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Metal complexes of pyridine-fused macrocyclic polyamines targeting the chemokine receptor CXCR4[†]

Sunil Hamal,^a Thomas D'huys,^b William F. Rowley,^a Kurt Vermeire,^b Stefano Aquaro,^c Brian J. Frost,^a Dominique Schols,^{*b} and Thomas W. Bell^{*a}

The chemokine receptor CXCR4 acts as a key cell surface receptor in HIV infections, multiple forms of cancer, and various other pathologies, such as rheumatoid arthritis and asthma. Macrocyclic polyamines and their metal complexes are known to exert anti-HIV activity, many acting as HIV entry inhibitors by specifically binding to CXCR4. Three series of pyridopentaazacylopentadecanes, in which the pyridine ring is fused to zero, one, or two saturated six-membered rings, were synthesized by manganese(II)-templated Schiff-base cyclization of triethylenetetramine with various dicarbonyl compounds. By evaluating these macrocyclic polyamines and their complexes with Mn²⁺, Cu²⁺, Fe³⁺, and Zn²⁺, we have discovered novel CXCR4-binding compounds. The MnCl₂ complex of a new pentaazacyclopentadecane with one fused carbocyclic ring (**11**) was found to have the greatest potency as an antagonist of the chemokine receptor CXCR4 (*IC*₅₀: 0.014 μM), as evidenced by inhibiting binding of CXCL12 to PBMCs (peripheral

^aDepartment of Chemistry, University of Nevada, Reno, NV, USA. E-mail: twb@unr.edu

^bDepartment of Microbiology and Immunology, Rega Institute for Medical Research, University of Leuven, Leuven, Belgium. E-mail: Dominique.Schols@rega.kuleuven.be

^cDepartment of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Rome, Italy

[†]Electronic supplementary information (ESI) available: Syntheses and characterization data for compounds **2**, **4**, **5**, and **10–21**. Numbered thermal ellipsoid plots and X-ray crystallographic data for three compounds, **2**, **4**, and **17**. CCDC 1062374-1062376. ¹H and ¹³C NMR spectra of compounds **6**, **8**, **12**, **15**, **18** and **21**.

blood mononuclear cells). Consequently, this compound inhibits replication of the CXCR4-using (X4) HIV-1 strain NL4-3 in the TZM-bl cell line with an IC_{50} value of 0.52 μM and low cytotoxicity (CC_{50} : > 100 μM). In addition, 18 other compounds were evaluated for their interaction with CXCR4 via their ability to interfere with ligand chemokine binding and HIV entry and infection. Of these, the metal complexes of the two more hydrophobic series with one or two fused carbocyclic rings exhibited the greatest potency. The Zn^{2+} complex **21** was among the most potent, showing that redox activity of the metal center is not associated with CXCR4 antagonist activity.

Introduction

The chemokine receptor CXCR4 belongs to the G-protein coupled receptor superfamily and acts as a key cell surface receptor in human immunodeficiency virus (HIV) infections, multiple forms of cancer, and various other pathologies, such as rheumatoid arthritis and asthma. CXCR4 regulates leukocyte trafficking in inflammation and has additional roles in the development of the hematopoietic, cardiovascular, and nervous systems during embryogenesis.¹ CXCR4 also serves in conjunction with CD4 as a coreceptor for entry of a number of specific (CXCR4-using or T-tropic) strains of HIV-1 and several low molecular weight CXCR4 antagonists have been shown to suppress HIV-1 replication by profoundly inhibiting the viral entry process.^{2,3} The drug combination therapy known as highly active anti-retroviral therapy, which often consists of reverse transcriptase inhibitors and a protease inhibitor, has markedly decreased HIV-1 associated morbidity and mortality.⁴ Yet, to anticipate emergence of drug-resistant viruses, the discovery of novel anti-HIV-1 agents with new mechanisms of action (e.g. entry inhibitors) is still needed to ensure effective anti-HIV-1 treatment. In addition, CXCR4 antagonists are also of interest for treatment of cancer,^{5,6}

rheumatoid arthritis, and WHIM (warts, hypogammaglobulinemia, infections and myelokathexis) syndrome.^{7,8}

Bicyclams, such as AMD3100 (Fig. 1a), are macrocyclic polyamines known to inhibit HIV infection by blocking CXCR4.^{9,10,11} AMD3100 is under investigation for counteracting opioid-induced hyperalgesia produced by chronic treatment with morphine¹² and for treating pancreatic cancer. CXCR4 antagonists like the bicyclams and their metal complexes can act as anti-tumor agents by preventing malignant cells from interacting with the tumor microenvironment, making them sensitive to chemotherapy.¹³ AMD3100 has also been approved in the USA for stem cell mobilization.¹⁴ It is of interest to determine whether other macrocyclic polyamines and their metal complexes can be designed with improved antagonist activity for CXCR4. Interestingly, the manganese-containing metalloenzyme superoxide dismutase (SOD) has been reported to inhibit HIV replication,¹⁵ while an SOD mimic consisting of the manganese complex of a pyridine-fused macrocyclic polyamine (M40401,^{16,17} a dimethyl analog of M40403,^{18,19} Fig. 1b) was reported to inhibit apoptosis in HIV-infected cells by reducing oxidative stress.²⁰

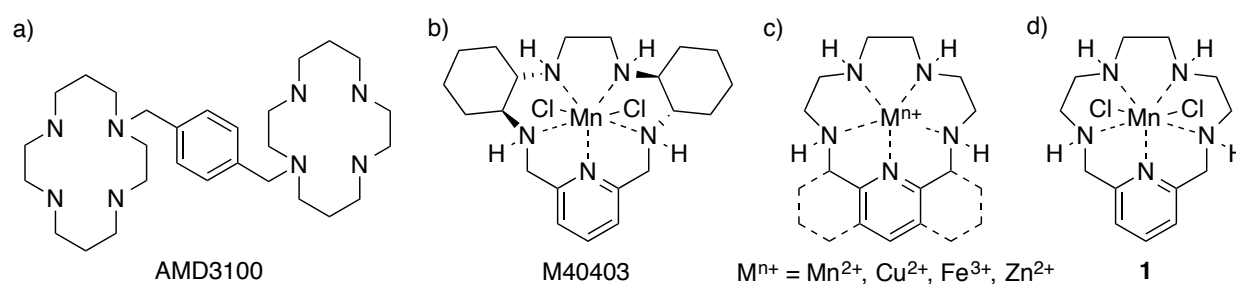


Fig. 1 Structures of bicyclam AMD3100 (a), SOD mimic M40403 (b), and compounds of interest in the current study (c), including parent MnCl_2 complex **1** (d).

