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Metallopharmaceutical Complexes Based on Vanadium as Potential Anti-hyperglycemic Agents

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Interaction between vanadium pentoxide and bioactive Schiff–bases led to the formation of vanadium (III/IV) complexes. Ten complexes have been characterized using mass, IR, UV-VIS and ESR spectra, magnetic moments and conductance measurements as well as elemental and thermal analyses. Magnetic moments and ESR measurements confirmed vanadium complexes have (III/IV) oxidation state. Conductivity measurements indicate that complexes are not electrolytes. A representative set of vanadium (III/IV) complexes in varying coordination environments have been tested as anti-hyperglycemic agents against type 2 diabetes mellitus in concentration doses (30 and 100 µM/Kg) using female albino rats. The pharmacological data showed that, complexes 1 and 8 produced significant decrease in blood glucose level. Furthermore, these two complexes showed an improvement in liver and kidney function after daily administration for two weeks.

Keywords: Vanadium complexes, ESR, Spectra, Magnetism, Pathological, type 2 diabetes, Anti-hyperglycemic

Introduction

The interest in the coordination chemistry of vanadium complexes had grown enormously over the last few decades due to their biological and industrial outcomes antimicrobial, spermicidal, anti-leukemia, antitumor and recently as insulin mimetic [1,2]. Oxo vanadium (V) complexes with N- and O-containing ligands have been extensively investigated in recent years with respect to their remarkable efficiency as insulin mimetic compounds [3-5]. The interaction of simple vanadium species with organic ligands having pharmacological activity is of particular interest. Schiff -base compounds have been widely used as versatile ligands in coordination chemistry [6-8] and they have shown interesting biological properties, such as antibacterial, antitumor, and antifungal activities [9-11] as well as catalytic properties [12-14]. Also, several vanadium compounds have been investigated in animal model systems as treatment for diabetes [15-16], and studies are ongoing in clinical trials in human beings with organic transition metal complexes [17]. In addition, human studies with vanadate have recently been reported [18-20], further documenting the notion that studies with vanadium compounds can be taken orally are very timely consumed. These compounds were recently reviewed and all the orally active compounds were found to contain vanadium in oxidation state IV. The toxicity of vanadium is correlated to its degree of oxidation (vanadyl/vanadate ion) and chemical form (organic\inorganic ligand) [21, 22].This has been demonstrated by experiments on animals, mainly rats and mice [23-24]. It appears that, both short-term (for a few days) and long-term (for a

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few months) administration of this element causes a variety of toxic effects such as hematological and biochemical changes (e.g., hemolysis and decrease in erythrocyte count/ hemoglobin level/ hematocrit index,), neurobehavioral injury (i.e. general activity and learning) [25], abnormalities in development and reproduction (e.g., embryo toxicity, teratogenicity) or morphological and functional lesions in liver, kidneys, bones, spleen and leukocytes [26]. Moreover, the most often observed side effects include loss of appetite and significant reduction of body weight (often leading to anorexia), weakness and nose bleeding, vomiting, diarrhea, dehydration, pulmonary hemorrhage, or death. However, it has also been reported that long term (5–12 months) administration of vanadium did not exert hematological, biochemical, or histopathological effects [27], and even lowered blood pressure and elevated plasma insulin levels in spontaneously hypertensive rats [28]. For this reason, the deep understanding about chemistry of metals within biological systems is very important [29]. Diabetes is characterized by hyperglycemia, altered lipids, carbohydrates, and proteins metabolism which affect the patient quality of life in terms of social, psychological well- being as well as physical ill health [30,31]. The World Health Organization reported that worldwide global population is in the midst of a diabetes epidemic. The people in Southeast Asia and Western Pacific are being under greater risk, and the majority of patients have type 2diabetes. Insulin resistance typically precedes the onset of type 2 diabetes and commonly accompanied by other cardiovascular risk factors such as dyslipidemia, hypertension, and prothrombotic factors [32]. Diabetes mellitus (DM), a leading non communicable disease with multiple etiologies, is considered as one of the five leading causes of death in the world. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [33]. DM is a clinically and genetically heterogeneous group of disorders, characterized by abnormally high blood glucose concentration. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Deficient supply of insulin cause abnormalities in carbohydrate, fat, and protein metabolism. These metabolic disturbances result in acute and long term diabetic complications, which are responsible for premature death and disability [34].Therefore, in that study of vanadium complexes their beneficial physiological function, their potential toxicity and side effect were considered. Searching literature revealed no published data on vanadium complex with oxidation state III used before as anti-hyperglycemic agents.

Experimental

Instrumentation and measurements: The vanadium complexes were analyzed for C, H and N at the Micro analytical center, Cairo University, Egypt. Standard analytical methods were used to determine the vanadium ion content [35-37]. 1H NMR and 13C NMR were carried out on Bruker spectrophotometer 400 MHz and 100 MHz, respectively, Faculty of Pharmacy, Cairo University, Cairo, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were obtained on Shimadzu Qp-2010 plus. FT-IR spectra of the complexes were measured using KBr discs by a Jasco FT/IR 300E Fourier transform infrared spectrophotometer covering the range 400-4000 cm^{-1}. Electronic spectra in the 200-900 nm regions were recorded on a Perkin-Elmer 550 spectrophotometer. The thermal analysis (DTA and TGA) were carried out on a Shimadzu DT-30 thermal analyzer from room temperature to 800 ºC at a heating rate of 10 ºC/ min. Magnetic susceptibilities were measured at 25ºC by the Gouy method using mercuric tetrathiocyanatocobaltate (II) as the magnetic susceptibility standard. Diamagnetic corrections were estimated from Pascal's constant [38]

The magnetic moments were calculated from the equation:

$$\mu_{eff.} = 2.84\sqrt{\chi_M^{corr} . T}$$

The molar conductance of 10^{-3} M solution of the complexes in DMSO was measured at 25 ºC with a Bibby conduct meter type MCl. The resistance measured in ohms and the molar conductivities were calculated according to the equation:

$$\Lambda_M = \frac{V \times K \times g}{Mw * \Omega}$$

Where: ΛM = molar conductivity /$\Omega^{-1}cm^2mol^{-1}$, V = volume of the complex solution/ mL, K = cell constant (0.92/ cm^{-1}), Mw

= molecular weight of the complex, g = weight of the complex/g, Ω = resistance/Ω. The ligands were prepared adopting the published procedure [39-43].

Chemistry (Synthesis of complexes 1-10)

Complex 1:

A mixture of ligand **1** (1.05 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in ethanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate which formed was filtered off and washed with ethanol.

Yield 85%, Yellow, m. p. > 300 ^{o}C. IR: 3430-3250, 3320 (H_2O, OH), 3550-3150, 3140-2750 (H-bond), 3175 (NH), 1367 (C-OH), 1673 (C=O), 1275 (C-O), 1614 (C=N), 1541, 749 (C=C_{Ar}), 576 (V-O), 518 (V-N), 1017 (V=O). ^{1}H-NMR: 4.84 (s, 2H, CH), 6.18 (s, 2H, OH), 6.93 (d, J= 6.8 Hz,2H, Ar-H), 7.16 (d, J= 5.2 Hz, 2H, Ar-H), 7.32 (d, J= 1.2 Hz, 2H, Ar-H), 7.65 (d, J= 1.2 Hz, 2H, Ar-H), 8.78 (s, 2H, CH=N), 11.07 (s, 2H, NH). ^{13}C-NMR: 76.0, 117.8, 118.5, 121.4, 127.5, 132.4, 146.0, 157.2, 175.5. M.S m/z: 536.00 (100.0%), 537.00 (19.5%), 538.00 (1.8%), 538.00 (1.8%), 536.99 (1.5%).Anal. Calcd for $C_{18}H_{18}N_4O_9V_2$ (535.8): C, 40.31; H, 3.35; N, 10.4; V, 19.01%. Found: C, 40.11; H, 2.86;

N, 10.4; V, 18.87%. Molar conductance, 12.6. Electronic abs (nm) λ_{max} = 290, 315, 425, 575, 628. Magnetic moment (μ_{eff}) = 2.53.ESR , $g_{\parallel}$ =2.0 , $g_{\perp}$ =1.95, $g_{iso}{}^{a}$ =1.97,$A_{\parallel}$(G) =85,$A_{\perp}$(G) =10,$A_{iso}{}^{b}$(G) =35, $g_{\parallel}$ / $A_{\parallel}$(cm^{-1})= 253.2.

