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# Rational design of gold(III)-dithiocarbamato peptidomimetics for the targeted anticancer chemotherapy

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ABSTRACT

As a further extension of our research work focusing on the development of gold(III)-dithiocarbamato dtc derivatives of oligopeptides as potential anticancer agents, complexes [$Au^{III}X_2$(dtc-Sar-L-Ser(*t*-Bu)-O(*t*-Bu))] (X = Br (**1a**)/Cl (**1b**)), [$Au^{III}X_2$(dtc-AA-$Aib_2$-O(*t*-Bu))] (AA = Sar (sarcosine, *N*-methylglycine), X = Br (**2a**)/Cl (**2b**); AA = D,L-Pro, X = Br (**3a**)/Cl (**3b**)), [$Au^{III}X_2$(dtc-Sar-$Aib_3$-O(*t*-Bu))] (X = Br (**4a**)/Cl (**4b**)), and [$Au^{III}X_2$(-dtc-Sar-$Aib_3$-Gly-OEt)] (X = Br (**5a**)/Cl (**5b**)) (Aib = "alpha"-aminoisobutyric acid, 2-methylalanine) were designed to obtain metal-based peptidomimetics that may specifically target two peptide transporters (namely, PEPT1 and PEPT2) upregulated in several human tumor cells. All the compounds were characterized by means of FT-IR and mono- and multidimensional NMR spectroscopy. According to *in vitro* cytotoxicity studies, complex [$Au^{III}Cl_2$(dtc-D,L-Pro-$Aib_2$-O(*t*-Bu))] (**3b**) turned out to be the most effective toward the four human tumor cell lines evaluated (PC3, 2008, C13, and L540), for which the $IC_{50}$ values were lower than cisplatin. Remarkably, it showed no cross-resistance with cisplatin itself and was proved to inhibit tumor cell proliferation by inducing almost exclusively late apoptosis/necrosis. Biological results are here reported and discussed in terms of the structure–activity relationship.



## 1. Introduction

Notwithstanding the therapeutic efficacy, conventional anticancer agents suffer from several drawbacks including high toxicity, chemical instability, unsuitable pharmacokinetic and pharmacodynamic profiles, no oral bioavailability, and poor specificity [1]. Consequently, much effort has been recently focusing on shifting from cytotoxic non-specific chemotherapies to molecularly-targeted rationally designed drugs [2], because of the potential for minimizing unwanted side-effects as well as for improving tumor selectivity while retaining the desirable therapeutic effectiveness [3].

Tumor cells express many biomarkers and receptors that can be specifically targeted. Thus, current research has been devoted to the development of efficient and innovative delivery systems in which conjugated drugs incorporate a tumor targeting group (carrier) and a cytotoxic agent (cargo). These systems should ideally reach the tissue of interest and deliver their cargo directly into the cells where it can exert its therapeutic activity [4].

So far, a number of strategies have been suggested as drug delivery systems, including liposomes, nanoparticles and polymers, but very few have reached the preclinical or clinical trial stage [5–8]. Among them, drug functionalization with specific peptides may offer higher flexibility over other approaches [9], and, accordingly, much attention has been recently given to peptide-based delivery systems targeting peptide transporters (PEPTs). The proton-coupled peptide transporters PEPT1 and PEPT2 are integral membrane carrier proteins that mediate the uptake of most possible physiologically occurring di- and tripeptides. The carriers are symporters that cotransport $H^+$ and substrates across cell membranes, the inwardly directed proton gradient providing the driving force for the accumulation of a substrate against its concentration gradient [10]. Besides their physiological substrates, PEPTs are also responsible for membrane transport of many pharmacologically active peptidomimetic drugs and prodrugs owing to their structural resemblance to di- and tripeptides [11,12]. They are expressed in some mammalian tissues/organs, including small intestine, kidney, pancreas, bile duct, liver, mammary glands, lung and choroid plexus, but, remarkably, seem to be overexpressed in some types of tumors [13]. In fact, cancer cells require larger amounts of peptide-bound amino acids for growth and metabolism and, consequently, peptide transporters might be upregulated [14]. Intriguingly, it was recently shown that PEPTs are largely overexpressed in several types of tumor cells but not in the

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healthy counterparts [15,16], thus representing a suitable target for the site specific delivery of pharmacologically active peptidomimetics.

