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Synthesis, Characterization, and Cytotoxic Activity of Au^{I} N,S-Heterocyclic Carbenes Derived from Peptides Containing L-Thiazolylalanine

Alejandro Gutiérrez,[a] M. Concepción Gimeno,*[a] Isabel Marzo,[b] and Nils Metzler-Nolte*[c]

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Thiazolium salts **2a–b** derived from a dipeptide Boc-Gly-(Thz-Ala)-OMe (**1b**) containing the nonproteinogenic amino acid L-thiazolylalanine (Thz-Ala) can be used to generate the corresponding N,S-heterocyclic carbene (NSHC) gold(I) (**3a–b**) and silver(I) (**4**) complexes. Reaction of the NSHC-gold(I) iodide **3b** with Boc-Cys-Gly-OMe gives access to the peptide bioconjugate **5**, which contains a NSHC-Au-(S-Cys) unit. Compounds **3–5** constitute the first coin metal NSHC-peptide complexes. All new compounds were comprehensively characterized by ^{1}H, ^{13}C and 2D-NMR spectroscopy, IR spectroscopy, and mass spectrometry. Their cytotoxicity was studied in vitro against three different tumor cell lines (A549, Jurkat T and MiaPaca2) and IC_{50} values in the low micromolar range (< 25 μM), and as low as 0.4 μM in the best case were observed. All new Au complexes show good stability and promising properties and, as a result, this novel type of gold(I) carbene complex opens possibilities for the design of new metal-based drugs with promising antitumor characteristics.

Introduction

Nowadays several metal-based drugs are employed for the treatment of various diseases.[1] Cisplatin,[2] for example, is successfully used clinically in the treatment of many types of cancer such as testicular or ovarian cancer, among others. However, drawbacks, such as lack of activity and selectivity against other types of cancer, development of resistance, and undesired toxic side-effects, must be overcome, and the preparation of new anticancer agents constitutes an active research field.[3]

The biological activity of gold compounds as antimicrobial agents was established more than a century ago.[4] The main use of gold compounds, however, has been for the treatment of rheumatoid arthritis, mostly by the well-known compound auranofin.[5] In the last decades, new gold complexes with promising anticancer,[6] antimicrobial,[7] anti-HIV,[8] fungicidal, and antiparasitic[9] activities have been prepared. Some of these gold(I) and gold(III) complexes seem to exhibit their excellent antiproliferative activities by a mechanism that differs from that of Cisplatin. Specifically, inhibition of mitochondrial enzymes, for example, thioredoxin reductase (TrxR), has been established as one important mode of action.[10] Such compounds are promising anticancer agents that possess a good pharmaceutical profile that could overcome the limitations mentioned before.

Diverse approaches have been employed towards the preparation of bioactive gold-based anticancer agents, such as in the synthesis of (a) derivatives of the auranofin lead structure,[11] (b) other gold(I) phosphine complexes,[12] (c) gold(III) complexes,[13] (d) dithiocarbamate complexes,[14] (e) porphyrin derivatives,[15] and (f) coordination of gold(I) or gold(III) complexes to biologically active molecules.[16]

Another novel class of compounds, namely gold complexes with N-heterocyclic carbene (NHC) ligands has attracted a lot of attention.[17] Carbenes are isolobal with phosphines, but they present several advantages such as better σ-donating ability as ligands, higher water- and air-stability, and ease of modification compared with phosphines, so that it is possible to tune their electronic and steric properties.[18] A large number of these NHC-gold(I) complexes functionalized with diverse ligands have been described in the last decades with interesting activities in fields such as catalysis[19] and, more recently, medicinal chemistry.[20] The majority of work has focused on the preparation of NHC

[a] Departamento de Química Inorgánica, Instituto de Síntesis Química y Catálisis Homogénea, Universidad de Zaragoza – CSIC,
50009 Zaragoza, Spain
http://conchita-gimeno.webs.com/
[b] Departamento de Bioquímica y Biología Molecular, Universidad de Zaragoza,
50009 Zaragoza, Spain
[c] Lehrstuhl für Anorganische Chemie I – Bioanorganische Chemie, Fakultät für Chemie und Biochemie, Ruhr-Universität Bochum,
Universitatsstraβe 150, 44801 Bochum, Germany
E-mail: nils.metzler-nolte@rub.de
www.chemie.rub.de/ac1

compounds derived from imidazolyl-2-ylidene, but strong interest also exists in the development of other heterocyclic systems.[21]

The replacement of a nitrogen for a sulfur atom in the imidazole ring results in the thiazole molecule. This heterocycle forms part of a range of peptides with biological activity,[22] and is also a critical motif in thiamine (vitamine B_1; Figure 1). Although thiamine is supposed to exist as free carbene during benzoin condensation,[23] no bioorganometallic thiamine-carbene metal complexes have been described, and thiazol derivatives could be used as much simplified bioorganometallic models.[24] In contrast to the intensely studied NHC systems based on imidazolyl-2-ylidene, thiazolyl-2-ylidene analogues are scarce. In thiazoles, only one nitrogen atom can be functionalized, in contrast to imidazole for which two nitrogen atoms can be substituted. To the best of our knowledge, NSHC derivatives of thiazole, benzothiazole, and methyl-thiazole have been reported with only a few metals; the majority were ruthenium(II)[25] and palladium(II),[26] and a few examples have been obtained for gold(I) and gold(III),[27,28] copper(I),[29] nickel(II),[30] manganese(0),[31] cromium(0), and tungsten(0).[32] The replacement of imidazole for the thiazole ring in histidine gives the nonproteinogenic amino acid L-thiazolylalanine (ThzAla).[33] Recently, Lemke et al. described the first NSHC-Rh^{III} and NSHC-Ru^{II} peptide conjugates bearing this interesting bioorganic derivative of thiazole.[34]

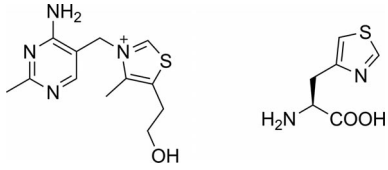


Figure 1. Important bioorganic thiazole derivatives: thiamine (vitamine B_1, left) and the amino acid L-thiazolylalanine (ThzAla, right).

