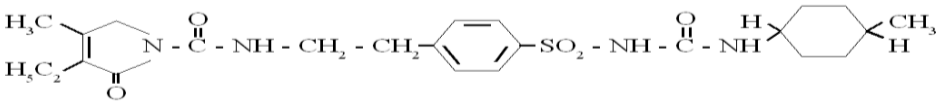


Fig. 1: Structural Formula of Glimepiride.

Experimental Section

All chemical used were of high purity analytical grade. Complete study has been carried out in using distilled water and other solvents used are ethanol and DMF. Pure sample of glimepiride molecular formula $C_{24}H_{34}N_4O_5S$ and molecular weight 490.622 was obtained from Sagun

Pharmaceutical Company, Indore in powered form, M.P. 212-214°C for the preparation of complexes, metal salts $CuCl_2.2H_2O$ and $FeCl_3.2H_2O$ was of Merck chemicals.

Ligand- metal ratio

To confirm the ligand- metal ratio, conductometric titrations using Monovariation method were carried out at 28°C. 0.01M solution of Glimepiride drug was prepared in DMF. Similarly, solution of metal salts $CuCl_2.2H_2O$ and $FeCl_3.2H_2O$ was prepared in the same solvent of 0.02M concentration. 20 mL of ligand was diluted to 200 mL with the same solvent. The ligand was titrated against metal salt solution using Monovariation method. Conductance was recorded after each addition. Graph is plotted between corrected conductance and volume of metal salt added. From the equivalence point in the graph it has been concluded that the complex formation has taken place in the ratio of 2:1 (L: M). Stability constants and free energy changes were also calculated using Job's method[7] of continuous variation modified by Turner and Anderson.[8]

Synthesis of complex

Complex was synthesized by mixing the solution (DMF) metal salt solutions with that of ligand in 1:2 molar ratios, respectively and refluxing the mixture for 3.5 to 4 h. The dark green precipitate of $[Cu(C_{24}H_{34}N_4O_5S)_2(H_2O)_2]$ and red brown precipitate of $[Fe(C_{24}H_{34}N_4O_5S)_2(H_2O)_2]$ formed was filtered, washed with DMF, dried and weighted, melting point of the complex was recorded.

RESULTS AND DISCUSSION

The synthesized complexes are stable solids, being soluble in DMF and DMSO and insoluble in all other organic solvents. Analytical data (Table-1) and Conductometric studies suggest 2:1 (L:M) ratio. Measured conductance values of the complex are too low to account for their electrolytic behavior. The magnetic studies indicate that the Cu(II) and Fe(III) complexes are octahedral structure (Table-2).

Table 1: Analytical data of Glimepiride and its Complexes.

Composition of Complex (m.w.)	Color	Yield (%)	M.P. (°C)	Elemental analyses (%) : Found (calculated)					
				C	H	N	M	S	H_2O
$C_{24}H_{34}N_4O_5S$ (490.622)	White	-	182	58.76 (58.79)	6.93 (6.97)	11.92 (11.95)	-	6.53 (6.58)	-
$(C_{24}H_{34}N_4O_5S)_2$ Cu.$2H_2O$ (1080.822)	Dark Green	70	250	55.07 (55.18)	6.48 (6.51)	9.90 (10.72)	5.98 (6.05)	6.01 (6.15)	3.36 (3.45)
$(C_{24}H_{34}N_4O_5S)_2$Fe.$2H_2O$ (1073.123)	Red Brown	72	227	22.94 (22.80)	2.73 (2.98)	4.46 (4.58)	2.23 (2.43)	2.57 (2.63)	3.38 (3.53)

Table 2: Stability Constant, Free Energy Change, Molar Conductance and Magnetic-Moment data of Complex.

Composition of Complex	Stability Constant log K (L/mol)	Free energy change - Δ F (Kcal/mol)	Molar conductance ($ohm^{-1}cm^2mol^{-1}$)	Magnetic moment (B.M.)
$(C_{24}H_{34}N_4O_5S)_2$ Cu.$2H_2O$	9.2324	12.4621	10.9	1.63
$(C_{24}H_{34}N_4O_5S)_2$ Fe.$2H_2O$	11.2006	15.6839	11.4	5.26

