J|A|C|S
COMMUNICATIONS

Published on Web 03/24/2006

A Mixed-Valent Ruthenium−Oxo Oxalato Cluster $Na_7[Ru_4(\mu_3\text{-}O)_4(C_2O_4)_6]$ with Potent Anti-HIV Activities

Ella Lai-Ming Wong,[†] Raymond Wai-Yin Sun,[†] Nancy P.-Y. Chung,[‡] Chen-Lung Steve Lin,[‡] Nianyong Zhu,[†] and Chi-Ming Che*,[†]

Department of Chemistry and Open Laboratory of Chemical Biology of the Institute of Molecular Technology for Drug Discovery and Synthesis, The University of Hong Kong, Pokfulam Road, Hong Kong, and Department of Surgery, The University of Hong Kong, Pokfulam Road, Hong Kong

Received November 21, 2005; E-mail: cmche@hku.hk

The medicinal properties of metal-based compounds have been and continue to be a subject of interest in drug discovery.[1] In the literature, metal-based compounds have found clinical applications with cisplatin being the most notable example.[2] While there has been extensive research on cytotoxic metal compounds, related studies on anti-viral metal-based compounds remain sparse. In literature, several classes of metal-based compounds, such as polyoxometalates[3] and metallocyclams,[4] and vanadium(IV),[5] zinc(II),[6] and gold(I)[7] complexes are known to display significant anti-HIV activities. Examples of ruthenium compounds which display anti-viral activities are sparse.[8] During the course of preparing $Na_3[Ru(C_2O_4)_3]$,[9] we obtained a polyanionic ruthenium−oxo oxalato cluster $Na_7[Ru_4(\mu_3\text{-}O)_4(C_2O_4)_6]$ (**1**). The structure of **1** bears some resemblances to polyoxometalates, where the peripheral surface of the latter is made up of anionic oxygen donor atoms. Recent reports revealed that polyanionic compounds,[10] as well as some spherical-shaped compounds, such as fullerene derivatives,[11] might have anti-HIV properties. This prompted us to examine the anti-HIV properties of **1**.

Compound **1** was synthesized in a reproducible manner by reacting $K_2[RuCl_5(H_2O)]$ and oxalic acid in an alkaline medium (pH ∼ 10). Dark green crystals of **1**·$8H_2O$ were obtained by slow diffusion of acetone to the aqueous reaction mixture. A small amount of $Na_3[Ru(C_2O_4)_3]$ (**2**) was also obtained as a light brown solid and was confirmed by X-ray crystallography (Figure S1). Compound **2** could be removed by repeated recrystallizations.

Single-crystal structure of **1** (Figure 1) is featured by a framework containing four ruthenium atoms and four oxygen atoms situated at alternate corners of a distorted cubane [Ru−O_{oxo} distance = 1.979(3)−2.052(3) Å]. Each ruthenium atom is bonded with 1.5 oxalate ligands. The Ru−$O_{oxalate}$ distances are within 2.051(3)−2.083(3) Å. Both the η^2- and μ_2-binding modes were observed for the oxalate ligands. Compound **1** is symmetrical with a C_2-axis. There are 3.5 sodium ions for each asymmetric unit. As a result, each formula unit contains 7 sodium ions, leading to a $Na_7[Ru_4(\mu_3\text{-}O)_4(C_2O_4)_6]$ formulation. Powder X-ray diffraction analysis on a bulk sample of **1** was performed. The experimental diffraction pattern matches with the simulated one based on the single-crystal structure, indicative of phase purity in the bulk sample (Figure S2).

Variable temperature magnetic susceptibility measurement was performed. The plots of χ versus T and $1/\chi$ versus T (T in absolute temperature, K) gave a straight line passing through the origin, and the Curie−Weiss Law was obeyed. At 300 K, the μ_{eff} of 1.67 μ_B reveals that **1** has one unpaired electron, consistent with the (III, III, III, IV) oxidation states of ruthenium in this complex. Mixed valency in **1** has also been inferred by the presence of a broad UV−visible absorption band at 753 nm (ϵ = 1300 $dm^3\ mol^{-1}\ cm^{-1}$) in H_2O. Compound **1** is electrochemically inactive from −500 to +800 mV at pH 7 in 0.1 M NaH_2PO_4 (Figure S10).

