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Novel 3-hydroxy-4-pyridinonato oxidovanadium(IV) complexes to investigate structure/activity relationships

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ABSTRACT

A previous evaluation of the insulin-like activity of three 3-hydroxy-4-pyridinonato oxidovanadium(IV) complexes raised questions about structure/activity relationships, namely the influence of the hydrophilic/lipophilic balance of the complex and the capacity of the ligand to stabilize the +4 oxidation state of vanadium ion, on achieving an positive effect. To address these questions, we synthesized six new oxidovanadium(IV) complexes with variable hydrophilic/lipophilic balance, obtained by introducing different substituents on the nitrogen atom, and used two 3-hydroxy-4-pyrones as starting reagents to provide *methyl* and *ethyl* groups in the *ortho* position of the ring. For the new and previously reported complexes, we studied the oxidation–reduction properties and insulin-like activity in terms of inhibitory effect on Free fatty acid (FFA) release in isolated rat adipocytes. The results obtained show that only one of the complexes, *Bis(3-hydroxy-1(H)-2-methyl-4-pyridonato)oxidovanadium(IV), VO(mpp)$_2$*, exhibits a significantly greater capacity to inhibit FFA release than $VOSO_4$ and consequently is worthy to be considered for further studies. The establishment of structure activity relationships was not attainable but this study brings new information about the influence of some properties of the compounds on the achievement of an insulin-like effect. The results reveal that: (i) the oxidation–reduction cycles of the complexes are identical; (ii) the presence of more lipophilic substituents on the nitrogen atom does not enhance insulin-like properties; (iii) a high solubility in water proved to be not sufficient for a positive activity in inhibiting FFA release; (iv) a small molecular size may be an important property for reaching the right targets.

1. Introduction

Diabetes Mellitus is a condition characterized by abnormal glucose levels with a tendency to hyperglycaemia, due to a relative or absolute deficiency of insulin. Unfortunately, many people still die as a direct consequence of the disease or its associated pathologies. The incidence of the disease has increased along the years and the number of patients is expected to be 300 million in 2025.

The first report of the effectiveness of a vanadium compound in improving the diabetic state of patients dates the year of 1899 [1], a time in which vanadium was expected to cure all kinds of diseases. However, only in the last quarter of the 20th century an active search of a vanadium compound that could be administered orally and produce insulin-like effects has been initiated. A large number of studies regarding the synthesis of novel vanadium complexes bearing different coordination spheres and its potential insulin enhancing properties has now been reported and several review papers collecting the overall information have been published [2–8]. The oxidovanadium(IV) complex of *ethylmaltol* (BEOV) has passed Phase I clinical trials in 2000 and recently Phase II clinical trials [9].

Our group has long been interested in the chemistry of 3-hydroxy-4-pyrone and 3-hydroxy-4-pyridinones and their complexes, as the ligands are synthetically versatile, have interesting structural and solvation properties and in particular have a strong affinity towards M(III) and M(II) metal ions forming a large variety of complexes [10]. Ligands and complexes find applications in many areas, namely the regulation of metal ions in the body and development of new drugs. In order to provide a new family of complexes with potential insulin enhancing properties we have

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synthesized and studied a set of oxidovanadium(IV) complexes of 3-hydroxy-4-pyridinones [11–17].

Some years ago we reported the first *in vitro* evaluation of insulin-like action of three oxidovanadium(IV) complexes derived from 3-hydroxy-4-pyridinones based on the inhibition of free fatty cid (FFA) release in isolated rat adipocytes [14]. The results obtained pointed out two complexes with a positive effect and raised some questions in what concerns the possible establishment of structure/activity relationships namely the influence of hydrophilic/lipophilic balance of the complex and the capacity of the ligand to stabilize the +4 oxidation state of vanadium ion.

In order to address those questions we synthesized six new complexes derived from ligands with the *methyl* and *ethyl* groups in the *ortho* position and with variable hydrophilic/lipophilic balance, provided by different substituents on the nitrogen atom of the heterocyclic ring. Here, we report the synthesis and characterization of the novel complexes and the study of oxidation–reduction cycles and insulin-like activity, in terms of inhibitory effect on free fatty acid release in isolated rat adipocytes, for the new and the previously described complexes. The *formulae* and abbreviations of the complexes under study are depicted in Fig. 1.

2. Experimental

2.1. Chemicals

The ligands were prepared according to established procedures described for 3-hydroxy-4-pyridinones [10,18,19]. All other chemicals were from Aldrich (*grade Puriss. P.A.*). The solvents were distilled under argon and degassed prior to use in order to avoid oxidation of the complexes.

