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Synthesis and characterization of oxidovanadium complexes as enzyme inhibitors targeting dipeptidyl peptidase IV

Ming-jin Xie, Ming-rong Zhu, Chun-Mei Lu, Yi Jin, Li-Hui Gao, Ling Li, Jie Zhou, Fan-fang Li, Qi Hua Zhao, Hong-Ke Liu, Peter J. Sadler, Carlos Sanchez-Cano

**Abstract**

Two oxidovanadium(IV) complexes carrying Schiff base ligands obtained from the condensation of 4,5-dichlorobenzene-1,2-diamine and salicylaldehyde derivatives were synthesised and characterised, including their X-ray crystallographic structures. They were evaluated as dipeptidyl peptidase IV (DPP-IV) inhibitors for the treatment of type 2 diabetes. These compounds were moderate inhibitors of DPP-IV, with IC \textsubscript{50} values of ca. 40 \textmu M. In vivo tests showed that complexes 1 and 2 could lower significantly the level of glucose in the blood of alloxan-diabetic mice at doses of 22.5 mg V·kg\textsuperscript{-1} and 29.6 mg V·kg\textsuperscript{-1}, respectively. Moreover, molecular modeling studies suggested that the oxidovanadium complexes 1 and 2 could fit well into the active-site cleft of the kinase domain of DPP-IV. To the best of our knowledge, this is the first report of vanadium complexes capable of inhibiting DPP-IV.

**1. Introduction**

Type 2 diabetes is a metabolic disease characterised by insulin resistance, hyperglycemia and hyperlipidemia. Dipeptidyl peptidase IV (DPP-IV) inhibitors are a new type of oral hypoglycemic agents for the treatment of patients with type 2 diabetes [1]. Inhibition of the enzyme DPP-IV results in decreased degradation of both glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) [2,3]. Consequently, inhibition of DPP-IV is rapidly emerging as a novel therapeutic approach for the treatment of type 2 diabetes, and potent DPP-IV inhibitors have recently been reported [4–7]. A number of X-ray structures of co-crystals of inhibitors and the DPP-IV enzyme have been reported, providing important structural information [8–11].

Vanadium compounds have long been recognised as phosphatase inhibitors, since vanadates are structurally similar to phosphates [12]. Numerous studies have demonstrated that vanadium compounds have insulin-enhancing effects and can improve the symptoms of diabetes in a variety of animal models [13–17]. Vanadyl sulfate and sodium metavanadate have been used in studies with human diabetic patients resembling phase II clinical trials [18–24]. These compounds showed moderate success in alleviating some of the symptoms of diabetes. Due to their wide availability as nutritional supplements they did not go through Phase I clinical trials. Additionally, the chelated complex bis(ethylmaltolato)oxidovanadium(IV) (BEOV) completed Phase I trials and a Phase Ila trial. Clinical trials were discontinued following further pre-clinical safety testing, in which it became clear that renal complications could be precipitated without any obvious warning signs, at slightly higher concentrations than were tried in human volunteers [25–27]. It has been reported that vanadium compounds exert insulin-enhancement effects and cell protection via a multiple mechanism involving inhibition of protein-tyrosine phosphatase 1B (PTP1B), activation of peroxisome proliferator-activated receptor (PPARs) – AMP-activated protein kinase (AMPK) signaling, and regulation of unfolded protein responses (UPRs) [28]. Vanadium complexes carrying...
tridentate Schiff base ligands selectively inhibit PTP1B, and oxidovanadium compounds carrying mixed ligands are highly potent (nanomolar activity) competitive inhibitors for PTP1B [29–31]. Additionally, vanadium complexes have also been shown to inhibit human islet amyloid polypeptide (hAPP) aggregation [32]. However, the mechanism of action of vanadium complexes ultimately leads to the observed insulin-sensitizing effects is not well understood [33–35]. Therefore, studying their role as enzyme inhibitors and their influence on glucose metabolism is of fundamental importance.

There appear to be no reports exploring the possible role of vanadium or other antidiabetic metal complexes as DPP-IV inhibitors. In this paper, we report two novel oxidovanadium complexes carrying Schiff base ligands obtained from the condensation of 4,5-dichlorobenzene-1,2-diamine and 4-R-salicylaldehyde (where R = H or diethylamine) that have been designed and synthesised as potential DPP-IV enzyme inhibitors. Their biological activity has been assessed by blood glucose-lowering assays, and their interaction with DPP-IV explored by biochemical assays using recombinant DPP-IV and molecular modeling.

