ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

An Integrated ESI-MS/EPR/Computational Characterization of the Binding of Metal Species to Proteins: Vanadium Drugs–Myoglobin Application †

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Table S1 Species identified in the ESI-MS spectra of the system V^{IV}O^{2+}/L-mimosinate.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Composition</th>
<th>Exptl m/z $^a$</th>
<th>Calcd m/z $^a$</th>
<th>Error (ppm) $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[VO(mim)$_2$+H]$^+$</td>
<td>C$<em>{16}$H$</em>{17}$N$_4$O$_9$V</td>
<td>460.04490</td>
<td>460.04407</td>
<td>1.8</td>
</tr>
<tr>
<td>[VO(mim)$_2$]$^{2-}$</td>
<td>C$<em>{16}$H$</em>{16}$N$_4$O$_9$V</td>
<td>229.51825</td>
<td>229.51839</td>
<td>-0.6</td>
</tr>
<tr>
<td>[VO(mim)$_2$(OH)+2H]$^-$</td>
<td>C$<em>{16}$H$</em>{17}$N$<em>4$O$</em>{10}$V</td>
<td>476.03973</td>
<td>476.03898</td>
<td>1.6</td>
</tr>
<tr>
<td>[VO(mim)$_2$+2H]$^-$</td>
<td>C$<em>{16}$H$</em>{18}$N$<em>4$O$</em>{10}$V</td>
<td>477.04744</td>
<td>477.04680</td>
<td>1.2</td>
</tr>
</tbody>
</table>

$^a$ The experimental and calculated m/z values refer to the monoisotopic representative peak. $^b$ Deviation in ppm from the calculated values, calculated as $10^6 \times [(\text{Exptl m/z} - \text{Calcd m/z})/\text{Calcd m/z}].
Table S2 $^{51}$V hyperfine coupling constants calculated at the level of theory BHandHLYP/6-311+g(d) for the possible bis-chelated V$^{IV}$O complexes formed by L-mimosinate.$^a$

<table>
<thead>
<tr>
<th>Complex</th>
<th>$A_x^{\text{calcd}}$</th>
<th>$A_y^{\text{calcd}}$</th>
<th>$A_z^{\text{calcd}}$</th>
<th>$A_z^{\text{exptl}}$</th>
<th>PD $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPY-5-12</td>
<td>-48.75</td>
<td>-57.32</td>
<td>-158.2</td>
<td>-169.1</td>
<td>-6.4</td>
</tr>
<tr>
<td>SPY-5-13</td>
<td>-48.53</td>
<td>-57.59</td>
<td>-158.0</td>
<td>-169.1</td>
<td>-6.5</td>
</tr>
<tr>
<td>OC-6-32</td>
<td>-59.17</td>
<td>-63.54</td>
<td>-163.8</td>
<td>-169.1</td>
<td>-3.1</td>
</tr>
<tr>
<td>OC-6-23</td>
<td>-57.37</td>
<td>-62.77</td>
<td>-162.9</td>
<td>-169.1</td>
<td>-3.7</td>
</tr>
<tr>
<td><strong>OC-6-34</strong> $^c$</td>
<td>-60.51</td>
<td>-64.32</td>
<td>-164.6</td>
<td>-169.1</td>
<td>-2.6</td>
</tr>
<tr>
<td><strong>OC-6-24</strong> $^c$</td>
<td>-59.40</td>
<td>-63.48</td>
<td>-164.4</td>
<td>-169.1</td>
<td>-2.8</td>
</tr>
</tbody>
</table>

$^a$ All the $A$ values are reported in $10^{-4}$ cm$^{-1}$. $^b$ Percent deviation (PD) with respect to the absolute experimental value calculated as: $100 \times \left[\frac{|A_z^{\text{calcd}} - A_z^{\text{exptl}}|}{|A_z^{\text{exptl}}|}\right]$. $^c$ With boldface text the most probable isomers are shown.
Table S3 Docking solutions for the interaction of VO(acac)$^+$ with myoglobin.

<table>
<thead>
<tr>
<th>Site</th>
<th>Residues</th>
<th>V–D $^a$</th>
<th>$F_{\text{max}}^{b}$</th>
<th>$F_{\text{mean}}^{c}$</th>
<th>Pop. $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^{\text{st}}$</td>
<td>His24; His119</td>
<td>2.108, 2.440</td>
<td>44.8</td>
<td>42.6</td>
<td>92%</td>
</tr>
<tr>
<td>2$^{\text{nd}}$</td>
<td>His82; Asp141</td>
<td>2.180, 2.452</td>
<td>34.5</td>
<td>30.7</td>
<td>64%</td>
</tr>
<tr>
<td>3$^{\text{rd}}$</td>
<td>Glu83; Asp141</td>
<td>2.181, 2.406</td>
<td>38.7</td>
<td>36.8</td>
<td>94%</td>
</tr>
<tr>
<td>4$^{\text{th}}$</td>
<td>His116; Gln124</td>
<td>2.683, 2.174</td>
<td>32.8</td>
<td>31.9</td>
<td>52%</td>
</tr>
</tbody>
</table>

