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Cytotoxic gallium complexes containing thiosemicarbazones derived from 9-anthraldehyde: Molecular docking with biomolecules

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ABSTRACT

We have synthesized a trio of gallium complexes bearing 9-anthraldehyde thiosemicarbazones. The complexes were assessed for their anticancer activity and their biophysical reactivity was also investigated. The three complexes displayed good cytotoxic profiles against two human colon cancer cell lines, HCT-116 and Caco-2. The IC_{50} ranged from 4.7 to 44.1 μM with the complex having an unsubstituted amino group on the thiosemicarbazone being the most active. This particular complex also showed a high therapeutic index. All three complexes bind strongly to DNA via intercalation with binding constants ranging from 7.46×10^4 M^{-1} to 3.25×10^5 M^{-1}. The strength of the binding cannot be directly related to the level of anticancer activity. The complexes also bind strongly to human serum albumin with binding constants on the order of 10^4–10^5 M^{-1} as well. The complexes act as chemical nucleases as evidenced by their ability to cleave pBR322 plasmid DNA. The binding constants along with the cleavage results may suggest that the extent of DNA interaction is not directly correlated with anticancer activity. The results of docking studies with DNA, ribonucleotide reductase and human serum albumin, however showed that the complex with the best biological activity had the largest binding constant to DNA.

1. Introduction

A number of metal ions, by way of coordination complexes, are currently being studied for use as chemotherapeutics. Among these metals, platinum and ruthenium are probably the most well-known. However, gallium is considered to be the second metal, (after platinum), to have generated much research interest [1], with detailed investigations occurring from the 1970s [2–7]. The attractiveness of gallium as a therapeutic agent stems primarily from its biological and chemical mimicry of iron. Gallium(III) has approximately the same charge density as iron(III) and show similar coordination behavior. Gallium salts are not readily bioavailable and various mechanisms have been proposed to explain the biological activity. Gallium affects iron-dependent processes as it competes with iron for binding to transferrin. This fact is also useful since cancer cells typically over-express transferrin receptors so that gallium may be delivered to such cells specifically and in high concentration. Gallium also interacts with ribonucleotide reductase [1], a key enzyme in DNA synthesis, by displacing iron [8,9].

The use of gallium salts, at least when administered orally, has its disadvantages. Gallium nitrate is used to treat hypercalcemia. However, it has poor pharmacokinetic properties as a result of its ease of hydrolysis. In general, stabilization of Ga(III) relative to hydrolysis can be achieved by coordination to chelating organic ligands. This could also possibly have the effect of increasing bioavailability and lipophilicity with an overall improvement in cytotoxicity [10]. Various types of ligand systems bearing N-, O- and/or S- donor atoms have been used. The most promising complexes being studied are based on the quinoline (tris(8-quinolinolato)gallium(III) designated as KP46) and maltol (tris(3-hydroxy-2-methyl-4H-pyran-4-onato)gallium(III)) scaffolds [11,12]. KP46 have successfully completed Phase I clinical trials [1].

One other common ligand scaffold that is being explored is the thiosemicarbazone system [13–15]. These ligands are known to be bioactive, showing a diverse range of biological behaviors including anticancer [16], antibacterial [17], and antiviral [18]. The biological properties of these chemicals can be modified by linkage to metal ions [19–21]. In this paper, we report on the synthesis of a series of three gallium complexes containing thiosemicarbazones from 9-

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anthraldehyde. We are reporting the results of the initial bioassays of these complexes versus two human colon cancer lines. In addition, their biophysical reactivity with DNA and human serum albumin was investigated and those results are also presented. To complete the paper, we also report on the results of molecular modelling of the complexes interacting with three potential biological targets.

2. Experimental

2.1. Material and methods

Analytical or reagent grade chemicals were used throughout. All the chemicals including solvents were obtained from Sigma-Aldrich or other commercial vendors and used as received. Microanalyses (C, H, N) were performed by Galbraith Laboratories, (Knoxville, TN). Proton NMR spectra were recorded in dimethylsulfoxide-d_6 on a Varian VNMRS-400 spectrometer operating at room temperature. The residual ^{1}H and ^{13}C present in DMSO-d_6 (2.50 and 39.51 ppm respectively) were used as internal references. IR spectra in the range 4000–500 cm^{-1} were obtained using the ATR accessory on a Nicolet 6700 FTIR spectrophotometer. Absorption spectra were recorded on an Agilent 8453A spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer. Viscosity studies were done using a Cannon-Manning semi micro-dilution viscometer (type 75, Cannon Instruments Co., State College, PA, USA). The conductivity measurements were made on an Accumet AB200 m. ESI-MS was recorded on an Advion CMS-L mass spectrometer.

2.2. Syntheses

The ligands were synthesized as previously described [22,23]. The complexes were synthesized as follows: The ligand, (3 molar equivalents), was suspended in approximately 25 mL of ethanol and the suspension heated to boiling. In the case of compound **3**, an equimolar amount (based on the thiosemicarbazone), of potassium methoxide was added to the reaction. To the boiling mixture was added drop-wise a solution of $Ga(NO_3)_3 \cdot xH_2O$ (1 M equivalent) in 5 mL ethanol. The yellow mixture was heated at reflux for 5 h during which time it became a bright yellow-orange color. The reaction mixture was allowed to cool to room temperature and then filtered to remove a small amount of a yellow solid. The filtrate was stripped of the solvent and the yellow-orange solid that resulted was washed with a small amount of water followed by extensive ether washes and then drying at the vacuum line.