M40403^{18,19} is one of a series of SOD mimetic manganese complexes investigated by MetaPhore Pharmaceuticals as potential therapeutic agents for inflammation and ischemia-reperfusion injury.^{21,22} Complexes of three metals (Fe, Mn, and Cu) are known to catalyze the

conversion of superoxide anion to oxygen and hydrogen peroxide, but manganese is the least toxic in mammals.^{21,22} Moreover, pyridine-containing macrocyclic polyamines were found to form very stable transition metal complexes, providing metabolic stability. Because of its high SOD-mimetic potency and favorable drug-like properties, M40403 was selected for clinical trials as an analgesic co-administered with an opioid for cancer pain and for prevention of dose limiting toxicities in treatment of melanoma or carcinoma with interleukin 2 (IL-2)²³ and was shown to be “safe and well-tolerated.”²⁴ M40403 was also found to prevent oral mucositis induced by radiation or chemotherapy in hamsters²⁵ and was approved in 2008 as an orphan drug for this use in cancer patients.²⁴ The purpose of this current study, based on the metal binding capacities of the bicyclams and their antiviral potency, was to determine whether metal complexes of macrocyclic polyamines similar to M40403 bind to CXCR4 and inhibit HIV-1 entry by this mechanism.

The new molecular scaffold of interest in the current study is illustrated in Fig. 1c. Here, the dashed lines indicate potential cyclohexane rings fused to the central pyridine ring. The parent manganese complex lacking additional fused rings, compound **1** (Fig. 1d), has been described previously.^{21,26-28} Compounds containing additional fused rings were designed to explore the effect of increased hydrophobicity on chemokine receptor interactions and to evaluate their potential CXCR4 binding and anti-HIV activity. Many cationic molecules bearing hydrophobic groups bind to the chemokine binding site of CXCR4 used for entry of HIV, and the chemokine CXCL12 binds to CXCR4 via a combination of electrostatic, hydrogen bonding and hydrophobic interactions.²⁹ Fusion of carbocyclic rings directly to the central pyridine ring in the target compounds facilitated chemical synthesis. Additional metal complexes in which the manganese atom is replaced by copper, iron, or zinc were of interest to determine if any anti-HIV activity is related to SOD mimetic activity, as zinc is not redox active and thus is not able to act as an SOD mimic.

Results and discussion

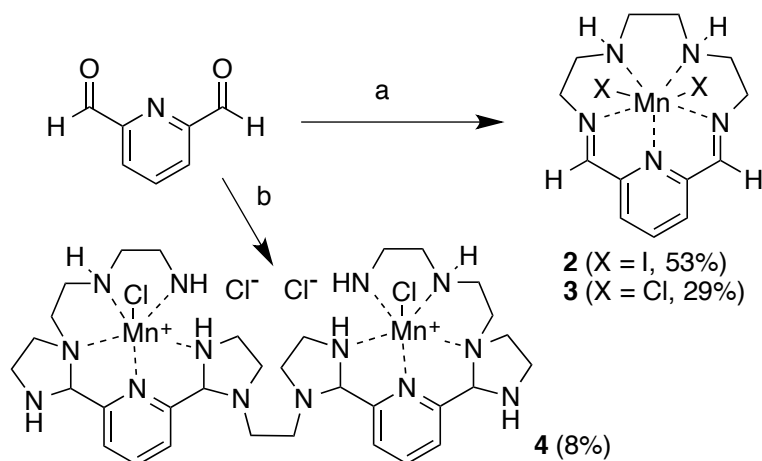
Chemistry

The initial synthetic target was the parent manganese complex **1**, lacking additional rings fused to the central pyridine ring (Fig. 1d). Two methods have been reported for synthesis of manganese complexes of pyridine-fused 15-membered macrocyclic pentamines: 1) Mn^{2+} templated condensation of 2,6-pyridinedicarboxaldehyde with triethylenetetramine to form the intermediate Schiff base macrocycle complex,³⁰ followed by reduction of the Schiff base moieties;²⁷ or, 2) synthesis of the metal-free macrocycle as described by Stetter³¹ (aka the Richman-Atkins method^{32,33}), followed by complexation of the manganese salt.^{21,26,28} The metal template method appears to work better for condensations of 2,6-diacetylpyridine,^{16,34–36} but it has been applied successfully to the corresponding dialdehyde by Dees *et al.* in their synthesis of **1**,²⁷ and by Malik *et al.* in their synthesis of Schiff base MnI_2 complex **2** (Scheme 1).³⁷ Drahos *et al.* later reported failure with Dees' method and used the Stetter approach.²⁸

The results of our efforts to prepare the Schiff-base precursor (**3**, Scheme 1) to macrocyclic manganese complex **1** are shown in Scheme 1. We first attempted to condense pyridine-2,6-dicarboxaldehyde³⁸ with triethylenetetramine by heating with manganese(II) chloride in methanol or ethanol, as described by Drew *et al.*,^{30,35} but we were not able to crystallize the desired product (**3**) from the crude mixture. Switching the template salt to manganese(II) acetate and adding aqueous potassium iodide enabled the isolation of the corresponding MnI_2 complex **2** in good yield.³⁷ A further attempt to prepare the MnCl_2 complex in methanol at room temperature resulted in the isolation of double Mn^{2+} complex **4** (Scheme 1), composed of two pyridine-2,6-dicarboxaldehyde and three triethylenetetramine moieties. The structures of **2** and **4** were determined by X-ray crystallography (*vide infra*). Dees *et al.* accomplished the two-step synthesis of **1** by condensation in boiling water,²⁷ without isolating intermediate Schiff-base complex **3**. By a variation of their method with methanol as a co-solvent, we were able to isolate the desired MnCl_2 complex **3** by column

chromatography. Attempts were made to template macrocyclizations with other metal cations (Cu^{2+} , Fe^{3+} or Zn^{2+}), but Mn^{2+} was most effective, so complexes of these metals were prepared later by demetallation of the Mn^{2+} complex and complexation of the free ligand.