Following this work, we have prepared the first NSHC-gold(I) conjugates derived from a dipeptide of this type. Cytotoxic activity is mainly attributed to the gold(I) atom, however, functionalization with amino acids and peptides[35] could improve the pharmaceutical profile of the complexes such as activity or selectivity, and decrease undesired toxic side effects. Amino acids and peptides are useful carriers that can deliver the metal to the target and could enable the complex to penetrate into the cell. Furthermore, our complexes could be more selective to abnormal cells due to the fact that tumoral cells overexpress amino acid receptors and also have increased requirements for nutrients.[36] In general, the concept of using bioorganometallic peptide bioconjugates for targeting of tumor cells has gained considerable attention.[37]

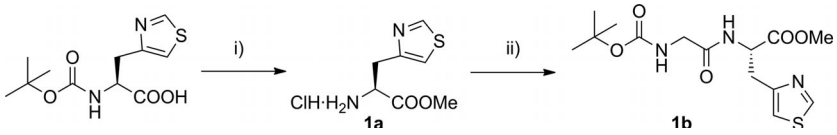
In this work, Au^I peptide derivatives of thiazolylalanine-containing peptides were prepared and their cytotoxic activity against three human tumor cell lines has been studied.

Results and Discussion

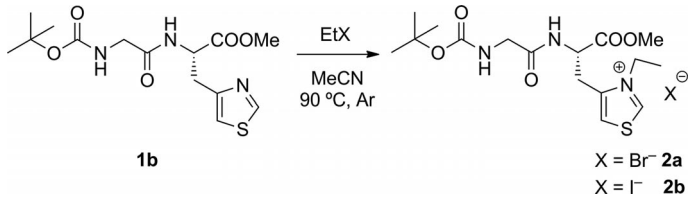
Synthesis and Characterization

The synthesis of dipeptide **1b** was readily achieved as shown in Scheme 1. In the first step, starting from commercially available Boc-L-thiazolylalanine, deprotection of the Boc amino protective group and esterification of the free carboxylic acid was carried out in the same step using $SOCl_2$. The ^1H NMR spectrum shows the disappearance of the signals corresponding to the Boc group at $\delta = 5.57$ and 1.47 ppm belonging to the carbamate and methyl protons, respectively, and the appearance of a new signal for the methyl ester at $\delta = 3.80$ ppm. The resulting amino ester derivative **1a** was obtained in quantitative yield and was coupled to Boc-Gly-OH by using the mixed anhydride method,[38] in which isobutyl chloroformate (IBCF) was employed as activating agent and *N*-methyl morpholine (NMM) as base. The desired dipeptide **1b** was obtained in high yield and in pure form after chromatographic purification. Formation of the new amide bond was confirmed by the appearance of a resonance at $\delta = 7.30$ ppm in the ^1H NMR spectrum and at $\delta = 169.4$ ppm in the ^{13}C spectrum.[28] The protons of the thiazol rings appear as doublets with a small characteristic coupling constant ($J = 2.0$ Hz) at $\delta = 8.73$ and 7.07 ppm (^1H NMR spectra) and at $\delta = 152.2$ and 115.8 ppm in the ^{13}C NMR spectra, which is consistent with previous reports and is a characteristic of this type of compounds.

The next step involved thiazolium salt formation by alkylation of the nitrogen atom of the thiazol ring. Attempts to carried out the alkylation by using previously reported conditions[39] with EtBr as alkylating agent in MeCN heated to reflux over three days gave the desired bromide thiazolium peptide salt **2a** only in very poor yield (< 5%); the remaining starting dipeptide was recovered by flash chromatography. In contrast to imidazole, the nitrogen atom of the thiazole is less basic and more difficult to alkylate, and therefore harsher conditions must be employed. As this reaction is of S_N2 type, it should be favored by the use of an alkyl halide with a better leaving group. Hence, performing the alklylation reaction with EtI[40] instead of EtBr gave the desired thiazolium iodide peptide salt **2b** in



Scheme 1. Synthesis of the L-thiazolylalanine-containing dipeptide **1b**. (i) $SOCl_2$, MeOH, (ii) Boc-Gly-OH, IBCF, NMM, THF.



