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Dedicated to Professor Alexandru T. Balaban on the occasion of his 85th anniversary

NEW MEMBERS OF THE *CINCHONA* ALKALOID FAMILY: SYNTHESIS, CHARACTERISATION AND ANTITUMOR EVALUATION OF NOVEL GOLD(I) COMPLEXES

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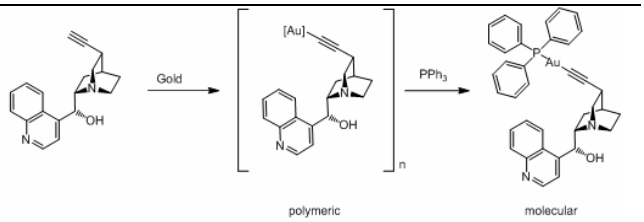
The synthesis of unpresented Gold(I) complexes related to *Cinchona* alkaloids derivatives is reported. The main target was the synthesis and biological evaluation of Gold(I)-PPh_3 complexes (**5 - 8**). This was achieved starting from the polymeric *Cinchona* alkaloids alkyne derivatives (**1 - 4**) in two steps. The target molecules **5 - 8** were investigated for antitumor activities *in vitro,* towards a panel of 12 cell lines using a monolayer cell survival and proliferation assay.



INTRODUCTION*

After the discovery of the antimalarial property of quinine, as the active compound isolated from cinchona bark, the *Cinchona* alkaloid family played an important role in medicinal chemistry since the early 17th century. Nowadays, over seven hundred metric tons are isolated annually from *Cinchona ledgeriana*, with important application in food and beverages industry (bitter additive) and in medicinal chemistry (antimalarial, muscle relaxant and antiarrhythmic).[1]

Quinuclidine nucleus is known to be a good mimic for the quaternary nitrogen from acetylcholine. The charged quaternary nitrogen can pass the blood brain barrier, unlike acetylcholine.[2,3] Derivatives of *Cinchona* alkaloids having aromatic substituents in the position 3 are capable of blocking M1, 5-HT3 and NK1-receptors[4-12] and can be active as inhibitors for squalene synthase.[13,14] Dimeric *Cinchona* alkaloids like dimeric quinine- and quinidine-based phthalazine- and pyrimidine-bridged are important ligands in the Asymmetric Dihydroxylation (AD) reaction.[15,16]

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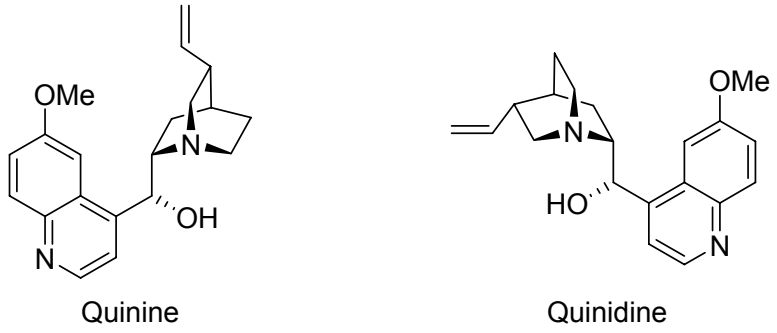


Fig. 1 – Main representatives of *Cinchona* alkaloids family.