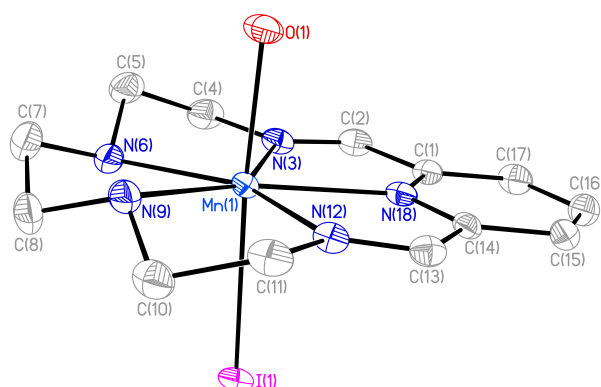
Scheme 1 Manganese(II) templated condensation of pyridine-2,6-dicarboxaldehyde with triethylenetetramine^a



^aReagents and conditions: (a) **2**: (1) $\text{Mn}(\text{OAc})_2$, $(\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2)_2$, MeOH, reflux, 6 h; (2) KI, H_2O , rt. **3**: MnCl_2 , $(\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2)_2$, MeOH, H_2O , reflux, 3 h. (b) MnCl_2 , $(\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2)_2$, MeOH, rt, 6 h.

Solid-state molecular structures of Mn^{2+} complexes **2** and **4** are shown in Fig. 2. Schiff-base macrocyclic MnI_2 complex **2** displays the expected seven-coordinate pentagonal bipyramidal geometry, although one of the axial iodide ligands is replaced by a water molecule and there is an additional non-coordinated water of crystallization. Halide ligands in Mn^{2+} complexes of pentadentate nitrogen macrocycles are known to dissociate from the metal center in aqueous solution.²⁷ In Mn^{2+} complex **4** the carbonyl groups form aminals, rather than imines, and the metal centers are only six-coordinate, perhaps due to steric congestion. Formation of such oligomeric aminals and other side products may limit yields and hinder crystallization of desired Mn^{2+} complexes of Schiff-base macrocycles.

a)



b)

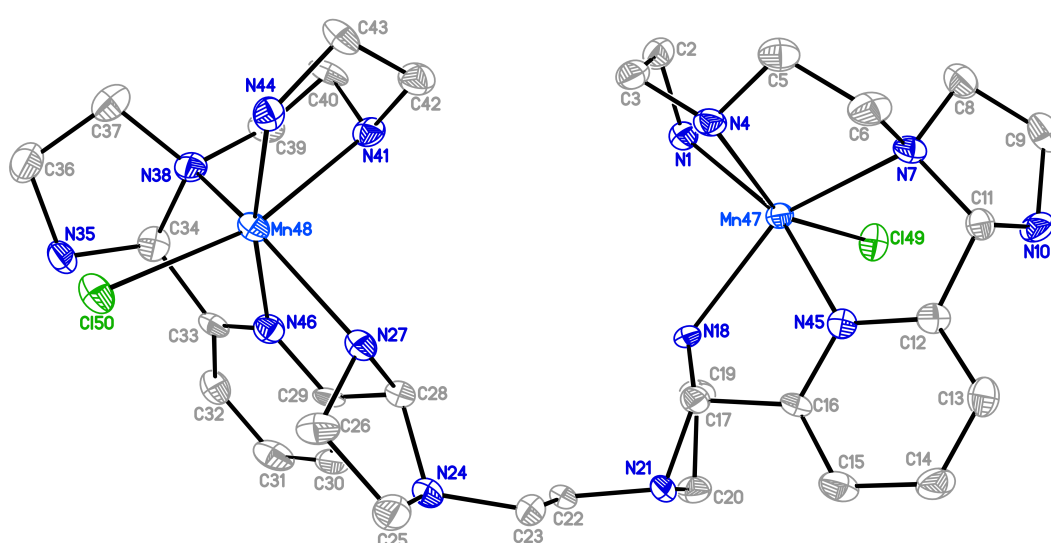
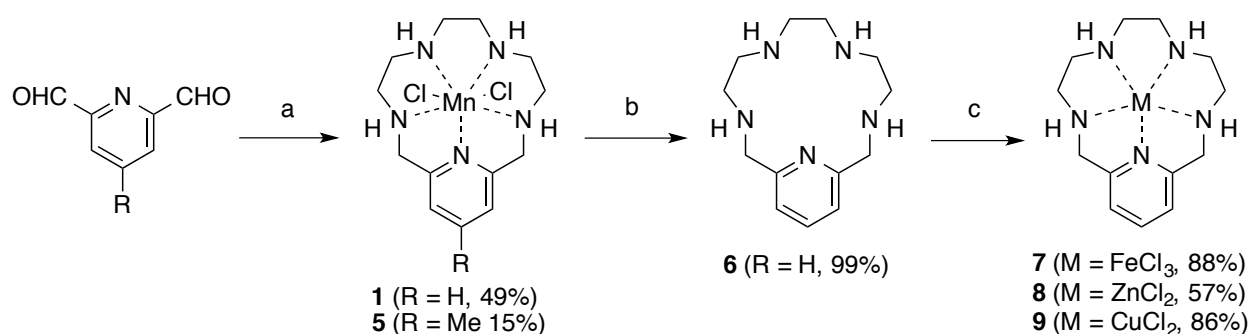


Fig. 2 Thermal ellipsoid representations of the Mn(II) complexes **2** (a) and **4** (b). Color scheme: C grey, N blue, O red, Cl green, I pink. Only the cationic portion of each complex is shown with an iodide and water omitted for **2** and two chlorides and two waters omitted for **4**. Hydrogen atoms have also been omitted for clarity. *Note:* the structure of **2** represents the molecule shown in Scheme 1 after replacement of one iodide ligand by water.

Syntheses of parent macrocyclic metal complexes lacking additional rings fused to the central pyridine ring are shown in Scheme 2. Since the yield of Schiff base macrocycle complex **3** was low and purification required column chromatography, crude **3** was reduced with sodium borohydride, as described by Dees *et al.*,²⁷ to give the parent MnCl₂ complex **1**. The yield of pure **1** for this two-step process was higher than for one-step synthesis of intermediate **3**, perhaps due to decomposition and/or solvent exchange of chloride during chromatography of **3**. The same sequence of reactions applied to 4-methyl-2,6-

pyridinediacarboxaldehyde³⁸ gave methyl substituted analog **5**, which was desired to test the effect of increased hydrophobicity. Metal-free parent ligand **6** was prepared in high yield by displacing Mn(II) from **1** with aq. HCl, followed by treatment with NaOH and filtration to remove precipitated metal salts. Reaction of **6** with FeCl₃, ZnCl₂, or CuCl₂ in ethanol gave corresponding complexes **7**, **8**, and **9** as precipitates in good to excellent yields.

