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Inhibition of the cathepsin cysteine proteases B and K by square-planar cycloaurated gold(III) compounds and investigation of their anti-cancer activity

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ABSTRACT

Gold(III) compounds have been examined for potential anti-cancer activity. It is proposed that the molecular targets of these compounds are thiol-containing biological molecules such as the cathepsin cysteine proteases. These enzymes have been implicated in many diseases including cancer. The catalytic mechanism of the cathepsin cysteine proteases is dependent upon a cysteine at the active site which is accessible to the interaction of thiophilic metals such as gold. The synthesis and biological activity of square-planar six-membered cycloaurated Au(III) compounds with a pyridinyl-phenyl linked backbone and two monodentate or one bidentate leaving group is described. Gold(III) cycloaurated compounds were able to inhibit both cathepsins B and K. Structure/activity was investigated by modifications to the pyridinyl-phenyl backbone, and leaving groups. Optimal activity was seen with substitution at the 6 position of the pyridine ring. The reversibility of inhibition was tested by reactivation in the presence of cysteine with a bidentate thiosalicylate compound being an irreversible inhibitor. Five compounds were evaluated for *in vitro* cytotoxicity against a panel of human tumor cell lines. The thiosalicylate compound was tested *in vivo* against the HT29 human colon tumor xenograft model. A modest decrease in tumor growth was observed compared with the untreated control tumor.

1. Introduction

With the introduction of the monomeric orally bioavailable anti-arthritic gold compound auranofin in 1985, and the success of the platinum-based anti-cancer drugs, there has been a great deal of interest in the use of gold compounds for cancer therapy [1–5]. There were early indications that auranofin had limited anti-tumor activity in *in vitro* systems and in the P388 leukaemia *in vivo*, however it was inactive against solid tumors. These observations led to the evaluation of related tertiary phosphine gold(I) complexes with thiosugar ligands. These also were found to be active against both the P388 leukaemia and B16 melanoma *in vitro* and P388 *in vivo* but again were inactive against a range of solid tumor models. More promising indications were achieved with a series of μ[-bis(diphenylphosphine) ethane]digold complexes. The bis(diphenylphosphine)ethane (dppe) entity is itself active, but the digold complexes were shown to have increased activity. The lead compound [dppe$(AuCl)_2$] was shown to rearrange to give the more stable tetrahedral complex $[Au(dppe)_2]^+$ [2,6,7]. Though this compound had marked activity against the P388 murine leukaemia [8], it had limited activity against solid tumor models. In spite of these early promising indications this compound was not entered for clinical trials due to problems with cardiotoxicity highlighted during pre-clinical toxicology studies [9].

The mechanism of action of the gold anti-arthritic drugs is most likely multi-factorial, resulting in modulation of the immune system, however early evidence suggested that the anti-tumor mechanism was due to the interaction with DNA [3]. Recent evidence, however, suggests alternative molecular targets for gold compounds with potential anti-tumor activity. These targets include histone deacetylase, mitochondria, and thioredoxin reductase [10–13].

Much of the early search for gold anti-tumor agents focused on Au(I) primarily because of the reactivity of Au(III) and resultant poor stability and toxicity. Our early work focused on Au(III). Au(III) was chosen as its complexes are square planar, with gold(III) being isoelectronic with Pt(II) [14]. The gold(III) structure was stabilized by using a single mononegative bidentate chelating ligand, 2-[(dimethylamino)methyl] phenyl, (damp). This ligand forms part of a 5-membered chelate ring with gold(III) in which the nitrogen of the amine and the carbon of the phenyl ring bond to the metal. The remaining two coordination positions are taken up by either two monodentate ligands such as chloride, acetate, or one bidentate ligand such as oxalate, malonate, and thiosalicylate [14,15]. These ligands are readily hydrolyzed allowing substitution reactions with biological molecules. In addition we adopted an integrated approach in our search for new metal-based cancer drugs.

