VANADYL-GLICLAZIDE COMPLEX; SYNTHESIS, CHRACTERIZATION AND PHARMACOLOGICAL STUDIES

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ABSTRACT*: This paper deals with the Pharmacological studies of oxovanadium (IV) complex with gliclazide [1-(Hexahydrocyclopenta(c)pyrrol-2(1H)-yl)-3-(p-tolylsulfonyl)urea], belonging to hypoglycemic sulphonylurea class of antidiabetic drugs. A number of nitrogen and oxygen donor atoms are present in this compound and therefore it can acts as a potential ligand for complex formation with transition metal ions. Keeping in view this property of gliclazide, Its complex with vanadyl ion is synthesized. Characterization of this complex is carried out by IR, atomic absorption and electronic spectroscopy. Elemental analyses (CHNS and metal analysis) and magnetic susceptibility measurements are also conducted to determine its structure. Based on various analytical and spectroscopic techniques an octahedral geometry has been proposed for VO(IV)-Gliclazide complex. The hypoglycemic activity of this complex is tested in erimental animals, rabbits, in which diabetes is induced by chemical alloxan. This complex shows amazing hypoglycemic actitivity. Further, this complex is also subjected to hypoglycemic, minimum inhibition concentration (MIC) and toxicity (LD_{50}) studies.*

Keywords: Gliclazide, Diabetes, vanadyl ion, complex, Pharmacology

INTRODUCTION

Transition metals occupy a prominent place in medicinal biochemistry. Due to presence of partially filled d- sub-shell they remain involve in coordination with neutral molecules or ions. This activity of transition metals has started the progress of metal based medications with promise pharmacological applications. The history of first metal based drug goes back to 1960, when an inorganic complex "cis-diammine-dichloroplatinum" (II) "(cisplatin)" was exposed and has proven to be a highly effective chemotherapeutic agent for treating various types of cancers [1].

Heterocyclic molecules including existing drugs containing electron donor atoms like N, O, S, and P etc. acts as ligand and may form coordination complexes with different transition metal ions. If such complexation causes chelation then extraordinary changes in the biological properties of both ligands and metal ions are carried out[2-4]. It has been found that In almost all the cases the resulting complexes (metal based drugs) are found to possess more potential and less toxicity with reference to the free ligand [5-8].

Antibiotics like bleomycin, streptonigrin and bacitracin etc perform effectively in presence of metal ions. Metalloantibiotics can interface with various types of biomolecules including DNA, RNA, proteins, receptors and lipids rendering them extraordinary bioactive [9].

Silver and silver containing compounds such as Ag-4-isopropyltopolone and silver (I) complexes 2-pyrollidone-2-carboxylic acid and Silver sulfadiazine etc. are found to be very effective antimicrobial agents[10-11]. Anti-parasitic activity of Chloroquine complex of transition metal ruthenium, $[RuCl_2(CQ)]_2$ has been found to be 2 to 5 times more active than chloroquine diphosphate in *in-vitro* without any acute toxicity[12].

Recently it has been found that vanadium oxide (V_2O_3) complexes of thiourea and vanadium substituted "polyoxotungstates" exhibit potent "anti-HIV" properties[13].Antibacterial and antifungal activities of simple Schiff bases are enhanced many fold upon coordination with Vanadium(IV) metal ion[14].

It is concluded from the above discussion that role of metal based drugs as theraputic compounds has become more and more pronounced. These complexes not only used as anti-cancer, anti-inflamatory, antibiotic,anti- parasitic compounds but also as Anti- Diabetic compounds.

Anti-diabetic compounds usually used to treat type 2 Diabetes Mellitus (DM). It is a state at which body cannot create insulin hormone which is essential for assimilation of glucose in to the body cells[15-16]. About 2 – 8 % population of the world is suffering from Diabetes.

More than 100 years ago, a vanadium based compound was surveyed for the treatment of diabetic patients. At that time blood glucose level was controlled by giving inorganic vanadium salts to diabetic patients. Vanadium is poorly absorbed in elemental form and is needed a high dose, which can cause some adverse effects. Researchers are therefore searching for alternate methodologies for the management of diabetes [17].