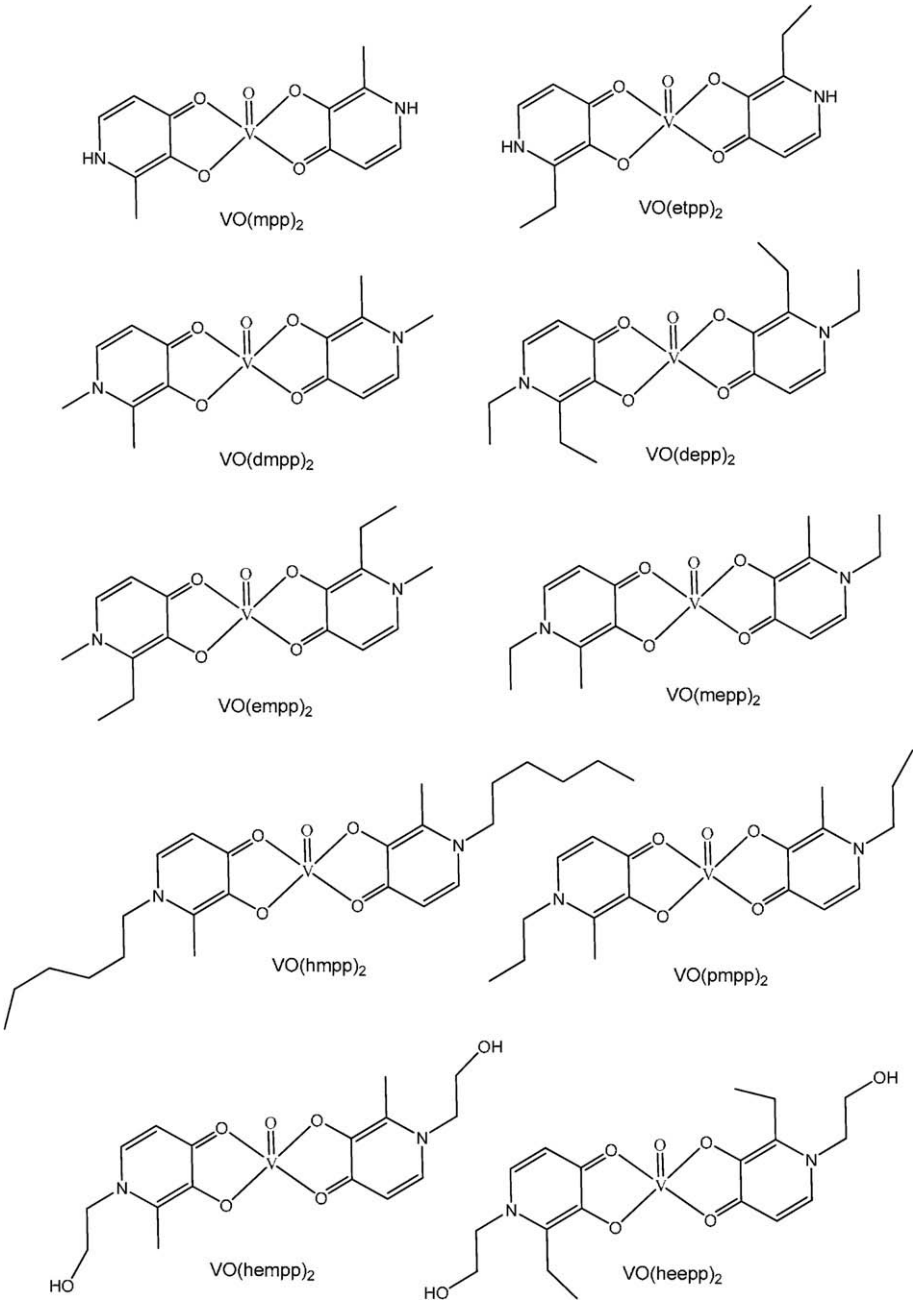


Fig. 1. Formulae and abbreviation of the oxidovanadium(IV) complexes with 3-hydroxy-4-pyridinones studied in this work.

2.2. Preparation and characterization of $[V^{IV}OL_2]$ complexes

The $[V^{IV}OL_2]$ complexes were prepared under anaerobic conditions using an argon/vacuum line and Schlenk type glassware. We prepared a solution of 20 mmol of $VOSO_4$ dissolved in the minimum amount of water required to complete dissolution and a solution of 20 mmol of the ligand dissolved in the minimum amount of water or an ethanol/water mixture (40:60), in the case of more lipophilic compounds. The two solutions were added slowly and the pH adjusted to 9 with a solution of NaOH. The reaction media was refluxed, under argon, for about 1 h. The blue/greyish powders that precipitate were filtered while the solution was still warm and dried in vacuum over P_2O_5. The yield is typically *ca* 70% and the compounds were characterized by elemental analysis, fast atom bombardment mass spectrometry (FAB), FTIR spectroscopy, UV/visible spectroscopy and EPR spectroscopy.

