Physiologically stable vanadium(IV) porphyrins as a new class of anti-HIV agents†

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The water soluble oxovanadium(IV) tetraarylporphyrin 1a has demonstrated excellent solution stability against glutathione reduction and high potency (5 μM, 97% inhibition) in inhibiting HIV-1 replication in Hut/CCR5 cells.

Vanadium is a physiologically essential element and its concentration in human serum is about 100 ng l^{-1}.[1] In biological media, this element has V(V)/V(IV) or V(IV)/V(III) redox interplay. Indeed, vanadium complexes have been known as potential therapeutics such as anticancer, spermicidal, and notably antidiabetic agents.[2] Besides, there is a growing interest in their antiviral properties. Recent studies showed that oxovanadium complexes of thiourea and vanadium substituted polyoxotungstates exhibit potent anti-HIV properties towards infected immortalized T-cells.[3]

Stability is an important issue in designing new anti-HIV agents. Previous studies have shown that the reported vanadium complexes are unstable in the presence of the biological reductant glutathione (GSH). Formation of vanadium–GSH complexes and their subsequent degradation are usually encountered.[4]

To circumvent these problems, we employed macrocyclic dianionic porphyrinato ligands to stabilize the vanadium ion. These ligands provide a rigid square planar scaffold and have been shown to avoid demetalation of the metal ion.[5] Herein we present the oxovanadium(IV) porphyrins **1a–e** (Fig. 1) which are stable in GSH containing solution and exhibit potent anti-HIV-1 activities in infected human Hut/CCR5 cells. Importantly, these vanadyl complexes showed low cytotoxicity to normal human peripheral blood mononuclear cells (PBMC).

Complexes **1a–e** (Fig. 1) were prepared according to a literature method with some modifications,[6] and were characterized by UV-vis, FT-IR, FAB-MS and elemental analyses (see ESI†). Their FT-IR spectra show a strong peak at around 1000 cm^{-1}, which is assigned to the V=O stretch. The mass spectra exhibit cluster peaks assignable to the corresponding parent ions. For the water soluble complex **1a**, prominent cluster peaks ascribable to the protonated species $[V(TASPP)(O)+ nH]^{n+}$ (where n= 1, 2, 3 or 4) also appear in its ESI-MS spectrum.† The molecular structure of **1b** has been established by X-ray crystallography.‡ The vanadium–oxygen distance (1.614(13) Å) is characteristic of the VO^{2+} unit.[7] The vanadium atom lies 0.51 Å out of the plane of the four nitrogen atoms, which is comparable to the related distance (0.53 Å) found in oxovanadium(IV) tetraphenylporphyrin (**1c**) reported in the literature.[8]

These vanadyl complexes exhibit excellent stability in common organic solvents such as CH_2Cl_2 and DMSO; no significant spectral change was observed for **1a** in over 7 days at room temperature. We also examined the aqueous stability of **1a** against glutathione (GSH) reduction. Unlike other non-porphyrinato oxovanadium analogues,[4] treatment of **1a** with GSH (2 mM) in Tris buffered saline (TBS) did not cause any spectral changes upon standing the solution for even up to 7 days at room temperature (Fig S3, ESI†). Likewise, treatment of **1b–e** with GSH also gave similar observation and no demetalation to give free porphyrin ligand (*e.g.* H_2TASPP) was found.

Complexes **1a–e** were evaluated for their inhibitory effects on HIV-1(BaL) replication in Hut/CCR5 cells. The viral contents were determined by measuring p24 antigen production in various cell cultures.[9] With the exception of **1e**, all vanadium porphyrins showed anti-retrovirus activities (with drug pretreatment before HIV infection, Fig. 2, or without drug pretreatment, Fig S4†) as compared to the vehicle control, whereas the water soluble analogue **1a** containing aminosulfonyl functional groups exhibited the highest potency at a 5 μM level with over 97% inhibition. Since the aqueous solubility of **1a** is similar to that of **1e**, but the anti-HIV potency is different at the 5 μM level (*cf.* **1a** = 97% inhibition, **1e** = 7% inhibition), we reason that the aqueous solubility of the vanadyl porphyrin may not be a major factor affecting its antiviral activities.