$^a$ Distance in Å; D = N, O. $^b$ Fitness value for the most stable pose of each cluster ($F_{\text{max}}$). $^c$ Mean Fitness value of the GoldScore scoring function for each cluster ($F_{\text{mean}}$). $^d$ Percent population of the cluster.
Fig. S1 ESI-MS spectra recorded in the systems: (a) Mb; (b) [VO(dhp)$_2$/Mb; (c) cis-[VO(mim)$_2$(H$_2$O)]$^{2-}$/Mb; (d) cis-[VO(ma)$_2$(H$_2$O)]/Mb; (e) [VO(acac)$_2$/Mb. Mb concentration was 5 μM and molar ratio V/Mb was 3/1.
Fig. S2 Deconvoluted ESI-MS spectrum of myoglobin (concentration 5 µM). Mass is expressed in Da.
Fig. S3 Zoom of the multipeak with charge +9 of (a) Mb and (b) system [VO(dhp)$_2$]/Mb. Mb concentration was 5 μM and molar ratio V/Mb was 3/1. The most intense peak due to the adducts [VO(dhp)$^+$]–Mb and [VO(dhp)$_2$]–Mb are indicated.
Fig. S4 Ultrazoom of the two most intense peaks with charge +9 detected in the systems: (a) Mb; (b) $[\text{VO(dhp)}_2]/\text{Mb}$; (c) cis-$[\text{VO(mim)}_2(\text{H}_2\text{O})]^2-/\text{Mb}$; (d) cis-$[\text{VO(ma)}_2(\text{H}_2\text{O})]/\text{Mb}$; (e) $[\text{VO(acac)}_2]/\text{Mb}$. Mb concentration was 5 μM and molar ratio V/Mb was 3/1.
Fig. S5 Most stable adducts predicted by docking methods for the interaction of the VO(dhp)$^+$ moiety with myoglobin: a) $SPY$-5-13-A-VO(dhp)(H$_2$O)$_2$ with His24 and His119; b) $SPY$-5-13-C-VO(dhp)(H$_2$O)$_2$ with His82 and Asp141 and c) $SPY$-5-13-C-VO(dhp)(H$_2$O)$_2$ with Glu83 and Asp141.
Fig. S6 Experimental (above) and calculated (below) isotopic pattern for the peak of $[\text{VO(mim)}_2]^2-$ revealed at m/z = 229.5 in the negative ESI-MS spectrum recorded on the system $\text{V}^{IV}\text{O}^{2+}/\text{mim}\ 1/2$ (V concentration 50 µM).
Fig. S7 Deconvoluted ESI-MS spectra recorded on the system containing VO(ma)$_2$ and myoglobin (50 µM): molar ratio 3/1 (top), 5/1 (centre) and 10/1 (bottom). With a and b the fragments VO(ma)$^+$ and VO(ma)$_2$ are indicated. Mass is expressed in Da.
Fig. S8 Deconvoluted ESI-MS spectra recorded on the system containing \([\text{VO(acac)}_2]\) and myoglobin (50 µM): molar ratio 3/1 (top) and 5/1 (bottom). With a and b the fragments \(\text{VO(acac)}^+\) and \(\text{VO(acac)}_2\) are indicated. Mass is expressed in Da.
Fig. S9 High field region of the X-band anisotropic EPR spectra recorded on frozen solutions (120 K) containing: a) V$^{IV}$O$^{2+}$/acac/Mel m 1/2/4; b) V$^{IV}$O$^{2+}$/acac/Mb 1/2/1; c) V$^{IV}$O$^{2+}$/acac/Mb 2/4/1; d) V$^{IV}$O$^{2+}$/acac/Mb 4/8/1; e) V$^{IV}$O$^{2+}$/acac/Mb 6/12/1; f) V$^{IV}$O$^{2+}$/acac/Mb 8/16/1; g) V$^{IV}$O$^{2+}$/acac/Mb 10/20/1 and h) V$^{IV}$O$^{2+}$/acac 1/2. V$^{IV}$O$^{2+}$ concentration was 1.0 × 10$^{-3}$ M. I and the dash-dotted lines indicate the $M_I = 7/2$ resonance of the species [VO(acac)$_2$].
Fig. S10 High field region of the X-band isotropic EPR spectra recorded on aqueous solutions (298 K) containing: a) $\text{V}^{IV}\text{O}^{2+}/\text{acac} \, 1/2$ and b) $\text{V}^{IV}\text{O}^{2+}/\text{acac/Mb} \, 1/2/1$. $\text{V}^{IV}\text{O}^{2+}$ concentration was $1.0 \times 10^{-3}$ M. I and the dash-dotted line indicate the $M_I = -7/2$ and $7/2$ resonances of the species [VO(acac)$_2$].
**Fig. S11** Cluster distribution for the interaction of [VO(acac)$_2$] with myoglobin. The six clusters are represented with different colors.
Scheme S1 Structures in aqueous solution and physiological pH of the bis-chelated V\textsuperscript{IV}O complexes formed by 1,2-dimethyl-3-hydroxy-4(1H)-pyridinonate, L-mimosinate, maltolate and acetylacetonate.
Scheme S2 Enantiomers of $[\text{VO(dhp)}(\text{H}_2\text{O})_2]^+$ (above) and $[\text{VO(ma)}(\text{H}_2\text{O})_2]^+$ (below).
Scheme S3 Possible isomers of the bis-chelated $^{IV}$O complex formed by L-mimosinate.