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This incorporated inorganic medicinal chemistry, *in vitro* screening using panels of human tumor-derived cell lines, *in vivo* testing using human tumor xenograft models, and mechanistic studies [16]. Compounds of this class were shown to be selectively cytotoxic across the tumor cell line panel, and the acetate complex was shown to have modest anti-tumor activity against *in vivo* tumor xenografts [15]. However, studies on the mechanism of action of the acetate complex revealed that the mechanism of action was significantly different from that of cisplatin, and that DNA was not the molecular target for the Au(III) complexes indicating that the analogy of d^8, square planar Au(III) and Pt(II) complexes do not extend to their interaction with cells [15]. ^{13}C NMR studies of model reactions with biological ligands showed that the damp complexes have a preference for S-donor ligands such as glutathione and cysteine, with only limited reactivity against nucleosides and their bases [17]. Though the activity of these compounds was modest compared with the platinum complexes the evidence suggested a new structural class of metal-based anti-tumor agents with a novel mechanism of action.

We therefore hypothesized that the molecular target for these compounds was a thiol-containing molecule. To this end we examined the inhibitory effect of these complexes on cathepsin cysteine proteases. Cysteine proteases have been implicated in the pathophysiology of several diseases [18–20] including inflammatory airway diseases [21], bone and joint disorders [22,23], parasitic diseases [24–26], and cancer [27–30]. The most extensively studied lysosomal cysteine protease is cathepsin B. Cathepsin B is capable of degrading components of the extracellular matrix in such diseases as muscular dystrophy and rheumatoid arthritis. Cathepsin B expression is located at the outer edge of solid tumors suggesting it is involved in degrading the tumor extracellular matrix thus playing a role in tumor metastasis [29]. Increased expression and secretion of cathepsin B have been shown to be associated with numerous human and experimental tumors, and have been proposed to be a prognostic marker for several types of cancer [31]. The exact role for cathepsin B in solid tumors has yet to be defined, but it has been proposed to be involved in metastasis, angiogenesis, and tumor progression [32]. Carcinoma cell invasion and metastasis can be inhibited by the nonspecific, irreversible, cysteine protease inhibitor E-64 [33]. Cathepsin B therefore presents itself as a possible therapeutic target for the control of tumor progression [34].

Cathepsin K is a validated drug target for bone diseases such as osteoporosis [35]. It is found in osteoclasts and plays a role in bone resorption [23,36–38]. A clear implication of cathepsin K in bone disease came from the observation that mutations in cathepsin K were responsible for the autosomal, recessive bone disorder pycnodysotosis characterized by osteosclerosis and short stature [39]. Cathepsin K has become a target for bone diseases such as osteoporosis and the identification of cathepsin K inhibitors has been the focus of intensive efforts by several laboratories in both industry and academia [40,41].

The cathepsin cysteine proteases contain a cysteine at their active site [19]. This cysteine acts in concert with a histidine, the two together forming a stable thiolate-imidazolium ion pair. Nucleophilic attack by the thiolate on the carbonyl carbon of the peptide bond results in the formation of an acyl intermediate. The collapse of the acyl intermediate results in the release of the cleaved peptide and regeneration of the active site. The cysteine thiol pKa of 8.3 is significantly reduced in the cathepsin active site due to the proximity of the active site histidine [42]. This results in a completely ionic thiolate group thus favoring the interaction of thiophilic metals such as gold, which have a high affinity for thiolates with a low pKa. We therefore hypothesized that a metal complex could inhibit a cysteine protease by ligand exchange with the thiol of the active site cysteine [43]. We have subsequently described a series of rhenium(V) oxorhenium complexes with inhibitory activity against cathepsin B which interacts with the thiol of the active site cysteine [44,45]. We therefore set out to investigate the activity of the Au(III)(damp) complexes against cathepsins B and K. In order to improve upon the modest anti-tumor activity observed with these complexes, and to explore their activity against cathepsin cysteine proteases, attempts were made to make further analogues of these structures. However, this was found to be synthetically challenging so focus was shifted away from 5-membered to 6-membered chelate rings. In this paper we describe the synthesis of square-planar cycloaurated Au(III) compounds, and compare their inhibitory effects against the two cathepsin cysteine proteases cathepsins B and K. We subsequently investigated their potential as anti-tumor agents evaluating *in vitro* cytotoxicity against a panel of human tumor cell lines and *in vivo* activity in a human tumor xenograft model.