The free H_2TASPP ligand (free based porphyrin of **1a**) and $Zn^{II}(TASPP)$ display less potent activity (33% and 6.4% inhibition, respectively). The porphyrin ligand is essential for the inhibitory activities, since vanadyl sulfate ($V^{IV}OSO_4$) showed a relatively low activity (36% inhibition) at the indicated concentration. We reason that the porphyrin ligand should stabilize vanadium(IV) and carry the VO^{2+} unit to the biological target.

As **1a** exhibited the highest potency in inhibiting HIV-1 replication, we turned to study the time- and dose-dependent manner of its anti-HIV activities. As shown in Fig. 3, **1a** shows dose-dependent anti-HIV activities in Hut/CCR5 cells. As

† Electronic Supplementary Information (ESI) available: Experimental details; syntheses and characterization of complexes **1a–e**; ESI-MS spectrum of **1a** (Fig. S1); ORTEP drawing of **1b** (Fig. S2); UV-vis spectra of **1b** in TBS in the presence of GSH (Fig. S3); percentage inhibition of HIV-1 replication in Hut/CCR5 cells by oxovanadium porphyrins (Fig. S4); percentage survival of Hut/CCR5 cells in the presence of oxovanadium porphyrins (Fig. S5); UV-vis spectral changes of H_2TASPP in TBS with increasing concentration of WETWWTEYWQ (Fig. S6); and UV-vis spectral changes of **1a** in TBS with increasing concentration of YCSSSKVVVR (Fig. S7). See http://www.rsc.org/suppdata/cc/b5/b503535j/

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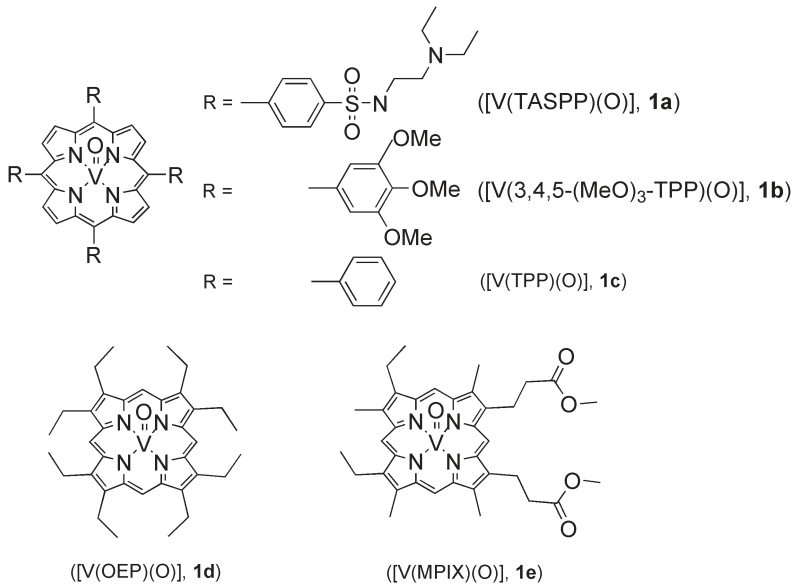

Fig. 1 Oxovanadium(IV) porphyrin complexes.