Complex 2:

A mixture of ligand **2** (0.48 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 83%, Yellowish green, m. p. >300 ^{o}C. IR: 3520-3310, 3300-3000 (H_2O, OH), 3570-3260, 3250-2650 (H-bond), 3190, 3350, 3230 (NH, NH_2), 1653 (C=O), 1313 (C-O), 611 (V-O), 500 (V-N), 1018 (V=O). ^{1}H-NMR: 4.22 (s, 4H, NH_2), 4.84 (s, 2H, CH), 9.08 (s, 2H, NH). ^{13}C-NMR: 75.7, 169.9. M.S m/z: 381.8 (100.0%), 364.97 (4.3%), 365.97 (1.8%), 364.96 (1.5%). Anal. Calcd for $C_4H_{16}N_4O_{10}V_2$ (381.8): C, 12.5; H, 4.1; N, 14.5; V, 26.6%. Found: C, 12.2; H, 3.92; N, 14.5; V, 26.6%. Molar conductance, 13.7. Electronic abs (nm) λ_{max} =290, 310, 365, 380, 495, 560, 615. Magnetic moment (μ_{eff}) = 2.45. ESR, $g_{iso}{}^{a}$ = 1.98 .

Complex 3:

A mixture of ligand **3** (1.06g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 92%, Yellow, m. p. > 300 ^{o}C. IR: 3510-3310 (H_2O), 3644-3265 (H-bond), 3215 (NH), 1276 (C-O), 1625, 750, 1124 (C=C_{Ar}), 591 (V-O), 515 (V-N), 1021 (V= O). ^{1}H-NMR: 1.5 (s, H, NH-N), 4.68 (s, H, CH=C), 6.69 (d, J= 2.4 Hz, 2H, Ar-H) , 6.73 (d, J= 2.8 Hz, 2H, Ar-H), 6.93-7.16 (m, 4H, Ar-H), 7.26 (d, J= 8.01, 2H, Ar-H), 7.32-7.65 (m, 4H, Ar-H), 8.55 (s, H, CH=N), 10.74 (s, H, NH-Ar), 11.62 (s, H, NH-Ar). ^{13}C-NMR: 81.7, 116.3, 117.8, 118.5, 121.4, 122.4, 125.5, 127.5, 129.5, 130.7, 132.4, 139.9, 146.0, 150.8, 157.2. M.S m/z: 428.11 (100.0%), 429.11 (22.7%), 430.11 (2.5%), 429.10 (1.5%). Anal. Calcd for $C_{21}H_{21}N_4O_3V$ (427.8): C, 58.8; H, 4.9; N, 13.08; V, 11.8%. Found: C, 58.53; H, 4.73; N, 12.8; V, 11.52%. Molar conductance, 14.7. Electronic abs (nm) λ_{max} =298, 320, 435, 560, 622.Magnetic moment (μ_{eff}) = 2.51. ESR, $g_{iso}{}^{a}$ = 1.96.

Complex 4:

A mixture of ligand **4** (0.90 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 80%, Brown, m. p. > 300 ^{o}C. IR: 3460-3510 (H_2O), 3520-3150 (H-bond), 3244, 3359 (NH), 1609 (C=O), 1290 (C-O), 1529, 748 (C=C_{Ar}), 604 (V-O), 480 (V-N), 1017 (V=O). ^{1}H-NMR: 5.0 (s, 2H, CH), 5.12 (s, 2H, NH-Ar), 6.85 (d, J= 1.2 Hz, 2H, Ar-H), 7.00 (d, J = 9.6 Hz, 2H, Ar-H), 7.40 (d, J= 5.6 Hz, 2H, Ar-H), 7.66 (d, J=7.8 Hz, 2H, Ar-H), 10.02 (d, 2H, NH-Ar).^{13}C-NMR: 73.3, 114.5, 118.9, 125.1, 125.4, 125.5, 149.5, 171.9. M.S m/z: 496.00 (100.0%), 497.00 (17.3%), 498.00 (1.6%), 497.00 (1.5%), 498.01 (1.4%). Anal. Calcd for $C_{16}H_{18}N_4O_8V_2$ (496.8): C, 38.4; H,3.66; N, 11.2; V,20.3%. Found: C, 38.0; H, 3.78; N, 10.8; V, 19.72%. Molar conductance, 16.1. Electronic abs (nm) λ_{max} =295, 318, 435, 570, 623.Magnetic moment (μ_{eff}) = 2.62. ESR, $g_{iso}{}^{a}$= 1.98.

Complex 5:

A mixture of ligand **5** (0.87 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 75%, deep green, m. p. > 300 ^{o}C. IR: 3380-3100, 3450 (H_2O, OH), 3610-3270 (H-bond), 2937 (NH), 1646 (C=O), 1328 (C-O), 1605, 1595 (C=N), 1545, 829 (C=C_{Ar}), 1128, 1017, 980 (N-OH), 644 (V-O), 526 (V-N), 1021 (V=O). ^{1}H-NMR: 2.07 (s, 6H, CH_3C=N), 2.42(s, 6H, CH_3C =O), 4.64 (d, J= 7.6 Hz, 2H, CH_2O), 7.11 (d, J= 6.01 Hz, 2H, Ar-H), 7.93 (d, J=8.01 Hz, 2H, Ar-H), 10.57 (s, 1H, NH), 10.87 (s, 1H, NH), 12.01 (s, 2H, NOH). ^{13}C-NMR: 27.8, 69.0, 114.4, 125.1, 128.5, 143.7, 144.9, 146.8, 147.7, 161.5, 163.2, 171.0, 195.0. M.S m/z: 620.09 (100.0%), 621.09 (20.5%), 622.09 (2.3%), 621.08 (2.2%), 622.09 (2.0%). Anal. Calcd for $C_{19}H_{30}N_6O_{11}V_2$ (619.8): C, 36.70; H, 4.87; N, 13.6; V, 16.5%. Found: C, 37.10; H, 4.65; N, 13.4; V, 16.1%. Molar conductance, 12.6.Electronic abs (nm) λ_{max} =290, 305, 435, 580, 625. Magnetic moment (μ_{eff}) = 2.57.ESR , $g_{\|}$=2.01 , $g_{\perp}$=1.95, g_{iso}^{a} =1.97,$A_{\|}$(G) =95,$A_{\perp}$(G) =10, A_{iso}^{b}(G) =38.3, $g_{\|}$ / $A_{\|}$(cm^{-1})= 225.8.