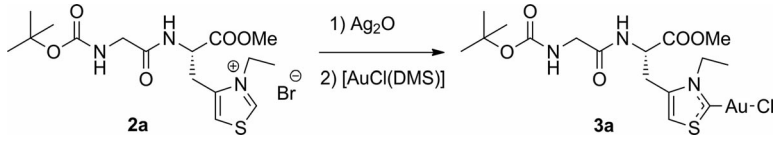
Scheme 2. Synthesis of the thiazolium peptide salts.

good yield (66% yield; Scheme 2). An attempt to improve further the yield by using the more reactive MeI failed, and a complex mixture of products that could not be readily separated was obtained, probably due to undesired side reactions with other functional groups in the molecule. The thiazolium salts show new resonances corresponding to the ethyl group in the ^{1}H NMR spectrum at δ = 4.68 and 1.70 ppm, and in the ^{13}C NMR spectrum at δ = 49.3 and 15.5 ppm, respectively. The signals arising from the thiazole ring appear strongly downfield shifted (with $\Delta\delta$ around 3 and 1 ppm for H-2 and H-5, respectively) in the ^{1}H NMR spectrum. This indicates that the heterocyclic ring possesses less electron density, and H-2 becomes more acidic after alkylation. Nevertheless, in the ^{13}C NMR spectrum the equivalent signal appears approximately 10 ppm more shielded and the signal of C-5 shifts ca. 10 ppm downfield.

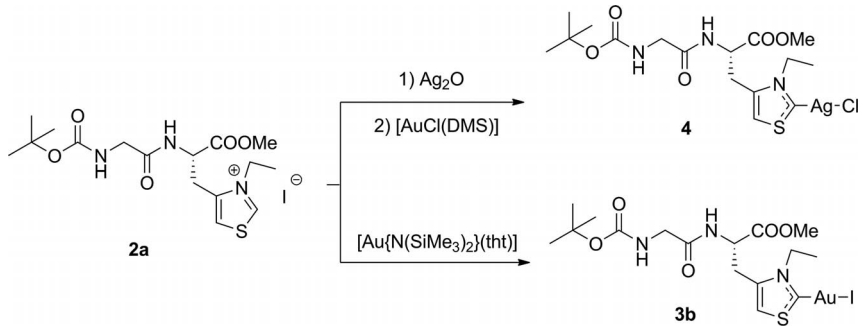
A number of methods are employed for the synthesis of N-heterocyclic metal carbene derivatives. Among these, the Ag_2O route,[41] which results in deprotonation of the imidazolium salt, followed by formation of the NHC-Ag^I complex in a first step and subsequent transmetallation to give other NHC-metal complexes, is extensively used. This method presents several advantages such as high yields, tolerance of other functional groups in the molecule and no need for rigorously anhydrous conditions or anhydrous solvents. In our case, the attempted formation of NSHC-Ag^I species was only partially successful, probably because the H-2 proton in the thiazole is more acidic but the resultant carbene is less nucleophilic compared to imidazole analogues. Therefore, carbene generation is more challenging from thiazolium salts than from imidazolium salts. Nevertheless, the reaction with Ag_2O, followed by transmetallation with [AuCl(DMS)] (DMS = dimethylsulfide) afforded the desired new NHSC-gold(I) complex **3a** (Scheme 3). The conjugate **3a** was obtained as an orange solid after chromatographic purification. The NMR spectrum shows all the resonances expected for a gold(I) carbene. In the ^{1}H NMR spectra, the H-2 proton disappears due to carbene formation. The other aromatic proton, H-5, appears as a singlet at δ = 7.16 ppm, around 1 ppm shielded compared with the thiazolium salt precursor. In the ^{13}C NMR spectrum, C-5 appears at δ = 120.8 ppm, around 5 ppm more shielded than the starting thiazolium salt. Notably, the carbon involved in the carbene bond appears at δ = 195.2 ppm, around 50 ppm downfield after carbene formation. The structure was further confirmed by 2D-NMR experiments including COSY, HSQC and HMBC, and IR and MS data are also consistent with the proposed structure.

Surprisingly, we were not able to obtain the desired NSHC-gold(I) iodide in satisfactory yield and purity by using the silver oxide method detailed above, starting from the thiazolium iodide salt **2b** instead of thiazolium bromide salt **2a**. It has been reported that thiazolium iodide salts can give rise to more complex structures because of the bridging capacity of the iodide ligand.[42] We did obtain the NSHC-Ag^I complex **4** after chromatographic purification (Scheme 4), but this compound did not undergo further transmetallation reactions. The NMR spectrum of **4** differed considerably from that of the NSHC-gold(I) analogue **3a**. The H-5 proton appears as a singlet at high field (δ = 5.86 ppm) and, in this case, the C-2 carbon, which is bonded to the silver atom, appears at δ = 172.1 ppm and C-5 at δ = 97.9 ppm. Halogen exchange with the solvent (chloride instead of iodide) is proposed based on the MS data, which is in agreement with previous reports.[43]

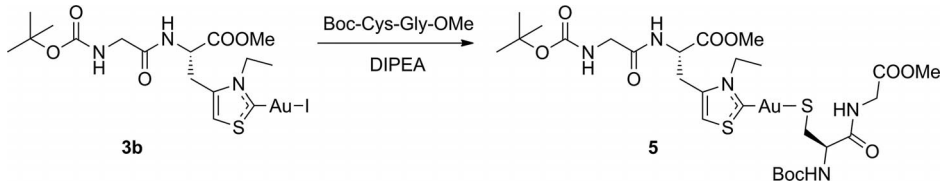
At this point, we tried to obtain the NSHC-gold(I) iodide by other methods. Hanh et al.[27] reported the use of a strong base such as potassium bis(trimethylsilyl)amide. In this case, the species [Au{N(SiMe$_3$)$_2$}(tht)] is generated in situ by metathesis reaction of K[N(SiMe$_3$)$_2$] and [AuCl(tht)] (tht = tetrahydrothiophene). The reaction with the thiazolium iodide salt resulted in deprotonation (and carbene generation), followed by gold(I) coordination in one pot (Scheme 4). By following this strategy, the desired compound **3b** was obtained as an orange solid in good yield and purity after flash chromatography. The ^{1}H and ^{13}C NMR spectra differ slightly from that of the chloride analogue **3a**. In this case, the most notable feature was the C-2 signal involved in the carbene bond, which appears at δ = 206.8 ppm. The characteristic Au-C vibration at 940 cm^{-1} in the IR spectrum clearly indicates carbene complex formation.