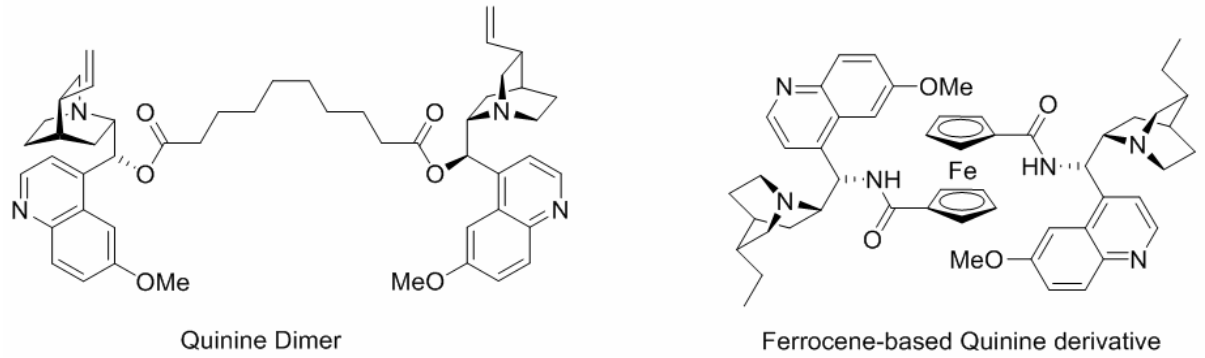


Fig. 2 – Examples of *Cinchona* alkaloid derivatives used in anticancer medicinal chemistry.

Although the antitumor activity of *Cinchona* alkaloids is not impressive (IC_{50} of quinine and quinidine for MCF-7 line *in vitro* is 40 respectively 113 μM), they have successfully been used in reversing of multidrug resistance (MDR) in the treatment of patients with marketed drugs such as vinblastine, doxorubicin or ethylprednisolone.[1,17] The most eloquent example is the Quinine dimer bridged by decanedioic acid (Fig. 2), a very active MDR, which can totally reverse the P-glycoprotein (P-gp)-mediated paclitaxel resistance phenotype and at the same time inhibit its transport in MCF-7/DX1 cell.[18]

Another reported *Cinchona* alkaloid-based antitumoral is the ferrocene-based quinine diamide (Fig. 2) which proved to be very active on most type of tumor cells with an IC_{50} values in the range between 0.72-1.70 μM.[19]

Taking into consideration the general biological activity of *Cinchona* alkaloids[20-23] we planned the synthesis and biological evaluation of some structurally diverse derivatives of these natural products.

As starting we choose the *Cinch* bases Cinchonidine and Cinchonine which are pseudo-enantiomeric to each other. The stereochemistry at the double bond makes them diastereometric.

Furthermore we chose the natural product based Quinuclidines Quincorine (QCI) and Quincoridine (QCD) in which the Quinol-4-yl rest of the *Cinch* bases are replaced by simple hydrogen (Figure 4). These compounds are also pseudo-enantiomeric.

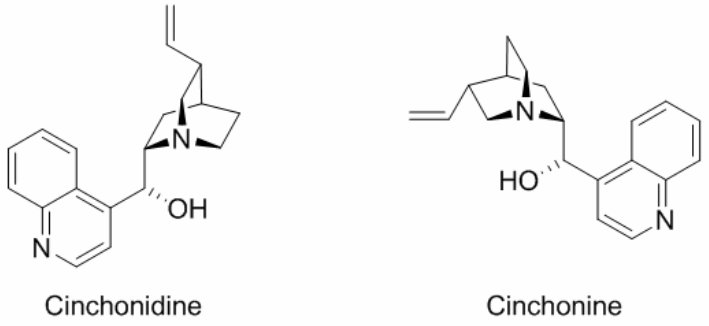
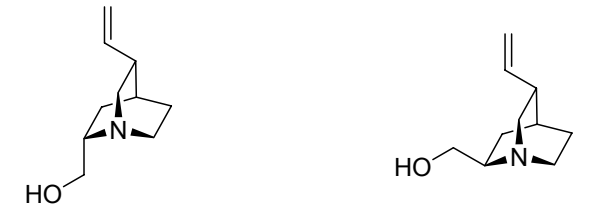


Fig. 3 – The *Cinch* bases Cinchonidine and Cinchonine.



Quincorine (QCI) Quincoridine (QCD)

Fig. 4 – QCI and QCD.

This gives two types of pairs of related structures. One is obvious, e.g. the *Cinch* bases have the same constitution. The other type of pair based on the stereochemistry at the Quinuclidine nucleus. Under this topic Cinchonidine and QCI build a pair.