2.2.1. Bis (3-hydroxy-1(H)-2-ethyl-4-pyridonato)oxidovanadium(IV), $VO(etpp)_2$

Anal. Calcd (Found): $C_{14}H_{16}N_2O_5V$: %C 48.99 (49.15); %H 4.70 (4.64); %N 8.16 (8.04) FTIR (KBr) ν (cm^{-1}): 3038 (ν_{OH}); 1607 ($\nu_{C=O}$); 1555–1499; ($\nu_{C=C}$); 1257, 1066 (ν_{C-O}); 708, 651 (ν_{V-O}); 980 ($\nu_{V=O}$); UV/visible (DMSO) ($\lambda_{máx}$ (nm) (ε M^{-1} cm^{-1})): 672 (149), 621 (65), 543 (80), 426 (150); mass spec. *m*/*z* (FAB): 344 (ML_2H^+).

2.2.2. Bis (3-hydroxy-1-(ethyl)-2-methyl-4-pyridonato)-oxidovanadium(IV), $VO(mepp)_2$

Anal. Calcd (Found): $C_{16}H_{20}N_2O_5V$: %C 51.75 (51.70); %H 5.44 (5.37); %N 7.55 (7.53) FTIR (KBr) ν (cm^{-1}): 3038 (ν_{OH}); 1607 ($\nu_{C=O}$); 1555–1499; ($\nu_{C=C}$); 1257, 1066 (ν_{C-O}); 708, 651 (ν_{V-O}); 967 ($\nu_{V=O}$); UV/visible (DMSO) ($\lambda_{máx}$ (nm) (ε M^{-1} cm^{-1})) 672 (149), 621 (65), 543 (80), 426 (150); mass spec. *m*/*z* (FAB): 372 (ML_2H^+).

2.2.3. Bis (3-hydroxy-1-(2-hydroxyethyl)-2-methyl-4-pyridonato)-oxidovanadium(IV), $VO(hempp)_2$

Anal. Calcd (Found): $C_{16}H_{20}N_2O_7V$: %C 47.82 (47.65); %H 5.00 (5.00); %N 6.91 (6.95) FTIR (KBr) ν (cm^{-1}): 3038 (ν_{OH}); 1607 ($\nu_{C=O}$); 1555–1499; ($\nu_{C=C}$); 1257, 1066 (ν_{C-O}); 708, 651 (ν_{V-O}); 970 ($\nu_{V=O}$); UV/visible (DMSO) ($\lambda_{máx}$ (nm) (ε M^{-1} cm^{-1})) 672 (149), 621 (65), 543 (80), 426 (150); mass spec. *m*/*z* (FAB): 444.1 (ML_2H^+).

2.2.4. Bis (3-hydroxy-1-(2-hydroxyethyl)-2-ethyl-4-pyridonato)-oxidovanadium(IV), $VO(heepp)_2$

Anal. Calcd (Found): $C_{18}H_{24}N_2O_7V$: %C 50.05 (50.12); %H 5.62 (5.61); %N 6.43 (6.49) FTIR (KBr) ν (cm^{-1}): 2970 (ν_{OH}); 1603 ($\nu_{C=O}$); 1550–1488; ($\nu_{C=C}$); 1227, 1087 (ν_{C-O}); 702, 625 (ν_{M-O}); 961 ($\nu_{V=O}$); UV/visible (DMSO) ($\lambda_{máx}$ (nm) (ε M^{-1} cm^{-1})) 700 (127), 608 (86), 536 (54), 434 (170); mass spec. *m*/*z* (FAB): 431.33 (ML_2H^+).

2.2.5. Bis (3-hydroxy-2-methyl-1-propil-4-pyridonato)-oxidovanadium(IV), $VO(pmpp)_2$

Anal. Calcd (Found): $C_{18}H_{24}N_2O_5V$: %C 54.43 (54.24); %H 5.78 (6.06); %N 6.92 (7.02) FTIR (KBr) ν (cm^{-1}): 1602 ($\nu_{C=O}$); 1344; ($\nu_{C=C}$); 1276, 978 (ν_{C-O}); 718, 576 (ν_{M-O}); 978 ($\nu_{V=O}$); UV/visible (DMSO) ($\lambda_{máx}$ (nm) (ε M^{-1} cm^{-1})) 711 (17), 617 (57), 550 (55), 405 (137); mass spec. *m*/*z* (FAB): 399.3 (ML_2H^+).

2.2.6. Bis (1-hexyl-3-hydroxy-2-methyl-4-pyridonato)-oxidovanadium(IV), $VO(hmpp)_2\ H_2O$

Anal. Calcd (Found) for: $C_{24}H_{36}N_2O_5V{\cdot}H_2O$: %C 57.89 (57.48); %H 7.55 (7.64); %N 5.54 (5.59) FTIR (KBr) ν (cm^{-1}): 1604 ($\nu_{C=O}$); 1340; ($\nu_{C=C}$); 1276, 978 (ν_{C-O}); 718, 574 (ν_{V-O}); 978 ($\nu_{V=O}$); UV/visible (DMSO) ($\lambda_{máx}$ (nm) (ε M^{-1} cm^{-1})) 712 (19), 616 (63), 550 (61), 402 (147); mass spec. *m*/*z* (FAB): 483.2 (ML_2H^+).