Infra-Red spectra

The synthesized complexes are Cu dark green and Fe red brownish crystal are characterized for Cu and Fe complexes. They are stable and soluble in DMSO; dilute alkali etc, in soluble in water and other organic solvents. IR spectrum of the ligand and the isolated complex were scanned in the range 4000-400 cm^{-1} and the probable assignments are given in table-3 and Fig. 1, 2, 3. The proposed structure for the complex (isolated) is also supported by IR absorption bands and characterized by the absorption (9-12) of NH group 3379 cm^{-1} in the ligand glimepiride shifted to 3329 cm^{-1} in Cu(II) glimepiride complex and 3321 cm^{-1} in Fe(III) glimepiride complex. The absorption of carbonyl (C=O) group at 1775 cm^{-1} in pure glimepiride and the metal complexes observed at 1705 cm^{-1} in Cu(II) complex and 1759 cm^{-1} in Fe(III) complex. The most important IR band of structural significance of the ligand appears at 1385 cm^{-1} which may be assigned to S=O which got shifted downwards at1313 cm^{-1} in Cu(II) complex and 1310 cm^{-1} in Fe(III) complex. The shift of the C=O and S=O by decreased frequency in the complex indicates that these groups are involved in the complexation. The linkage through sulphone -O- and amide -O- atom was further supported by the appearance of a band in the far-IR reason at 660 cm^{-1} in Cu(II) complex and 652 cm^{-1} in Fe(III) complex that may be assigned to M-O frequency. Additional band in the complex reason of Cu(II) at 1439 cm^{-1} and Fe(III) at 1439 cm^{-1} compared with IR spectra of free ligand has been tentatively assigned to six membered enolic ring structure modified to chelate ring formation in complex. A strong band in the reason of 3436 cm^{-1} and 3453 cm^{-1} indicate the presence of co-ordinated water for Cu-glimepiride complex, Fe-glimepiride complex.

Table 3: IR bands and their assignments for glimepiride and its Complexes in cm^{-1}.

Composition of Complex	$\nu_{(N-H)}$ (cm^{-1})	$\nu_{(C=O)}$ (cm^{-1})	$\nu_{(S=O)}$ (cm^{-1})	$\nu_{(SO2N)}$ (cm^{-1})	$\nu_{(M-O)}$ (cm^{-1})	$\nu_{(H2O)}$ (cm^{-1})
$(C_{24}H_{34}N_4O_5S)$	3375	1770	1385	1277	-	-
$(C_{24}H_{34}N_4O_5S)_2$ $Cu.2H_2O$	3329	1705	1313	1230	660	3436
$(C_{24}H_{34}N_4O_5S)_2Fe.2H_2O$	3321	1759	1310	1226	652	3453

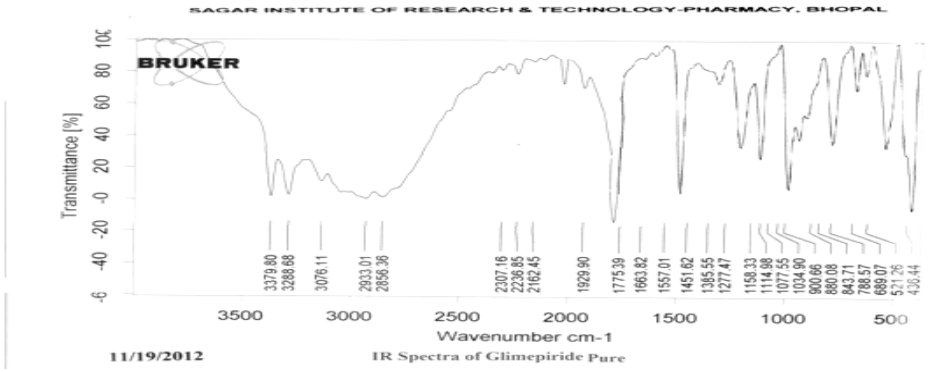


Fig. 1: IR Spectra of Glimepiride.