Scheme 3. Synthesis of the NSHC-gold(I) chloride (**3a**).



Scheme 4. Synthesis of the NSHC-Ag^{I} chloride **4** and the NSHC-gold(I) iodide **3b**.



Scheme 5. Synthesis of the peptide bioconjugate **5**.

Finally, substitution of the iodide ligand by a previously synthesized dipeptide, Boc-Cys-Gly-OMe, using DIPEA as base, yielded the peptide bioconjugate **5** as a pale-yellow solid in pure form and good yield after chromatographic purification (Scheme 5). The ^{1}H and ^{13}C NMR spectra are difficult to interpret because of overlapping resonances and, furthermore, the complex appears as a mixture of two rotamers (1:0.4 ratio). Nevertheless, all signals could be assigned on the basis of 2D-NMR experiments. The IR and MS spectra further confirmed the proposed structure.

Cytotoxic Activity

The cytotoxic activities of two selected compounds, NSHC-gold(I) iodide **3b** and bioconjugate **5**, were assayed by the MTT method against three different human tumor cell lines: A549 (lung carcinoma), Jurkat (T-cell leukaemia), and MiaPaca2 (pancreatic carcinoma). Cells were exposed to different concentrations of each compound for a total of 24 h. In all cases, care was taken to ensure that the final concentration of dimethyl sulfoxide (DMSO) was below 0.5%, at which level this solvent has no appreciable toxicity by itself. IC_{50} values were calculated from dose-response curves obtained by nonlinear regression analysis.

The IC_{50} values are collected in Table 1. The two NSHC-gold(I) conjugates exhibited good cytotoxicity in vitro against all three tumor cell lines. Only the IC_{50} value of the bioconjugate **5** in A549 was outside the low micromolar range (> 25 μM), whereas it was around 25 μM for the other cell lines. The carbene complex with iodide instead of the thiolate was more potent in all cases. The Jurkat cell line appears to be most sensitive to these compounds, and the A549 cell line showed more resistance to the complexes. Notably, complex **3b** exhibits excellent cytotoxicity against the A549 cell line, with IC_{50} values in the submicromolar range. Taken together, it seems that the iodide complex is more potent than the thiolate derivative. This may be due to the higher lability of the Au–I bond compared with the Au–S bond, or may simply be a consequence of the higher lipophilicity of **3b**.

Table 1. IC_{50} values against three human tumor cell lines after 24 h incubation. Mean value ± standard error is given, experiments were performed in quadruplicate.

	A549	MiaPaca2	Jurkat
3b	0.4 ± 0.01	16.6 ± 0.2	6.2 ± 0.1
5	>25	24.8 ± 0.1	ca. 25

Conclusions

Alkylation of dipeptide **1b**, containing L-thiazolylalanine, gave thiazolium salts **2a** (bromide) and **2b** (iodide). From these compounds, the first NSHC-gold(I) chloride **3a** or iodide **3b** bearing this nonproteinogenic amino acid were obtained by employing the well-established Ag_2O route or direct carbene formation by deprotonation with a strong base (KHMDS). Reaction of **3b** with a cysteine-containing dipeptide gave bioconjugate **5**, a NSHC-gold(I) thiolate. All new compounds were comprehensively characterized by spectroscopic methods (NMR, IR and MS) and constitute the first examples of this type of gold(I) bioorganometallic complexes derived from a thiazole peptide moiety. The biological evaluation of **3b** and **5** revealed that they are cytotoxic in vitro against human tumor cell lines, with some differential activity. This work gives access to a novel and scarcely explored class of NHC derivatives. Further studies will focus on elucidating possible modes of action and identify molecular targets of the complexes described herein.[47]

Experimental Section

General: All manipulations were routinely carried out under Ar by using common Schlenk techniques. Solvents were purified by standard procedures immediately prior to use. Boc-β-(4-thiazolyl)-Ala-OH, Boc-Gly-OH, NMM, IBCF and KHMDS were purchased from Aldrich, Fluka or Bachem and used without further purification. The starting gold(I) compounds [AuCl(DMS)][45] and [AuCl(tht)][46] were prepared as previously reported. Mass spectra were recorded with a Bruker Esquire 3000 Plus, with the electrospray (ESI) technique and with a Bruker Microflex (MALDI-TOF). ^{1}H and ^{13}C{H} NMR spectra and 2D NMR experiments were recorded at room temperature with a Bruker Avance 400 spectrometer (^{1}H, 400 MHz, ^{13}C, 100.6 MHz) or with a Bruker Avance II 300 spectrometer (^{1}H, 300 MHz, ^{13}C, 75.5 MHz), with chemical shifts (δ, ppm) reported relative to the solvent peaks of the deuterated solvent.[21] Multiplicities are abbreviated by standard nomenclature, "app. t" means apparent triplet. The RPMI 1640 (Roswell Park Memorial Institute) cell culture medium, fetal bovine serum (FBS) were purchased from Lonza Co. MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was purchased from Sigma Chemical. MTT was dissolved (5 mg/mL) in phosphate buffer at pH 7.2. Fluorescence intensity measurements were carried out with a PTI QM-4/206 SE Spectrofluoremeter (PTI, Birmingham, NJ) with right angle detection of fluorescence using a 1 cm path length quartz cuvette. IR spectra were measured as neat samples on ATR accessories to Perkin-Elmer FTIR or Bruker Tensor 27 instruments.