Ga(ATSC)$_3$, ***1***. Yield: yellow solid, 161 mg (44%). Elemental analysis for $C_{48}H_{36}GaN_9S_3$; calc./found: C 63.72/63.33, H 4.01/4.47, N 13.93/14.24. IR (cm^{-1}): 3436, 3212, 3152, 2981, 1599, 1585, 1484, 1407, 1282, 1161, 1070, 943, 883, 842, 823, 782, 730. ^{1}H NMR (ppm): 11.65, 9.35, 8.69, 8.58, 8.56, 8.31, 8.29, 8.18, 7.71, 7.56–7.66.

Ga(EtATSC)$_3$·0.25C$_2$H$_5$OH ***2***. Yield: yellow solid, 248 mg (67%). Elemental analysis for $C_{54.5}H_{49.5}GaN_9O_{0.25}S_3$; calc./found: C 65.43/65.71, H 4.99/5.38, N 12.60/12.67. IR (cm^{-1}): 3354(w), 3342, 3153, 2978, 2929, 2876, 1623, 1533, 1473, 1416, 1299, 1265, 1219, 1158, 1087, 1047, 1018, 943, 891, 840, 812, 785, 729. ^{1}H NMR (ppm): 11.65, 9.28, 8.70, 8.50, 8.48, 8.23, 8.25, 7.55–7.65, 3.58, 1.16.

Ga(PhATSC)$_3$·H$_2$O ***3***. Yield: yellow solid, 185 mg (36%). Elemental analysis for $C_{66}H_{50}GaN_9OS_3$; calc./found: C 68.87/69.18, H 4.38/4.72, N 10.95/11.13. IR (cm^{-1}): 3309, 3129, 3046, 2985, 1622, 1593, 1541, 1531, 1442, 1416, 1398, 1304, 1259, 1194, 1069, 1017, 936, 889, 841, 783, 733. ^{1}H NMR (ppm): 12.01, 9.99, 9.41, 8.75, 8.59, 8.16, 7.56–7.66, 7.28–7.34.

2.3. Cell culture

The cell lines were two human colon cancer cells: HCT116 (human colon carcinoma) and Caco-2 (human epithelial colorectal adenocarcinoma). In addition, normal human colon cells CCD-18Co (human colon fibroblasts), were included. All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained at the University of Rhode Island. Caco-2 cells were grown in EMEM medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1% v/v antibiotic solution (Sigma). HCT-116 cells were grown in McCoy's 5a medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 2% v/v HEPES and 1% v/v antibiotic solution. CCD-18Co cells were grown in EMEM medium supplemented with 10% v/v fetal bovine serum, 1% v/v nonessential amino acids, 1% v/v L-glutamine and 1% v/v antibiotic solution and were used from PDL = 26 to PDL = 35 for all experiments. (PDL is the population doubling levels). The cells were maintained at 37 °C in an incubator under a 5% CO_2/95% air atmosphere at constant humidity and maintained in the linear phase of growth. The pH of the culture medium was determined using pH indicator paper (inside the incubator. The complexes were solubilized in DMSO (<0.5% in the culture medium) by sonication and were filter-sterilized (0.2 μm) prior to addition to the culture media. Control cells were also run in parallel and subjected to the same changes in medium.

2.4. Cytotoxicity assay

The assay was carried out as described previously [24] to measure the IC50 values for the complexes as reported before [22].

2.5. DNA-interaction studies

All the experiments involving the interaction of the complexes with calf-thymus (ct) DNA were carried out in TRIS buffer (5 mM Tris, 50 mM NaCl, pH 7.20). Stock solutions of ct-DNA were prepared by dissolving commercial nucleic acids in buffer and stored at 4 °C for 24 h. The DNA solution was diluted appropriately and the concentration of the diluted solutions (per nucleotide phosphate) was determined spectrophotometrically using the molar absorption coefficient of 6600 M^{-1} cm^{-1} at 260 nm [25]. The purity of the solutions was checked by observing a ratio of ≥1.8 for the absorbances at 260 nm–280 nm [26]. The DNA stock solutions were stored at 4 °C and used within 4 days after their preparation. Milli-Q water (18.2 μS/cm) was used in all experiments.

2.5.1. Viscosity measurements

The viscosity of DNA solutions was measured in the presence and absence of the complexes, in a water bath maintained at 31.0 ± 0.1 °C. The DNA concentration in each solution was 100 μM, while the complex concentration was varied from 0 to 40 μM. Data are presented as $(\eta/\eta_0)^{1/3}$ versus 1/R, where R = [DNA]/[complex], η is the viscosity of DNA in the presence of the complex and η_0 is the relative viscosity of DNA alone. Relative viscosity values were calculated from the observed flow time of DNA solution (t) and for the flow time of buffer (t_0), using the expression $\eta_0 = (t - t_0)/t_0$. Flow time of each sample was measured three times and an average flow time was used.

2.5.2. Absorbance titration experiments

Spectroscopic titrations were carried out at room temperature to determine the binding affinity between the complexes and ct-DNA. A constant concentration of the complexes (1.0×10^{-5} M) was treated with aliquots of a concentrated solution of the DNA.