Complex 6:

A mixture of ligand **6** (1.28 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 94%, Dark brown, m.p. > 300 ^{o}C. IR: 3400-3310 (H_2O), 3575-3240 (H-bond), 3445 (NH), 1644 (C=O), 1326 (C-O), 1605, 1595 (C=N), 1564, 829 (C=C_{Ar}), 1128, 1022, 985 (N-OH), 598 (V-O), 548 (V-N), 1021 (V=O). ^{1}H-NMR: 2.07 (s, 6H, CH_3C=N) , 2.42 (s, 6H, CH_3C=O), 4.66 (d, J = 7.55 Hz, 2H, CH_2O), 5.23(d, J = 1.2 Hz, 1H, CHCH_2O), 6.01 (s,1H, CHNH) 7.09 (d, J = 6.8 Hz, 2H, Ar-H), 7.11 (d, J = 2.01 Hz, 2H, Ar-H), 7.31 (d, J =8.4 Hz, 2H, Ar-H), 7.47 (d, J = 6.4 Hz,2H, Ar-H), 8.05 (d, J = 4.0 Hz, 2H, Ar-H), 8.27 (d, J = 2.8 Hz, 2H, Ar-H), 10.02 (s, 1H, NHC=O), 11.44 (s, 1H, NHC=OAr), 12.01 (s, 2H, NOH). ^{13}C-NMR: 15.9, 27.8, 66.6, 114.4, 118.8, 123.5, 125.9, 126.5, 128.5, 129.1, 129.6, 132.2, 133.8, 143.7, 153.9, 161.5, 164.7, 167.6, 195.0. M.S m/z: 770.13 (100.0%), 771.14 (33.5%), 772.14 (2.7%), 772.14 (2.7%), 772.14 (2.3%), 771.13 (2.2%). Anal. Calcd for $C_{31}H_{36}N_6O_{11}V_2$ (795.8): C, 48.5; H, 4.73; N, 10.9; V, 13.3%. Found: C, 48.23; H, 4.76; N, 10.72; V, 12.98%. Molar conductance, 12.2. Electronic abs (nm) λ_{max} =298,318,445,565,620. Magnetic moment (μ_{eff}) = 2.45. ESR, g_{iso}^{a} = 1.96.

Complex 7:

A mixture of ligand **7** (1.0 g, 0.002 mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 70%, Yellowish green, m. p. > 300 ^{o}C. IR: 3600-3282 (H-bond), 3138 (NH), 1638 (C = O), 1290 (C-O), 1610, 1592 (C=N), 1510, 820 (C=C_{Ar}), 1225, 1015, 985 (N-OH), 606 (V-O), 554 (V-N), 1021 (V=O). ^{1}H-NMR: 2.07 (s, 3H, CH_3C=N), 2.42 (s, 3H, CH_3C=O), 6.80 (s, 1H, NH-Ar), 6.81 (s, 1H, CH-N) 6.84-6.92 (m, 4H, Ar-H), 7.0-7.73 (m, 4H, Ar-H), 7.45 (s, 1H, NHCHO), 9.6 (s, 1H, NOH). ^{13}C-NMR: 15.9, 27.8, 113.4, 119.3, 119.7, 121.2, 123.9, 124.8, 125.2, 126.2, 127.7, 131.6, 134.1, 134.3, 143.7, 149.8, 153.9, 195.0. M.S m/z: 419.08 (100.0%), 420.08 (19.5%), 420.08 (1.8%), 421.09 (1.8%). Anal. Calcd for $C_{18}H_{18}N_5O_4V$ (419.8): C, 51.56; H, 4.3; N, 16.2; V, 12.1%. Found: C, 51.7; H, 3.98; N, 16.2; V, 11.95%. Molar conductance, 11.7. Electronic abs (nm) λ_{max} =290,308,442,575,626. Magnetic moment (μ_{eff}) = 2.42. ESR, g_{iso}^{a} = 1.97.

Complex 8:

A mixture of ligand **8** (1.47 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 70%, Green, m. p. > 300 ^{o}C. IR: 3600-3240 (H-bond), 3076 (NH), 1632 (C=O), 1264 (C-O), 1602, 1595 (C=N), 1457, 827 (C=C_{Ar}), 1264, 1130, 1015, 990 (N-OH), 591 (V-O), 499 (V-N), 1021 (V=O). ^{1}H-NMR: 2.07 (s, 6H, CHC=N), 2.42 (s, 6H, CH_3C=O), 5.0 (s, 2H, CH), 7.09 (d, J = 6.8 Hz, 2H, Ar-H), 7.31 (d, J = 8.4 Hz, 2H, Ar-H) ,7.47 (d, J = 6.4 Hz, 2H, Ar-H) ,8.27 (d, J = 2.8 Hz, 2H, Ar-H), 9.36 (s, 2H, NOH), 10.02 (s, 2H, NHC=O). ^{13}C-NMR: 16.7 , 27.5 , 73.3, 118.8, 123.5, 125.9, 129.1, 132.2 ,133.8, 143.7, 153.9, 171.9, 195.0. M.S m/z: 684.06 (100.0%), 685.06 (28.1%), 686.07 (2.7%), 685.06 (2.2%), 686.06 (2.1%), 686.07 (1.1%). Anal. Calcd for

$C_{26}H_{26}N_6O_{10}V_2$ (683.8): C, 45.6; H, 3.8; N, 12.2; V, 14.8%. Found: C, 45.11; H, 3.43; N, 11.78; V, 14.42%. Molar conductance, 10.9. Electronic abs (nm) λ_{max} =292, 315, 385, 465, 565, 635. Magnetic moment (μ_{eff}) = 2.51. ESR , $g_{\parallel}$ =1.99 , $g_{\perp}$ =1.95, $g_{iso}{}^{a}$ =1.96,$A_{\parallel}$(G) =90,$A_{\perp}$(G) =8.0,$A_{iso}{}^{b}$(G) =35.3, $g_{\parallel}$ / $A_{\parallel}$(cm^{-1})= 239.7.

Complex 9:

A mixture of ligand **9** (1.74 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 96%, Black, m. p. > 300 °C. IR: 3321 (OH), 3630-3110 (H-bond), 1395 (C-O), 1274 (C-O), 1604 (C=N), 1595, 755 (C=C_{Ar}), 1395 (N-OH), 590 (V-O), 516 (V-N), 1016 (V=O). ^{1}H-NMR: 1.36 (q, J = 4.0 Hz, 6H, $\underline{CH_3}CH_2O$), 1.81 (t, J = 2.4 Hz, 4H, $\underline{CH_2}CH$), 1.94 (s, 6H, CH_3C=N), 2.08 (d, J = 2.5 Hz, 2H, $\underline{CH}CH_2$), 3.66 (t, J = 3.6 Hz, 4H, $\underline{CH_2}CH_3$), 6.81 (d, J = 3.2 Hz, 4H, Ar-H), 6.92 (d, J = 4.0 Hz, 4H, Ar-H), 6.97 (d, J = 8.0 Hz, 4H, Ar-H), 7.17 (d, J = 5.2 Hz, 4H, Ar-H). ^{13}C-NMR: 15.3, 18.7, 21.2, 38.9, 61.4, 115.5, 119.8, 122.6, 128.6, 137.0, 151.5, 164.6, 171.3. M.S m/z: 767.15 (100.0%), 768.15 (40.0%), 769.16 (5.1%), 769.16 (2.7%), 769.15 (1.6%), 768.15 (1.1%). Anal. Calcd for $C_{37}H_{37}N_4O_8V_2$ (767.8): C, 58.2; H, 4.86; N, 7.3; V, 12.9%. Found: C, 57.82; H, 5.01; N, 7.1; V, 13.27%. Molar conductance, 11.2. Electronic abs (nm) λ_{max} =296, 310, 425, 562, 618. Magnetic moment (μ_{eff}) = 1.76. ESR, $g_{iso}{}^{a}$ = 2.11.

