### **Supplementary Material**

#### for

# "Physiologically Stable Vanadium(IV) Porphyrins as a New Class of Anti-HIV Agents"

#### Suk-Yu Wong, Raymond Wai-Yin Sun, Nancy P.-Y. Chung, Chen-Lung Lin and Chi-Ming Che\*

#### Part I. Experimental Section

**Materials**. All chemicals, except otherwise noted, were purchased from Sigma-Aldrich Chemical Co.  $CH_2Cl_2$  and quinoline were freshly distilled prior to use. Analytical grade organic solvents and double distilled deionized water were used throughout the experiments. *meso*-Tetrakis[4-(*N*-(*N*,*N*-diethylamino)ethyl)aminosulfonyl]phenylporphyrin (H<sub>2</sub>TASPP) was prepared according to literature method [B. Meunier and F. Cosledan, *PCT Int. Appl.*, 2002, WO 2002004454]. *meso*-Tetraphenylporphyrin (H<sub>2</sub>TPP) and *meso*-tetrakis(3,4,5trimethoxyphenyl)porphyrin (H<sub>2</sub>(3,4,5-(MeO)<sub>3</sub>-TPP)) were synthesized and purified by the literature methods [(a) A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour and L. Korsakoff, *J. Org. Chem.*, 1967, **32**, 476; (b) E. Keinan, E. Benory, S. C. Sinha, A. Sinha-Bagchi, D. Eren, Z. Eshhar and B. S. Green, *Inorg. Chem.*, 1992, **31**, 5433].

**Physical Measurements**. All absorption spectra were recorded on a Perkin-Elmer Lambda 900 UV-vis spectrophotometer. Infrared spectra were recorded as Nujol mulls on a Nicolet model 20 FXC FT-IR spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a Finnigan MAT95 mass spectrometer using 3-nitrobenzyl alcohol as matrix. Electrospray ionization (ESI) mass spectra were recorded on a Finnigan LCQ mass spectrometer.

*X-ray crystal determination*. Crystals of **1b** were obtained by slow diffusion of diethyl ether into a CH<sub>2</sub>Cl<sub>2</sub> solution of **1b**. A purple crystal having dimensions  $0.3 \times 0.25 \times 0.2$  mm mounted in a glass capillary was used for data collection at -20°C on a MAR diffractometer with a 300 mm image plate detector using graphite monochromatized Mo-K<sub> $\alpha$ </sub> radiation ( $\lambda = 0.71073$  Å). Data collection was made with 2° oscillation step of  $\varphi$ , 600 seconds exposure time and scanner distance at 120 mm. 90 images were collected. The images were interpreted and intensities integrated using program DENZO [Z. Otwinowski and W. Minor. In *Processing of X-ray Diffraction Data Collected in Oscillation Mode, Methods in Enzymology*, C. W. Carter and R. M. Sweet Jr., Eds.; Academic Press.: 1997; Vol. 276, p. 307-326]. The structure was solved by direct methods employing SHELXS-97 program [G. M. Sheldrick, *SHELX97. Programs for Crystal Structure Analysis* 

(*Release 97-2*). University of Goetingen, Germany, 1997]. V and many non-H atoms were located according to the direct methods. The positions of the other non-hydrogen atoms were found after successful refinement by the full-matrix least-squares using program SHELXL-97.