2. Experimental

2.1. Materials and methods

All reagents and solvents were purchased commercially as reagent grade and used without further purification unless otherwise stated. Double-distilled water was used to prepare buffer solutions. Vanadyl sulfate hydrate was purchased from the Sigma-Aldrich Chemical Co., and EPR tubes from Wilmad Labglass.

2.2. Preparation of oxidovanadium complexes

The synthetic routes are shown in Scheme 1.

2.2.1. Preparation of [(1E,1′E)-N,N′-((4,5-dichloro-1,2-phenylene)-bis(1-(2-(λ3-oxazolyl)phenyl)methanimine))oxidovanadium (IV)]

Salicylaldehyde (0.94 g, 5 mmol) dissolved in ethanol was added dropwise to 4,5-dichloro-1,2-phenylenediamine (3 mmol, 0.51 g) in ethanol at room temperature. The mixture was gradually heated to 333 K and maintained at this temperature for 4 h. Then VOSO4·5H2O (1 mmol, 0.254 g) was added to this solution. The mixture was stirred for about 30 min. The green precipitate was collected and dissolved in dimethyl sulfoxide (DMSO)/dichloromethane (2:1 v/v). Crystals suitable for X-ray diffraction were obtained by slow diffusion of ether into DMSO/dichloromethane (2:1 v/v) solution of the product at 277 K. Yield: 56.5%.

Anal. Calcd. for C20H12Cl2N2O3V: %C, 53.3; %H, 2.7; %N, 6.2. Found: %C, 53.6; %H, 2.4; %N, 6.6. UV-vis (DMSO) \( \lambda_{\text{max}} = 310 \text{ nm} \) (\( e = 18,204 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)), 325 nm (\( e = 17,844 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)), 340 nm (\( e = 18,204 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)), 370 nm (\( e = 5707 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)), 415 nm (\( e = 3868 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)). IR (KBr disk): \( \nu (\text{cm}^{-1}) = 1601 \) (\( \nu_{\text{C}} - \nu_{\text{N}} \)), 965 (\( \nu_{\text{C}} - \nu_{\text{O}} \)), 598 (\( \nu_{\text{C}} - \nu_{\text{O}} \)).

2.2.2. Preparation of [(4,4′,4″-(1E,1′E)-((4,5-dichloro-1,2-phenylene)bis(methanylylidene))bis(N,N-diethyl-3-(λ3-oxazolyl)aniline))-oxidovanadium(IV)]

4-(Diethylamino)salicylaldehyde (9 mmol, 1.85 g) dissolved in ethanol (15 mL) was added dropwise to 4,5-dichloro-1,2-phenylenediamine (3 mmol, 0.51 g) in ethanol (30 mL) at room temperature. The mixture was gradually heated to 333 K and maintained at this temperature for 4 h. Then VOSO4·5H2O (1 mmol, 0.254 g) was added into this solution. The mixture was stirred for about 30 min. The green-yellow precipitate was collected and dissolved as before. Crystals suitable for X-ray diffraction were obtained by slow diffusion of ether into a DMSO solution of the product at 277 K. Yield: 45.4%.

Anal. Calcd. for C35H39Cl4N9OV3: %C, 53.7; %H, 5.4; %N 8.4. Found: %C, 53.3; %H, 5.5; %N, 8.6. UV-vis (DMSO) \( \lambda_{\text{max}} = 376 \text{ nm} \) (\( e = 4104 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)), 396 nm (\( e = 5363 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)), 448 nm (\( e = 7793 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \)). IR (KBr disk): \( \nu (\text{cm}^{-1}) = 1655 \) (\( \nu_{\text{C}} - \nu_{\text{N}} \)), 978 (\( \nu_{\text{C}} - \nu_{\text{O}} \)), 567 (\( \nu_{\text{C}} - \nu_{\text{O}} \)).