Synthesis of New Compounds

Synthesis of 1a: To a solution of Boc-(L)-ThzAla-OH (0.39 g, 1.42 mmol) in MeOH (20 mL) cooled in an ice bath, $SOCl_2$ (0.206 mL, 2.84 mmol) was added. The mixture was stirred at 65 °C for 24 h, then the solvent was evaporated under reduced pressure. The residue was dissolved in water and lyophilized to give **1a** in quantitative yield as a white solid. ^{1}H NMR (D_2O, 200 MHz, 25 °C): δ = 9.44 (d, J = 2.0 Hz, 1 H, H-2), 7.75 (d, J = 2.0 Hz, 1 H, H-5), 4.55 (app. t, J = 6.8 Hz, 1 H, $C_{\alpha,ThzAla}H$), 3.80 (s, 3 H, OCH_3), 3.58 and 3.54 (dd, J = 6.4, 2.4 Hz, 2 H, $C_{\beta,ThzAla}H_2$) ppm. ^{13}C NMR (D_2O, 200 MHz, 25 °C): δ = 172.6 (*C*OOMe), 159.1 (C, *C*4), 152.8 (CH, *C*2), 113.3 (CH, *C*5), 52.3 ($C_{\alpha,ThzAla}H$), 51.9 (O*C*H$_3$), 29.3 ($C_{\beta,ThzAla}H_2$).

Synthesis of 1b: To a solution of Boc-Gly-OH (0.18 g, 1 mmol) in THF (10 mL), *N*-methylmorpholine (0.11 mL, 1 mmol) and IBCF (0.13 mL, 1 mmol) were added. In a second flask, **1a** (0.22 g, 1 mmol) was suspended in THF (10 mL) and triethylamine (0.14 mL, 1 mmol) was added. Both suspensions were combined and the mixture was stirred for 2 h at room temp. The mixture was filtered and the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 mL) and washed with water (30 mL). The organic phase was dried with Na_2SO_4, filtered, and evaporated under reduced pressure. Purification by column chromatography (EtOAc/hexane, 1:1) gave **1b** (0.290 g, 0.85 mmol, 85%) as a white solid. TLC: R_f = 0.4 (EtOAc/hexane, 3:7).^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 8.73 (d, J = 2.0 Hz, 1 H, H-2), 7.30 (m, 1 H, $CONH_{ThzAla}$), 7.07 (d, J = 2.0 Hz, 1 H, H-5), 5.15 (m, 1 H, $CONH_{Gly}$), 4.93 (m, 1 H, $C_{\alpha,ThzAla}H$), 3.83 (dd, J = 5.4, 6.8 Hz, 2 H, $C_{\alpha,Gly}H_2$), 3.69 (s, 3 H, OCH_3), 3.35 and 3.34 (app. t, ABM system, J = 5.6 Hz, 2 H, $C_{\beta,ThzAla}H_2$), 1.43 (s, 9 H, $C_{Boc}H_3$). ^{13}C NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 171.4 (*C*OOMe), 169.4 ($CONH_{ThzAla}$), 153.0 ($CONH_{Gly}$), 152.2 (CH, C-2), 115.8 (CH, C-5), 80.2 (C, C_{Boc}), 52.3 (OCH_3), 51.8 ($C_{\alpha,ThzAla}H$), 41.0 ($C_{\alpha,Gly}H_2$), 32.6 ($C_{\beta,ThzAla}H_2$), 28.4 ($C_{Boc}H_3$) ppm. MS (ESI$^+$): *m*/*z* calcd. for $[M + H]^+$ 344.1; found: 344.0.

Synthesis of 2a: EtBr (2 mL) was added to a suspension of **1b** (0.1 g, 0.29 mmol) in MeCN (1 mL) under an argon atmosphere and the mixture was heated to reflux (90 °C) and stirred for 72 h. The crude product was directly purified by column chromatography [EtOAc/hexane, 1:1 (to recover unreacted **1b**), then MeOH/CH_2Cl_2, 10%] to give **2a** (< 5%) as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 10.58 (d, J = 2.2 Hz, 1 H, H-2), 8.27 (d, J = 7.8 Hz, 1 H, $CONH_{ThzAla}$), 8.18 (d, J = 2.2 Hz, 1 H, H-5), 5.64 (m, 1 H, $CONH_{Gly}$), 4.86 (m, 1 H, $C_{\alpha,ThzAla}H$), 4.67 (m, 2 H, N*C*H_2CH$_3$), 3.80 (s, 3 H, OCH_3), 3.72 (m, 2 H, $C_{\alpha,Gly}H_2$), 3.49 (m, 2 H, $C_{\beta,ThzAla}H_2$), 1.68 (t, J = 7.2 Hz, 3 H, NCH$_2$C*H*$_3$), 1.43 (s, 9 H, $C_{Boc}H_3$) ppm. MS (ESI$^+$): *m*/*z* calcd. for $[M]^+$ 372.1; found: 372.0.