Scheme 2 Syntheses of parent macrocyclic metal complexes and free ligand (**6**)^a

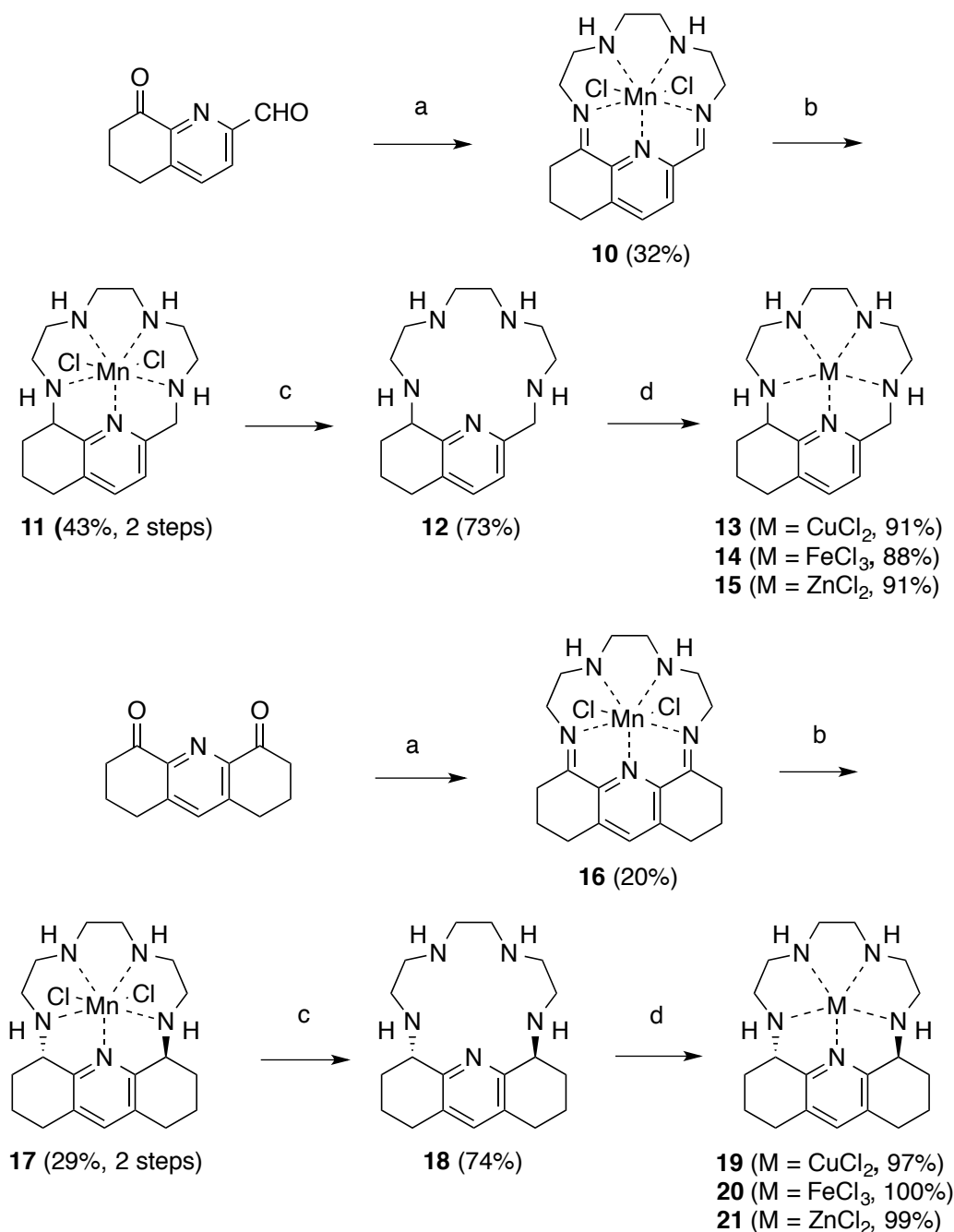


^aReagents and conditions: (a) (1) MnCl₂, (CH₂NHCH₂CH₂NH₂)₂, H₂O, reflux, 3 h; (2) NaBH₄, EtOH, 50 °C, 5 h; (3) LiCl, MeOH; (4) NaCl, H₂O. (b) (1) **1**, HCl, H₂O, rt, 30 min; (2) NaOH, H₂O. (c) M, EtOH.

Syntheses of metal complexes of macrocycles with one or two saturated six-membered rings fused to the pyridine ring (tetrahydroquinolines or octahydroacridines) are shown in Scheme 3. MnCl₂ templated condensation of 6,7-dihydro-8(5*H*)-quinolinone-2-carboxaldehyde³⁹ or 2,3,7,8-tetrahydroacridine-4,5-(1*H*,6*H*)-dione⁴⁰ with triethylenetetramine carried out in boiling water or aqueous ethanol afforded Schiff base macrocycle complexes **10** or **16** in modest yields after purification by column chromatography. As observed in the parent series, reaction of the crude intermediate with sodium borohydride gave the reduced products **11** or **17** in higher overall yield for two steps. Metal-free ligands **12** and **18** were obtained in good yields by the same protonation/deprotonation sequence used for parent ligand **6**, then the complexes of CuCl₂ (**13** and **19**), FeCl₃ (**14** and **20**), and ZnCl₂ (**15** and **21**) were prepared in excellent yields. All new compounds were characterized by mass spectrometry, infrared spectroscopy, and combustion microanalysis, as well as ¹H/¹³C NMR spectroscopy, except for complexes of the paramagnetic metal ions, Mn(II) and Cu(II). The

^1H and ^{13}C NMR spectra of zinc complexes **15** and **21** showed two species in solution, possibly a mixture in which one of the axial chloride ligands is replaced by water, as seen in the solid state structure of MnI_2 complex **2** (Fig. 2a).

Scheme 3 Syntheses of tetrahydroquinoline and octahydroacridine containing macrocyclic metal complexes and metal-free ligands (**12** and **18**)^a



^aReagents and conditions: (a) MnCl_2 , $(\text{CH}_2\text{NHCH}_2\text{CH}_2\text{NH}_2)_2$, heat. (b) (1) NaBH_4 , EtOH, 50 °C; (2) LiCl, MeOH. (c) (1) HCl, H_2O , rt, 30 min; (2) NaOH, MeOH. (d) M, EtOH.

Octahydroacridine-containing ligand **18** has two symmetry related stereocenters and therefore can exist in meso or d,l diastereomers. Single crystals of the of Mn(II) complex **17** were obtained and the crystal structure was determined by X-ray crystallography, showing only the d,l diastereomer. The solid-state structure of this complex shown in Fig. 3 displays pentagonal bipyramidal geometry about the metal center with two axial chloride ligands and proves the relative configuration of the ligand. Bulk samples of **17** appear to consist of only one isomer by tlc and hplc analysis. Free ligand **18** also apparently consists of a single isomer by NMR and chromatographic analysis, so the d,l ligand structure is assigned to all products **17** – **21**.