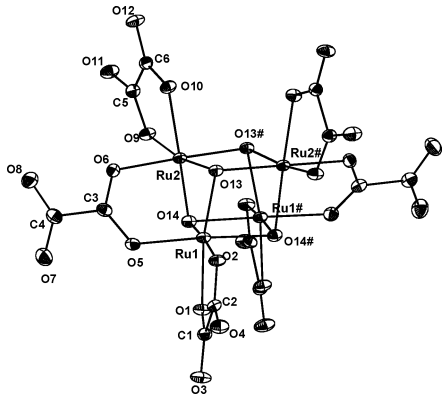

Figure 1. ORTEP drawing of complex anion of **1**. Selected intramolecular bond distances (Å): Ru(1)−O(14) 1.980(3); Ru(1)−O(13) 2.014(3); Ru(1)−O(14)# 2.029(3); Ru(1)−O(1) 2.058(3); Ru(1)−O(2) 2.051(3); Ru(1)−O(5) 2.054(3); Ru(2)−O(13) 1.979(3); Ru(2)−O(14) 1.988(3); Ru(2)−O(13)# 2.052(3); Ru(2)−O(9) 2.071(3); Ru(2)−O(10) 2.075(3); Ru(2)−O(6) 2.083(3).

Compound **1** has also been characterized by ESI-MS (Q-TOF, Figure S6), which shows cluster peaks with m/z centered at 1003.42, 1025.43, 1047.40, 1069.42, 1091.40, 1113.41, and 1135.40, attributable to $[Ru_4O_4(C_2O_4)_6{\cdot}H_{(6-n)}{\cdot}Na_n]^-$ (n = 0−6) with ruthenium in oxidation states of (III, III, III, IV). This result suggests that the basic cubane unit of the cluster should be intact in the gas phase. Cubane-like clusters with a M_4O_4 core are well established. In the literature, the "Ru_4O_4" core unit is usually observed in complexes with benzene or arene ligands, for example, the benzene−cubane tetracation $[(\eta^6\text{-}C_6H_6)_4Ru_4(OH)_4]^{4+}$ with ruthenium atoms in oxidation states of (II, II, II, II).[12] The mixed oxidation states (III, III, III, IV) in **1** are seldom reported for clusters with $[Ru_4(\mu_3\text{-}O)_4]$ core unit.[12a]

Solution stability is an important issue in drug development. Most reported polyoxometalates are unstable in water; they degrade or rearrange to a complex mixture of inorganic products, making the investigation of the mode of drug action difficult.[3b] In this study, the stability of **1** under physiologically relevant condition (PBS, pH 7.4 with 2 mM glutathione) was monitored by UV−visible spectrophotometry. No spectral change was observed for the solution upon standing for 7 days.

Compound **1** was evaluated for its anti-viral activity toward R5-tropic HIV-1(BaL) infection/replication. Hut/CCR5 cells were preincubated with **1** and followed by viral infection. After substantial washing with PBS and incubation with **1** for 3 or 7 days, final viral contents were determined by measuring the p24 antigen

[†] Department of Chemistry and Open Laboratory of Chemical Biology.
[‡] Department of Surgery.

10.1021/ja057883k CCC: $33.50

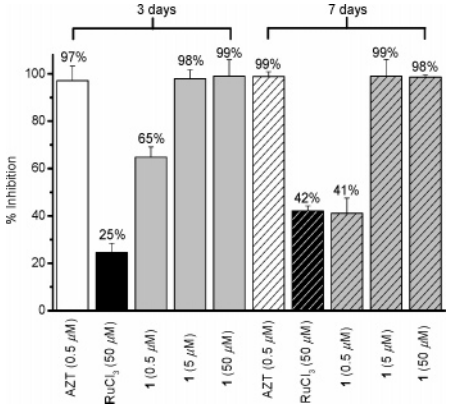

Figure 2. Percentage inhibition of HIV-1(BaL) replication of **1** in Hut/CCR5 cells after (a) 3 day and (b) 7 day incubation.