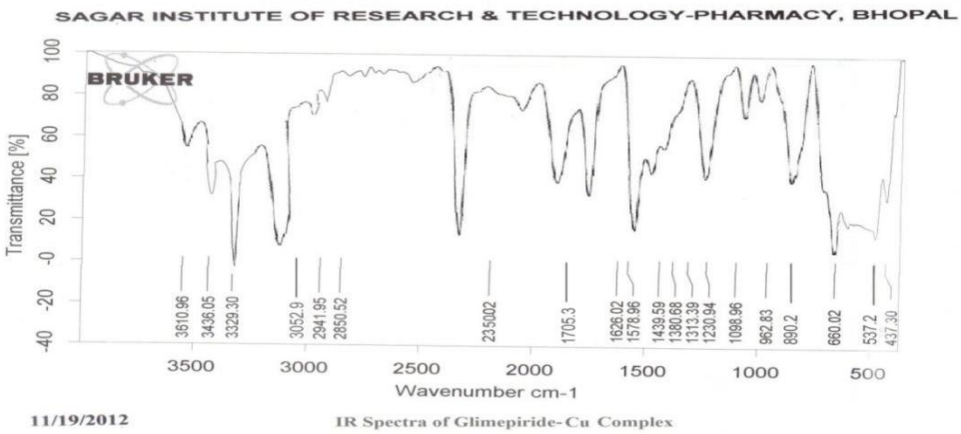


Fig. 2: IR Spectra of Glimepiride - Cu Complex.

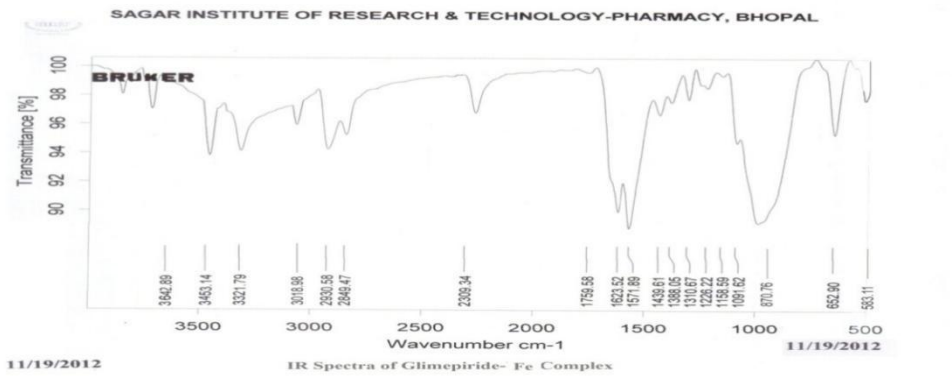


Fig. 3: IR Spectra of Glimepiride - Fe Complex.

Electronic Spectral Study

The electronic spectra of Cu(II) complexes exhibit a two broad, band in reason 13537 and 18723 cm^{-1}. The bands observed are assigned to the transition LMCT, $^2T_2g \leftarrow {}^2Eg$ respectively, suggesting the presence of Cu(II) complex expected tetragonally distorted octahedral geometry.[13,14] It is further supported by the high μeff value in the range 1.63 B.M.

The electronic spectra of Fe(III) complexes exhibit a two broad, band in reason 10432 and 17123 cm^{-1}. The bands observed are assigned to the transition $^5Eg \leftarrow {}^5T_2g$ charge transfer respectively, suggesting the presence of Fe(III) complex expected octahedral geometry. It is further supported by the high μeff value in the range 5.26 B.M.

Table 4: Electronic Spectral Bands, Magnetic Moment Values and Possible Assignments of Glimepiride and their Metal Complexes.

S. No.	Complexes	Magnetic Moment (μeff) value (B.M.)	Electronic Absorption Bands (cm^{1})	Possible Assignment	Geometry
1	$[Fe(GLM)_2 (H_2O)_2]$	5.26	10432 17123	$^5Eg \leftarrow {}^5T_2g$ Charge transfer	Octahedral
2	$[Cu(GLM)_2(H_2O)_2]$	1.63	13537 18723	$^2T_2g \leftarrow {}^2Eg$	Octahedral

Scanning Electron Micrograph (SEM) of Ligand and its Complexes

Particle size analysis

To find out the maximum efficiency of the drugs and their metal complexes, studies on the particle size analysis are being considered very helpful. The particle size of the metal complexes are smaller than their respective sulphonyl ureas i.e., glimepiride (Fig. 4-6 and table-5).

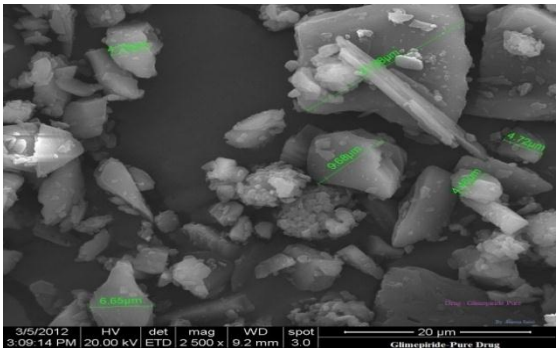


Fig. 4: Pure Glimepiride.