In 2000, a vanadium-based drug compound "(bis(ethylmaltolato)oxovanadium(IV)",was recommen- -ded for clinical trial by "Medeval Ltd.,Manchester, UK"[18].Complexes of vanadium with organic compounds have been demonstrated to show less toxicity, with enhanced solubility and "lipophilicity". Insulin-mimetic properties have been shown by various vanadium complexes [19].

Vanadium complexes such "bis(pyridine-2-carboxylato),oxovanadium (IV) $[VO(pic)_2]$",Vanadyl complexes with "maltol" "(3-hydroxy-2-methyl-4-pyrone)" and "kojic acid" "(3-hydroxy-2-hydroxymethyl-4-pyrone)" etc. have shown insulin type action with low toxicity and suggested for clinical use in humans[20-22].

Metforminare and phenformine belonging to biguanides class of oral hypoglycemic drugs are frequently used in these days to treat non-insulin dependent diabetes mellitus (NIDDM). Highly colored, air-stable VO(IV) complexes of these drugs,

[VO(metf)2] and [VO(phenf)2] have been synthesized and found to be potential insulin-enhancing compounds[23].

Transition Metal complexes of different sulphonylurea drugs have been prepared, characterized and tested for their antidiabetic activity [24-27] but very few examples of formation of VO(IV) complexes with sulphonylureas are reported in literature [28]. However, synthesis, characterization and hypoglycemic studies of VO(IV)-GCZ complex have rarely been reported in literature. Therefore in continuation of our previous work [28] we report here the results of our study pertaining to the synthesis, characterization and pharmacological investigation of VO(IV)-Gliclazide complex.

MATERIAL AND METHOD

All chemicals utilized in this work were of analytical grade imported from "Merck, Germany" Fluka, Switzerland and "BDH Chemicals England". Pure drug compound "Gliclazide" was obtained from "E.Merck Co Germany".

"IR spectra (in KBr)" were taken out on "Shimadzu FTIR 4200 infrared spectrophotometer".

^{1}H-NMR and ^{13}C-NMR "(in DMSO-d_6)" spectra were recorded on "Bruker 14.1T NMR spectrometer" that functions at 600 MHz frequency.

Investigation of elements present in this complex was conducted on "CHNS" analyzer "Exeter Analytical CE-440".

Analysis of metal in this complex was carried out on "atomic absorption spectrophotometer model AA-680" operational with "GFA-4B Graphite Furnace Atomizer" with "ASA Arsenic analyzer"by applying an accepted procedure [29].

"Melting point apparatus", "Mel-Temp MP-D", "Mitamura Rikon Kogyo Japan" was used to find out the melting/decomposition point the complex and ligand drug itself. "Sealed capillary tube" process was adapted to do this.

"Absorption spectra" of metal complex and pure ligand were obtained through "Perkin- Elmer Lambda 20 spectrometer".Magnetic moment, "μ_{eff}" of this complex was obtained by "Chyo Balance MSB - 10" [29].

SYNTHESIS AND CHARACTERIZATION

Gliclazide

"IR: (KBr, cm^{-1}) 3375 (s, NH Amide.), 3210 (NH thionyl), 1704 (C=O), 1591 (C-N)" 1345, 1163 (SO_2). UV (λ_{max} DMSO, nm), (ε x 10^3): 3.86 (30674), 2.76 (36101), 3.21 (40000), 2.32 (45045). δ_H (DMSO-d_6);1.2-1.7 (m, Heterocyclic ring), 2.3 (s, CH_3), 7.4 (d, H^3, H^4), 7.6 (d, H^1, H^2), 8.1 (b, N^a–H) and 10.0 (b, N^b–H). δ_C (DMSO-d_6); 61.73

Oxovanadium(IV)-Gliclazide Complex

The result of analysis of the elements (CHNS and metal) fits nicely into the molecular formula $K_2[VO(C_{15}H_{21}N_3O_3S)(OH_2)(OH)_2)].3H_2O$, suggesting an octahedral environment around the metal ion if ligand acts in a bidentate mode.