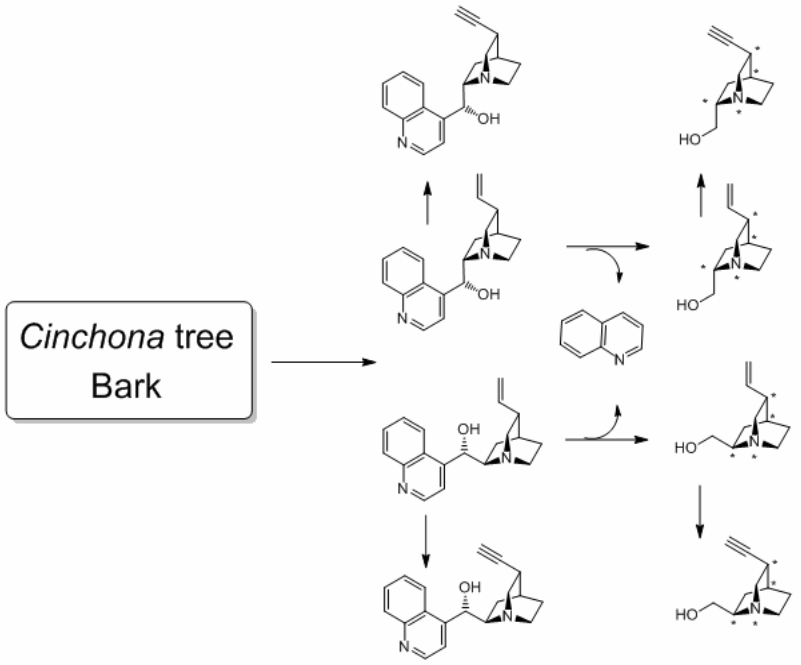
RESULTS AND DISCUSSION

As precursors for the gold(I) complexes we choose the alkyne derivatives of the *Cinch* bases, QCI and QCD. 10,11-Didehydrocinchonine, 10,11-Didehydrocinchonidine, 10,11-Didehydroquincorine, 10,11-Didehydroquincoridine were synthesized according to known protocols (scheme 1).[24-27]

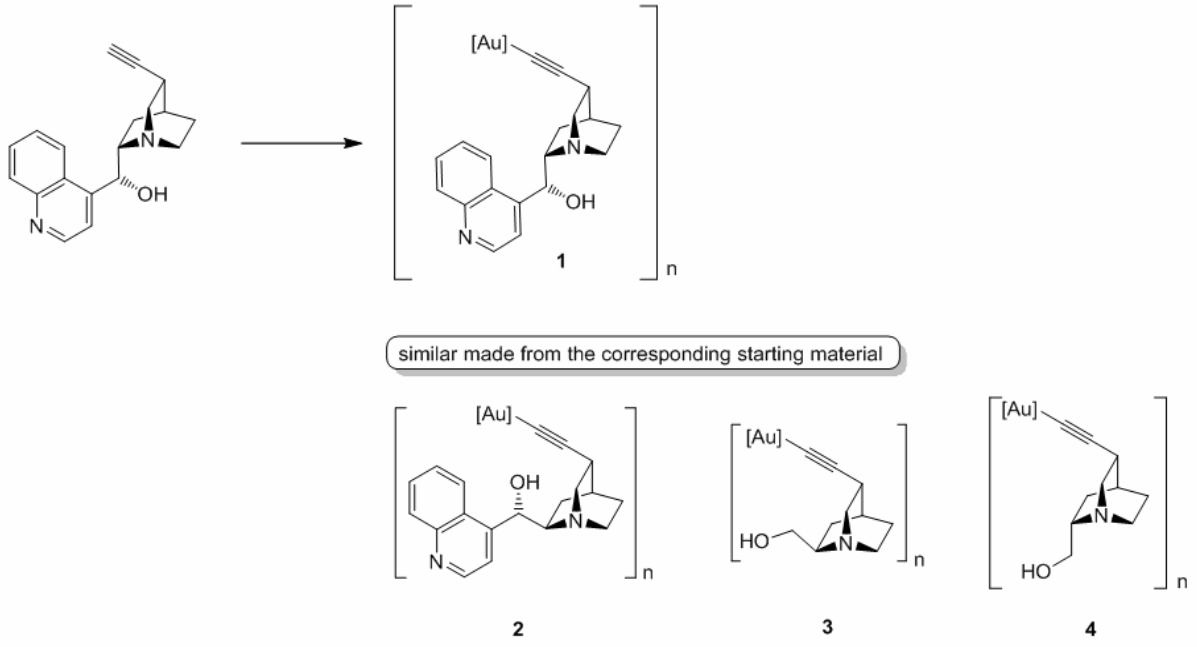
Having as prime precursors of the *Cinchona* alkaloids alkyne derivatives by hand we planned to synthesize a series of *Cinchona* alkaloids gold(I)-complexes. The synthesis of the novel alkenyl gold(I)-PPh_3 complexes was performed with modifications of already related synthetic routes changing the alkyne precursor.[28-32]