2. Methods

2.1. Chemistry

All reactions were carried out under ambient conditions unless otherwise noted. Diethyl ether and tetrahydrofuran (THF) were dried by refluxing over sodium in the presence of benzophenone. $NaAuCl_4 \cdot 2H_2O$ (containing 49.2−49.6% Au), $AgSO_3CF_3$, Ni(dppp)Cl_2 (dppp = 1,3-bis(diphenylphosphino)propane), 2-anilinopyridine 2-bromopyridine, 2-bromo-3-methylpyridine, 2-bromo-4-methylpyridine, 2-bromo-5-methylpyridine, 2-bromo-6-methylpyridine, 2-bromo-3-methoxypyridine, 2-bromo-6-methoxypyridine, 2-(4-chlorobenzyl)pyridine, 2-bromo-3-pyridinol, iodomethane, 2-bromopropane, 1bromobutane, 1-bromohexane, benzyl bromide, benzyl chloride, 3-methoxybenzyl bromide, 2-methoxybenzyl chloride, propionyl chloride, butyric anhydride, 4-fluorobenzoyl chloride, 4-methoxybenzoyl chloride, tert-butyldimethylsilyl chloride, 2-methylbenzyl alcohol, chloroacetone, p-cresol, 3-aminopropionitrile, benzylmethylamine, bis-(2-methoxy-ethyl)amine, butylamine, diethylamine, diisopropylamine, glycine methyl ester hydrochloride, isopropylamine, pyrrolidine, piperidine, BuLi (2.5 M in hexanes), MeMgCl (3.0 M in THF), EtMgCl (3.0 M in THF), PhMgCl (3.0 M in THF), *N*-bromosuccinimide (NBS), 2,2-azobisisobutyronitrile (AIBN), $SOCl_2$, PBr_3, Mg turnings, and $[Bu_4N]F \cdot 3H_2O$ were all purchased from commercial sources. 6-Phenoxypyridin-3-ylamine, 6-phenoxy-nicotinic acid, 5-bromo-2-bromopyridine and 5-methyl-2-phenoxy pyridine were prepared following the procedures developed previously. ^1H and ^{13}C measurements were performed on a Bruker Avance 300 spectrometer. NMR spectra were referenced to residual solvent (^1H and ^{13}C with δ(tetramethylsilane (TMS)) = 0 ppm). The splitting of proton resonances in the reported ^1H NMR spectra is defined as s = singlet, d = doublet, t = triplet, and m = multiplet, dt = doublet of triplet, and dd = doublet of doublet. Mass spectra were obtained on a Bruker Esquire-LC 00052 mass spectrometer with an electrospray interface. Elemental analyses were performed at Atlantic Microlab, Inc. Norcross, Georgia, USA.

The ((dimethylamino)methyl)phenyl compounds **1**–**3** [15] and compounds **4** [25] **7**, **9**, **10**, **29**, **30**, **31**, **32**, **33** [46] **37** and **38** [25] were synthesized as previously described. The remaining novel compounds were prepared using a general procedure for cycloauration, as illustrated in Fig. 1 Scheme 1 and as reported previously [47], with the exception of compound **6** which was obtained with a modified method as shown in Fig. 1 Scheme 2.

The general method for cycloauration is as follows. A solution of the pyridine ligand (HL) (1.00 mmol) in CH_3CN (5 mL) was added to a solution of $NaAuCl_4$ (1.00 mmol) in H_2O (20 mL), forming a yellow suspension. The suspension was stirred at room temperature (10 min-24 h depending on the compound), and a yellow solid was collected by filtration. After being washed with H_2O and a small amount of diethyl ether the yellow solid was suspended in mixed CH_3CN/H_2O (50 mL, 1/5 in v/v). The suspension was stirred at room temperature for 10 min, and was then heated at reflux. The reaction mixture was cooled to room temperature, and a solid was collected by filtration and washed with H_2O. The solid was extracted with hot acetone or CH_2Cl_2, and the extract was filtered through a filter paper