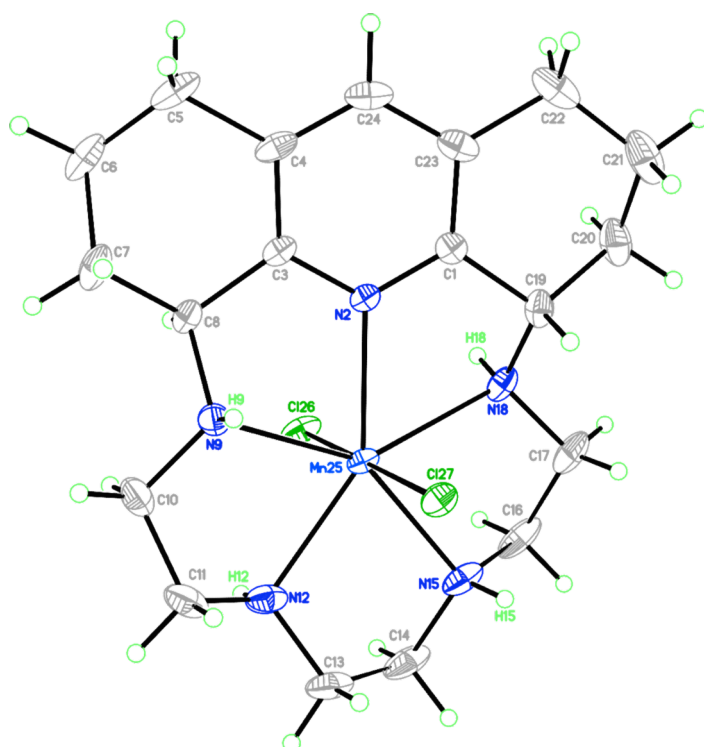


Fig. 3. Solid state structure of the d,l diastereomer of MnCl₂ complex **17**. Color scheme: C grey, N blue, Cl green.

Biological evaluation

To determine the ability of the SH compounds to inhibit the CXCL12/CXCR4 axis, a direct CXCL12 binding inhibition assay was performed with PBMCs (peripheral blood mononuclear cells) to study the competitive binding of the compounds on the CXCR4 receptor. The results are presented in Table 1. Manganese complex **11** with one fused six-

membered ring was the most potent inhibitor overall, with an IC_{50} value of 14 nM. The corresponding metal-free ligand (**12**) and its Cu, Fe and Zn complexes (**13-15**) were also active, but less potent (IC_{50} : 0.13 – 1.1 μ M). All of the corresponding compounds with no fused ring (**1**, **6** and **7-9**) were less potent. While Mn complex **17** with two fused rings was less potent than **11**, the Cu, Fe and Zn complexes in this series (**19-21**) were more potent than their single fused-ring analogs (**13-15**).

Table 1 Inhibitory effects of the compounds in CXCL12 chemokine binding and HIV-1 NL4-3 (X4) replication assays, as well as cellular toxicities.

Compound	CXCL12 binding	Anti-HIV-1 activity	Cytotoxicity
	IC_{50} (μ M) ^a	IC_{50} (μ M) ^b	CC_{50} (μ M) ^c
1	3.1 \pm 0.7	17.2 \pm 2.9	> 100
3	2.0 \pm 0.4	17.0 \pm 3.4	> 100
5	13.5 \pm 5.4	3.0 \pm 0.4	21.5
6	55.7 \pm 10.3	66.0 \pm 23.9	40.1
7	1.9 \pm 0.2	2.8 \pm 0.8	> 100
8	2.5 \pm 0.4	18.6 \pm 2.4	> 100
9	7.6 \pm 0.5	67.7 \pm 10.8	> 100
10	0.107 \pm 0.006	1.1 \pm 0.2	> 100
11	0.014 \pm 0.002	0.52 \pm 0.05	> 100
12	0.177 \pm 0.005	0.86 \pm 0.28	30.9
13	0.21 \pm 0.03	1.8 \pm 0.4	> 100
14	1.1 \pm 0.2	0.25 \pm 0.04	> 100
15	0.13 \pm 0.02	1.1 \pm 0.1	> 100
16	2.5 \pm 0.3	1.9 \pm 0.4	99.1
17	0.05 \pm 0.01	1.6 \pm 0.2	> 100
18	0.32 \pm 0.03	1.4 \pm 0.2	> 100

19	0.08 ± 0.02	0.40 ± 0.04	> 100
20	0.25 ± 0.08	0.20 ± 0.03	> 100
21	0.051 ± 0.005	0.27 ± 0.05	> 100

^a IC_{50} : 50% inhibitory concentration of the compounds to inhibit CXCL12-CXCR4 binding on PBMCs after 15 min of drug treatment.

^b IC_{50} : 50% inhibitory concentration of the compounds to inhibit HIV-1 NL4-3 replication in TZM-bl cells after 48 h of drug treatment.

^c CC_{50} : 50% cytotoxic concentration of the compounds in TZM-bl cells after 48 h of drug treatment.

Next, the *in vitro* anti-HIV potencies of the SH compounds towards the CXCR4-using (X4) HIV-1 strain NL4-3 were evaluated in a Tat-inducible, luciferase-based HIV-1 infection assay with the TZM-bl cell line. As seen from Table 1, the strongest inhibitory activity (IC_{50} : ca. 0.20 μ M) was noted for **14** and **20**, which are Fe³⁺ complexes with one or two fused rings, respectively. Zinc complex **21** in the two fused-ring series had equal potency to **14**, within experimental error. Cytotoxicity was also determined and the values are given in Table 1. The only significant toxicity issues were with the more polar metal-free polyamines **6** and **12** and the Mn²⁺ complex bearing a methyl group in the para position of the pyridine ring, **5**. Overall, the anti-HIV-1 potencies of the SH compounds generally increased with the degree of hydrophobicity imparted by the number of saturated six-membered rings fused to the pyridine ring in the complex. In the most hydrophobic series, complexation with Cu, Zn or Fe (in **19** – **21**) generally improved the antiviral activity of the complex as compared to Mn (in **17**), or the metal-free ligand (**18**). Other azamacrocyclic complexes of Zn²⁺ and Cu²⁺ have been reported to exhibit potent anti-HIV activity by binding CXCR4.⁴¹

The bicyclam AMD3100 was included in these experiments as a control, and gave IC_{50} values of 26 ± 3 nM for CXCL12 binding inhibition and 0.22 ± 0.02 nM for anti-HIV activity, thus showing approximately 100-fold greater potency in the anti-HIV assay than in the CXCL12 binding assay. This may be attributed largely to the experimental differences

between these two assays using different cell types. Interestingly, the potencies of the SH compounds are more similar in these two assays and in some cases the CXCL12 binding inhibition potencies are more than 10-fold higher than the anti-HIV potencies, particularly for the more hydrophobic Mn complexes **11** and **17**. This difference between relative potencies in the two assays suggests that the SH compounds may bind differently to the CXCR4 binding site, relative to AMD3100. The binding pocket of CXCR4 is relatively open and consists of two subpockets of different sizes.⁴² Several anionic amino acid side chains line the pockets, forming salt bridges with ligands, including CXCL12 and small molecules. It has been established that small molecules, including bicyclams, monocyclams, and noncyclams, have multiple modes of binding to the chemokine binding pocket.^{7,43} Finally, the submicromolar potencies observed for Zn complexes **15** and **21** and the more modest potencies of the metal-free ligands (**6**, **12**, and **18**) show that the CXCR4 binding and anti-HIV activities of these compounds do not depend on redox activity of the metal center and therefore are not related to SOD activity.