The first step of the plan was the generation and isolation of the alkenyl gold(I) polymers (**1** - **4**) as depicted in Scheme 2. By reacting the gold $AuCl(SMe_2)$ with the corresponding *Cinchona*-alkynyl derivative, in the presence of base (triethylamine) at ambient temperature in the absence of light we managed to produce and isolate the corresponding neutral oligomers **1** - **4** as pale-yellow solids (Scheme 2). With the knowing that in pure state this kind of compounds have the tendency to explode[33-37] and the fact that they are insoluble in most of the organic solvents we did not attempt to make any other purification. The IR spectrum showed a specific band around 2000 cm^{-1} which can be attributed to the $\nu(C\equiv C)$ stretching mode.[38]

The supposed intermolecular interaction of the single unit is illustrated in Fig. 5. The Gold atom is covalent-bonded to the alkynyl moiety of one ligand. Furthermore it is coordinated to triple bond of a neighbored molecule in way of a side on bond.



Scheme 1 – Synthetic derivatives from *Cinchona* alkaloids.



Scheme 2 – Synthesis of gold(I)-polymeric derivatives **1**-**4**.

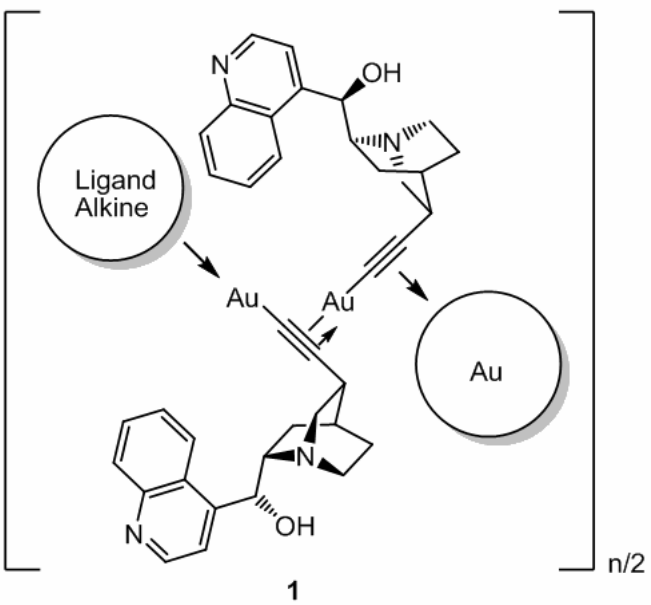


Fig. 5 – Suggested intermolecular interaction of the gold(I) with a second unit.

This suggestion is underlined by the ESI mass spectrometry. The main fragment which is observed is a tetrameric oligomer (Fig. 6). For us this proves the oligomeric / polymeric nature of the product and furthermore it shows that no alternative ligand is coordinated to the metal core.

One of the most efficient ways to synthesize alkynyl-gold(I) derivatives is the depolymerisation of the neutral homoleptic alkynyl-gold(I) polymers. This involves the presence of a good *σ*-donor ligand (phosphines, halides, isocyanates). We choose for this step the neutral triphenylphosphine ligand and after stirring in dry dichloromethane for 60 minutes, the insoluble alkynyl-gold(I) polymer disappeared and the corresponding alkynyl-gold(I)-PPh_3 derivatives were isolated (scheme 3). After precipitation with hexane were the compounds **5**-**8** fully characterised. The reactions occur with moderate to good yields (55-92%) and good purity (at least 95% based on the NMR spectroscopy).