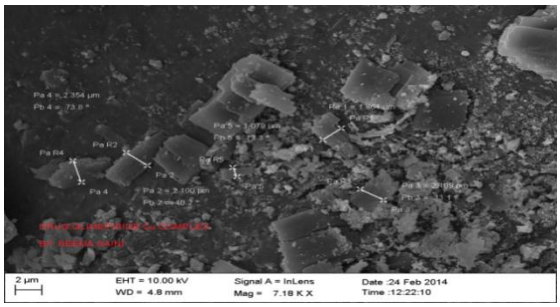


Fig. 5: Glimepiride-Cu complex.

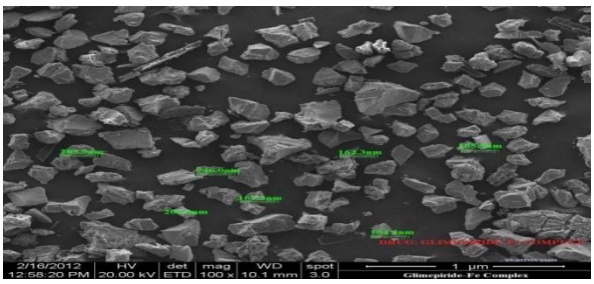


Fig. 6: Glimepiride-Fe complex.

Table 5: Particle Size Measurement of Glimepiride and its Metal Complexes.

S.No.	Name of Sample	Particle Size	Appearance
1	Glimepiride Pure Ligand	20 μm	Heavy compect crystals
2	GLM-Cu Complex	2 μm	Small spherical particleform
3	GLM- Fe Complex	2 μm	Small particles

Anti- Diabetic Activity

In the present study, the activity is done on Glimepiride-metal complexes. The isolated glimepiride-metal complex was found to be more potent as compared to the parent drug. Hence as compared to standard synthetic drug the glimepiride metal complex is having more hypoglycemic activity.

Experimental Procedure

Chemicals

Streptozotocinwas purchased from Hamidia. All other chemicals used for this study were of analytical grade.

Oral Glucose Tolerance Test

The hypoglycemic effect of pure synthetic glimepiride and its Cu and Fe complexes were investigated on blood sugar levels of both male and female Wister albino rats by oral glucose tolerance test.[15,17]

Procedure: The glucose tolerance test was performed by feeding glucose (2g/Kg) in the form of solution orally to the all animals (Group 1 to 5 except Group-1). Treatment was given orally 1.5 hrs before (single treatment) eliciting the glucose tolerance test. The blood sample was collected from tail vein at 0, 30, 60 and 90 min. after administration of glucose.[18,19]

Animals were divided into twelve groups containing six animals in each.

Grouping and Dosing

Group	Dosing and treatment
I	Normal Control, (Vehicle only), 1ml/100gm
II	Diabetic Control, Glucose 2g/Kg, p.o.
III	Pure Glimepiride (0.1mg/Kg p.o.)+ Glucose 2g/ Kg, p.o.
IV	Glimepiride $FeCl_3$ Complex (0.1mg/Kg p.o.) + Glucose 2g/Kg, p.o.
V	Glimepiride $CuCl_2$ Complex (0.1mg/Kg p.o.) + Glucose 2g/Kg, p.o.

Streptozotocin Induced Diabetes

Procedure: The diabetes was induced in the animals by a single intraperitoenal injection of a freshly prepared Streptozotocin (STZ). STZ solution of 10 mg/ ml was prepared in ice-cold citrate buffer 0.1 M, pH 4.5 kept in ice and was administer at a dose of 50mg/Kg-body weight.

Grouping and Dosing

Group	Dosing and treatment
1	Normal Control (NC) (vehicle only), 1ml/100gm
2	Diabetic Control (DC), Streptozotocin 50g/Kg, i.p. on 1st day.
3	Pure Glimepiride (0.1mg/Kg p.o.) once daily for 7 days and Streptozotocin 50g/Kg, i.p. on 1st day.
4	Glimepiride $FeCl_3$ Complex (0.1mg/Kg p.o.) once daily for 7 days and Streptozotocin 50g/Kg, i.p. on 1st day.
5	Glimepiride $CuCl_2$ Complex (0.1mg/Kg p.o.) once daily for 7 days and Streptozotocin 50g/Kg, i.p. on 1st day.

Sample collection for estimation of blood glucose

Blood samples were collected by tail vein and blood glucose levels were estimated using an electronic glucometer (Gluco chek).