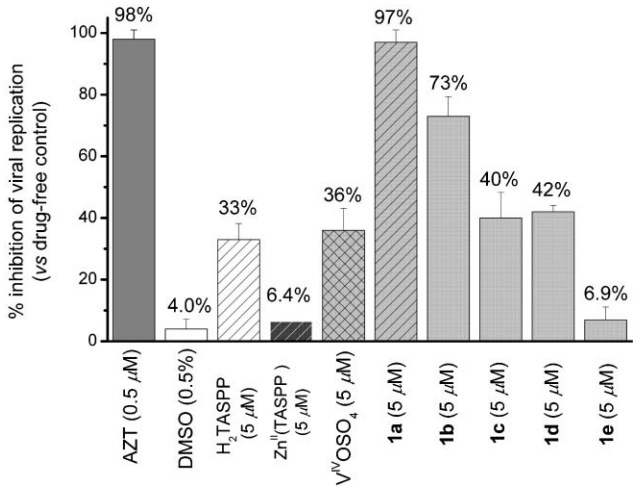

Fig. 2 Percentage inhibition of HIV-1(BaL) replication in Hut/CCR5 cells (7 days) by oxovanadium(IV) porphyrins and related complexes.

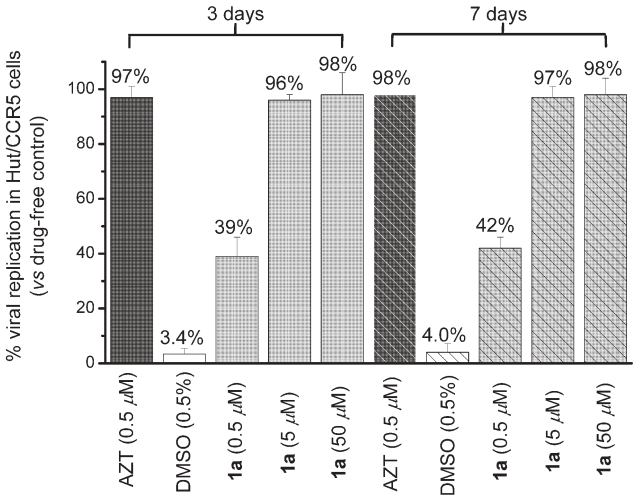

Fig. 3 Percentage inhibition of HIV-1(BaL) replication in Hut/CCR5 cells (3 and 7 days) by **1a** and related complexes.

incubation progressed from 3 to 7 days, similar dose-dependent inhibitory properties were observed with the dose concentration inhibiting HIV-1 replication by 50% (IC_{50} value) equal to 0.75 μM (*cf.* $IC_{50(3\ days)}$ = 0.82 μM).

In order to determine whether the test compounds were endowed with selective antiviral activity instead of killing the host Hut/CCR5 cells, we evaluated the cell viability of the oxovanadium(IV) porphyrins in Hut/CCR5 cells in parallel by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[10] Fig S5 (see ESI†) depicts that complexes **1a–e** did not exert significant acute cytotoxicities to the Hut/CCR5 cells, with >80% cell survival being registered at complex concentration up to 50 μM, which corresponds to 10-fold of the concentration required for effective anti-HIV-1 activities. These results highlight that the cytotoxicity of the oxovanadium(IV) porphyrins cannot account for the antiviral properties. Furthermore, we tested the acute cytotoxicity of the complexes to human PBMC, which are the host cells for HIV-1 replication *in vivo*. Similar to the results that we tested on Hut/CCR5 cells, the oxovanadium(IV) complexes showed low cytotoxicity to the normal PBMC (Fig. 4).

HIV-1 reverse transcriptase (RT) is one of the major targets for anti-HIV drugs,[11] and binding of vanadium complexes to RT has been reported.[3a] In this study, the activity of the oxovanadium(IV) porphyrin **1a** toward HIV-1 RT inhibition was measured by using an ELISA method developed by Eberle and Seibl.[12] Upon treatment of HIV-1 RT in lysis buffer (2 ng, 128.7 μL) with **1a** (5 and 50 μM) dissolved in TBS at 37 °C, significant RT inhibition (~38 and 73%, respectively) was observed after 30 min incubation compared with the drug-free control (*i.e.*, 0% inhibition).