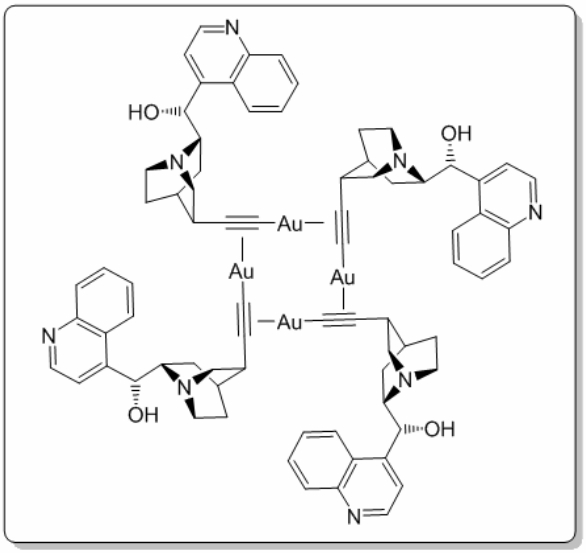
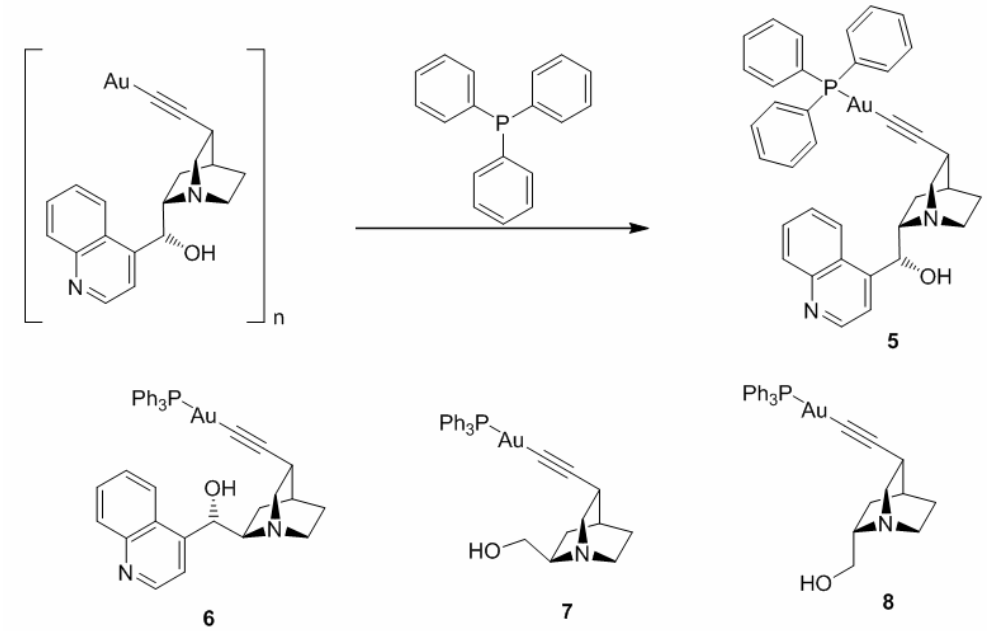


Fig. 6 – Main fragment of the ESI spectrometry measurement.



Scheme 3 – Synthesis of alkynyl-gold(I)-PPh_3 derivatives **5-8**.

ANTI-TUMOR ACTIVITY

Having isolated the Cinchona related gold(I) compounds (**5-8**) we tested their biological proprieties as antitumor agents. In this sense the *in vitro* anti-tumor activity of the compounds **5, 6, 7** and **8** was assessed in a panel of 12 human tumor cell lines by using a monolayer cell survival and proliferation assay. As shown in Fig. 3, anticancer potency as reflected by the geometric mean IC_{50} value was in the range from 0.78 μM (**5**) to 4.92 μM **(8**). Individual IC_{50} values for the most active compound **5** were between 0.31 μM (PAXF 1657, pancreatic cancer) and 1.9 μM (LXFA 629, lung cancer).

The IC_{50} mean graph presentation of compound **5** (Fig. 8) illustrates the selectivity profile of this compound with above average activity towards the cell lines PAX 1657, MAXF 401, MEXF 462 and OVXF 899.