Statistical analysis

The data are expressed as mean ± SEM. The results were analyzed statistically by software (Graph pad instate 3) using ANOVA followed by Turkey's multiple comparison test. The minimum level of significance was fixed at $p<0.05$.

Table 6: Effect of Various Complexes of Glimepiride on Blood Glucose Levels on Glucose Loaded Normal Rats.

Groups	Treatment	Blood Glucose (mg/dL)				% Increase In Blood glucose
	(mg/Kg body weight)	0 min. Fasting	30 min.	60 min.	90 min.	
I	Normal Control (NC) (vehicle only)	90.45±3.44	92.12±2.13	92.25±2.66	90.32±3.67	-
II	Diabetic Control(DC)/ Control group +Glucose (2mg) +Vehicle	88.78±3.76	182.45±2.44	142.49±2.74	120.67±2.43	20.49
III	Pure Glimepiride (0.1mg/Kg p.o.)	77.5±3.88	96.35±2.32	108.43±2.25	94.2±2.44	15.26
IV	Glimepiride- $FeCl_3$ Complex (0.1mg/Kg p.o.)	75.6±3.86	91.52±2.56	98.0±2.34	82.5±2.66	6.70
V	Glimepiride- $CuCl_2$ Complex (0.1mg/Kg p.o.)	74.45±3.55	86.35±5.35	92.65±2.64	80.29±2.56	4.30

Values are, mean ± SEM from a group of six animals.

Table 7: Effect of Various Complexes of Glimepiride on Blood Glucose Levels on Glucose Loaded Normal Rats.

Groups	Treatment	Blood Glucose (mg/dL)			
	(mg/Kg body weight)	**Initial**	**1 h**	**3 h**	**6 h**
I	Diabetic Control(DC)/Control group +Glucose (2mg) +Vehicle	224.8±1.76	210.4±1.44	212.4±1.70	225.6±2.43
II	Pure Glimepiride (0.1mg/Kg p.o.)	222.4±1.88	195.3±1.31	123.4±1.26	97.2±1.42
III	Pure Gliclazide (2mg/Kg p.o.)	220.8±1.45	193.3±1.36	120.4±1.75	96.22±1.10
IV	Glimepiride- $FeCl_3$ Complex (0.1mg/Kg p.o.)	223.6±1.84	191.5±1.55	116.0±2.32	84.5±1.68
V	Glimepiride- $CuCl_2$ Complex (0.1mg/Kg p.o.)	221.4±1.57	190.3±1.35	115.6±1.69	83.2±1.53

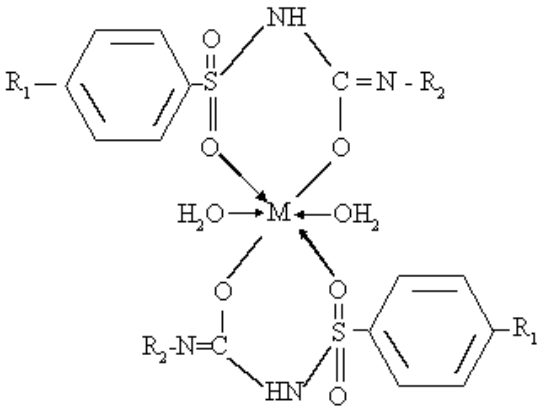
Values are mean ± SEM from a group of six animals.

Oral glucose tolerance and streptozotocin induced anti diabetic model were employed for determining the anti-diabetic activity. The pharmacological test indicates that the blood sugar level effectively as compared to the parent drugs.

The continuous treatment of the complexes and pure drug for a period of 7 days produced a significant decrease in the blood sugar levels of diabetic rats. The reports published by various authors, increase in glucose levels has been attributed to the destruction of β cells by Streptozotocin. Damage to the Beta cells is associated with the liberation of stored insulin after which the insulin synthesis is stopped leading to a persistent diabetic state. Since insulin is no longer available, glucose absorption is impaired leading to hyperglycaemia. Experimental studies revels that, Glimepiride –Fe(III) & Cu(II) complexes were found more effective than pure drug.

CONCLUSION

The studied complexes of Fe(III) & Cu(II) with Glimepiride show 2:1 Ligand: Metal ratio which is confirmed by elemental analysis. Both metals show the through sulphone -O- and amide -O- atom. On the basis of analytical and spectral investigations octahedral geometries are proposed to Fe(III) & Cu(II) complexes of Glimepiride. Possible general structure of complexes is presented in following figure



(M= Fe, Cu)

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