The synthesis of complexes, $VO(mpp)_2$, $VO(dmpp)_2$ and $VO(empp)_2$, which are also used in the present work, has already been described and the structure of these compounds was analyzed by extended X-ray absorption fine structure (EXAFS) [17].

2.3. Spectroscopic and analytical measurements

Solutions of the complexes under study were prepared in DMF and DMSO under anaerobic conditions. EPR spectra were recorded in frozen solution at 100 K using an X-band (9.15 GHz) Bruker EMX spectrometer. The spectra were simulated with the computer suite program Bruker WinEPR/SimFonia. UV/visible spectra were obtained with a Unicam-UV 300 spectrometer and deconvoluted with Origin. Elemental analysis (C, H, N) were performed at the Microanalytical Laboratory of the University of Manchester, UK. Fast atom bombardment (+FAB) mass spectra were obtained using a Kratos concept double focusing mass spectrometer at the University of Santiago.

2.4. Cyclic voltammetry (CV)

Cyclic voltammetry of the VO(IV) complexes (0.5–1.0 mM) was performed in DMF, at room temperature using an Autolab PGSTAT20 potentiostat/galvanostat. We used a three-electrode cell composed by a Pt disk electrode with an area of 0.0314 cm^2 as the working electrode, a Pt gauze electrode as the counter electrode and a Ag/AgCl (1 M NaCl Metrohm, Ref. 6.0724.140) as the reference electrode. Tetrabutylammonium perchlorate (TBAP) was used as the supporting electrolyte. The ferrocene/ferrocenium redox couple was used as internal standard and under the experimental conditions used in the present work, $E_{1/2}$ for Fc/Fc^+ couple was 0.46 V in DMF. Prior to use, the Pt working electrode was polished with an aqueous suspension of 0.05 μm alumina (Beuhler) on a Master-Tex (Beuhler) polishing pad, then rinsed with water and acetone and dried. All solutions were de-aerated in the cell by a stream of argon. In all experiments we have used the following parameters: scan rates in the interval 0.05–0.5 V s^{-1} and the potential limits were 1.0 and −0.1 V.

2.5. Animal studies

Male Wistar rats were sacrificed under anesthesia with ether. The adipose tissues were removed, chopped with scissors and digested with collagenase for 60 min at 37 °C in Krebs Ringer bicarbonate buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$: pH 7.4), containing 2% BSA (bovine serum albumin). The obtained adipocytes were then separated from undigested tissues by filtration through nylon mesh and washed three times. The metal complexes were dissolved in saline or DMSO at various concentrations (final conc.; 0.1, 0.25, 0.5, and 1 mM) by ultrasonic disintegrating and glucose (final conc.; 5 mM) were added to the isolated rat adipocytes. The resulting suspensions were incubated at 37 °C for 30 min. Finally, epinephrine solution (final conc.; 10 μM) was added to the reaction mixtures, and the resulting solutions were incubated at 37 °C for 180 min. The reactions were stopped by soaking in ice water, and the mixtures were centrifuged at 3000 rpm for 10 min. FFA (free fatty acids) levels in the outer solution of the cells were determined using an FFA kit (NEFA C-test WAKO, Wako Pure Chemicals). The *in vitro* insulin-mimetic activity of the complexes was evaluated by the IC_{50} value, which defines the concentration of the complex required for 50% inhibition of FFA release from the isolated rat adipocytes treated with epinephrine.

All animal experiments in the present study were approved by the Experimental Animal Research of Kyoto Pharmaceutical University (KPU) and were performed according to the Guideline for Animal Experimentation of KPU.

3. Results and discussion

3.1. Chemistry

Ligands of the 3-hydroxy-4-pyridinone type are generally prepared by reaction of 3-hydroxy-4-pyrones and primary amines using either a direct method or a synthetic route in which the hydroxyl group is protected according to the nature of the amine [10,18,19]. In order to provide a set of ligands that could be used to investigate the influence of hydrophilic/lipophilic balance and the effect of electron donation in position 2 of the heterocyclic ring our approach was to use two different pyrones, 3-hydroxy-2-methyl-4-pyrone and 3-hydroxy-2-ethyl-4-pyrone and make them react with a set of primary amines of variable lipophilicity. In this way, we produced a set of ligands with variable hydrophilic/lipophilic balance, which have different solubility in water, different molecular size and different ability to stabilize the +4 oxidation state. The corresponding oxidovanadium(IV) complexes also exhibit variable properties as can be inferred from their *formulae* (Fig. 1).