The Cinconidine and the QCI derivatives have the same stereochemistry at the quinuclidinic bicycle. Although they differ in their absolute activity they show a similar selectivity to the single cell types. That means that the selectivity depends on the stereochemistry.

compound	unit	Cell lines												Geom. mean IC_{50} [μM]
		CXF HT-29	GXF 251	LXFA 629	LXFL 529	MAXF 401	MEXF 462	OVXF 899	PAXF 1657	PRXF 22Rv1	PXF 1752	RXF 486	UXF 1138	
5	μM	1,76	1,05	1,90	0,79	0,38	0,44	0,62	0,31	0,91	0,83	1,07	0,72	0,78
6	μM	3,83	1,20	3,06	1,86	1,10	1,51	5,51	1,60	1,99	1,79	3,11	2,04	2,13
7	μM	3,90	3,15	3,67	3,80	0,96	1,58	6,05	1,04	2,73	3,24	4,28	2,04	2,66
8	μM	11,43	6,30	9,84	5,78	3,05	3,87	3,00	1,94	5,46	5,42	5,48	4,36	4,92

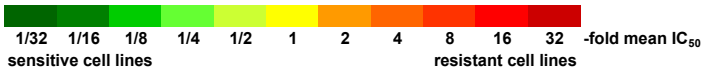


Fig. 7 – Heatmap presentation of absolute IC_{50} values for compounds **5, 6, 7** and **8** in a panel of 12 human tumor cell lines.

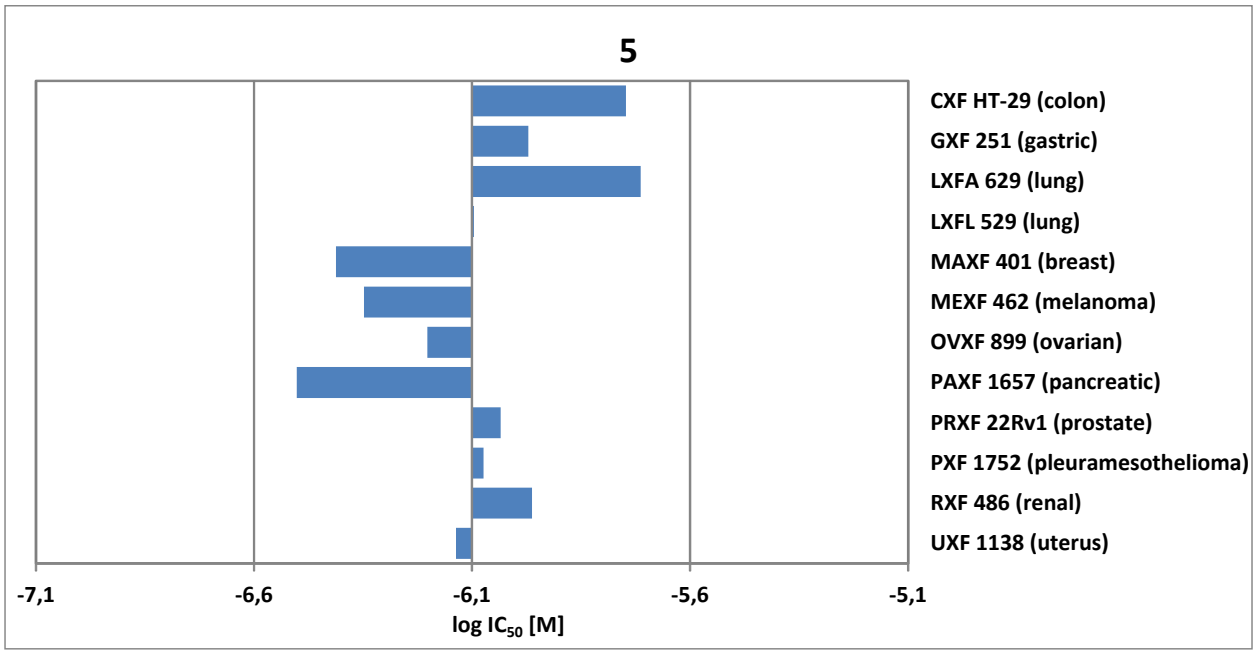


Fig. 8 – Selectivity profile of compound **5**.