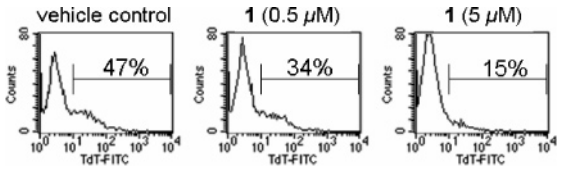

Figure 3. Flow cytometric data of TUNEL assay showing that **1** (7 day treatment) exhibited cytoprotective activity toward HIV-1(BaL) infected Hut/CCR5 cells.

production in various cell cultures.[13] As compared to vehicle control, **1** (5 μM, 3 day incubation) exhibited promising anti-HIV-1 activity with over 98% inhibition of viral replication (Figure 2). The dose-dependent manner of its anti-HIV activity has been demonstrated (i.e., 65% inhibition at 0.5 μM and 98% inhibition at 50 μM). Since **1** exhibited similar anti-HIV activity in the cases for 3 and 7 day treatment, prolonged incubation of **1** with the HIV infected cells did not alter its anti-HIV-1 activity. For comparison, $RuCl_3$, though it is nontoxic to HeLa and CCD-19Lu cell lines (IC_{50} > 100 μM) in 3 days MTT assay, showed a lower inhibitory activity (i.e., 25% inhibition at 50 μM) in HIV-1 replication. This suggests that the auxiliary ligand environment of **1** plays an important role in its anti-HIV property.

The anti-viral property of **1** toward X4-tropic HIV-1(III_B) was also evaluated (Figure S7). Similar inhibitory activities were observed for **1** in GHOST/CXCR4 cells when compared with that in Hut/CCR5 cells. Compound **1** also demonstrated promising anti-HIV (X4-tropic) activity toward peripheral blood mononuclear cells (PBMCs), though a slightly lower potency (80% inhibition) was observed at 5 μM level. To sum up, **1** is effective in inhibiting both kinds of R5- and X4-tropic viruses.

Human T-cell is known to undergo apoptotic cell death after HIV infection,[14] while successful anti-HIV agents/regimens would significantly drop the viral load and hence reduce apoptosis. In this study, we evaluated the cytoprotective activity of **1** toward HIV-1 infected Hut/CCR5 cells by TUNEL (Terminal Uridyl-Nucleotide End Labeling) assays. Flow cytometric data (Figure 3) showed that **1** at 0.5 μM would reduce the percentage of apoptosis from 47 to 34%. There was a significant drop in percentage from 47 to 15% at 5 μM (the effective anti-HIV concentration). We reason that **1** would inhibit viral replication in Hut/CCR5 cells and hence reduce HIV-associated apoptosis.

To confirm whether **1** was endowed with selective anti-viral activity instead of killing the host cells, the cell viabilities of Hut/CCR5, GHOST/CXCR4, and PBMCs were determined in parallel by MTT assay.[15] The result showed that **1** did not exert significant acute toxicity to the host cells, with >90% of cell survival being registered at a concentration up to 50 μM (Figure S8).

To delineate the possible anti-HIV mechanism, the inhibitory activity of **1** toward HIV-1 reverse transcriptase (RT), one of the major targets for anti-HIV-1 agents, was evaluated and quantified by the ELISA method.[16] Results showed that **1** could reduce the HIV-1 RT activity by half at nanomolar concentration, with IC_{50} = 1.9 nM (Figure S9). Notably, the observed anti-HIV RT activity of **1** is more effective than the commonly used HIV-1 RT inhibitor, AZT-TP (IC_{50} = 68 nM) by more than 10-fold. It appears that RT can be a possible target for **1** to execute its anti-HIV activity.

In summary, a nontoxic mixed-valent tetranuclear ruthenium−oxo oxalato cluster which exhibits anti-viral activities toward R5- and X4-tropic HIV-1 and cytoprotective activity toward HIV-1 infected cells was prepared and structurally characterized.