Complex 10:

A mixture of ligand **10** (2.05 g, 0.002mol) and vanadium pentoxide (1.0 g, 0.005 mol) in methanol (25 mL) was refluxed with stirring for 3 h. The mixture was concentrated and the precipitate formed was filtered off and washed with ethanol.

Yield 93%, Yellowish green, m. p. >300 °C. IR: 3434 (OH), 3630-3320, 3310-2750 (H-bond), 1389 (C-OH), 1670 (C=O), 1279 (C-O), 1623 (C=N), 1530, 728 (C=C_{Ar}), 588 (V-O), 511 (V-N), 1023 (V=O). ^{1}H-NMR: 1.36 (q, J = 4.0 Hz, 6H, $\underline{CH_3}CH_2O$), 1.81 (t, 2.4 Hz, 4H, $\underline{CH_2}CH$), 1.94 (s, 6H, CH_3C=N), 2.08 (d, 2.5 Hz, 2H, $\underline{CH}CH_2$), 3.66 (d, J = 3.6 Hz, 4H, CH_2O) , 7.65 (d, J = 9.2 Hz, 4H, Ar-H), 7.88 (d, J = 10.4 Hz, 4H, Ar-H), 8.04 (d, J = 8.4 Hz, 4H, Ar-H), 8.15 (d, J = 8.4 Hz, 4H, Ar-H). ^{13}C-NMR: 15.3, 18.7, 21.2, 38.9, 61.4, 116.3, 124.1, 126.5, 128.2, 135.2, 144.5, 149.7, 164.6, 166.5, 171.3. M.S m/z: 878.12 (100.0%), 879.12 (44.3%), 880.13 (9.6%), 880.13 (2.5%), 879.12 (1.5%), 881.13 (1.1%). Anal. Calcd for $C_{41}H_{36}N_4O_{12}V_2$ (878.8): C, 56.5; H, 4.13; N, 6.38; V, 11.40%. Found: C, 55.72; H, 4.01; N, 6.14; V, 10.98%. Molar conductance, 10.6. Electronic abs (nm) λ_{max} =298, 316, 425, 580, 610. Magnetic moment (μ_{eff}) = 1.63. ESR, $g_{iso}{}^{a}$ =2.12.

Biological Screening:

Choice of the effective compound at minimal oral dose:

To Screen the potential anti-hyperglycemic effect of the ten newly synthetized vanadium complexes, normal overnight food-deprived female rats (190-220 g weight) were given 30 or 100 µmol/kg (dissolved in 1% tween 80) orally by gastric intubation. A drop of blood was taken from the tip of tail before and one hour after administration. Animals were then given 6 g/kg glucose (10%) orally, and blood samples were taken at 0.5, 1.0, 1.5 and 2.0 h after glucose administration for determination of blood glucose level (oral glucose tolerance test, OGTT). OGTT was also carried out 30 min after oral administration of compounds 1 & 8.

Investigation of the effects of Compound 1 & 8 in STZ-induced hyperglycemia:

Hyperglycemia was induced in overnight food-deprived female rats by administration of streptozotocin (STZ, 50 mg/kg, i.p., in citrate buffer). Rats were given sucrose (1.5%) in drinking water for two days. In the third day, blood glucose level was measured, and animals with hyperglycemia (above 230 mg%) were chosen. Animals were then injected s.c. with insulin (2.0 U/animal) daily for one week. Compounds 1 & 8 were given (as a single or repeated oral doses for 14 days) to overnight food-deprived female rats and blood glucose level was measured at 0.5, 1.0, 1.5, and 2.0 h after administration. OGTT was also performed 30 min after oral administration of compounds 1 & 8. Sixteen days after chronic administration of compounds 1 and 8, animals were over-night food deprived, blood samples were taken for determination lipid profile, liver and kidney function using commercially available kits and according to the manufacturer instructions before and 2 hours after oral administration.

Statistical analysis:

The results are presented as means + SD and

compared with paired or unpaired t test and one-way ANOVA followed by Tukey post-hoc test where appropriate. The area under blood glucose level versus time (AUC) of OGTT was calculated as a percent of control.

Results and Discussion

Chemistry:

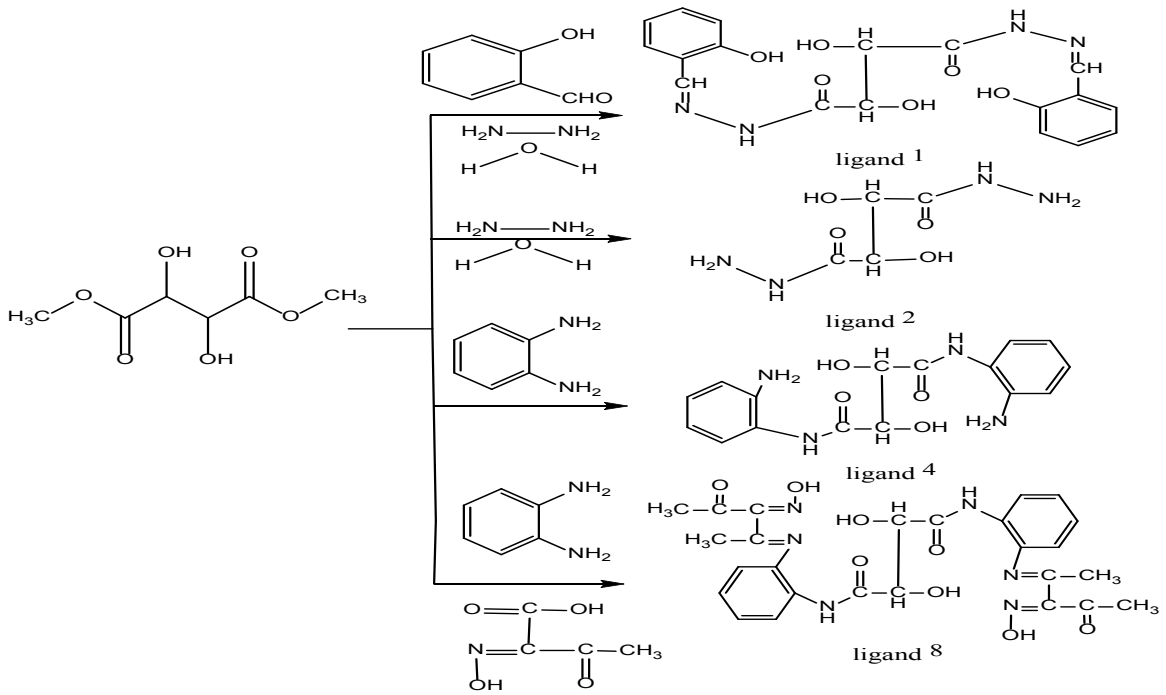
Synthesis of the ligands (1-10) is illustrated in schemes 1, 2, 3, 4 and 5.

First, dimethyl tartrate was used as starting material in the formation of ligands **1**, **2**, **4** and **8**. As it reacted with hydrazine hydrate followed by salicylaldehyde, hydrazine hydrate only, o-phenylenediamine only or o-phenylenediamine followed by 3-(hydroxyimino)pentane-2,4-dione yielded ligands **1**, **2**, **4** and **8** respectively scheme 1. Ligand **3** was prepared by adding equimolar amounts of ethyl chloroacetate and aniline. The mixture was refluxed with hydrazine hydrate and salicylaldehyde (scheme 2).

