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Tetra-chloro-(bis-(3,5-dimethylpyrazolyl)methane)gold(III) chloride: An HIV-1 reverse transcriptase and protease inhibitor

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ABSTRACT

The title compound ([3,5-Me_2bpzaH$_2$][$AuCl_4$]Cl, **1**) (Me_2bpza = bis(3,5-dimethylpyrazolyl)acetic acid), was prepared by reacting H[$AuCl_4$] with 3,5-Me_2bpza; and spectroscopically and structurally characterized. In the solid state structure of **1**, the pyrazolyl ligand is doubly protonated to form two strong charge assisted hydrogen bonds of the type N^+—H···Cl^- with the single chloride anion whilst the [$AuCl_4$]$^-$ anion remains discrete. The anti-HIV-1 activity of **1** was determined by a colorimetric direct enzyme reverse transcriptase (RT) assay and a fluorogenic protease (PR) assay. Compound **1** significantly ($p < 0.05$) inhibited RT over a concentration range of 5–250 μM and inhibited HIV-1 protease at 100 μM. Compound **1** inhibited two very important HIV-1 enzymes (RT and PR) in direct enzyme assays and therefore warrants further evaluation.

1. Introduction

There currently is great interest in the identification of compounds exhibiting activity against human immunodeficiency virus (HIV) and the acquired immunodeficiency syndrome (AIDS) owing to the high mortality rates. The inhibition of reverse transcriptase (RT, the key enzyme involved in the conversion of viral RNA to DNA) is crucial for arresting the replication of HIV. One of the first potent anti-RT drugs was a nucleoside analog called 3′-azido-3′-deoxylthymidine (AZT) but the development of HIV strains resistant to this drug and others warrants the search for alternative RT inhibitors [1]. HIV protease is responsible for the maturation and production of infectious virions [2] and has also been a crucial target for anti-HIV drug development. The US food and drug administration has approved 21 anti-retroviral drugs to date with eight of them being nucleoside RT inhibitors and three non-nucleoside RT inhibitors [3]. These together with nine protease inhibitors and a fusion inhibitor are combined in drug cocktails to form highly active anti-retroviral therapy (HAART). HAART has so far helped to combat drug resistance, increase effectiveness of drugs [4] and has also led to a dramatic decline in morbidity and mortality from AIDS [5]. It is, however, still faced with shortcomings, such as not completely eliminating the disease [6]. HAART is also affected by drug resistance and intolerable side effects, for example lipodystrophy syndrome [7].

Metal-containing drugs like cisplatin have been shown to exert their therapeutic activities by blocking DNA synthesis [8]. For this reason metal compounds have also been probed for potential anti-HIV therapeutic actions. The few existing examples of metal-based anti-viral compounds include ruthenium complex Na_7-[Ru_4(μ-O)$_4$(C_2O_4)$_6$] [9,10], eilatin ruthenium complexes [11], and oxovanadium(IV) porphyrin complexes [10] which have been shown to possess anti-viral activity toward HIV-1. Anti-arthritic drugs aurothioglucose and aurothiomalate have been shown to possess anti-HIV activity specifically by inhibiting RT [12]. Gold cyanide, [$Au(CN)_2$]$^-$, a common metabolite in patients treated with gold based drugs, is also reported to exert anti-HIV activity by inhibiting RT [13]. In addition, clinical observations indicated that an AIDS patient, who was being treated with auranofin for psoriatic arthritis, experienced an increased CD4+ T lymphocyte count [14]. These types of reports have triggered interest in the use of metal-based compounds as anti-HIV agents. All the examples mentioned thus far are gold(I) based metallodrugs.

There is scant information on the use of gold(III) complexes as anti-HIV agents with the exception of the recently reported gold(III) complexes of porphyrins, Schiff-bases, bis(pyridyl)carboxamides and bis(pyridyl)sulfonamides [15], which have been shown to be very active anti-HIV compounds. Owing to our interest in

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synthesis of pyrazolyl based metal complexes for various therapeutic applications, we set out to explore the anti-HIV activity of bis(pyrazolyl)methanegold(III) compounds. In this paper we report the synthesis, characterization, and anti-HIV activity of the novel gold salt [3,5-Me_2bpzaH$_2$][$AuCl_4$]Cl (**1**) specifically targeting RT and PR. Notably, inhibition of HIV-PR has not been previously reported for gold. Gold(I) compounds have been shown to interact with sulfhydryl groups found in the active site of an enzyme [16] and consequentially we pursued the anti-protease activity of **1** with the hope that it would inhibit PR similarly or by some other mechanism.

2. Chemistry

2.1. Materials and Instrumentation

All commercial chemicals other than those described were used as received. Bis(3,5-dimethylpyrazolyl)methane [17] and $HAuCl_4{\cdot}4H_2O$ [18] were synthesized according to literature methods. All manipulations of air-and/or moisture sensitive compounds were performed under dry, deoxygenated nitrogen atmosphere using Schlenk techniques. IR spectra were recorded as nujol mulls using NaCl pellets on a Perkin-Elmer, paragon 1000 PC FTIR spectrophotometer. ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker Avance DPX 300 spectrometer (300 MHz) in $CDCl_3$ at room temperature. ^{1}H chemical shifts were referenced to the signals of the residual protons and carbon of the NMR solvents and are quoted in ppm. Splitting pattern; s = singlet.