Acknowledgment. We acknowledge the financial support from The University of Hong Kong (University Development Fund), and the Areas of Excellence Scheme (AoE/P-10/01) administered by the University Grants Committee (HKSAR, China). We are thankful to Drs. J.-S. Huang and C. C.-L. Wong for their helpful discussions.

Supporting Information Available: Experimental procedures, characterization data, and complete ref 10. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Orvig, C.; Abrams, M. J. *Chem. Rev.* **1999**, *99*, 2201−2203. (b) Abrams, M. J.; Murrer, B. A. *Science* **1993**, *261*, 725−730.
(2) Rosenberg, B.; VanCamp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385−386.
(3) (a) Rhule, J. T.; Hill, C. L.; Judd, D. A.; Schinazi, R. F. *Chem. Rev.* **1998**, *98*, 327−357. (b) Hill, C. L.; Weeks, M. S.; Schinazi, R. F. *J. Med. Chem.* **1990**, *33*, 2767−2772.
(4) Liang, X.; Sadler, P. J. *Chem. Soc. Rev.* **2004**, *33*, 246−266.
(5) (a) D'Cruz, O. J.; Dong, Y.; Uckun, F. M. *Biochem. Biophys. Res.* **2003**, *302*, 253−264. (b) Wong, S.-Y.; Sun, R. W.-Y.; Chung, N. P.-Y.; Lin, C.-L.; Che, C.-M. *Chem. Commun.* **2005**, 3544−3546.
(6) Aoki, S.; Kimura, E. *Chem. Rev.* **2004**, *104*, 769−787.
(7) Shapiro, D. L.; Masci, J. R. *J. Rheumatol.* **1996**, *23*, 1818−1820.
(8) (a) Luedtke, N. W.; Hwang, J. S.; Glazer, E. C.; Gut, D.; Kol, M.; Tor, Y. *ChemBioChem* **2002**, *3*, 766−771. (b) Mishra, L.; Sinha, R.; Itokawa, H.; Bastow, K. F.; Tachibana, Y.; Nakanishi, Y.; Kilgore, N.; Lee, K.-H. *Bioorg. Med. Chem.* **2001**, *9*, 1667−1671.
(9) Che, C.-M.; Kwong, S.-S.; Poon, C.-K.; Lai, T.-F.; Mak, T. C. W. *Inorg. Chem.* **1985**, *24*, 1359−1363.
(10) Yamamoto, N.; et al. *Mol. Pharmacol.* **1992**, *42*, 1109−1117.
(11) Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510−6512.
(12) (a) Scaccianoce, L.; Braga, D.; Calhorda, M. J.; Grepioni, F.; Johnson, B. F. G. *Organometallics* **2000**, *19*, 790−797. (b) Gould, R. O.; Jones, C. L.; Robertson, D. R.; Tocher, D. A.; Stephenson, T. A. *J. Organomet. Chem.* **1982**, *226*, 199−207.
(13) (a) Trkola, A.; Paxton, W. A.; Monard, S. P.; Hoxie, J. A.; Siani, M. A.; Thompson, D. A.; Wu, L.; Mackay, C. R.; Horuk, R.; Moore, J. P. *J. Virol.* **1998**, *72*, 396−404. (b) Sun, R. W.-Y.; Chen, R.; Chung, N. P.-Y.; Ho, C.-M.; Lin, C.-L. S.; Che, C.-M. *Chem. Commun.* **2005**, 5059−5061.
(14) Meyaard, L.; Otto, S. A.; Jonker, R. R.; Mijnster, M. J.; Keet, R. P. M.; Miedema, F. *Science* **1992**, *257*, 217−219.
(15) (a) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63. (b) Wong, E. L.-M.; Fang, G.-S.; Che, C.-M.; Zhu, N. *Chem. Commun.* **2005**, 4578−4580.
(16) Eberle, J.; Seibl, R. *J. Virol. Methods* **1992**, *40*, 347−356.

JA057883K