Synthesis of 2b: The reaction of **1b** (0.1 g, 0.29 mmol) with EtI (2 mL) in the same way as described for **2a** gave **2b** (0.0955 g, 0.19 mmol, 66%) as a white solid; TLC: R_f = 0.6 (CH_2Cl_2/MeOH, 9:1). ^{1}H NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 10.40 (d, J = 2.2 Hz, 1 H, H-2), 8.24 (d, J = 1.8 Hz, 1 H, H-5), 7.99 (d, J = 8.0 Hz, 1 H, $CONH_{ThzAla}$), 5.70 (m, 1 H, $CONH_{Gly}$), 4.98 (m, 1 H, $C_{\alpha,ThzAla}H$), 4.68 (q, J = 6.8 Hz, 2 H, N*C*H_2CH$_3$), 3.82 (m, 2 H, $C_{\alpha,Gly}H_2$), 3.79 (s, 3 H, OCH_3), 3.62 (m, 2 H, $C_{\beta,ThzAla}H_2$), 1.70 (t, J = 7.2 Hz, 3 H, NCH$_2$C*H*$_3$), 1.42 (s, 9 H, $C_{Boc}H_3$) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 170.8 (*C*OOMe), 170.1 ($CONH_{ThzAla}$), 156.2 ($CONH_{Gly}$), 145.0 (CH, C-2), 124.8 (CH, C-5), 80.0 (C, C_{Boc}), 53.4 (OCH_3), 50.5 ($C_{\alpha,ThzAla}H$), 49.3 (N*C*H$_2$CH$_3$), 44.1 ($C_{\alpha,Gly}H_2$), 29.0 ($C_{\beta,ThzAla}H_2$), 28.4 ($C_{Boc}H_3$), 15.5 (NCH$_2$*C*H$_3$) ppm. MS (ESI$^+$): *m*/*z* calcd. for $[M]^+$ 372.1; found 372.0.

Synthesis of 3a: Ag_2O (0.0095 g, 0.041 mmol) was added to a solution of **2a** (0.037 g, 0.082 mmol) in CH_2Cl_2 (20 mL) and the mixture was stirred overnight at room temp. in the dark. Then, [AuCl(DMS)] (0.024 g, 0.082 mmol) was added and the mixture was stirred at room temp. for 24 h. The mixture was filtered through Celite and the solvent was evaporated under reduced pressure. The crude reaction product was purified by column chromatography (MeOH/CH_2Cl_2, 10%) to give **3a** (0.021 g, 0.035 mmol, 42.6%) as an orange powder; TLC: R_f = 0.8 (CH_2Cl_2/MeOH, 9:1). IR: $\tilde{\nu}$ = 3284 (s, CONH and OCONH), 1741 (s, COOMe), 1706 (s, OCONH), 1674 (s, CONH), 1520 and 1434 (w, thiazol), 1165 and 1023 (s, C–O), 950 (s, Au–C) and 334 (s, Au–Cl) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 7.36 (d, J = 7.5 Hz, 1 H, $CONH_{ThzAla}$), 7.35 (s, 1 H, H-5), 5.31 (app. t, J = 3.6 Hz, 1 H, $CONH_{Gly}$), 4.90 (m, 1 H, $C_{\alpha,ThzAla}H$), 4.52 (m, 2 H, N*C*H_2CH$_3$), 3.76 (m, 2 H, $C_{\alpha,Gly}H_2$), 3.76 (s, 3 H, OCH_3), 3.38 and 3.24 (dd, ABM system, J = 12.0, 3.9 Hz and J = 12.0, 6.0 Hz, 2 H, $C_{\beta,ThzAla}H_2$), 1.54 (t, J = 5.4 Hz, 3 H, NCH$_2$C*H*$_3$), 1.43 (s, 9 H, $C_{Boc}H_3$) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 195.2 (C, C-2), 170.4 (*C*OOMe), 170.2 ($CONH_{ThzAla}$), 154.9 ($CONH_{Gly}$), 143.6 (C, C-4), 120.8 (CH, C-5), 80.6 (C, C_{Boc}), 53.2 ($C_{\alpha,ThzAla}H$), 50.8 (N*C*H$_2$CH$_3$), 50.7 (OCH_3), 44.2 ($C_{\alpha,Gly}H_2$), 30.6 ($C_{\beta,ThzAla}H_2$), 28.3 ($C_{Boc}H_3$), 16.3 (NCH$_2$*C*H$_3$) ppm. HRMS (ESI–): *m*/*z* calcd. for $[M - H]^-$ 602.0785; found: 602.0796.

Synthesis of 4: The reaction of **2b** (0.499 g, 1 mmol), Ag_2O (0.116 g, 0.5 mmol) and [AuCl(DMS)] (0.295 g, 1 mmol) following the procedure described for **3a**, gave **4** (0.134 g, 0.26 mmol, 36%) as a yellow solid; TLC: R_f = 0.9 (CH_2Cl_2/MeOH, 9:1). IR: $\tilde{\nu}$ = 3280 (s, CONH and OCONH), 1743 (s, COOMe), 1709 (s, CONH), 1648 (s, CONH), 1517 and 1435 (w, thiazol), 1162 and 1024 (s, C–O), 951 (s, Ag–C) and 333 (s, Ag–Cl) cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 7.44 (d, J = 8.1 Hz, 1 H, $CONH_{ThzAla}$), 5.86 (s, 1 H, H-5), 5.57 (app. t, J = 5.7 Hz, 1 H, $CONH_{Gly}$), 4.76 (dd, J = 13.8, 7.5 Hz, 1 H, $C_{\alpha,ThzAla}H$), 3.76 (m, 2 H, N*C*H_2CH$_3$), 3.76