2.3. X-ray crystallography

X-ray diffraction data for complexes 1 and 2 were collected at 298 K using a Bruker Smart Apex II CCD detector with graphite monochromator (0.04 mm; Mo Ka radiation. For complex 1, a total of 12,051 reflections were collected, with 4252 reflections being unique. For complex 2, a total of 17,511 reflections were collected, with 5573 reflections being unique. The crystal structures of 1 and 2 were solved by direct method SHELXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHELXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. The crystal data and structure refinements are given in Table 1.

2.4. IR spectroscopy

Infrared spectra were recorded as KBr pellets in the range 4000–400 cm\(^{-1}\) on a Perkin-Elmer Paragon 1000 Fourier-transform spectrometer.

2.5. EPR spectroscopy

EPR spectra were recorded at ambient temperature on a Bruker EMX (X-band) spectrometer. Liquid samples were contained in quartz capillary (I.D. 1.0 mm; O.D. 1.2 mm; Wilmad Labglass) sealed with T-Blu Tac®, placed inside larger quartz tubes (O.D. 2.0 mm) to achieve easy and accurate positioning of the sample inside the resonator. Measurements were made on DMF solutions or on powders at 300 K. Data were fitted using the Easyspin version 5.1.9 software [36].

2.6. DPP-IV inhibition assay

Recombinant DPP-IV was mixed with different concentrations of complexes 1 or 2 (45–0.6 μM) in the assay buffer (100 mM Tris-HCl, 150 mM NaCl, 1.0 mg/mL BSA, pH 8.2). The enzyme reaction was started by addition of a glycy-l-proline-p-nitroanilide tosylate solution and the change in absorbance at 405 nm was monitored for 30 min using a microplate reader (Rayto, Rt-6100, China) [37]. Inhibition constants (IC\(_{50}\)) were calculated by fitting the dose-dependent inhibition curves based on the means of several replicate experiments.

2.7. Molecular modeling

To investigate the binding mode of vanadium complexes 1 and 2 to the DPP-IV, the advanced docking program Autodock 3.0.3 was used to dock automatically the inhibitor to the kinase domain of the enzyme. The crystal structure of the kinase domain of DPP-IV in a complex with its inhibitor W61 (PDB entry code 3VJM) was recovered from Brookhaven Protein Database (PDB). The missing atoms and residues were modeled in Sybyl 6.8.17. Atomic charges were taken as Kollman- united-atom [38] for the macromolecule and Gasteiger–Marsili [39] for the inhibitor. To explore the binding mode of compounds to the kinase domain of DPP-IV, the advanced docking program Autodock 3.0.3 [40] was used to dock automatically the inhibitor to the kinase domain of the enzyme. The Lamarckian genetic algorithm (LGA) was applied to analyze inhibitor–enzyme interactions. A Solis and Wets local search performed the energy minimization on a user-specified proportion of the population. The docked conformations of the inhibitor were generated after a reasonable number of evaluations. The whole docking operation in this study can be summarised as follows. First, the kinase domain of DPP-IV was checked for polar hydrogens and assigned for partial atomic charges, then a PDBQ file was created, and the atomic solvation parameters were assigned for the macromolecule. Meanwhile,
some of the torsion angles of the inhibitor that would be explored during molecular docking stage were defined, allowing the conformation search for the ligand during the docking process. Second, the grid map with 60 × 60 × 60 points and a spacing of 0.375 Å was calculated using the AutoGrid program in order to evaluate the binding energies between the inhibitor and the macromolecule. Third, some important parameters for LGA calculations were reasonably set (see Box 1), not only the atom types, but also the generations and the number of runs for the LGA algorithm were edited and properly assigned according to the requirements of the Amber force field. The maximum number of generations, energy evaluations and docking runs were set to 3.0 × 10^5, 1.5 × 10^7, and 30, respectively. Finally, the docked complex of the inhibitor–enzyme for the inhibitor was selected according to the criterion of interaction energy combined with geometrical matching quality.

Additionally, to identify the binding sites in DPP-IV, the grid size was set to 100 × 100 × 100 points along the X-, Y-, and Z-axes with a 0.375 Å grid spacing. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method. Each run of the docking operation was terminated after a maximum of 2,500,000 energy evaluations.
water. Diabetic mice were generated by a single intraperitoneal injection of alloxan. Mice were allowed free access to standard food for laboratory purposes.

Diabetic mice were generated by a single intraperitoneal injection of alloxan. Mice were allowed free access to standard food for laboratory purposes.