Conclusions

Three series of pyridine-containing pentaazacyclopentadecanes were synthesized by Mn²⁺-templated Schiff-base cyclizations of triethylenetriamine with various dicarbonyl compounds in which the pyridine ring was fused to zero, one, or two saturated six-membered rings. By evaluating these macrocyclic polyamines and their complexes with Mn²⁺, Cu²⁺, Fe³⁺, and Zn²⁺, we have discovered novel CXCR4-binding compounds with anti-HIV activity. The Mn²⁺ complex of a new pentaazacyclopentadecane with one fused carbocyclic ring (**11**) has the greatest potency as an inhibitor of the chemokine receptor CXCR4 (*IC*₅₀: 0.17 μM), as evidenced by inhibiting binding of CXCL12 to PBMCs (*IC*₅₀: 0.014 μM). Consequently, **11** inhibits replication of the CXCR4-using (X4) HIV-1 strain NL4-3 in the TZM-bl cell line with an *IC*₅₀ value of 0.53 μM and has low cytotoxicity (*CC*₅₀: >100 μM). In addition, 18

other compounds were evaluated for their interaction with CXCR4 and their interference with natural ligand binding and HIV entry and infection. Of these, the metal complexes of the most hydrophobic series with two fused carbocyclic rings exhibited the greatest potency. The Zn²⁺ complex (**21**) was among the most potent, showing that redox activity of the metal center is not associated with CXCR4 inhibitory activity. The molecular mode of binding between these compounds and CXCR4 has not yet been elucidated, but many CXCR4 inhibitors contain one or more pyridine rings and highly basic nitrogen atoms that are protonated at physiologic pH.^{3,7,37,38} The pyridine rings and the 2-3 positive charges of these new compounds suggest that they may bind in a similar manner.

CXCR4 is well established as a target of great importance for therapeutic compounds. Some of the experimental SH compounds, as described above, effectively disrupt the CXCL12-CXCR4 axis and also prevent the entry of HIV into cells expressing CXCR4. Taken together, results of the binding and anti-HIV assays performed in this article appear very promising. In the future, *in vivo* experiments will be needed to further validate the efficacy of these experimental SH compounds, and site directed mutagenesis together with molecular modeling and docking studies might be used to determine the manner in which these compounds interact with the ligand-binding pocket of CXCR4.

The authors declare no competing financial interest. D.S. and T.W.B. contributed equally.

Experimental section

Chemistry

All reactions were performed under an atmosphere of dry nitrogen. All starting materials, reagents, and solvents were used as purchased without further purification, unless otherwise noted. Anhydrous methanol, acetonitrile and THF were distilled from Mg powder, CaH₂ or Na, respectively. Triethylenetetramine hydrate used in Mn-templated condensation reactions

contained 4.7 eq. of H₂O by ¹H NMR analysis. Water used in the formation of metal complexes was deionized then distilled at atmospheric pressure. Solutions of 2 M HCl in methanol were prepared by adding 15 mL of con. aq. HCl to 75 mL of methanol. Silica (32-63 μm), from Sorbent Technologies was used for column chromatography. Samples for microanalysis were dried under vacuum (0.1 mm) at 78 °C for 48 hours, unless otherwise mentioned. Elemental analysis was performed by NuMega Resonance Laboratories. Melting points were measured in open glass capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Nicolet Protégé 460 spectrometer. ¹H and ¹³C NMR spectra were taken on Varian Unity Plus NMR instruments at the indicated operating frequencies. Mass spectrometry (MS) was performed with a Waters ZQ electrospray ionization (ESI) quadrupole micromass spectrometer (capillary 3.0 kV). X-ray crystallographic data were obtained on a Bruker APEX CCD diffractometer equipped at low temperature (100 ± 1 K) using Mo K α radiation ($\lambda = 0.71073 \text{ \AA}$) and a detector-to-crystal distance of 4.94 cm. Data collection for complexes was optimized utilizing the APEX2 software with a 0.5° rotation about ω between frames, and an exposure time of 10 s per frame. Data integration, correction for Lorentz and polarization effects, and final cell refinement were performed using SAINTPLUS, and corrected for absorption using SADABS. The structures were solved using direct methods followed by successive least squares refinement on F2 using the SHELXTL.⁴⁴ All non-hydrogen atoms were refined anisotropically and hydrogen atoms placed in calculated positions.

Synthesis of 3,6,9,12,18-pentaazabicyclo[12.3.1]octadeca-1(18),2,12,14,16-pentaene dichloromanganese(II) (3)

A mixture of 0.51 g (3.7 mmol) of pyridine-2,6-dicarboxaldehyde,³⁸ 0.74 g (3.7 mmol) of MnCl₂•4H₂O, 15 mL of water and 1 mL of methanol was stirred at 50 °C for 1.5 h. The resulting pale yellow solution was cooled to room temperature and stirred as a solution of 0.85 g (3.7 mmol) of triethylenetetraamine hydrate in 1 mL of water was added dropwise

over 10 min. The reaction mixture was boiled under reflux for 3 h then stood at room temperature overnight. The resulting brown mixture was filtered the filtrate concentrated to dryness by rotary evaporation (bath temp. 60 °C). The residue was dried (0.1 mm) overnight to give a reddish brown solid. A 1.21 g sample of the crude product was purified by column chromatography, eluting with 2.5:97.5 to 15:85 (v/v) methanol/dichloromethane, yielding 0.34 g (29%) of pure **3**. MS (ESI⁺) *m/z* 335 (M-Cl, 100%); 299 (M-H-2Cl, 50%). IR (neat, cm⁻¹) 3285 (w), 3232 (w), 3058 (w), 2915 (w), 2849 (w), 1653 (w), 1584 (w), 1455 (w), 1428 (w), 1327 (w), 1273 (w), 1183 (w), 1142 (w), 1106 (m), 1042 (s), 1013 (m), 973 (m), 876 (m), 858 (s), 826 (s), 807 (m), 796 (s), 753 (w), 660 (m), 637 (m). Anal. Calcd. for C₁₃H₁₉Cl₂MnN₅: C, 42.07; H, 5.16; N, 18.87. Found: C, 42.46; H, 5.31; N, 18.52.