(m, 2 H, $C_{\alpha,Gly}H_2$), 3.70 (s, 3 H, OCH_3), 2.95 and 2.85 (dd, ABM system, diastereotopic protons, J = 16.2, 6.0 Hz and J = 16.8, 8.1 Hz, 2 H, $C_{\beta,ThzAla}H_2$), 1.39 (s, 9 H, $C_{Boc}H_3$), 1.20 (t, J = 7.2 Hz, 3 H, NCH_2CH_3) ppm. ^{13}C NMR ($CDCl_3$, 200 MHz, 25 °C): δ = 172.1 (C, C-2), 170.9 (*C*OOMe), 169.7 ($CONH_{ThzAla}$), 156.0 ($CONH_{Gly}$), 131.0 (C, C-4), 97.9 (CH, C-5), 80.0 (C, C_{Boc}), 52.6 (OCH_3), 50.4 ($C_{\alpha,ThzAla}H$), 44.0 ($C_{\alpha,Gly}H_2$), 38.1 (NCH_2CH_3), 30.9 ($C_{\beta,ThzAla}H_2$), 28.1 ($C_{Boc}H_3$), 14.1 (NCH_2CH_3) ppm. HRMS (ESI^+): *m*/*z* calcd. for $[M - Cl]^+$ 478.0560; found: 478.1234.

Synthesis of 3b: To a solution of $K[N(SiMe_3)_2]$ (0.044 g, 0.22 mmol) in THF (2 mL), [AuCl(tht)] (0.07 g, 0.22 mmol) in THF (2 mL) was added under an argon atmosphere. The mixture was stirred for 5 min, then a suspension of **2b** (0.100 g, 0.20 mmol) in THF (2 mL) was added. The reaction mixture was stirred at room temp. under an argon atmosphere for 2 h, then filtered through Celite and the solvent was evaporated under reduced pressure. Purification by column chromatography ($MeOH/CH_2Cl_2$, 10%) and recrystallization (CH_2Cl_2/hexanex, 1:5) gave **3b** (0.082 g, 0.12 mmol, 54%) as an orange solid; TLC: R_f = 0.8 (CH_2Cl_2/MeOH, 9:1). IR (KBr): $\tilde{\nu}$ = 3312 (s, CONH and OCONH), 1741 (s, COOMe), 1668 (s, CONH and OCONH), 1519 and 1433 (w, thiazol), 1162 (s, C–O), 940 (s, Au–C) and 334 (s, Au–I) cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 7.16 (s, 1 H, H-5), 6.98 (d, J = 6.4 Hz, 1 H, $CONH_{ThzAla}$), 5.06 (m, 1 H, $CONH_{Gly}$), 4.82 (m, 1 H, $C_{\alpha,ThzAla}H$), 4.46 (m, 2 H, NCH_2CH_3), 3.69 (m, 2 H, $C_{\alpha,Gly}H_2$), 3.69 (s, 3 H, OCH_3), 3.31 and 3.15 (dd, J = 15.6, 4.8 Hz and J = 15.6, 7.2 Hz, 2 H, $C_{\beta,ThzAla}H_2$), 1.47 (t, J = 6.8 Hz, 3 H, NCH_2CH_3), 1.35 (s, 9 H, $C_{Boc}H_3$) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz, 25 °C): δ = 206.8 (C, C-2), 170.3 (*C*OOMe), 170.0 ($CONH_{ThzAla}$), 156.2 ($CONH_{Gly}$), 143.4 (C, C-4), 120.3 (CH, C-5), 80.9 (C, C_{Boc}), 53.3 ($C_{\alpha,ThzAla}H$), 51.0 (OCH_3), 50.4 (NCH_2CH_3), 44.6 ($C_{\alpha,Gly}H_2$), 30.8 ($C_{\beta,ThzAla}H_2$), 28.3 ($C_{Boc}H_3$), 16.4 (NCH_2CH_3) ppm. HRMS (ESI^+): *m*/*z* calcd. for $[M + Na]^+$ 718.0117; found: 718.0124.