The IR spectrum of this complex was compared with that of the ligand and found that most of the bands of the ligand remained unshifted while few were shifted upon complexation with metal. The band due to N–H stretch in the ligand (3210 cm^{-1}) disappeared upon coordination with VO(IV). The major band shift was observed in case of C=O stretching. In this complex, the C=O band of the ligand (1704 cm^{-1}) was shifted to lower frequency (1695 cm^{-1}) upon coordination. Further C - N stretch band of the –SO_2NCO– moiety of the ligand (1591 cm^{-1}) was transferred to 1596 cm^{-1} in complex.

(C1), 21.08 (C11), 24.20 (C2, C3, C4), 127.54 (C9), 129.34 (C8), 137.42 (C10), 143.59 (C7), 152.07 (C6).

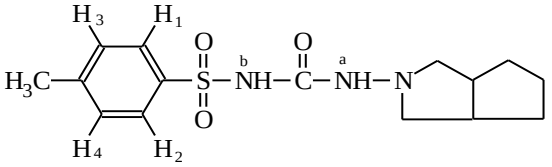
Vanadyl (IV)- Gliclazide Complex

Gliclazide 3.23 g (10 mmol) and KOH 0.56 g (10 mmol) were dissolved in methanol (50 ml) with constant stirring under reflux. To this mixture an aqueous solution of 3.53 g (10 mmol) hydrated vanadyl (IV) sulphate was added drop wise. The resulting mixture was stirred further for ten hours. A dirty green product was obtained after overnight stay at 0^0C. The product was filtered out and washed with acetone and ether solvents respectively. Finally it was dried by keeping it at room temperature. Yield: 71 %.

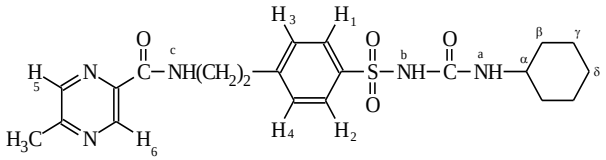
It is dirty green powder, mp 135 °C (dec.). Melting and decomposition of complex resulted a weak molecular ion peak at low electron volt area at *m*/*z* 653.7. "IR: (KBr, cm^{-1})" 3265 "(s, NH, str.), 1695 (C=O, str.), 1596 (C–N, str.) 1350, 1161 (SO_2, unsym, sym str.)", 770 "(M–O)" and 570 (M–N). UV"(λ_{max} DMSO, nm),(ε x 10^3)": 2.30 (14326), 5.99 (31847). Complex is paramagnetic in nature having μ_{eff} (B.M) 1.97. *"Anal.Calcd"*. for $K_2[VO(C_{15}H_{21}N_3O_3S)(OH_2)(OH)_2].3H_2O$: C, 31.35; H, 5.44; N, 7.31; S, 5.57; M, 8.87; Found: C, 31.03; H, 4.91; N, 6.96; S, 4.94; M, 8.37.

RESULTS AND DISCUSSION

The complex of VO(IV), with gliclazide (GCZ) drug ligand (**I,II**) was synthesized by reacting equimolar quantities (1:1) of ligand and metal salts in methanol/water solution and characterized by its elemental (CHNS and metal) analysis and different spectroscopic techniques like absorption, IR, UV-visible etc. Magnetic susceptibility measurement for this complex was also carried out.

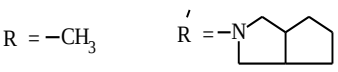


(I)



(II)

(III)



The band of stretching frequency "$\nu_{asy}(SO_2)$" and "ν_{sym} (SO_2)" found at 1345 cm^{-1} and 1163 cm^{-1} in the ligand remained un-shifted for VO(IV)-GCZ complex which suggests that sulphonyl group is not involved in coordination. The new bands which were probably because of

"M–O" and "M–N" stretch appeared at 770 cm^{-1} and 570 cm^{-1} in the spectrum of complex. In this complex a characteristic M=O str. was observed at 922 cm^{-1}. In this complex the GCZ acts as bidentate ligand and coordinates through deprotonated nitrogen atom and carbonyl oxygen of $–SO_2NCO-$ moiety. The remaining coordination sites are satisfied by OH/OH_2 group to give an octahedral geometry to the molecule of VO(IV)-GCZ complex.