EXPERIMENTAL

Cinchonine, Cinchonidine, Quincorine and Quincoridine were purchased from Buchler GmbH, Germany. All other reagents were purchased from other commercial sources and used without further purification. Solvents were of analytical grade.

^{1}H-NMR, ^{13}C-NMR and ^{31}P-NMR spectra were recorded at room temperature on a Bruker Avance 200 operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C. Chemical shifts (δ) are reported relative to tetramethylsilane. In the case of multiplets, the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet. IR spectra were recorded with a Bruker Vertex 70 ATR. Mass spectra were recorded on a Finnigan MAT 8400-MSS and Finnigan MAT 4515. High resolution mass spectra were recorded on a Finnigan MAT 95 XP.

GENERAL PROCEDURE

Synthesis of 10,11-Didehydrocinchonidine-gold(I) polymer (1)

To a solution of 514 mg of 10,11-Didehydrocinchonidine (1.76 mmol) in CH_2Cl_2 (20 mL) were successively added triethylamine (1 mL) and 519 mg

$AuCl(SMe_2)$ (1.76 mmol). The reaction mixture was stirred for 60 minutes at room temperature. The resulting solution was concentrated under reduced pressure to 10 mL and MeOH (10 mL) was added to precipitate a yellow-green powder which was filtered, washed with Et_2O (2 x 5 mL) and dried. Yield*:* 92% (790 mg, 1.61 mmol).
IR (ATR): 1/λ = 3116, 3097, 3067, 2965, 2927, 2871, 1616, 1593, 1519, 1463, 1424, 1410, 1391, 1347, 1319, 1282, 1244, 1193, 1138, 1091, 1032, 986, 970, 956, 890, 854, 775, 743, 696, 673, 632, 602, 551 cm^{-1}.

***Synthesis of 10,11-Didehydrocinchonine-gold polymer* (2)**

To a solution of 257 mg of 10,11-Didehydrocinchonine, (0.88 mmol) in CH_2Cl_2 (10 mL) were successively added triethylamine (0.5 mL) and 260 mg $AuCl(SMe_2)$, (0.88 mmol). The reaction mixture was stirred for 60 minutes at room temperature. The resulting solution was concentrated under reduced pressure to 5 mL and MeOH (5 mL) was added to precipitate a yellow-green powder which was filtered, washed with Et_2O (2 x 5 mL) and dried. Yield*:* 94% (403 mg, 0.82 mmol).
IR (ATR): 1/λ = 3266, 3067, 2931, 2866, 2164, 1591, 1511, 1452, 1320, 1238, 1094, 1022, 936, 852, 808, 759, 634, 607, 537 cm^{-1}.

***Synthesis of 10,11*-Didehydroquincoridine-gold(I) polymer (3)**

To a solution of 165 mg of 10,11-Didehydroquincoridine (1 mmol) in CH_2Cl_2 (10 mL) were successively added triethylamine (0.5 mL) and 295 mg $AuCl(SMe_2)$ (1 mmol). The reaction mixture was stirred for 60 minutes. The resulting solution was concentrated under reduced pressure to 5 mL and MeOH (5 mL) was added to precipitate a yellow powder which was filtered, washed with Et_2O (2 x 5 mL) and dried. Yield*:* 92% (332 mg, 0.92 mmol).
IR (ATR): 1/λ = 3335, 2929, 2866, 1979, 1646, 1452, 1384, 1321, 1255, 1198, 1134, 1100, 1068, 1014, 931, 909, 862, 817, 751, 626, 591 cm^{-1}.

***Synthesis of 10,11-Didehydroquincorine-gold(I) polymer* (4)**

To a solution of 165 mg of 10,11-Didehydroquincorine (1 mmol) in CH_2Cl_2 (10 mL) were successively added NEt_3 (0.5 mL) and 295 mg [$AuCl(SMe_2)$], (1 mmol). The reaction mixture was stirred for 60 minutes at room temperature. The resulting solution was concentrated under reduced pressure to 5 mL and MeOH (5 mL) was added to precipitate a yellow powder which was filtered, washed with Et_2O (2 x 5 mL) and dried. Yield*:* 89% (321 mg, 0.89 mmol).
IR (ATR): 1/λ = 3329, 2931, 2879, 2602, 2496, 1982, 1633, 1476, 1447, 1397, 1326, 1172, 1100, 1036, 996, 935, 811, 747, 604 cm^{-1}.