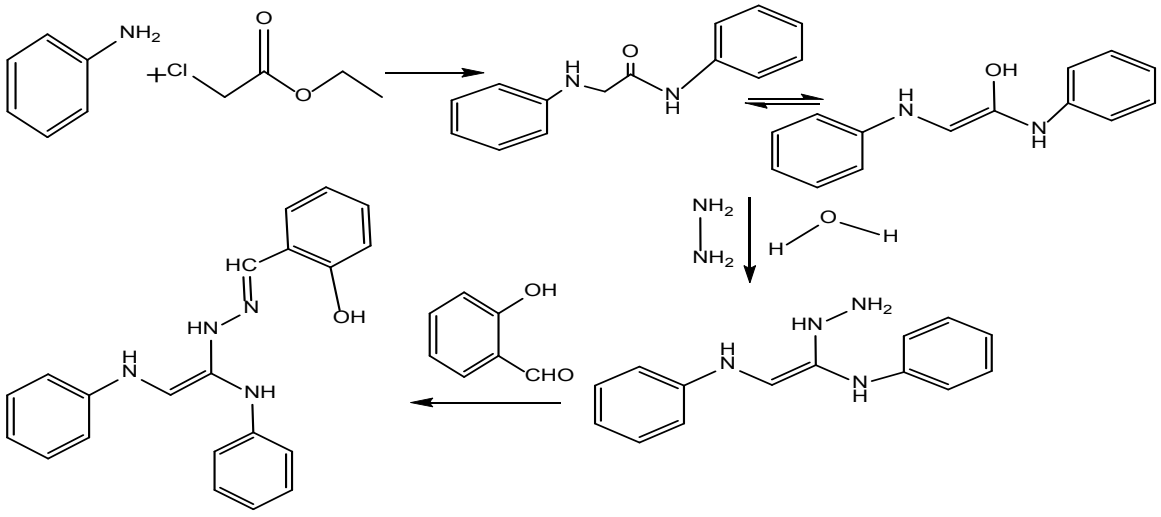
Ethyl chloroacetate with methyl *p*-hydroxybenzoate sodium salt were considered the synthons for preparing ligands **5** and **6**. In ligand **5**, hydrazine hydrate was added in second step but for ligand **6** o-phenylenediamine was added. The last step, 3-(hydroxyimino) pentane-2, 4-dione was added for both ligands (scheme 3). Ligand **7** was prepared by adding o-phenylenediamine to 3-(hydroxyimino) pentane -2, 4-dione (scheme 4).

Finally, Ligands **9** and **10** were prepared by refluxing equimolar amounts of ethyl acetoacetate and 1, 2-dibromoethane and o-aminophenol or o-amino benzoic acid respectively (scheme 5).

All new analog complexes were prepared by adding ligands to vanadium pentoxide to give the Vanadium complexes. The proposed structures of vanadium complexes are shown in figure.**1**. The new complexes (**1**, **2**, **3**, **4**, **8**, **9** and **10**) were confirmed by elemental analysis and also confirmed by disappearance of OH signal in ^{1}H-NMR spectra 11.11, 6.18, 11.11, 6.18, 6.18, 9.85 and 12.75 respectively. Also confirmed by the difference between mass spectra of ligands and vanadium complexes.

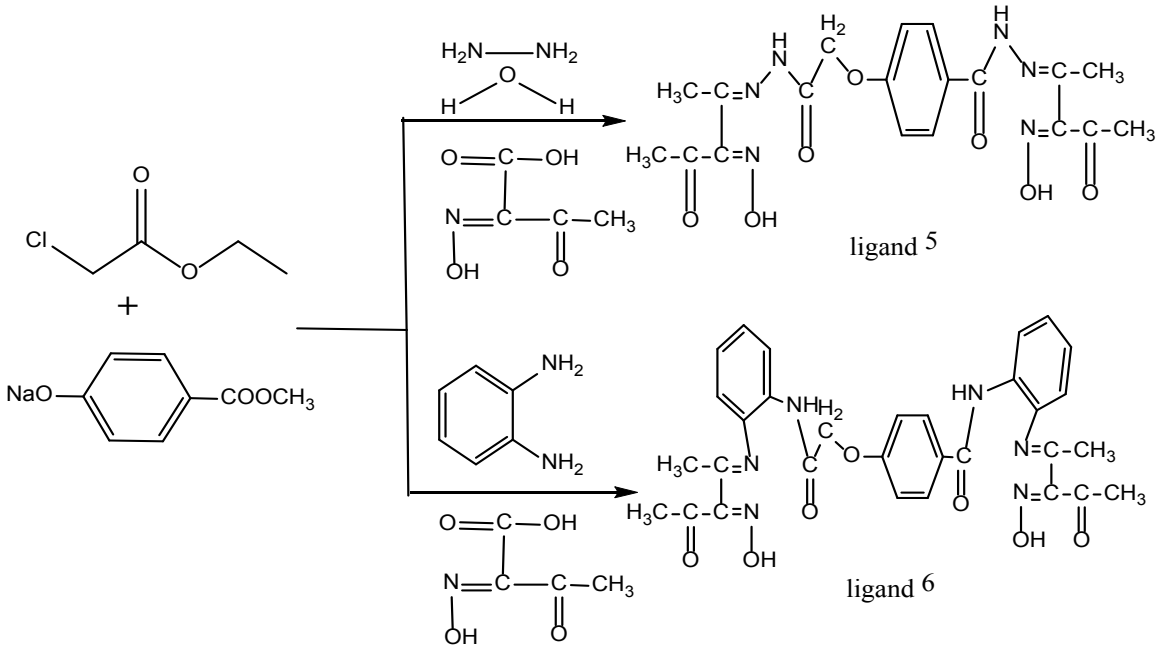


Scheme.1: Synthesis of ligands (1, 2, 4 and 8)



ligand 3

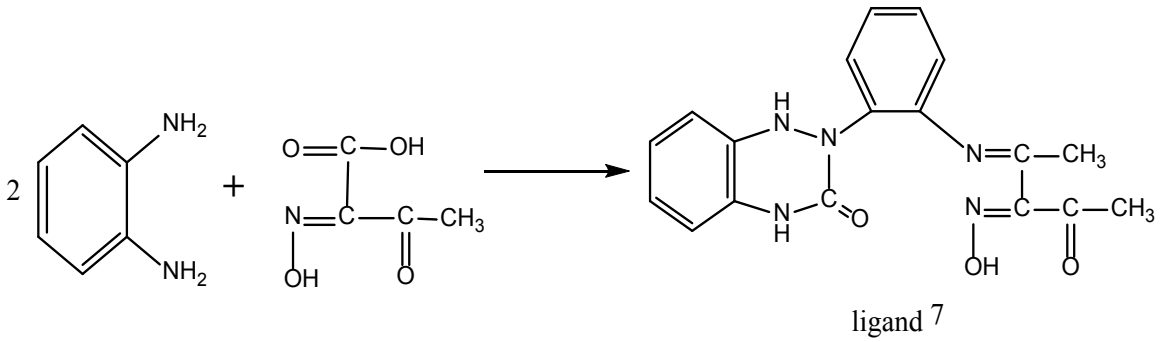
Scheme.2 Synthesis of ligand (3)