Absorption spectrum of VO(IV)-Gliclazide complex in DMSO solution consists of only one strong band at 14,326 cm^{-1} (ε = 230 L mol^{-1} cm^{-1}). In addition to this, an intense band at 31,847 cm^{-1} (ε = 5990 L $mol^{-1}cm^{-1}$) was also observed that may be assigned to a charge transfer band. Oxovanadium (IV) is a d^1 system. Its absorption spectrum in octahedral environment usually consists of a band due to an electronic transition; $^2T_{2g} \rightarrow {}^2Eg$. However due to Jahn Teller distortion and low symmetry of the complex, usually 2 or 3 absorption bands are observed [30] which are assigned to the transitions $^2B_{2g} \rightarrow {}^2A_{1g}$- (14000––18000 cm^{-1}) and $^2B_{2g} \rightarrow {}^2E_{1g}$ (20,000-24,000 cm^{-1}) respectively. The observed band in the spectrum of VO(IV)-GCZ complex at 14,326 cm^{-1} is therefore assigned to the transition $^2B_{2g} \rightarrow {}^2A_{1g}$. The second band may be masked under the envelop of high energy charge transfer band at 31,847 cm^{-1} (5990 L mol^{-1} cm^{-1}) extending into visible region.

Magnetic susceptibility studies of this complex reveal its paramagnetic nature. The measured effective magnetic moment (μ_{eff}) for this complex is 1.95 B.M which shows one unpaired electron in this complex[31]. This corresponds to the μ_{eff} value obtained for the VO(IV)-GCZ octahedral complex. Therefore it further confirmed an octahedral structure for this complex (**III**).

HYPOGLYCEMIC ACTIVITY

The complex of VO (IV) with gliclazide have shown significant hypoglycemic activity. Hypoglycemic effect of VO (IV)-GCZ complex has been compared with the standard drug and control. The variation of mean blood glucose level of control and compounds treated groups of "alloxan diabetic rabbits" is reported in Table 1.

When VO(IV)-GCZ complex was administered to all the groups of alloxan diabetic rabbits a considerable hypoglycemic activity was observed. After two hours the (BGL) of complex treated animals and drug (GCZ) treated groups was 401.12 ±7.15 and 366.20 ±6.76 respectively compared to control group 445.60 ±12.68. After four hours, the BGL of complex and drug loaded groups was 324.46 ±5.94 and 285.60 ±3.57. During this time the decrease in blood glucose level was greater in drug (GCZ) treated group than VO(IV)-GCZ complex.

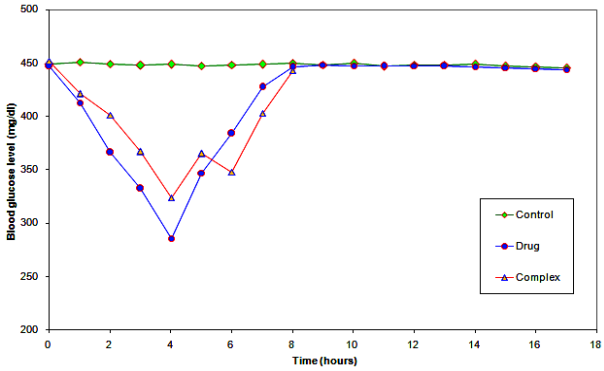


Fig.1. Change in blood glucose (mg/dl) of alloxan diabetic rabbits treated with gliclazide(GCZ) drug and VO(IV)-GCZ complex.