#### **Biological Studies and Measurement.**

*Materials*. HIV-1(BaL) stock (300 ng/mL of p24 protein) and Hut/CCR5 cells were the gifts from Dr. D. Tao (Institute of Molecular Medicine, University of Oxford, UK). Peripheral blood mononuclear cells (PBMC) were prepared from buffy coat obtained from the Hong Kong Red Cross Blood Transfusion Service. Each unit of the buffy coat prepared from whole blood (450 mL) contained approximately  $5 \times 10^8$  cells. To isolate the PBMC, the buffy coat (15 mL) was transferred to an centrifuge tube (50 mL) under sterile condition and was diluted by RPMI culture medium in a 1:1 (v/v) ratio. Ficoll® solution (15 mL) was gently added to the diluted blood and the mixture was centrifuged at 800 rpm for 25 min at room temperature. The PBMC at the interface were washed five times with cold RPMI culture medium. The washed PBMC were spun at first 500 rpm for 7 min, then at 250 rpm for 7 min and finally at 200 rpm to obtain a cell pellet. Hut/CCR5 cells and PBMC were maintained in the RPMI 1640 medium (Life Technologies, Inc.) supplemented with fetal bovine serum (10%, v/v), *L*-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100  $\mu$ g/mL), and were incubated at 37 °C in a 5% CO<sub>2</sub>/ 95% air humidified atmosphere. The peptides (WETWWTEYWQ) and (YCSSSKVVVR) were purchased from Biopeptide Co. (San Diego, CA) and were used as received.

Anti-HIV-1 assay of the oxovanadium(IV) porphyrin complexes. Stock solutions (10 mM) of the vanadium complexes were prepared in sterile water (for **1a**) or DMSO (for **1b–e**) and diluted to desired concentration in growth medium. Hut/CCR5 cells in 24-well plate ( $5 \times 10^5$  cells/well) were pretreated with compounds (400  $\mu$ L) for an hour at 37°C and subsequently infected in triplicate with a panel of CCR5-tropic HIV-1 isolates (with the addition of 5  $\mu$ L of HIV-1 stock to each well, total p24 content = 1500 pg) for 3 hour. Following infection, the cells were washed to remove residual viral inoculums and cultured in the presence of compounds (400  $\mu$ L) for 3 or 7 days. Culture supernatants were harvested and viral replication was measured by determination of viral p24 antigen concentration by ELISA (HIV-1 p24 antigen Kit, Beckman Coulter). Compounds were tested in triplicate at 3 concentrations ranging from 0.5–50  $\mu$ M.

*Cell viability assay of the oxovanadium(IV) porphyrin complexes.* Assays on the cell viability of Hut/CCR5 cells and PBMC were conducted in 96-well flat-bottomed microtitre plates. The supplemented culture medium (90  $\mu$ L) with cells (1×10<sup>6</sup> cells per mL) was added into a 96-well plate. Vanadium complexes with concentrations from 0.5–100  $\mu$ M were dissolved in the culture medium (10  $\mu$ L) or with 1% DMSO (for **1b–e**), and the solutions were subsequently added into a

set of wells. Control well contained only supplemented media (100  $\mu$ L). Microtitre plates were incubated at 37°C in a 5% CO<sub>2</sub>/95% air humidified atmosphere for a further 7 days. All the assays were run in parallel with a negative control (i.e., vehicle control) and a positive control with cisplatin as cytotoxic agent.

Assessment of the cell viability was carried out using a modified method of Mosmann based 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay [T. Mosmann, J. *Immunol. Methods*, 1983, **65**, 55]. At the end of each incubation period, MTT solution (10  $\mu$ L, Cell Proliferation Kit I, Roche) was added into each well and the cultures were further incubated for 4 h at 37°C in a 5% CO<sub>2</sub>/95% air humidified atmosphere. Then a solubilizing solution (100  $\mu$ L) was added into the wells to lyse the cells and solubilize the formazan complex formed. The microtitre plates were maintained in a dark, humidified chamber overnight. The formation of formazan was measured with a microtitre plate reader at 550 nm and the percentages of cell survival were determined. The cytotoxicity was evaluated based on the percentage cell survival in a dose-dependence manner relative to the negative control.