ligand 5

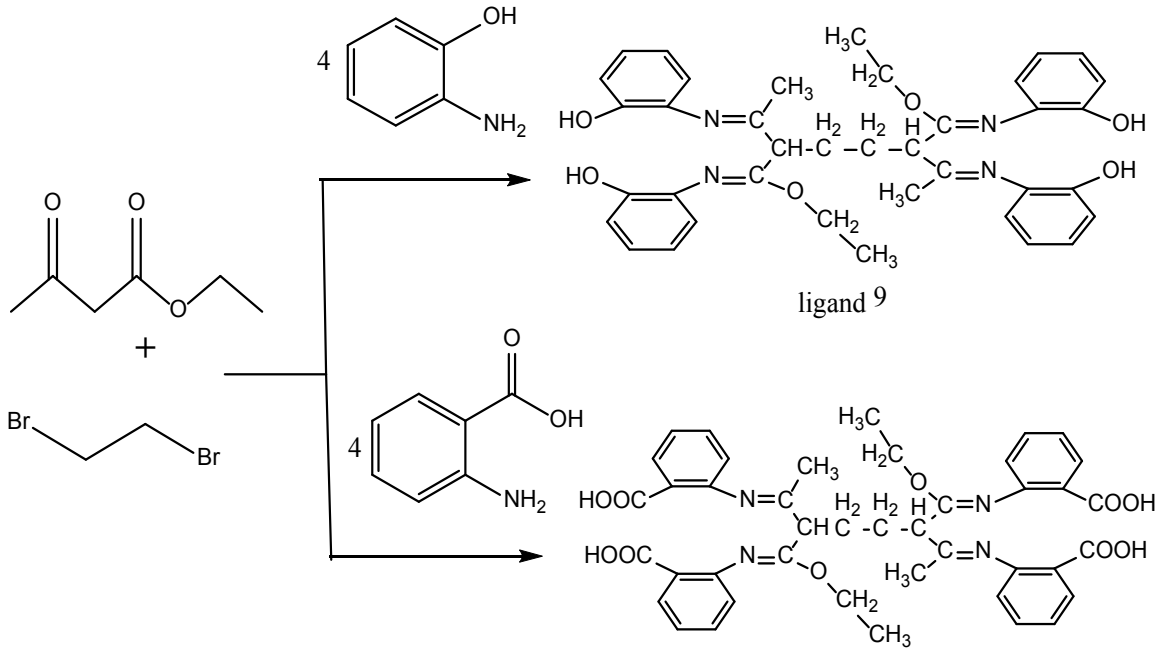
ligand 6

Scheme.3 Synthesis of ligands (5, 6)

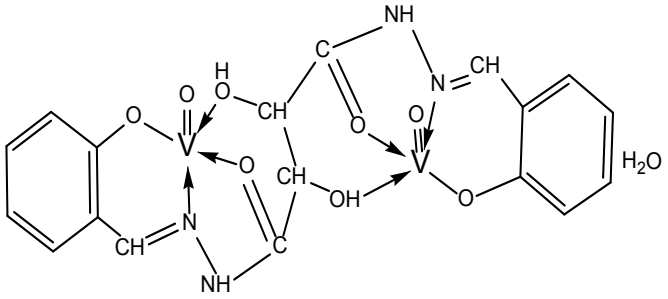


ligand 7

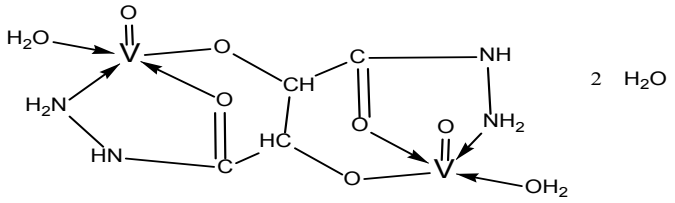
Scheme.4 Synthesis of ligand (7)



Scheme.5 Synthesis of ligands (9, 10)



complex 1



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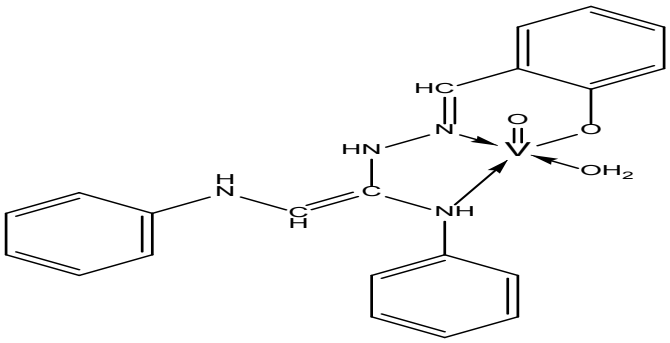

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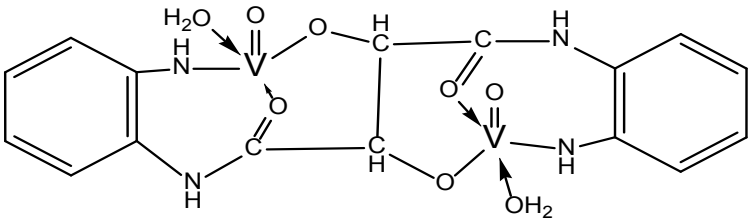

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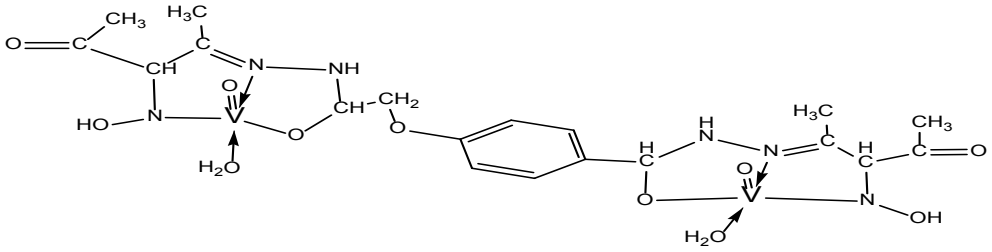
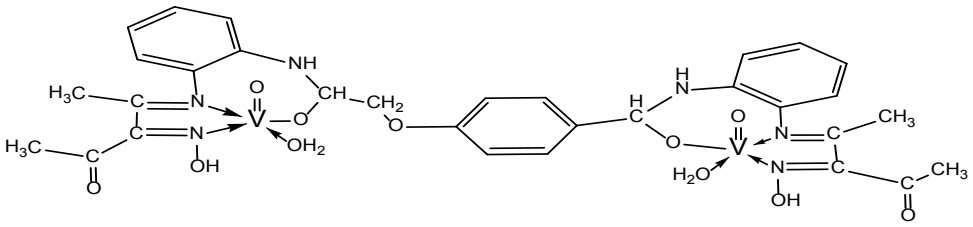
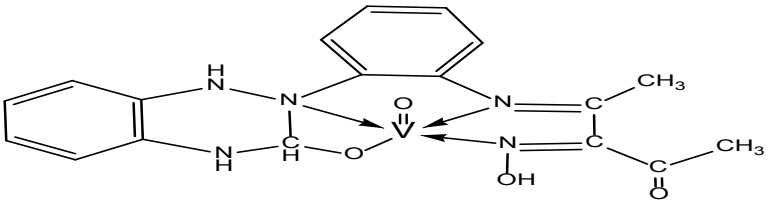