Among the metal-based anticancer agents, gold complexes have been gaining increasing attention due to their capability to strongly inhibit tumor cell growth by exploiting mechanisms of action different from clinically established platinum drugs [17–20]. In this context, in recent years gold(III)-dithiocarbamato derivatives have been carving out their space in such a research area [21,22]. "First-generation" compounds of the type [$Au^{III}X_2$(dtc)] (X = Cl, Br; dtc = various dithiocarbamates) were shown to exert excellent *in vivo* anticancer activity on xenografts [23,24] coupled with negligible acute toxicity and an almost total lack of nephrotoxic side-effects [25].

Despite the positive outcomes achieved, alternative routes have been explored to improve the therapeutic effectiveness of this class of complexes as well as to provide their active delivery into the target tumor site. Consequently, we have been focusing on "second-generation" gold(III)-dithiocarbamato derivatives of oligopeptides as improved intracellular drug transfer and delivery systems supported by peptide transport proteins. The rationale of our research was to design gold(III) complexes which could preserve both the antitumor properties and the reduced toxic side-effects of the "first-generation" analogues, together with an enhanced bioavailability and tumor selectivity owing to the peptide-mediated cellular internalization provided by PEPTs. In this regard, we have recently reported on preliminary results related to some novel gold(III)-dipeptidedithiocarbamato derivatives having general formula [$Au^{III}X_2$(dtc-Sar-AA-O(*t*-Bu))] (X = Cl, Br; Sar = sarcosine (*N*-methylglycine); AA = Gly, Aib ("alpha"-aminoisobutyric acid, 2-methylalanine), L-Phe), and *in vitro* cytotoxicity and subsequent biological studies confirmed the goodness of such a designing strategy [26]. Remarkably, to the best of our knowledge, that was the first time that metal-based peptidomimetic anticancer agents were synthesized on purpose to target peptide transporters.

In the attempt to improve and tune tumor selectivity, we have been extending our evaluation to gold(III)-dithiocarbamato derivatives of additional selected small peptides. Accordingly, we report here on the synthesis and characterization of a handful of novel oligopeptides (from di- to pentapeptides, namely H-Sar-L-Ser(*t*-Bu)-O(*t*-Bu), H-Sar-$Aib_2$-O(*t*-Bu), H-D,L-Pro-$Aib_2$-O(*t*-Bu), H-Sar-$Aib_3$-O(*t*-Bu) and H-Sar-$Aib_3$-Gly-OEt) and the corresponding gold(III) compounds of the type [$Au^{III}X_2$(pdtc)] (X = Cl, Br; pdtc = oligopeptidedithiocarbamate). *In vitro* cytotoxicity and apoptosis induction studies were carried out toward human prostate cancer (PC3), ovarian adenocarcinoma (2008/C13) and Hodgkin's lymphoma cell lines (L540), including cisplatin-resistant cancer cells (C13) so as to check the possible occurrence of cross-resistance with platinum drugs. Biological results are compared to those obtained for the previous dipeptide analogues and discussed.

## 2. Experimental

### 2.1. Materials

Sarcosine, NMM, palladium catalyst (10% on activated charcoal), TEA (Fluka), IBCF (Lancaster), DMSO, DMSO-$d_6$, acetone-$d_6$, $CDCl_3$, carbon disulfide, HOBt, DMAP, PI, trypan blue (Aldrich), cisplatin (Pharmacia and Upjohn), isobutene (Siad), ZOSu, EDC (Iris Biotech), Z-Aib-OH, Z-L-Ser(*t*-Bu)-OH, Z-D,L-Pro-OH, HCl·H-Gly-OEt (Bachem), potassium tetrachloro- and tetrabromoaurate(III) dehydrate (Alfa Aesar), RPMI medium, penicillin, streptomycin, L-glutamine, (Cambrex Bio Science), and FBS (Gibco) were of reagent grade or comparable purity and were used as supplied. All other reagents and solvents were used as purchased without any further purification.

### 2.2. Instrumentation

Melting points were determined using a Stuart SMP10 apparatus and were not corrected.

Thin layer chromatography was performed on silica gel Merck Kieselgel $60F_{254}$ precoated glass plates. Retention factors ($R_f$) were measured using either chloroform/ethanol 9:1 ($R_{f1}$) or 1-butanol/acetic acid/water 3:1:1 ($R_{f2}$) or toluene/ethanol 7:1 ($R_{f3}$) as eluents. Spots were visualized by direct UV irradiation at 254 nm or developed by exposure to either iodine vapors or NaClO/ninhydrin chromatic reaction as appropriate. Flash column chromatography was performed on MercK Kiesegel 60 silica gel (40–63 μm, 230–400 mesh) as a stationary phase.