Paterson and co-workers had proposed that metalloporphyrins such as zinc(II) protoporphyrin IX (Zn^{II}(PPIX)) and iron(II) haematoporphyrin mediated cell free *in vitro* HIV-1 RT inhibition

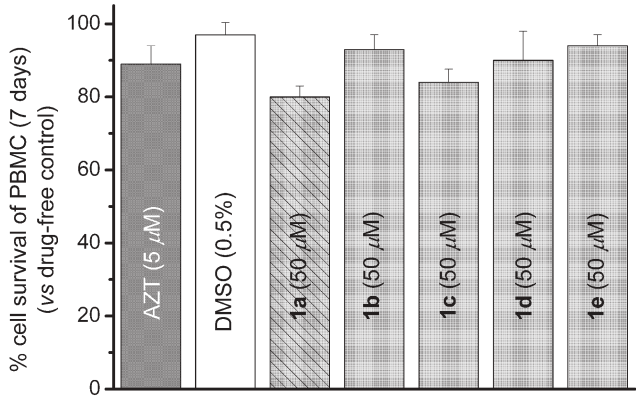

Fig. 4 Percentage survival of PBMC (7 days) in the presence of oxovanadium porphyrin complexes (50 μM).

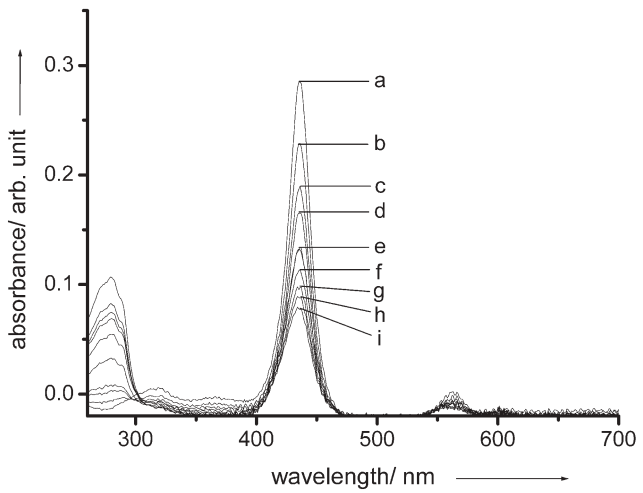

Fig. 5 UV-vis spectral changes of **1a** (5 μM) in TBS with increasing concentration of WETWWTEYWQ (r = [WETWWTEYWQ]/[**1a**]): (a) r = 0, (b) r = 0.21, (c) r = 0.43, (d) r = 0.64, (e) r = 0.85, (f) r = 1.06, (g) r = 1.27, (h) r = 1.48, (i) r = 1.90.

and the inhibition could be related to binding of the metalloporphyrin to the connection domain sequence 398–407 (WETWWTEYWQ).[13] In this work, a UV-vis absorption titration study revealed that **1a** binds to the peptide in aqueous buffer medium with a binding constant (K_b) equal to (4.0 ± 0.5) × 10^5 dm^3mol^{-1} at 292 K (Fig. 5). A comparable K_b value ((1.3 ± 0.1) × 10^5 dm^3mol^{-1} at 292 K) was obtained for the Zn^{II}(PPIX)–WETWWTEYWQ interaction. As a control experiment, we found that the free porphyrin ligand showed a much lower binding affinity (K_b = (2.3 ± 0.2) × 10^4 dm^3mol^{-1} at 292 K, Fig S6†) than that of the **1a** to the connection domain sequence (WETWWTEYWQ). However, **1a** can also bind strongly to a random peptide sequence (YCSSSKVVVR) with a comparable binding constant of (6.8 ± 0.3) × 10^5 dm^3mol^{-1} at 292 K (Fig S7†). Given the strong binding affinity to the random peptide sequence, we reason the binding of **1a** to the connection domain of the HIV-1 RT may not be specific.

In summary, the oxovanadium(IV) porphyrins **1a–e**, are stable in aqueous solutions containing biological reductant GSH, and exhibit potent anti-HIV-1 properties toward infected Hut/CCR5 cells. Complex **1a** also showed inhibitory activity toward HIV-1 reverese transcriptase *in vitro*. An on going study is to elucidate the underlying mechanism of these metalloporphyrins in inhibiting viral replication.