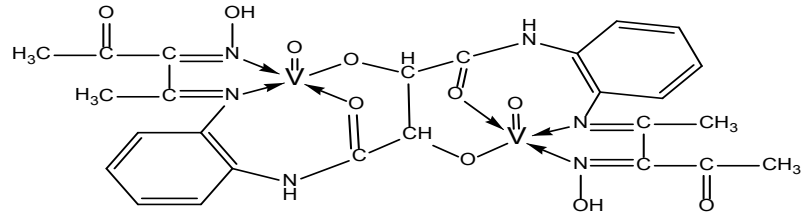
Complex 5



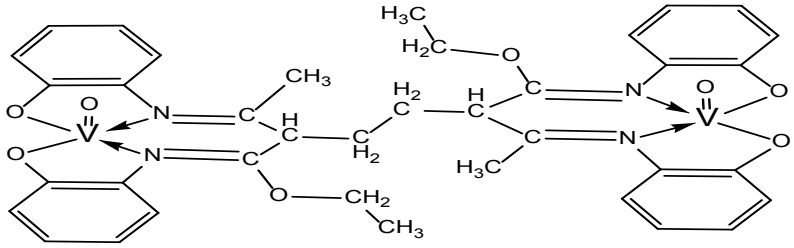
complex 6



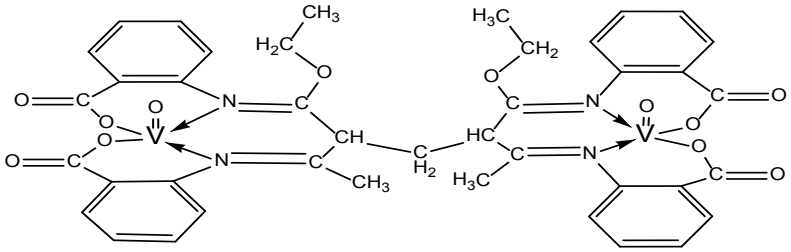
Complex 7



Complex 8



Complex 9



Complex 10

Biological Screening :

Choice of the most effective compound at minimal oral dose:

Animal experiments were carried out according to the guidelines of the Ethics Committee of Faculty of Science, Menoufia University (MUFS/S/CH/1/18). OGTT was carried out one hour after oral administration of 30 or 100 µmol /kg to normal rats of the ten complexes. Only complexes **1**, **4** and **8** showed noticeable reduction in OGTT. The effect was not dose related and that of complexes 1 and 8 was better 30 min after administration

(Figure **.2**). From figure. **2**, it can be seen that the three compounds did not alter fasting blood glucose level before OGTT, but significantly decreased it 120 min after OGTT ($p<0.01$) compared with the control values.

Investigation of the effects of Compounds 1 and 8 on STZ-induced hyperglycemia:

Effect after single administration: A single oral administration of 30 µmol/kg of complexes **1** and **8** to STZ-induced hyperglycemic rats did not show any statistical significant difference in blood glucose level at different times, up to 2 hours, when compared with the vehicle treated controls or those given metformin 7.7 mmol/kg (1000 mg/kg) and their initial values before administration (Table.**1**). When area under blood glucose level (AUC) was calculated, Complexes **1** and **8** showed a non-significant reduction in AUC to 88.3 and 88.9 % of the control values, respectively. Metformin, however, produced a reduction of AUC to 78.5% of the control ($p<0.05$) (Table.**1**).

On the other hand, a single oral administration of 30 or 100 µmol/kg of complexes **1** and **8**, 30 min before OGTT (Figure.**3**), showed that fasting blood glucose level was lower in the group given complex **1** (100 µmol/kg) than that of the control group as well as that of the group given complex **8** (100 µmol/kg). Hundred and twenty min after OGTT, all treated groups, including metformin showed reduction in blood glucose level when compared with the control group. BG was

significantly lower in the groups given complexes **1** and **8** (30 µmol/kg) when compared with their respective group taken 100 µmol/kg or that given metformin, 120 min after OGTT (Figure. **3**). The effect was also calculated as a % of control AUC. Metformin and 30 µmol/kg of compound **1** showed 77% and 78% reduction, respectively ($p<0.01$). Complex **1** (100 µmol/kg) and complex **8** (30 µmol/kg) showed reduction of 82% and 81%, respectively ($p<0.05$), (Figure. **3**).

Effect after repeated administration: Repeated oral administration of complexes **1** and **8** (30 umol/kg) for 14 days to STZ-induced hyperglycemic rats showed a reduction in fasting BG in the group given complex **8** more than **1**, which was statistically significant ($p<0.05$) 60 to 120 min after administration (Figure .**4A**). The effect of repeated administration for 16 days, however, did not show any significant difference between the two treated groups during a OGTT (Figure. **4B**).

Effect on liver and kidney function as well as on lipid profile of Diabetic rats after repeated administration:

When STZ-induced hyperglycemic rats were given repeated daily oral administration of complexes **1** and **8** (30 µmol/kg), and liver and kidney function as well as lipid profile was measured in over-night food-deprived rats before and after treatment; it was found that both complexes **1** and **8** produced a significant reduction in GOT, GPT, total bilirubin and creatinine. Complex **8** but not complex **1** also decreased HDL (Table.**2**).

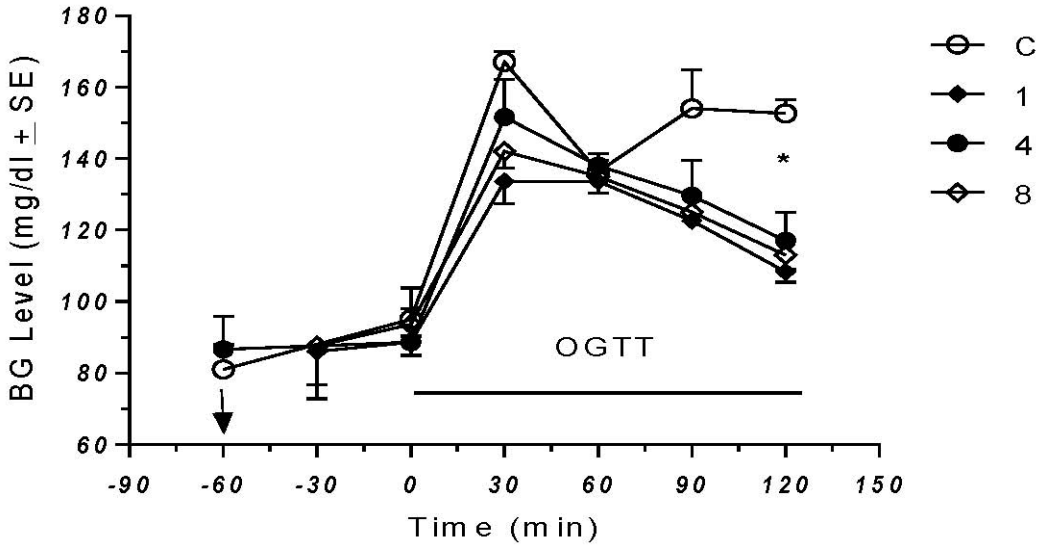


Figure. **2**: Effect of Complexes **1, 4** and **8** on blood glucose (BG) level in normal non-diabetic rats 30 or 60 min after oral administration of 30 µmol/kg compared with the control (C) and at 30, 60, 90 and 120 min after 6 gm/kg oral glucose (OGTT). Results are shown as mean ± SE of three animals and compared using one-way ANOVA followed by Tukey post hoc test.

*: $P<0.01$, compared with the control group.

Blood samples were taken from the retro-orbital sinus after over-night food deprivation before and after 16 daily doses of complexes **1** and **8** of STZ-induced hyperglycemic female rats. Number of animals/group is 3 rats.

The after treatment values were compared with before values for each animal using paired t test.

Table. 1: Effect of Complexes 1, 8 and metformin on blood glucose level in streptozotocin-induced diabetic rats for 120 min after oral administration, compared with the control group.