Inhibition studies of HIV-1 reverse transcriptase (RT) by oxovanadium(IV) complexes. Assays on the *in vitro* HIV-RT inhibitory activities were conducted by using a commercial assay kit (Reverse Transcriptase Assay, Chemiluminescent, Roche). Complex **1a** and AZT-TP were dissolved in PBS (1.3  $\mu$ L) and mixed with a set of HIV-1 RT in the lysis buffer (2 ng, 128.7  $\mu$ L) at 37°C (30 min). The ELISA assays were conducted by following the manufacturer's instructions [J. Eberle and R. Seibl, J. Virol. Methods, 1992, **40**, 347]. The HIV-1 RT activities were evaluated based on the percentage luminescence of the solutions in a dose-dependent manner relative to the negative control (untreated population).

Absorption Titration. A solution of **1a** (5  $\mu$ M) in Tris buffered saline (TBS, 3000  $\mu$ L) was placed in a thermostatic cuvette in a UV-vis spectrophotometer. Aliquots of a millimolar stock peptide (WETWWTEYWQ or VCSSSKVVVR) solution were added to the solution. Absorption spectra were recorded after equilibration for 10 min per aliquot until saturation point has reached. The binding constant ( $K_b$ ) was determined according to the literature method [R. W.-Y. Sun, W.-Y. Yu, H. Sun and C.-M. Che, *Chembiochem*, 2004, **5**, 1293].

#### Preparation of oxovanadium(IV) porphyrin complexes.

Complexes **1a–e** were prepared according to the literature method with some modifications [S. Bencosme, M. Labady and C. Romero, *Inorg. Chim. Acta*, 1986, **123**, 15]. In general, a mixture of VO(acac)<sub>2</sub> (1.5 mmol) and free porphyrin (0.15 mmol) in quinoline (20 mL) was heated under reflux for 6 h; complete metalation was checked by disappearance of the Q-band using UV-vis spectrophotometry. Upon removal of solvent by vacuum distillation, the solid residue was dissolved

in  $CH_2Cl_2$  (3 mL) and chromatographed on a neutral 90-alumina packed column with  $CH_2Cl_2$ /hexane (1:1, v/v) to elute the impurities. The oxovanadium(IV) porphyrin product was eluted using a  $CH_2Cl_2$ /MeOH (99:1, v/v) mixture. A purple solid was obtained after removal of solvent. The complex was recrystallized from a  $CH_2Cl_2$ /pentane (for **1a**) or  $CH_2Cl_2$ /diethyl ether (for **1b–e**) mixture.

(1a) Yield: 150.0 mg (72%). IR (Nujol, cm<sup>-1</sup>): 1006 ( $v_{V=0}$ ). UV-vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{max}$ /nm (log  $\varepsilon$ ): 423 (5.29), 547 (4.03). m/z=1392 ( $M^+$  + H), 697 ( $M^{2+}$  + 2H), 465 ( $M^{3+}$  + 3H) and 349 ( $M^{4+}$  + 4H). Anal. Calcd. for C<sub>68</sub>H<sub>84</sub>N<sub>12</sub>O<sub>9</sub>S<sub>4</sub>V•CH<sub>2</sub>Cl<sub>2</sub> (%): C, 56.08; H, 5.82; N, 11.38. Found: C, 55.99; H, 5.93; N, 11.57.

(1b) Yield: 123.1 mg (79%). IR (Nujol, cm<sup>-1</sup>): 1003 ( $v_{V=O}$ ). UV-vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{max}$ /nm (log  $\varepsilon$ ): 429 (5.10), 548 (3.92). m/z=1040 ( $M^+$ ). Anal. Calcd. for C<sub>56</sub>H<sub>52</sub>N<sub>4</sub>O<sub>13</sub>V (%): C, 64.68; H, 5.39; N, 5.00. Found: C, 65.05; H, 5.50; N, 5.07.

(1c) Yield: 86.6 mg (85%). IR (Nujol, cm<sup>-1</sup>): 1005 ( $\nu_{V=0}$ ). UV-vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{max}$ /nm (log  $\varepsilon$ ): 422 (5.96), 546 (4.54). *m/z*=680 (*M*<sup>+</sup>). Anal. Calcd. for C<sub>44</sub>H<sub>28</sub>N<sub>4</sub>OV (%): C, 77.76; H, 4.12; N, 8.25. Found: C, 77.16; H, 4.23; N, 7.81.