After six hours the BGL of complex loaded group was 348.20 ±3.96 and of GCZ treated group was 383.40±1.07 compared to control 447.20 ± 12.13. At this time decrease in BGL was greater in complex treated group than drug treated group. After eight hours the blood glucose level (BGL) of both complex and drug loaded groups (443.00 ±3.46 and 445.80 ±9.6) reaches to minimum i.e. almost equal to the BGL of control group (446.20 ±13.21).

Table-1. Change in Mean Blood Glucose Level (mg/dl) of Alloxan Diabetic Rabbits Treated with 1.0 mg/kg B.W of Gliclazide Drug and VO(IV)-GCZ Complex.

Time	Aloxan Diabetic Control	Treated with Standard GCZ	Treated with VO(IV)-GCZ
0 hr	447.80 ± 12.72	446.80 ± 6.37	452.00 ± 8.86
1hr	449.40 ± 14.08	412.20 ± 2.00	421.20 ± 4.32
2 hr	445.60 ± 12.68	366.20 ± 6.76	401.12 ± 7.15*
3 hr	446.40 ± 11.83	331.80 ± 4.20	367.78 ± 8.16*
4 hr	447.00 ± 13.34	285.60 ± 3.57	324.46 ± 5.94
5hr	447.20 ± 11.68	346.20 ± 2.23	365.40 ± 9.45**
6 hr	447.20 ± 12.33	383.40 ± 1.07	348.20 ± 3.96*
7 hr	446.40 ± 12.30	626.60 ± 7.53	403.00 ± 3.67*
8 hr	446.20 ± 13.21	445.80 ± 9.60	443.00 ± 3.46

* $p < 0.001$ Significant relative to GCZ., **(GCZ = Gliclazide)**
** $p < 0.01$ Significant relative to GCZ.
*** $p < 0.05$Significant relative to GCZ.

These observations (Table-1) show that under similar experimental conditions, both the compounds (GCZ and VO(IV)-GCZ) complex show hypoglycemic activity. However, at the end of two hours, the fall in glucose level was greater in case of GCZ drug (18%) than VO(IV)-GCZ complex (11%). Similar behaviour was observed after four hours (GCZ drug, 35%, VO(IV)-GCZ 28%).

But after six hours the lowering of blood glucose level (BGL) in complex treated group was greater (23%) than in drug (GCZ) treated group (14%). After six hours hypoglycemic

activity of both gliclazide drug and vanadyl gliclazide complexes slow down steeply and reaches to minimum after eight hours. During this interval (6-8 hrs) BGL of the complex treated group remained low compared to drug treated group upto the end of experiment (8 hrs). The profiles of various curves obtained are shown in Fig.1.

These observations prove that VO(IV)-GCZ complex shows significant hypoglycemic activity compared to control but less than that of the parent drug (GCZ). It is therefore concluded that VO(IV)-GCZ complex showed slower onset of activity with prolonged time interval with reference to parent drug(GCZ)

TOXICITY (LD_{50})

Drugs used in therapeutic, usually show certain adverse affects, but selective toxicity of certain chemicals and biological substances may make them useful therapeutic agents **[**32**].**

Table-2: Toxicity (LD_{50}) Data of VO(IV)-GCZ Complex

S.No	Compounds	Toxicity (LD_{50}) g/kg B.W.
1.	VO(IV)-GCZ	2.538

Hence, before introduction of a new drug, it is necessary to observe their toxicity with a view to find its therapeutic index, usually in terms of LD_{50}. For this purpose, metal based drug compound was also trialed for its toxicity (LD_{50}) and is reported in table 2. LD_{50} value for gliclazide is reported to be greater than 3g/kg b.w., orally [33]

LD_{50} value for vanadyl (IV)-gliclazide complex was found to be 2.538 g/kg b.w. orally in rats. This value shows that toxicity of this complex is more than the parent drug (3g/kg b.w.) VO(IV)-GCZ complex therefore is proved to be slightly more toxic compared to the parent drug(GCZ).