2.1.1. Synthesis of tetra-chloro-(bis-(3,5-dimethylpyrazolyl)methane) gold(III)chloride (*1*)

To a solution of bis(3,5-dimethylpyrazolyl)methane (0.09 g, 0.44 mmol) in CH_2Cl_2 (20 mL) was added $HAuCl_4{\cdot}4H_2O$ (0.18 g, 0.44 mmol). The resultant suspension was stirred for 2 h leading to the formation of a clear yellow solution. This solution was concentrated to *ca.* 10 mL *in vacuo* and layered with hexane (4 mL) to produce golden crystals suitable for single crystal X-ray analysis. Yield = 0.20 g (74.1%). ^{1}H NMR: ($CDCl_3$): δ 7.61 (s, 2H, 2-pz); 6.42 (s, 2H, 4-pz); 5.96 (s, 2H, CH_2); 2.55 (s, 6H, 5-pz); 2.29 (s, 6H, 3-pz). ^{13}C{^{1}H} NMR: ($CDCl_3$): 146.8 (C(3-pz); 140.5 (C(5-pz)); 106.4 (C(4-pz)); 71.4 (C(CH_2)); 13.3 (C(3-CH_3)); 10.8 (C(5-CH_3)). IR (Nujol cm^{-1}): 3069 (υ_{N-H}), 1560 ($\upsilon_{C=N}$). Anal. Calc. for $C_{13}H_{24}AuCl_5N_4$: C, 25.57; H, 3.96; N, 9.18%. Found: C, 25.27; H, 3.88; N, 8.86%.

2.2. Crystal structure solution and refinement

A crystal of **1** was mounted in oil on a glass fiber and data collection was performed on a Bruker CCD-1000 diffractometer with Mo Kα (λ = 0.71073 Å) radiation and the diffractometer to crystal distance of 4.9 cm. The reflections were successfully indexed by an automated indexing routine built in the SMART program. These highly redundant datasets were corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equiv. measurements. A successful solution by the direct methods provided all non-hydrogen atoms from the *E*-map. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients [19].

3. Bioassays

3.1. Reverse transcriptase assay

The gold(III) complex **1** was tested for reverse transcriptase (RT) inhibitory activity against a purified recombinant HIV-1 RT (Merck, Darmstadt, Germany) using the cell free reverse transcriptase colorimetric kit (Roche Diagnostics, Mannheim, Germany). This assay is used to obtain a quantitative measure of the RT activity in cell culture supernatant and in direct enzyme inhibition screening assays. The assay takes advantage of the ability of RT to synthesize DNA starting from a template/primer hybrid poly (A) $\times$ oligo $(dT)_{15}$ and uses digoxigenin (DIG) and biotin labeled nucleotides. We used the enzyme directly by transferring 20 μL (0.2U) of it and 20 μL of reaction mixture {reconstituted template (template/primer hybrid poly (A) $\times$ oligo $(dT)_{15}$} and diluted nucleotide (tris–HCl (50 mM, (pH 7.8) with DIG-dUTP, biotin-dUTP and dTTP) to microfuge tubes containing 20 μL of samples (5–250 μM) followed by a 1 h incubation at 37 °C. The samples were then transferred to appropriate wells of streptavidin coated plates and incubated for 1 h at 37 °C. The plate was washed five times with 250 μL of wash buffer and 200 μL of anti-DIG-POD (antibody to DIG conjugated to peroxidase) working solution (200 mU/mL) added. A further incubation (37 °C, 1 h) was done followed by five rinses using wash buffer. A 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) substrate solution (200 μL) was transferred to all wells of the plate and incubated at room temperature (15–25 °C) for 15 min, which led to development of a sufficient green color for photometric detection. Control samples included an untreated sample of enzyme, reaction mixture only, and a reaction mixture with a known inhibitor of RT. The plate was read on a plate reader (Synergy, BioTek) at 405 nm with a reference wavelength of 492 nm. Percentage inhibition was calculated based on the formula: 100 − [(Test reagent absorbance/positive control absorbance) $\times$ 100)].

3.2. HIV protease assay

This assay makes use of a fluorogenic HIV protease substrate 1 with structure Arg-glu-(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys-(DABCYL)-Arg, (Sigma-Aldrich, Missouri USA) where EDANS is 5-(2-aminoethylamino)-1-napthalene sulfonate] and DABCYL is 4′-dimethylaminoazobenzene-4-carboxylate). This substrate is a synthetic peptide sequence that contains a cleavage site (Tyr-Pro) for HIV protease as well as two covalently modified amino acids for the detection of cleavage [20]. The assay was performed according to procedures by Lam et al. [21]. The fluorogenic substrate was dissolved in DMSO to make a 1 mM stock. The stock fluorogenic substrate was diluted to 16 μM using assay buffer (0.1 M sodium acetate, 1 M NaCl, 1 mM EDTA, 1 mM dithiothreitol and 1 mg/mL bovine serum albumin, pH 4.7). An aliquot of the substrate (16 μM, 49 μL) and 1 μL of HIV-1 PR solution (1 μg/μL; Bachem, Switzerland) were added to the reaction mixture in an assay buffer in the presence or absence (untreated control) of compound **1** to a final volume of 100 μL; with two concentrations (25 and 100 μM) of compound **1**. This mixture was incubated at 37 °C for 1 h in black 96 well fluorescence assay plates (Scientific group, South Africa). A 10 μg/mL of acetyl pepstatin (Bachem-UK) was used as a positive control for inhibition of HIV-1 protease. The fluorescence intensity was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a Synergy microplate spectrofluorometer (BioTek, Analytical & Diagnostic Products, South Africa). A blank treatment consisted of assay buffer only, whilst an untreated control of enzyme and substrate was also included. Data was analyzed using the Gen5™ software (ADP, South