2.8. Glucose modulation studies

Male ICR mice weighting 25–30 g were obtained from Chengdu Dossy Experimental Animals Co. Ltd. Sichuan, China. Mice were allowed free access to standard food for laboratory purposes.

Diabetic mice were generated by a single intraperitoneal injection of alloxan. Mice were allowed free access to standard food for laboratory purposes.

Table 1
Crystallographic data for complexes 1 and 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Empirical</td>
<td>C2H2ClN2O2V</td>
<td>C2H2ClN2O2SV</td>
</tr>
<tr>
<td>Formula weight</td>
<td>450.16</td>
<td>674.48</td>
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<tr>
<td>Temperature (K)</td>
<td>298(2)</td>
<td>298(2)</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>0.71073</td>
<td>0.71073</td>
</tr>
<tr>
<td>Crystal size (mm)</td>
<td>0.26 × 0.19 × 0.12</td>
<td>0.28 × 0.21 × 0.14</td>
</tr>
<tr>
<td>Space group</td>
<td>P2(1)/n</td>
<td>P2(1)/n</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>α (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>β (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>a (Å)</td>
<td>10.4674(11)</td>
<td>16.4371(16)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>9.1813(19)</td>
<td>19.0776(18)</td>
</tr>
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<td>c (Å)</td>
<td>12.051/4252</td>
<td>15.6911/4252</td>
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<tr>
<td>Z</td>
<td>4</td>
<td>4</td>
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<td>Dcalc (Mg/cm³)</td>
<td>1.653</td>
<td>1.411</td>
</tr>
<tr>
<td>Absorption coefficient (mm⁻¹)</td>
<td>1.372</td>
<td>0.592</td>
</tr>
<tr>
<td>F(000)</td>
<td>908</td>
<td>1396</td>
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<td>Limiting indices</td>
<td>−10 ≤ h ≤ 13</td>
<td>−17 ≤ h ≤ 19</td>
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<tr>
<td></td>
<td>−11 ≤ k ≤ 12</td>
<td>−12 ≤ k ≤ 12</td>
</tr>
<tr>
<td></td>
<td>−25 ≤ l ≤ 24</td>
<td>−22 ≤ l ≤ 22</td>
</tr>
<tr>
<td>Reflections collected/unique</td>
<td>12,051/4252(R(int)</td>
<td>17,511/5573(R(int))</td>
</tr>
<tr>
<td>Completeness to theta = 25.15</td>
<td>94.40%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on F²</td>
<td>Full-matrix least-squares on F²</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>4252/0/253</td>
<td>5573/0/385</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>1.01</td>
<td>1.045</td>
</tr>
<tr>
<td>Final R indices</td>
<td>R1 = 0.0399, wR2 = 0.0957</td>
<td>R1 = 0.0539, wR2 = 0.1485</td>
</tr>
<tr>
<td></td>
<td>[I &gt; 2σ(I)]</td>
<td>[I &gt; 2σ(I)]</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0650, wR2 = 0.0990</td>
<td>R1 = 0.0763, wR2 = 0.1628</td>
</tr>
<tr>
<td>Largest diff. peak and hole(e. Å)</td>
<td>0.307 and −0.306</td>
<td>0.589 and −0.698</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. Synthesis and the X-ray crystal structure

Vanadyl complexes 1 and 2 were synthesised by the routes shown in Scheme 1. The X-ray crystal structures of complexes 1 and 2 (Fig. 1) show that they are structurally similar to related complexes described previously [42,43]. Bond lengths and angles around the vanadium centre are shown in Tables S1 and S2.

Similarly to other VO(salen) compounds [44,45], complexes 1 and 2 are best described as having closely square-pyramidal geometry, because of their τ values of 0.06 and 0.05. The τ parameter measure the distortion of a square-pyramidal structure toward trigonal bipyramidal [46]. The basal plane of complexes 1 and 2 is constituted by the Schiff base ligand, which acts as a tetradentate ligand through its imine N atoms and its deprotonated phenolate O atoms. Torsional angles show that atoms N(1)/N(2)/O(2)/O(3) are approximately in one plane, with O1 atoms located at the axial positions. The V=O bond length is 1.596(6) Å, which is typical for five-coordinate vanadyl species, and V=O2 and V=O3 bond lengths in 1 and 2 are all between 1.9162(17) and 1.944(2) Å, typical of single bonds. Additionally, the bond angles in the basal plane are smaller than the vertex angles. This is due to the position of the vanadyl(IV) center, which lies above the basal plane of the complex (0.6022 Å in 1, and 0.5901 Å in 2).