The oxidovanadium(IV) complexes were synthesized using the experimental procedure previously described by us [11,17]. The complexes are isolated as blue/greyish powders and the Elemental Analysis results are consistent with VOL_2 or $VOL_2{\cdot}H_2O$ stoichiometries. In the solid state the compounds are stable under aerobic conditions. If the compounds are filtered while the solution is still hot it is not obligatory to perform the synthesis under an argon atmosphere although we usually do it in order to prevent oxidation.

As had already been observed for the complexes synthesized previously, $VO(mpp)_2$, $VO(dmpp)_2$ and $VO(empp)_2$, no crystals suitable for X-ray Diffraction analysis have been isolated, a difficulty which is attributed to the fact that in some solvents the complexes undergo rapid oxidation and two isomeric forms are present [11].

The structure of compounds $VO(mpp)_2$, $VO(dmpp)_2$ and $VO(empp)_2$ was analyzed by us using EXAFS spectroscopy [17]. The results obtained indicate that the non-hydrated compounds, $VO(dmpp)_2$ and $VO(empp)_2$, are five-coordinate, showing one oxygen atom at 1.60 Å and four oxygen atoms at 1.95 Å. The compound $VO(mpp)_2$ was isolated with a bound water molecule detected at 2.3 Å and occupying the sixth coordination position. The vanadium–oxygen bond lengths do not vary significantly between the various pyridinone complexes, thus showing that the local structure around the metal centre is not appreciably modified by changing the substituents on the ring nitrogen atom. An identical conclusion was obtained from an EXAFS study of a group of iron(III) complexes of similar 3-hydroxy-4-pyridinones [20] and both analyses corroborate the fact that the hidrophilic/lipophilic balance of the 3-hydroxy-4-pyridinone complexes can be tuned without significantly altering the local structure around the metal centre.

At room temperature the compounds are soluble in water or water/alcohol mixtures, alcohols, N,N-dimethylformamide (DMF) and DMSO. Under anaerobic conditions the complexes remain in the +4 oxidation state but undergo oxidation in the presence of air. In order to illustrate this fact the UV/visible spectra of a DMF solution of the compound $VO(mpp)_2$ are shown, in Fig. 2 under anaerobic conditions and in the presence of air after 24 and 48 h exposure. The spectrum obtained after 48 h, exhibiting an intense

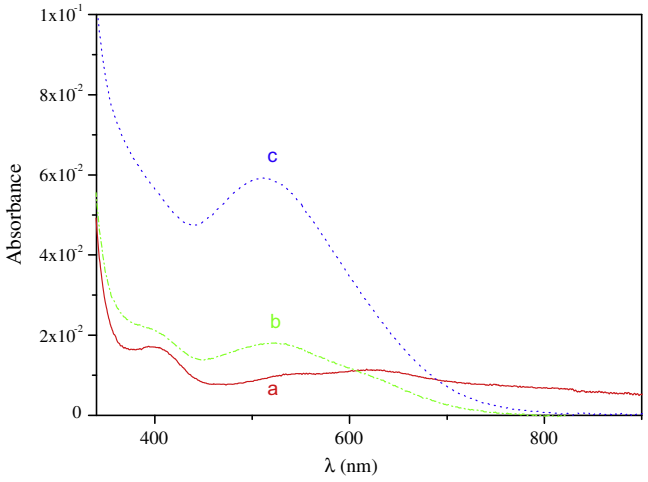


Fig. 2. Electronic absorption spectrum of $VO(mpp)_2$ in a DMF solution recorded in the following conditions: (a) anaerobic conditions; (b) exposed to air for 24 h, (c) exposed to air for 48 h.

charge transfer band at 512 nm, is identical to the one obtained by chemical oxidation of the vanadium(IV) complex with potassium peroxodisulfate and is assigned to the species $[VO_2(mpp)_2]^+$. Moreover, the rate of the oxidation process is solvent-dependant, being instantaneous in water and alcohols and slower in DMF and DMSO as can be gathered by the changes observed in the UV/visible and EPR spectra which loose intensity and become silent after a few days.

The characterization of the electronic structure of the complexes in solution has been achieved by a combined study of UV/Visible spectroscopy and EPR in frozen solution. Electronic spectra are characterized by four absorption bands in the visible region, three of which are clearly distinguished and a fourth one which is noticeable as a shoulder and that can be resolved by spectra deconvolution as shown in Fig. 3. For a square-pyramidal geometry of C_{4v} symmetry, with the *z*-axis taken as the vanadium–oxygen double bond and the *x*- and *y*-axes along the equatorial bonds, three transitions are expected $^2B_2(d_{xy}) \rightarrow {}^2E(d_{xz}, d_{yz})$, $^2B_2(d_{xy}) \rightarrow {}^2B_1(d_{x^2-z^2})$ and $^2B_2(d_{xy}) \rightarrow {}^2A_1(d_{z^2})$. The presence of a fourth transition is due to the splitting of the $^2E(d_{xz}, d_{yz})$ state in lower symmetry.