Optical rotations were measured at 546 nm in spectrophotometric grade methanol at 20 °C using a Perkin-Elmer 241 polarimeter equipped with a Haake D thermostat and a cell with an optical pathway of 10 cm.

CHNS elemental analyses were carried out on either a Fisons EA1108 or a Thermo Scientific FLASH 2000 CHNS-O microanalyzer.

Positive ion electrospray mass spectra (ESI-MS) were obtained by means of an ESI-TOF Mariner 5220 mass spectrometer (PerSeptive Biosystems). The samples were prepared in a 1:1 mixture of water/methanol containing 0.1% formic acid, to a final concentration of 0.1–1 mM. Samples were injected directly and the ions produced in an atmospheric pressure ionization (API) ESI ion source. The source temperature was 413 K, and the drying gas ($N_2$) flow rate was 300 L $h^{-1}$. A potential of 4.3 kV (capillary voltage) was applied to the probe tip, and the ions accelerated at 10, 15, 20 or 30 eV. Mass spectra were recorded over the scan range 100–4000 Da at a scan rate of 5 s and a resolution of 2 *m/z*. Data acquisition and processing were carried out using Data Explorer TM version 4.0 (Applied Biosystems).

FT-IR spectra were recorded in nujol on a Nicolet Nexus 870 spectrophotometer (1000 scans, resolution 2 $cm^{-1}$) for the range 50–600 $cm^{-1}$, and in solid KBr on either a Nicolet 55XC or a Perkin-Elmer 580B spectrophotometer (32 scans, resolution 2 $cm^{-1}$) for the range 400–4000 $cm^{-1}$. Data processing was carried out using OMNIC version 5.1 (Nicolet Instrument Corporation).

All NMR spectra were acquired in the appropriate deuterated solvent at 298 K on a Bruker Avance DRX300 spectrometer using a BBI [$^1$H,X] probe-head equipped with *z*-field gradients. Data processing was carried out using MestReNova version 6.2 (Mestrelab Research S.L.).

Typical acquisition parameters for 1D $^1$H NMR spectra ($^1$H: 300.13 MHz): 16 transients, spectral width 7.5 kHz, using 32 k data points and a delay time of 5.0 s. Spectra were processed using exponential weighting with a resolution of 0.5 Hz and a line-broadening threshold of 0.1 Hz.

Typical acquisition parameters for 2D [$^1$H, $^{13}$C] HMBC NMR spectra ($^1$H: 300.13/$^{13}$C: 75.48 MHz): 512 transients of 32 scans/block, spectral width 7.5/18.8 kHz, 2 k/2 k data points and a delay time of 2.0 s. Sequences were optimized for $^1J$($^{13}$C, $^1$H) = 145 Hz/$^nJ$($^{13}$C, $^1$H) = 5 Hz with no $^1$H decoupling. Spectra were processed by using sine-square weighting with a resolution of 1.0/3.0 Hz and a line-broadening threshold of 0.3/1.0 Hz.

$^1$H and $^{13}$C chemical shifts were referenced to TMS at 0.00 ppm *via* internal referencing to the residual peak of the deuterated solvent employed [27].

Thermogravimetric (TG) and differential scanning calorimetry (DSC) curves were recorded with a TA Instruments thermobalance equipped with a DSC 2929 calorimeter. Measurements were carried out in the range 25–1100 °C in alumina crucibles under air (flux rate 30 $cm^3$ $min^{-1}$) at a heating rate of 5 °C $min^{-1}$, using alumina as a reference.

Crystals of HCl·H-Sar-$Aib_3$-O(*t*-Bu) were obtained as trihydrates from a methanol/diethyl ether mixture. A plate (*ca.* 0.40 × 0.40 × 0.07 $mm^3$) was glued on the tip of a glass fiber. Single crystal diffraction data were collected at 293(2) K with a Philips PW1100 four-circle diffractometer in the θ–2θ scan mode using graphite-monochromated Cu Kα radiation (λ = 1.54178 Å). Intensities were corrected for Lorentz and polarization effects, as well as for absorption, the latter being based on a psi-scan. The structure was solved by direct methods (SIR2002)