3.2. Infrared spectroscopy

Vanadyl complexes 1 and 2 exhibit a sharp band at ca. 966 cm⁻¹ due to the ν(V=O) mode [47]. The C≡N stretching frequencies for the ligands occur at ca. 1620 cm⁻¹ and are shifted to lower frequencies (1600–1605 cm⁻¹) in the complexes, indicating the coordination of the imine nitrogens to vanadium [48].

3.3. UV–visible spectroscopy

The VO-complexes were only moderately soluble in methanol, but highly soluble in DMSO. The spectra of the ligands show several sharp absorption maxima in the UV region at approximately 390 nm (ligand 1), and 352 and 400 nm (ligand 2), assignable to π → π* and n → π* transitions (see Fig. S1). Upon complexation, the π → π* transitions shifted to longer wavelengths, while the n → π* transition merged with an additional broad intense band at approximately 415 nm (complex 1) and 438 (complex 2) due to ligand-to-metal charge transfer from the phenolate oxygen to empty d orbitals of vanadium.
3.4. EPR spectroscopy of VO-complexes

EPR is a useful tool to investigate the nature of the V(IV) centre in oxidovanadium (IV) complexes [49]. The X-band EPR spectra of the complexes were recorded as DMF solutions and powder at 298 K (Fig. S2 and S3). V^{IV} has a 3d1 outer shell configuration and 51V (99.8% abundance) has a nuclear spin quantum number I = 7/2 giving rise to the observed hyperfine coupling (8 lines) characteristic of oxidovanadium (IV) complexes [50–52]. Furthermore, g0 and A0 values obtained (1: g0 = 1.972; A0 = 98 G; 2: g0 = 1.974; A0 = 96.6 G) are in accordance with the correlation previously established for square-pyramidal vanadyl(IV) complexes with equatorial ligand fields [53].

3.5. DPP-IV inhibition assays by oxidovanadium complexes

DPP-IV is a protein located in the extracellular membrane, but it can also be found in human plasma as a soluble form lacking the trans-membrane region [54]. Some of DPP-IV inhibitors are in clinical trials as a new therapy for non-insulin-dependent diabetes mellitus (Type 2 diabetes). The therapeutic benefit is derived from reduced activation of incretins GLP-1 and GIP by DPP-IV, thus stimulating greater insulin production, and control the level of glucose in blood in patients with type 2 diabetes [55,56]. Several studies have investigated the efficiency of drugs for the inhibition of DPP-IV [1], but as yet none has involved V compounds.

Both complexes were tested for their ability to inhibit DPP-IV using Gly-Pro-p-nitroanilide as the substrate. The enzyme and complexes were preincubated for 30 min prior to the initiation of the enzymatic reaction by the addition of the substrate. IC50 values were obtained by fitting the dose-dependent inhibition curves and was defined as the concentration of the complex needed to reduce the activity of the enzyme by 50%. The IC50 values for complexes 1 and 2 are very similar, 38 and 45 μM respectively (Table 2), indicating that the presence of the NEt2 substituent has little effect on the activity of the complexes. These results indicate moderate inhibition of DPP-IV, although the observed effect is considerably lower than that of clinically-used sitagliptin.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Sitagliptin (^a)</td>
<td>0.018 ± 0.06</td>
</tr>
</tbody>
</table>

\(^a\) Sitagliptin as a positive control was used to evaluate the selected model.

3.6. Molecular modeling study of the interaction of complexes 1, 2 with DPP-IV

Molecular docking was performed to explore the possible binding of complexes 1 and 2 to the kinase domain of DPP-IV. The blind docking mode with the lowest binding free energy is shown in Figs. 2–3, and S4–S5. The interaction energies between amino residues and complexes 1 and 2 are listed in Tables S3 and S4.