The EPR spectra obtained in frozen solution are typical of VOL_2 complexes and are characteristic of the interaction of one unpaired

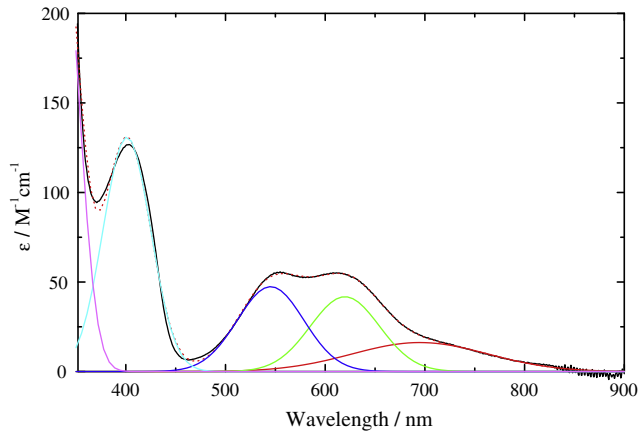


Fig. 3. Electronic absorption spectrum of $VO(mpp)_2$ in a DMF solution and its deconvolution into four components. The solid line is the experimental spectrum and the dashed, dot-dashed and dot-dot-dashed lines are deconvoluted spectra.

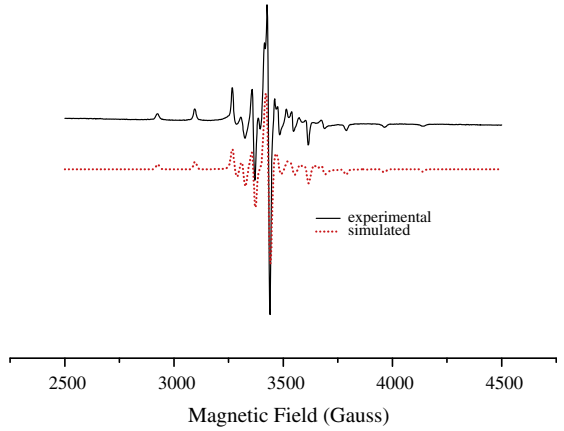


Fig. 4. Frozen solution (100 K) EPR spectrum of VO(mepp)$_2$ in a DMF solution. Experimental spectrum (top, solid line), simulated spectrum (bottom, dotted line).

electron with an atom with a nuclear spin of 7/2, Fig. 4. The EPR spectra obtained for all the compounds studied are almost identical. The experimental spectra have been simulated assuming three different g values and matching of experimental and simulated spectra has been obtained for a set of values shown in Table 1 for the compound VO(mepp)$_2$.

The observation of a four band pattern in the UV/visible spectra together with EPR spectra characterized by three different g values is a criterion to reveal the existence of a distortion of the $V^{IV}O$ complexes from a square-pyramidal towards a trigonal-bipyramidal geometry [11]. According to the parameters obtained for the electronic and EPR spectra, we conclude that the new oxidovanadium(IV) complexes described in this work exhibit a square planar geometry with a moderate distortion towards a trigonal-bipyramidal geometry as has been observed for the other oxidovanadium(IV) complexes with 3-hydroxy-4-pyridinones.

The UV/visible spectroscopy and the EPR results obtained confirm that for the complexes studied in this work the properties of the metal centre are not significantly changed as the hydrophilic/lipophilic balance of the ligand is modified by introducing different substituents on the heterocyclic nitrogen atom and we consider that the new complexes exhibits structures identical to those previously studied by EXAFS [17].

In order to get insight on the oxidation–reduction cycles of the complexes and to understand if this behavior is influenced by the changes in the substituents of the ligands we studied the redox properties of the complexes, that were enough soluble within the experimental conditions, by cyclic voltammetry in DMF. The results obtained are very similar for all the complexes and are shown in Table 2 for the complex VO(mepp)$_2$. As an example the voltammogram of the complex VO(mepp)$_2$ is shown in Fig. 5.

All the studied complexes exhibit one anodic wave and the corresponding cathodic wave. For the majority of the complexes another ill defined and low intensity pre-anodic wave is observed. Analysis of the CV data in the redox process observed for all the VO complexes reveals: (a) a linear dependence of i_p with $\upsilon^{1/2}$; (b) anodic–cathodic peak potential separation (ΔE) that is higher than that of the Fc^+/Fc couple; (c) variation of the scan rate between 50 and 500 mV s^{-1} showed a decrease in ΔE values; (d) i_{pc}/i_{pa} ratios are close to 1, but show a slight increase with the scan rate.