ANTIBACTERIAL ACTIVITY

On the basis of results obtained from the above discussion, it was considered of interest from clinical point of view to compare the toxicity of this complex with standard streptomycin sulphate. Antibacterial action of this complex was tested against various strains of microorganisms such as *Escheria coli(C), Staphylococus eureus, Staphylococus coagulase, Escheria coli and Streptococus and "gram negative bacteria" as "Pseudomonas" (C), Escheria coli, Salamonella thphi(R).Pseudomonas, "Escheria coli", Enterobacteria coloacae, Enterobacteria faecalis, Proteus mirabilis, Klepsiella pneumonia and Salimonella sensitive*, using only one concentration 100 μg/cm^3 of this complex. Results of this study are reported in Table 3.

Based on this study it is concluded that VO(IV)-GCZ complex has not shown any activity against the organisms used in this study compared to streptomycin sulphate.

MINIMUM INHIBITION CONCENTRATION

Antibacterial study of this complex was done in "DMSO/water" solution using concentration range of 10-1280 μg/cm^3. It is because this complex rendered insoluble in water. This complex was first dissolve in DMSO and then water is added to get required dilutions. Finally the minimum inhibition concentration (MIC) was determined against bacteria referred above. MIC was additionally led utilizing blank solvent for checking inhibitory action of the solvent on these microbes. MIC information are given in Table 4.

VO(IV)-GCZ complex found to possess a very weak inhibition effect against different gram positive and gram negative bacterial strains.

This complex has shown very weak inhibitory effect against Pseudomonas (C), S. typhi (R), Etn. coloacae, Klepsiella pneumonia and Salimonella sensitive (MIC 1280 μg/cm^3). and showed weak inhibitory effect against gram positive bacteria Staph. aureus (MIC 280 μg/cm^3) against which it does exhibit some activity

CONCLUSION:

Analytical and spectral investigation of VO(IV)-Gliclazide complex is in accordance with the proposed structures. Pharmacological investigations reveal that this complex has not shown antibacterial activity against the organisms tested having low toxicity (LD_{50}) and MIC. Vanadium-containing compound have been surveyed clinically for the use in treatment of human diabetic patients. It is one of the few VO(IV) complexes synthesized with sulphonyl urea drug (GCZ). After having undergone a comprehensive clinical trial, this complex can be utilized as convincing and fascinating metal based medication. A thorough examination can see if the complex is secured to use as "Metal Based Drug" for type 2 diabetes mellitus.

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Table-3: Antibacterial Activity of VO(IV)-GCZ Complex against Gram Positive and Gram Negative Bacteria.

Standard organism = Streptomycin in Sulphate, Concentration =100 μg/cm^3 Solvent = DMSO

Compound	*Zone of Inhibition of Organisms*						
	Gram Negative bacteria			***Gram Positive bacteria***			
	E. coli	***B. bron-chiseptica***	***S. cerevisiae***	***Staph. aureus***	***M. luteus***	***M. flavus***	***B. subtilis***
VO(IV)-GCZ	–ve	–ve	–ve	–ve	–ve	–ve	–ve
Streptomycin Sulphate Standard	22 mm	20 mm	18 mm	25 mm	20 mm	18 mm	27 mm

Slightly +ve = Less than 15 mm –ive = No activity at 100 μg/cm^3 concentration

Table-4: Minimum Inhibitory Concentration Data of Metal Complexes against Different Bacteria (MIC μg/cm^3).

Compounds	Gram Negative Bacteria										Gram positive		
	1	2	3	4	5	6	7	8	9	10	11	12	13
Co-GCZ	1280	–	1280	–	–	1280	–	–	1280	1280	280	1280	1280

*1. Pseudomonas (C)** *2. E. coli (C)** *3. Salmonella typhi (R)* *4. Pseudomonas*
5. E. coli *6. Ent. Cloacae* *7. Ent. Faccalis* *8. Proteus mirabilis* *9. Klepsiella pneumoniae* *10. Salmonella sensitive* *11. Staph. aureus* *12. Staph. Coagulase*
13. Streptococcus **(* = mutant gene of the bacteria ¶ = resistant gene of the bacteria)**

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