Synthesis of 3,6,9,12,18-pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene dichloromanganese(II) (1)^{21,26–28}

A mixture of 4.44 g (32.9 mmol) of pyridine-2,6-dicarboxaldehyde,³⁸ 6.57 g (33.2 mmol) of MnCl₂•4H₂O, and 150 mL of water was stirred at 50 °C for 1 h. The resulting pale yellow solution was cooled to room temperature and stirred as a solution of 7.50 g (32.6 mmol) of triethylenetetraamine hydrate in 15 mL of water was added dropwise over a period of 50 min. The reaction mixture was boiled under reflux for 3 h then stood at room temperature overnight. The resulting brown mixture was filtered and the filtrate was concentrated to dryness by rotary evaporation (bath temp. 60 °C). The residue was dried (0.1 mm) overnight to yield the Schiff base-manganese complex, as a reddish brown solid. A solution of this intermediate in 300 mL of ethanol was stirred at room temperature as 10 g (0.27 mol) of NaBH₄ was added in two portions. The resulting suspension was stirred at room temperature for 2 h, then at 50 °C for 5 h. The solvent was removed by rotary evaporation, the residue was stirred for 10 h with 400 mL of a 0.5 M solution of LiCl in methanol, and the solvent was again removed by rotary evaporation. Water (180 mL) and 28 g (0.47 mol) of NaCl were added. The mixture was stirred until all solid dissolved, then extracted with

dichloromethane (4 x 50 mL). The combined extracts were dried (Na_2SO_4), filtered, and concentrated to dryness by rotary evaporation. Drying (0.1 mm) gave 7.75 g (63%) of a light brown solid. The crude product was purified by column chromatography, eluting with 9:1 (v/v) dichloromethane/methanol, yielding 6.09 g (49%) of **1** as a white solid, mp 255-265 °C (dec). MS (ESI⁺) m/z 339 (M-Cl, 65%), 152 (M-2Cl(2+), 100%). IR (neat, cm^{-1}) 3453 (w), 3276 (w), 3209 (w), 2897 (w), 2851 (w), 1602 (w), 1577 (w), 1457 (m), 1336 (w), 1297 (w), 1203 (w), 1118 (m), 1012 (m), 934 (w), 885 (m), 847 (m), 801 (s). Anal. Calcd. for $\text{C}_{13}\text{H}_{23}\text{Cl}_2\text{MnN}_5$: C, 41.61; H, 6.18; N, 18.67. Found: C, 41.46; H, 6.43; N, 18.87.

Synthesis of 3,6,9,12,18-pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene (**6**)

A solution of 1.21 g (3.23 mmol) of **1** in 40 mL of 2 M aq. HCl was stirred for 0.5 h at room temperature then concentrated to dryness by rotary evaporation (bath temp. 40 °C, 50-100 mm). The residue was stirred with 16.5 mL of 5 M aq. NaOH for 5 h, then 5 g of NaCl was added with stirring. The mixture was filtered and the filtrate was extracted with chloroform (1 x 20 mL, 2 x 10 mL). The combined extracts were dried (Na_2SO_4), filtered, and concentrated to dryness by rotary evaporation. Drying (0.1 mm) gave 0.80 g (99%) of **6** as a white solid, mp 62-65 °C. A sample for microanalysis was obtained by stirring 0.49 g of the solid in 20 mL of ether, filtering, rotary evaporating the filtrate, and drying the resulting residue (0.1 mm), yielding 0.26 g (32%) of pure **6** as a white solid, mp 64-65 °C. ¹H NMR (CDCl_3 , 400 MHz) δ 7.54 (t, $J = 7.6$ Hz, 1 H, H16), 7.03 (d, $J = 7.4$ Hz, 2 H, H15,17), 3.90 (s, 4 H, H2,13), 2.85 (m, 4 H, H4,11), 2.78 (m, 4 H, H5,10), 2.73 (s, 4 H, H7,8). ¹³C NMR (CDCl_3 , 100 MHz) δ 159.0, 136.8, 120.8, 54.1, 48.9, 48.8, 48.7. MS (ESI⁺) m/z 272 (M+Na, 20%), 250 (M+H, 100%). IR (neat, cm^{-1}) 3312 (w), 3273 (w), 3049 (w), 2870 (m), 2791 (m), 1671 (w), 1586 (w), 1572 (m), 1448 (s), 1129 (m), 1120 (m), 1070 (m), 1045 (m), 875 (m), 816 (m), 759 (m), 709 (s). Anal. Calcd. for $\text{C}_{13}\text{H}_{23}\text{N}_5$: C, 62.62; H, 9.30; N, 28.09. Found: C, 62.26; H, 9.03; N, 27.70.

Synthesis of 3,6,9,12,18-pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene trichloroiron(III) (7)

To a solution of 30 mg (0.12 mmol) of **6** in 2 mL of ethanol, a solution of 20 mg (0.12 mmol) of FeCl₃ in 3 mL of ethanol was added dropwise. The resulting mixture was swirled for 0.5 h at room temperature. The solvent removed by rotary evaporation. The residue was washed with 5 mL of ether then stirred with 60 mL of dichloromethane. Filtration, evaporation of the filtrate and drying the resulting residue (0.1 mm) gave 44 mg (88%) of **7** as a green solid. MS (ESI⁺) *m/z* 375 (M-Cl, 18%), 339 (M-H-2Cl, 100%), 303 (M-2H-3Cl, 30%). IR (neat, cm⁻¹) 3554 (w), 3379 (w), 3218 (w), 3148 (w), 2920 (w), 2870 (w), 1610 (m), 1578 (w), 1477 (m), 1451 (m), 1296 (w), 1277 (w), 1211 (w), 1160 (m), 1106 (m), 1090 (m), 1055 (m), 1030 (m), 1017 (m), 970 (s), 929 (s), 884 (m), 846 (m), 793 (m), 568 (m), 530 (m). Anal. calcd. for C₁₃H₂₃Cl₃ FeN₅•2H₂O: C, 34.88; H, 6.08; N, 15.65. Found: C, 35.18; H, 5.71; N, 15.93.