Table 1
Spin Hamiltonian parameters for the compound VO(mepp)$_2$.

g_x	1.985 ± 0.002	A_x	46 ± 1 × 10^{-4} cm^{-1}
g_y	1.978 ± 0.003	A_y	53 ± 1 × 10^{-4} cm^{-1}
g_z	1.952 ± 0.004	A_z	158 ± 1 × 10^{-4} cm^{-1}

Table 2
Voltammetric data for the compound VO(heepp)$_2$ (DMF, 0.1 M TBAP). Scan rates 500, 200, 100, 50 mV s^{-1}.

υ/mV s^{-1}	E_{pa}/V	E_{pc}/V	ΔE/V	$E_{1/2}$/V	i_{pa}/i_{pc}
500	0.57 ± 0.06	0.27 ± 0.04	0.30 ± 0.11	0.42 ± 0.02	0.92 ± 0.13
200	0.54 ± 0.06	0.29 ± 0.04	0.25 ± 0.11	0.42 ± 0.02	0.98 ± 0.12
100	0.53 ± 0.08	0.29 ± 0.04	0.23 ± 0.14	0.41 ± 0.02	1.00 ± 0.12
50	0.50 ± 0.08	0.31 ± 0.03	0.20 ± 0.10	0.41 ± 0.02	1.08 ± 0.12

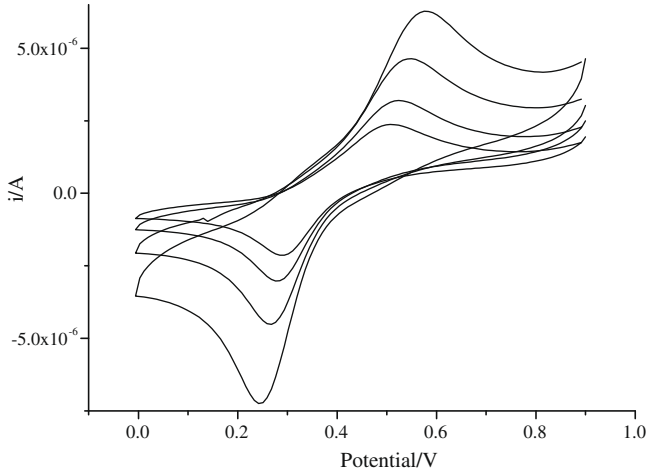


Fig. 5. Cyclic voltammogram of VO(heepp)$_2$ (DMF, 0.1 M TBAP). Scan rates 500, 200, 100, 50 mV s^{-1}.

These results suggest that the process is diffusion-controlled, but with a certain degree of irreversibility [21]. That is in agreement with a quasi-reversible V(IV) → V(V) oxidation process which is expected, since it can be foreseen that changes in the coordination sphere of the complex may occur in the oxidation–reduction process, namely the exchange of solvent molecules. In a previous study such changes have been observed in the EPR spectra of DMSO solutions [11].

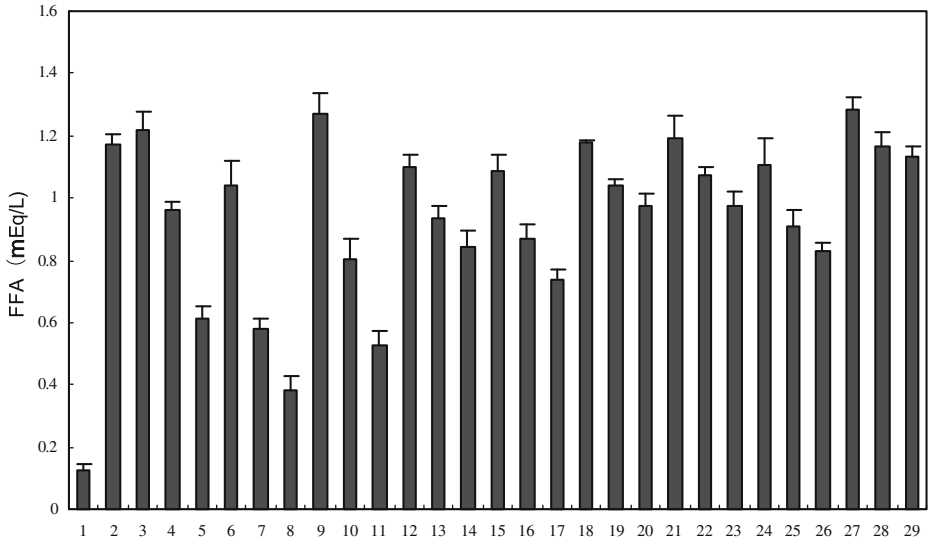
The similarity of the CV results observed for all the compounds signifies that the redox properties of the oxovanadium(IV) complexes are not significantly modified by varying the lipophilicity of ligand and that the redox process may be essentially assigned to the metal centre.

3.2. Insulin-mimetic properties

It is known from *in vivo* studies that the serum level of FFA is a good index to know the degree of *diabetes mellitus* and also that both serum glucose and FFA levels in streptozotocin-induced diabetic rats (STZ-rats) are normalized by vanadyl treatment. Vanadyl ion enhances glucose uptake in peripheral adipocytes, therefore, the use of adipocytes gives valuable information on the mechanism of vanadium-dependent normalization of both serum glucose and FFA levels in STZ-rats [22–25].