(1d) Yield: 80.7 mg (76%). IR (Nujol, cm<sup>-1</sup>): 1000 ( $v_{V=0}$ ). UV-vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{max}$ /nm (log  $\varepsilon$ ): 407 (5.41), 533 (4.17). *m/z*=600 (*M*<sup>+</sup>). Anal. Calcd. for C<sub>36</sub>H<sub>44</sub>N<sub>4</sub>OV (%): C, 72.04; H, 7.34; N, 9.34. Found: C, 72.54; H, 6.99; N, 9.52.

(1e) Yield: 49.5 mg (50%). IR (Nujol, cm<sup>-1</sup>): 988 ( $v_{V=0}$ ). UV-vis (CH<sub>2</sub>Cl<sub>2</sub>)  $\lambda_{max}$ /nm (log  $\varepsilon$ ): 406 (5.85), 532 (4.46), 571 (4.76). *m/z*=660 (*M*<sup>+</sup>). Anal. Calcd. for C<sub>36</sub>H<sub>42</sub>N<sub>4</sub>OV (%): C, 65.48; H, 6.37; N, 8.49. Found: C, 64.66; H, 6.22; N, 8.49.

# Part II. Figures

Figure S1 ESI mass spectrum showing prominent cluster peaks ascribable to the protonated

species of **1a**:  $[V(TASPP)(O)+nH]^{n+}$  where n=1 (a), 2 (b), 3(c) and 4 (d).



Figure S2 ORTEP drawing of [V<sup>IV</sup>(3,4,5-(MeO)<sub>3</sub>-TPP)(O)]·3H<sub>2</sub>O with atom-numbering scheme. The central V and O atoms are disordered over two positions, and only one of the two orientations is shown. Hydrogen atoms are omitted for clarity and water hydrogens were not located. Thermal ellipsoids are drawn at the 30% probability level. Selected bond distances (Å): V–O(1), 2.033(5); V–N(1), 2.032(5); V–N(2), 2.033(5); V–N(1\*), 2.033(5) and V–N(2\*), 2.033(5). Selected bond angles (°): N(1)-V-N(1\*), 89.8(3); N(1)-V-N(2\*), 177.31(19); N(1\*)-V-N(2\*), 90.1(2); N(1\*)-V-N(2), 90.1(2); N(1\*)-V-N(2), 177.31(19) and N(2\*)-V-N(2), 90.1(3).



**Figure S3** UV-vis spectra of **1a** (5  $\mu$ M) in TBS containing GSH (2 mM) at time= 0 day (solid



line) and time = 7 days (dotted line).

**Figure S4** Percentage inhibition of HIV-1(BaL) replication in Hut/CCR5 cells (7 days) by



oxovanadium(IV) porphyrins (without drug pre-treatment before HIV-1 infection).

## **Figure S5** Percentage survival of Hut/CCR5 cells in the presence of oxovanadium(IV)



porphyrin complexes.

**Figure S6** UV-vis spectral changes of  $H_2$ TASPP (8  $\mu$ M) in TBS with increasing concentration

of WETWWTEYWQ (r = [WETWWTEYWQ]/[1a]): (a) r= 0, (b) r= 0.13, (c) r=



Figure S7 UV-vis spectral changes of  $1a (8 \mu M)$  in TBS with increasing concentration of

YCSSSKVVVR (r = [YCSSSKVVVR]/[1a]): (a) r= 0, (b) r= 0.13, (c) r= 0.26, (d)

r=0.40, (e) r=0.53, (f) r=0.66, (g) r= 0.79, (h) r=0.92, (i) r= 1.05, (j) r=1.70 and (k) r=

2.34.