Synthesis of 3,6,9,12,18-pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene dichlorozinc(II) (8)

To a solution of 84 mg (0.34 mmol) of **6** in 0.5 mL of ethanol, a solution of 46 mg (0.33 mmol) of ZnCl₂ in 2 mL of ethanol was added dropwise. A white precipitate dissolved upon swirling. The solvent was removed by rotary evaporation to give a colorless oil. Diethyl ether (5 mL) was added and the resulting white solid collected by filtration, rinsed with 2 mL of ether and dried (0.1 mm). A solution of this solid in dichloromethane was washed with saturated aq. NaCl, dried (Na₂SO₄), filtered, and concentrated to dryness by rotary evaporation. The resulting residue was dried (0.1 mm), giving 75 mg (57%) of **8** as a white solid. ¹H NMR (CDCl₃, 500 MHz) δ 7.79 (t, *J* = 8 Hz, 1 H, H16), 7.21 (d, *J* = 8 Hz, 2 H, H15,17), 4.47 (br, 2 H, H2,13), 3.81 (br, 4 H, NH), 3.61 (br, 2 H, H2',13'), 3.22 (br, 6 H, H5,7,8,10), 2.69 (s, 6 H, H5',7',8',10'). ¹³C NMR (CDCl₃, 125 MHz) δ 154.5, 140.0, 121.6, 49.8, 47.1, 45.8, 44.1. MS (ESI⁺) *m/z* 348 (M-Cl, 90%), 312 (M-H-2Cl, 100%). IR (neat, cm⁻¹) 3474 (w), 3303 (w), 3240 (w), 3217 (w), 3175 (w), 3139 (w), 2912 (w), 2867 (w), 1626

(w), 1604 (m), 1581 (w), 1465 (m), 1439 (m), 1336 (w), 1119 (m), 1083 (m), 1020 (s), 959 (s), 941 (s), 796 (s). Anal. calcd. for $C_{13}H_{23}Cl_2N_5Zn \cdot H_2O$: C, 38.68; H, 6.24; N, 17.35. Found: C, 38.40; H, 6.42; N, 17.13.

Synthesis of 3,6,9,12,18-pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene dichlorocopper(II) (9)

To a solution of 89 mg (0.36 mmol) of **6** in 1.5 mL of ethanol, a solution of 48 mg (0.35 mmol) of copper(II) chloride in 1 mL of ethanol was added dropwise. The resulting mixture was swirled for 0.5 h at room temperature. The solvent was removed by rotary evaporation and residue was dried (0.1 mm) to give a blue solid. A solution of this solid in 9 mL of dichloromethane was filtered and the filtrate was evaporated to obtain 116 mg (86%) of **9** as a blue solid. A sample for microanalysis was prepared by slow diffusion of ether into the solution of the complex in 1 mL of ethanol. The resulting precipitate was filtered and dried (0.1 mm) to give 93 mg (69%) of pure **9** as a blue solid. MS (ESI⁺) *m/z* 347 (M-Cl, 15%), 311 (M-H-2Cl, 100%). IR (neat, cm^{-1}) 3324 (w), 3217 (w), 3142 (w), 3080 (w), 2881 (w), 1608 (w), 1584 (w), 1477 (s), 1452 (w), 1420 (m), 1357 (w), 1339 (w), 1076 (s), 1041 (s), 1012 (w), 983 (m), 942 (s), 915 (m), 844 (w), 831 (w), 809 (w), 778 (w), 675 (w). Anal. calcd. for $C_{13}H_{23}Cl_2 CuN_5 \cdot 0.5H_2O$: C, 39.75; H, 6.16; N, 17.83. Found: C, 39.31; H, 6.10; N, 17.85.

Biological Evaluation

CXCL12^{AF647} binding assay⁴⁵

Peripheral blood mononuclear cells (PBMCs) were isolated out of buffy coats from healthy donors, derived from the blood transfusion center (Red Cross, Belgium) by density centrifugation. The anonymous donors signed informed consent agreements allowing their blood to be used for scientific research. The cells were cultured in RPMI-1640 medium supplemented with 10% FCS and 1% l-glutamine. PBMCs were stimulated with 2 $\mu g/ml$

phytohemagglutinin (PHA) for 3 days at 37°C. The cells were washed once with assay buffer (Hanks' balanced salt solution with 20 mM HEPES buffer and 0.2% bovine serum albumin, pH 7.4) and then incubated for 15 min at room temperature with the SH compounds diluted in assay buffer. Subsequently, fluorescent CXCL12^{AF647} (25 ng/ml) was added to the compound-incubated cells. The cells were incubated for 30 min at room temperature. Thereafter, the cells were washed twice in assay buffer, fixed in 1% paraformaldehyde in PBS, and analyzed on a FACSArray flow cytometer equipped with a 635-nm red diode laser (Becton Dickinson, San Jose, CA, USA). The percentages of inhibition of CXCL12^{AF647} binding were calculated according to the formula: $[1 - ((\text{MFI} - \text{MFI}_{\text{NC}}) / (\text{MFI}_{\text{PC}} - \text{MFI}_{\text{NC}}))] \times 100$, where MFI is the mean fluorescence intensity of the cells incubated with CXCL12^{AF647} in the presence of the inhibitor, MFI_{NC} is the mean fluorescence intensity measured in the negative control (i.e., autofluorescence of unlabeled cells), and MFI_{PC} is the mean fluorescence intensity of the positive control (i.e., cells exposed to CXCL12^{AF647} alone).

HIV infection assay^{46,47}

TZM-bl cells were seeded in transparent 96-well plates at 10⁴ cells per well in DMEM (Dulbecco's Modified Eagle Medium; Life Technologies, Waltham, MA, USA) with 10% Fetal Bovine Serum (FBS) and 10 mM HEPES. Subsequently, compounds were added and the cell/compound mixture was incubated at 37 °C. After 30 min, virus was added at 100 pg p-24 per well. HIV-1 NL4-3 (CXCR4-using, X4) was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, NIAID, NIH). After 48 h of incubation, the assay plates were analyzed. For the analysis, steadylite plus substrate solution (PerkinElmer, Waltham, MA, USA) was added to the assay plates. The luminescent signal of the lysed cell suspension was analyzed in white 96-well plates on a SpectraMax L luminescence microplate reader (Molecular Devices, Sunnyvale, CA, USA) after a 10 min incubation period in the dark. Luciferase activity induced by HIV-1 Tat protein expression

was measured as an assessment of the amount of HIV replication. The 50% cytotoxic concentration (CC_{50}) of compounds was determined from the reduction of viability of uninfected TZM-bl cells exposed to the compounds, as measured by the MTS/phenazine ethosulfate (PES) method described above.

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