	Blood Glucose Level (mg/dl ± SD)					AUC as a % of control
Time after admin	**0**	**30**	**60**	**90**	**120**	
Control	484 ± 37.1	448 ± 24.3	413 ± 21.6	414 ±2 6.2	420 ±50	100
Comp-1, 30 µmol/kg	446 ±55.6	417 ±46.7	395 ±58.4	336 ± 89.7	305 ±84.7	88.3
Comp-8, 30 µmol/kg	391 ± 23.2	417 ± 63.5	386 ±27.8	353 ±27.6	368 ±17.2	88.9
Metformin, 7.7 mmol/kg	346 ± 30.3	395 ± 32.6	361 ± 25.4	290 ± 23.4	274 ± 34.5	78.5*

Results are presented as mean + SD of 3 rats.

*: P < 0.05 compared with the control group using one-way ANOVA followed by Tukey post hoc test.

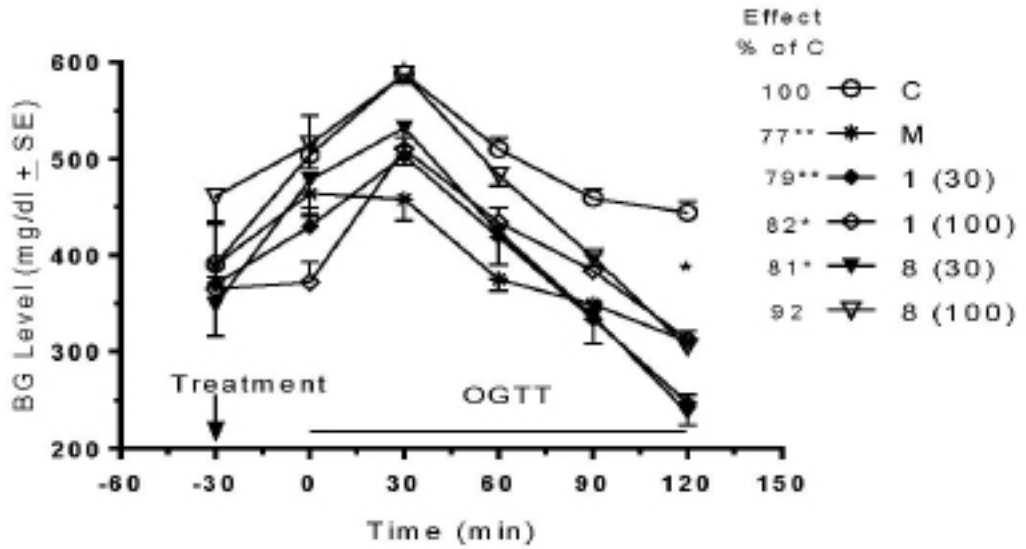


Figure. 3: Effect of two dose levels (30 and 100 µmol/kg, orally) of complexes 1 and 8 on blood glucose (BG) level in STZ-induced diabetic rats 30 min before and after oral glucose tolerance test (OGTT) compared with the control (C) and metformin (7.7 mmol/kg, orally).

Results are shown as mean ± SE of three animals and compared usingone-way ANOVA followed by Tukey post hoc test. Effect % of C: AUC as a % of Control.

***: P<0.05, **: P<0.01, compared with the control group.**

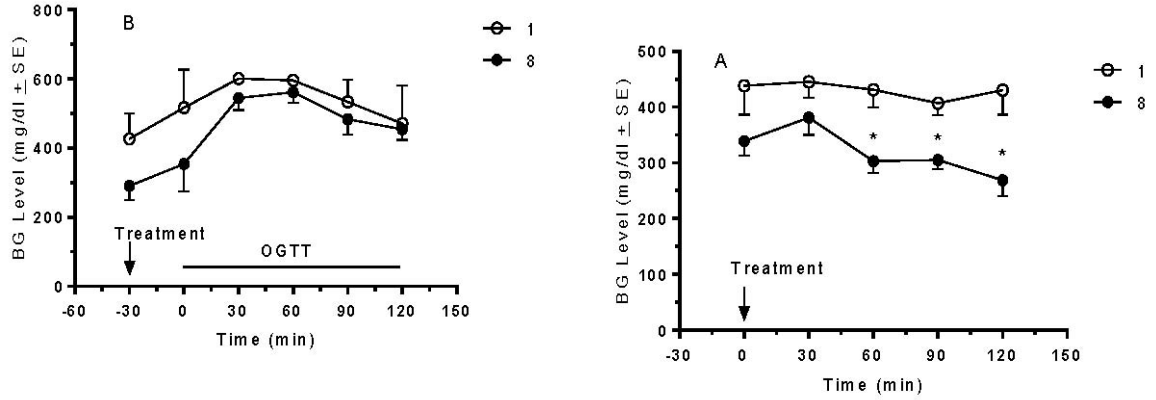


Figure.4: Effect of Complexes 1 and 8 (30 umol/kg) when given as a daily oral dose for 14 days on fasting blood glucose level (BG) of STZ-induced hyperglycemic rats (A), and on OGTT 30 min after administration for 16 days (B).

Results are shown as mean ± SE of three animals and compared using unpaired Students t test.

***: P<0.05, compared with respective time point.**

Table .2: Effect of Complexes 1 and 8 (30 µmol/kg) when given as a daily oral dose for 16 days on Liver and kidney functions as well as lipid profile of STZ-induced hyperglycemic rats.

	Complex 1			**Complex 8**		
			Liver function			
	Before	**After**	**P value**	**Before**	**After**	**P value**
GOT	141.7 ± 3.58	42.4 ± 19.2	**0.0149**	126.5 ± 4.17	34.7 ± 4.8	**0.0012**
GPT	78 ± 7.9	22.2 ± 5.2	**0.0181**	73. ± 26.72	16.5 ± 1.06	**0.0663**
ALP	681 ± 276.8	764 ±222.3	0.794	432 ± 144.5	346.6 ± 71.6	0.406
GGT	13.4 ± 3.52	13.1 ± 2.1	0.943	13.3 ± 1.09	13.1 ± 0.2	0.713
Albumin	3.7 ± 0.69	3.1 ± 0.12	0.289	3.7 ± 0.28	3.14 ± 0.3	0.216
T. bilirubin	1.0 ± 0.14	0.48 ± 0.05	**0.0225**	1.1 ± 0.04	0.54 ± 0.07	**0.0013**
T. protein	7.8 ± 0.73	7.4 ± 0.48	0.145	8.0 ± 0.17	7.3 ± 1.11	0.469
			Kidney function			
Urea	50.2 ± 6.05	37.5 ± 0.5	0.079	47.9 ± 1.25	44.6 ± 7.1	0.44
Creatinine	0.6 ± 0.03	0.88 ± 0.1	**0.0207**	0.5 ± 0.02	0.76 ± 0.07	**0.0288**
			Lipid profile			
Cholesterol	104.2 ±14.41	105.9±11.6	0.830	102.7±10.6	94.1±5.28	0.109
Triglycerides	90.47 ±11.92	140±45.7	0.247	109±30.4	156.8±11.07	0.175
HDL	39.63±1.79	32.2±3.4	0.129	38.5±2.4	28.8±1.9	**0.048**
LDL	46.47±15.07	45.6±19.7	0.950	42.3±12.4	33.8±5.3	0.204
VLDL	18.08±2.38	28±9.1	0.247	21.8±6.08	31.3±2.2	0.175
T. Lipids	454.67±3.83	505.9±40.3	0.163	471.7±25.8	510.8±12.2	0.215

Conclusion :

The current study included synthesis and characterization of ten vanadium complexes. These complexes have been tested as antihyperglycimic agents, complexes **1** and **8** among all tested metallo-compounds showed the best activity with decrease in blood glucose level at oral doses (30 and 100 μM/Kg) in female albino rats. Furthermore, these two compounds showed an improvement in liver and kidney function after daily administration for two weeks.

Acknowledgments

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