Figs. 2 and S4 show that complex 1 is buried at one end of the active site of the kinase domain of the DPP-IV enzyme. Hydrogen bonds are formed in the active site between the axial oxygen from the oxidovanadium moiety and Asn 710, and one of the hydroxyl groups from the salicylaldimine moieties and Ser 630 residues. Additionally, our model suggests a π-cation interaction between the 4,5-dichloro-1,2-phenylene and nitrogen atoms from the arginine 125 residue, and π-π stacking interaction between the phenyl ring from one salicylaldimide moiety and the tyrosine 666 residue. Finally, the interaction energies between the enzyme residues and complex 1 indicate that Pro-109, Ile-107 and Lys-463 interact effectively with complex 1 (Fig. 2, and Table S3).

The simulated interaction between complex 2 and the active site of the kinase domain of DPP-IV is shown in Fig. 3 and Fig. S5. Our calculations suggest the presence of hydrogen bonds between the oxygens from both salicylaldimine moieties and the Tyr 547 residue. Additionally, there seem to be π-cation interactions (Arg 669 and 125, respectively) and π-π stacking (Phe 357, His 740 and Tyr 547) between the 4,5-dichloro-1,2-phenylene and one of the salicylaldimine moieties of 2 and the active site of DPP-IV. Interaction energies between the enzyme residues and complex 2 indicate effective interaction with Pro-109, Pro-159 and Ile-107 (Fig. 3 and Table S4).

3.7. Effect of complexes 1 and 2 on blood glucose in alloxan-induced diabetic mice

Complexes 1 and 2 were administered intragastrically to alloxan-diabetic mice for 3 weeks. Metformin was used as positive control. This drug is the only therapeutic agent that has been demonstrated to reduce macrovascular events in type 2 diabetes [57]. The results showed that complex 1 (22.5 mg V·kg\(^{-1}\); 198.8 mg·kg\(^{-1}\)) and complex 2 (29.6 mg·V·kg\(^{-1}\); 344.1 mg·kg\(^{-1}\)) could significantly lower the level of serum glucose in the alloxan-diabetic mice (Fig. 4 and Table S5), when compared with the diabetic control group (P < 0.05). After three weeks of treatment, the extent of glucose reduction observed was...
similar to the effect produced by the treatment of mice with metformin (250 mg·kg\(^{-1}\)), although none of the compounds administered was able to reduce blood glucose to the levels observed for non-diabetic mice (Table S5). Throughout the experiment, there was no significant difference in body weight among treated and control diabetic mice (Fig. S6). This suggested that 1 and 2 were not toxic at the doses used.

These results are in agreement with evidence previously reported; our compounds show moderate to low activity when compared with similar complexes carrying chlorido substituents on ligands coordinated to V [58–60].

4. Conclusions

Two oxidovanadium (IV) Schiff base complexes with N,N’-1,2-phenylenediamine -bis(salicyladimine) ligands carrying different substituents were synthesised and evaluated as inhibitors of dipeptidyl peptidase IV (DPP-IV) for the treatment of type 2 diabetes. The X-ray structures of complexes 1 and 2 showed that the V(IV) is penta-coordinate and in a distorted square-pyramidal environment. Both complexes significantly inhibit DPP-IV in vitro. Molecular modeling shows that complexes 1 and 2 fit well into the active-site cleft of the kinase domain of DPP-IV. Moreover, in vivo tests show that 1 (22.5 mg V·kg\(^{-1}\)) and 2 (29.6 mg V·kg\(^{-1}\)) are as effective in reducing the levels of serum glucose in alloxan-diabetic mice as the clinical drug metformin (250 mg·kg\(^{-1}\)). However, oxidovanadium complexes are known to degrade in the gastrointestinal tract into insoluble and partially soluble vanadate species (which are known phosphatase antagonists that can be used to treat diabetic symptoms). Therefore, further experiments will be needed to determine if 1 and 2 can arrive intact in the plasma of treated mice. Nevertheless, these results constitute the first report of vanadium complexes capable of inhibiting DPP-IV in vitro, and provide an approach for exploring new design concepts for vanadium agents for incretin-based therapy.

Acknowledgements

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Appendix A. Supplementary data

Crystallographic data for 1 and 2 have been deposited in the Cambridge Crystallographic Data Centre (deposition numbers CCDC 1437003 and 1437007, respectively). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax: (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk). Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jinorgbio.2017.06.014.

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