Synthesis of 5: To a solution of **3b** (0.0468 g, 0.067 mmol) in acetone (4 mL), DIPEA (0.012 mL, 0.067 mmol) and Boc-Cys-Gly-OMe (0.0197 g, 0.067 mmol) were added, and the mixture was stirred for 24 h (the solution changed from orange to green, indicating thiolate coordination to gold). The solvent was evaporated under reduced pressure and the crude material was purified by column chromatography (CH_2Cl_2/MeOH, 9.5:0.5) to give **5** (0.0349 g, 0.040 mmol, 61%); TLC R_f = 0.5 (CH_2Cl_2/MeOH, 9.5:0.5). IR: $\tilde{\nu}$ = 3314 (s, CONH and OCONH), 1742 (s, COOMe), 1663 (s, CONH and OCONH), 1513 and 1435 (w, thiazol), 1160 (s, C–O), 942 (s, Au–C) cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz, 25 °C): δ (rotamers mixture, 1:0.4 ratio) = 8.13 [m, 1 H, $CONH_{Gly}$(dipeptide)], 7.45 (m, 1 H, $CONH_{ThzAla}$), 7.38 (s, 1 H, H-5), 5.91 (B) and 5.66 (A) [m and d, J = 9.6 Hz, 1 H, $CONH_{Cys}$(dipeptide)], 5.45 (m, 1 H, $CONH_{Gly,ThzAla}$), 4.95 (m, 1 H, $C_{\alpha,ThzAla}H$), 4.95 (A) and 4.30 (B) [m, 1 H, $C_{\alpha,Cys}H$(dipeptide)], 4.60 (m, 2 H, NCH_2CH_3), 4.02 [dd and m, ABX system, diastereotopic protons, J = 17.7 and 6.3 Hz, 2 H, $C_{\alpha,Gly}H_2$(dipeptide)], 3.87 (m, ABX system, 2 H, $C_{\alpha,Gly,ThzAla}H_2$), 3.77 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.36 [m, 2 H, $C_{\beta,Cys}H_2$(dipeptide)], 3.06 (A) and 2.91 (A), and 2.65 (B) (dd and m, J = 14.4, 3.6 Hz, J = 14.4, 7.5 Hz, 2 H, $C_{\beta,ThzAla}H_2$), 3.69 (m, 2 H, $C_{\alpha,Gly}H_2$), 1.53 (t, J = 6.9 Hz, 3 H, NCH_2CH_3), 1.42 (s, 18 H, $C_{Boc}H_3$) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz, 25 °C): δ (rotamers mixture) = 207.1 (C, C-2), 171.9 (A) and 171.8 (B) ($CONH_{ThzAla}$), 170.8 (A) and 170.4 (B) [$CONH_{Gly}$(dipeptide)], 170.3 (A) and 170.0 (B) (*C*OOMe), 169.6 (A) and 169.3 (B) (*C*OOMe), 155.6 ($CONH_{Gly,ThzAla}$), 155.6 [$CONH_{Cys}$(dipeptide)], 143.9 (C, C-4), 120.5 (CH, C-5), 80.5 (C, C_{Boc}), 80.3 (C, C_{Boc}), 58.1 (B) and 50.9 (A) [$C_{\alpha,Cys}H$-(dipeptide)], 54.5 (B) and 53.8 (A) ($C_{\alpha,ThzAla}H$), 53.1 (OCH_3), 52.2 (OCH_3), 50.4 (NCH_2CH_3), 46.6 ($C_{\alpha,Gly,ThzAla}H_2$), 44.3 (A) and 36.2 (B) ($C_{\beta,ThzAla}H_2$), 41.3 (A) and 40.9 (B) [$C_{\alpha,Gly}$(dip)H_2], 36.8 (B) and 30.3 (A) ($C_{\beta,Cys}H_2$), 28.3 ($C_{Boc}H_3$) and 16.4 (NCH_2CH_3) ppm. HRMS (ESI^+): *m*/*z* calcd. for $[M + H]^+$ 860.2268; found: 860.2292.

Cell Culture: Jurkat (leukaemia) and MiaPaca2 (pancreatic carcinoma) cell lines were maintained in RPMI 1640, whereas A549 (lung carcinoma) cells were grown in DMEM (Dulbecco's Modified Eagle's Medium). Both media were supplemented with 5% fetal bovine serum (FBS), 200 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. Media for A549 cells were also supplemented with 2.2 g/l Na_2CO_3, 100 μg/mL pyruvate, and 5 mL nonessential amino acids (Invitrogen). Cell cultures were maintained in a humidified atmosphere of 95% air/5% CO_2 at 37 °C.

Cytotoxicity Assay by MTT: The MTT assay was used to determine the cell viability as an indicator for the sensitivity of the cells to the complexes.[44] Exponentially growing cells were seeded at a density of approximately 1×10^5 cells/mL (A549, MiaPaca2) or 3×10^5 cells/mL (Jurkat), in a 96-well flat-bottomed microplate and, after 24 h, they were incubated with the compounds. The complexes were dissolved in DMSO and tested in concentrations ranging from 0.5 to 100 μM and in quadriplicate. Cells were incubated with the test compounds for 24 h at 37 °C, then, 10 μL/well of MTT (5 mg/mL) was added and plates were incubated for 1–3 h at 37 °C. Finally, 100 μL/well *i*PrOH (0.05 M HCl) was added. The optical density was measured at 570 nm using a 96-well multiscanner autoreader (ELISA). The IC_{50} was calculated by nonlinear regression analysis using Origin software (Origin Software, Electronic Arts, Redwood City, California, USA).

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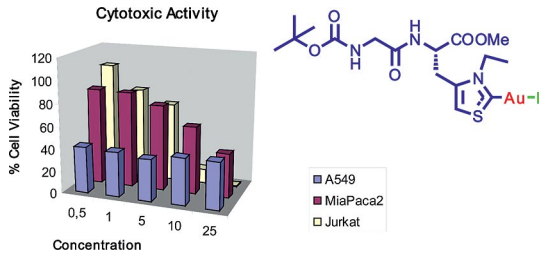
Gold Antitumor Agents

A. Gutiérrez, M. C. Gimeno,* I. Marzo, N. Metzler-Nolte* 1–9

Synthesis, Characterization, and Cytotoxic Activity of Au^{I} N,S-Heterocyclic Carbenes Derived from Peptides Containing L-Thiazolylalanine

Keywords: Antitumor agents / Carbenes / Drug design / Gold / Medicinal chemistry / Metal-based drugs / Peptides



The first organometallic gold(I) bioconjugates of a thiazole-based peptide are reported. The N,S-heterocyclic carbene gold(I) derivatives were prepared from the corresponding thiazolium peptide salts either by following the Ag_2O route or by reaction with a strong base. The new compounds display excellent in vitro cytotoxicity with IC_{50} values as low as 0.4 μM.