The insulin enhancing potential of the vanadium complexes with 3-hydroxy-4-pyridinones prepared in this work was tested performing *in vitro* experiments in which the inhibition of FFA release in rat adipocytes was measured in the presence of the complexes.

The results obtained for the complexes which were enough soluble together with those obtained for a blank and for vanadyl sulfate are shown in Fig. 6. In order to evaluate the dependence of the inhibitory effect on concentration, solutions of three



1. blank, 2. control, 3. VS 0.1 mM, 4. VS 0.5 mM, 5. VS 1 mM,
6. VM 0.1 mM, 7. VM 0.5 mM, 8. VM 1 mM, 9. VEt 0.1 mM, 10. VEt 0.5 mM,
11. VEt 1 mM, 12. VDm 0.1 mM, 13. VDm 0.5 mM, 14. VDm 1 mM, 15. VMe 0.1 mM,
16. VMe 0.5 mM, 17. VMe 1 mM, 18. VEm 0.1 mM, 19. VEm 0.5 mM, 20. VEm 1 mM,
21. VDe 0.1 mM, 22.VDe 0.5 mM, 23. VDe 1 mM, 24. VHem 0.1 mM, 25. VHem 0.5 mM,
26. VHem 1 mM, 27. VHee 0.1 mM, 28. VHee 0.5 mM, 29. VHee 1 mM

$VOSO_4$: VS, $VO(mpp)_2$: VM, $VO(etpp)_2$: VEt, $VO(dmpp)_2$: VDm, $VO(mepp)_2$: VMe, $VO(empp)_2$: VEm, $VO(depp)_2$: VDe, $VO(hempp)_2$: VHem, $VO(heepp)_2$: VHee.

Fig. 6. Inhibitory effects of $VOSO_4$ and oxidovanadium(IV) complexes with 3-hydroxy-4-pyridinone derivatives on FFA release from rat adipocytes treated with epinephrine. Data are expressed as the means ± SDs for three experiments.

different concentrations of each complex have been used and the values of IC_{50}, 50% inhibition concentration, are summarized in Table 3.

It is noticeable from Fig. 6 that the complexes $VO(mpp)_2$ (bars 6, 7, 8) and $VO(etpp)_2$ (bars 9, 10, 11) have a considerable dose dependant response comparable with $VOSO_4$ (bars 3, 4, 5) and that the complex $VO(mpp)_2$ shows a better performance than the inorganic salt. All the other complexes have a very poor effect when compared to that observed for $VOSO_4$. Surprisingly, the values obtained for the complexes $VO(dmpp)_2$ and $VO(empp)_2$ are different from the previously obtained in an independent study [14] and in particular we could not find a response for the latter complex although several attempts have been tried.

Solubility in water by itself does not seem to be the only property required for positive activity as the compounds $VO(hempp)_2$ and $VO(heepp)_2$ are at least as soluble $VO(mpp)_2$ and $VO(etpp)_2$. Also, the different behavior in what concerns inhibition of FFA release cannot be accounted for by considering a different behavior/speciation in aqueous solution, as it is well known that the stability constants of complexes formed by the same metal ion with 3-hydroxy-4-pyridinones of variable hydrophilic/lipophilic balance are not significantly modified.

Regarding the establishment of structure/activity relationships, unfortunately we could not get insight in this subject from the present results, although we perceive that a high solubility in water and a small molecular size seem to be important properties for achieving a positive effect. In what concerns oxidation–reduction properties we found that the redox cycles of the complexes are not significantly changed by the presence of more electron donating groups in the ligand as can be gathered from results of parent complexes derived from the pyrones *with methyl* and *ethyl* groups in the *ortho* position.

As a final conclusion we think that the most important result is the fact that the present results do confirm that the complex $VO(mpp)_2$ shows a significantly better effect than the inorganic salt and is worthy to be considered for animal studies.

Table 3
Values of IC_{50} (mM) for inhibition of FFA in rat adipocytes.

Compound	IC_{50} (mM) this work	IC_{50} (mM) Ref. [14]
$VOSO_4$	0.74 ± 0.04	3.20
$VO(mpp)_2$	0.53 ± 0.04*	0.56
$VO(etpp)_2$	0.75 ± 0.06	Not tested
$VO(empp)_2$	None	0.56
$VO(mepp)_2$	2.98 ± 0.6	Not tested
$VO(dmpp)_2$	12.5 ± 5.5	1.36
$VO(depp)_2$	None	Not tested
$VO(hempp)_2$	8.26 ± 3.3	Not tested
$VO(heepp)_2$	None	Not tested

* $p < 0.05$ vs. $VOSO_4$.

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