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Notes and references

‡ *Crystal data*: [V(3,4,5-(MeO)$_3$-TPP)(O)]·3H$_2$O: $C_{56}H_{58}N_4O_{16}V$, M = 1094, monoclinic, $C2/m$, a = 16.297(3), b = 27.009(5), c = 8.878(2) Å, β = 115.11(3)°, V = 3538.5(12) Å^3, T = 253 K, Z = 2, μ = 0.197 mm^{-1}, 5611 collected reflection, 1976 independent reflection, R indices (all data) R = 0.11, wR = 0.32. CCDC 268109. See http://www.rsc.org/suppdata/cc/b5/b503535j/ for crystallographic data files in CIF format.

1 D. C. Crans, J. J. Smee, E. Gaidamauskas and L. Yang, *Chem. Rev.*, 2004, **104**, 849.
2 (*a*) K. H. Thompson and C. Orvig, *Coord. Chem. Rev.*, 2001, **219**, 1033; (*b*) P. Ghosh, O. J. D'Cruz, R. K. Narla and F. M. Uckun, *Clin. Cancer Res.*, 2000, **6**, 1536; (*c*) K. H. Thompson, J. H. McNeill and C. Orvig, *Chem. Rev.*, 1999, **99**, 2561; (*d*) O. J. D'Cruz, P. Ghosh and F. M. Uckun, *Biol. Reprod.*, 1998, **58**, 1515; (*e*) A. Bishayee and M. Chatterjee, *Brit. J. Cancer*, 1995, **71**, 1214.
3 (*a*) O. J. D'Cruz, Y. Dong and F. M. Uckun, *Biochem. Biophys. Res. Commun.*, 2003, **302**, 253; (*b*) S. Shigeta, S. Mori, E. Kodama, J. Kodama, K. Takahashi and T. Yamase, *Antiviral Res.*, 2003, **58**, 265.
4 (*a*) T. Kiss, T. Jakusch, J. C. Pessoa and I. Tomaz, *Coord. Chem. Rev.*, 2003, **237**, 123; (*b*) A. J. Tasiopoulos, A. N. Troganis, A. Evangelou, C. P. Raptopoulou, A. Terzis, Y. Deligiannakis and T. A. Kabanos, *Chem. Eur. J.*, 1999, **5**, 910.
5 See for examples: (*a*) R. W.-Y. Sun, W.-Y. Yu, H. Sun and C.-M. Che, *Chembiochem*, 2004, **5**, 1293; (*b*) C.-M. Che, R. W.-Y. Sun, W.-Y. Yu, C.-B. Ko, N. Zhu and H. Sun, *Chem. Commun.*, 2003, 1718; (*c*) Y. Li, J.-S. Huang, Z.-Y. Zhou and C.-M. Che, *Chem. Commun.*, 2003, 1362.
6 A. D. Adler, F. R. Longo, F. Kampas and J. Kim, *J. Inorg. Nucl. Chem.*, 1970, **32**, 2443.
7 R. Guilard and C. Lecomte, *Coord. Chem. Rev.*, 1985, **65**, 87.
8 M. G. B. Drew, P. C. H. Mitchell and C. E. Scott, *Inorg. Chim. Acta*, 1984, **82**, 63.
9 A. Trkola, W. A. Paxton, S. P. Monard, J. A. Hoxie, M. A. Siani, D. A. Thompson, L. Wu, C. R. Mackay, R. Horuk and J. P. Moore, *J. Virol.*, 1998, **72**, 396.
10 T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55.
11 H. Mitsuya, R. Yarchoan and S. Broder, *Science*, 1990, **249**, 1533.
12 J. Eberle and R. Seibl, *J. Virol. Methods*, 1992, **40**, 347.
13 E. G. Argyris, J. M. Vanderkooi, P. S. Venkateswaran, B. K. Kay and Y. Paterson, *J. Biol. Chem.*, 1999, **274**, 1549.