***Synthesis of 10,11-Didehydrocinchonidine-Gold(I)-PPh_3 complex* (5)**

To a suspension of 100 mg of 10,11-Didehydrocinchonidine-gold(I) polymer (**1**) (0.2 mmol) in CH_2Cl_2 (15 mL) was added 54 mg of PPh_3 (0.2 mmol). The reaction mixture was stirred for 60 minutes at room temperature and filtered through anhydrous $MgSO_4$. The solution was concentrated under vacuum to 1 mL and then hexane (10 mL) was added. After stirring for 15 hours, the suspension was filtered and the solid was air dried to give a white solid. Yield*:* 72% (108 mg, 0.144 mmol).
IR (ATR): 1/λ = 3048, 2922, 2879, 2861, 2711, 2582, 1591, 1507, 1481, 1436, 1330, 1308, 1236, 1212, 1158, 1102, 1024, 997, 946, 883, 853, 820, 772, 746, 711, 692, 648, 632, 613, 536 cm^{-1}. ^1H NMR (300.1 MHz, $CDCl_3$): δ = 8.81 (d, J = 4.5 Hz, 1H), 8.09-8.76 (m, 2H), 7.62-7.54 (m, 2H), 7.52-7.33 (m, 16H), 5.66 (d, J = 4.8 Hz, 1H), 3.94(bs, 1H, OH), 3.51-3.28 (m, 2H), 3.26-3.14 (m, 1H), 2.99-2.88 (m, 1H), 2.75-2.66 (m, 1H), 2.61-2.46 (m, 1H), 2.11-1.96 (m, 3H), 1.82–1.62 (m, 2H), 1.45-1.31 (m, 1H) ppm. ^{13}C NMR (75.47 MHz, $CDCl_3$): δ = 150.08, 149.25, 148.11, 134.12, 134,11, 131.32, 130.02, 129.07, 128.92, 126.53, 125.89, 123.31, 118.22, 87.53, 71.57, 59.69, 59.33, 42.70, 29.05, 28.13, 26.38, 22.41 ppm. ^{31}P NMR (121.49 MHz): δ = 42.78 ppm. MS (HR-ESI): calcd. for $C_{37}H_{34}AuN_2OPH^+$ ($M + H^+$) 751.2147 (100), 752.2180 (40), 753.2214 (10); found 751.2146 (100), 752.2175 (40), 753.2234 (10).

***Synthesis of 10,11-Didehydrocinchonine-Gold(I)-PPh_3 complex* (6)**

To a suspension of 100 mg of 10,11-Didehydrocinchonine-gold polymer (**2**) (0.2 mmol) in CH_2Cl_2 (15 mL) was added 54 mg of PPh_3 (0.2 mmol). The reaction mixture was stirred for 60 minutes at room temperature and filtered through anhydrous $MgSO_4$. The solution was concentrated under vacuum to 1 mL and then hexane (10 mL) was added. After stirring for 15 hours, the suspension was filtered and the solid was air dried to give a white solid. Yield*:* 65% (98 mg, 0.13 mmol).

IR (ATR): 1/λ = 3220, 3052, 2943, 2800, 2750, 2522, 1591, 1572, 1510, 1480, 1461, 1436, 1326, 1237, 1130, 1101, 1027, 997, 958, 862, 838, 750, 693, 617, 537 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 8.83 (d, J = 4.6 Hz, 1H), 8.12-7.99 (m, 2H), 7.62-7.58 (m, 2H), 7.49-7.36 (m, 16H), 5.71 (m, J = 5.8 Hz, 1H), 3.62 (bs, 1H, OH), 3.44 (m, 1H), 3.37 (m, 1H), 3.21 (m, 1H), 2.98 (m, 1H), 2.83 (m, 1H), 2.74 (m, 1H), 2.54 (m, 1H), 2.25 (m, 1H), 2.18 (m, 1H), 1.65 (m, 1H), 1.41 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ = 150.12, 149.09, 148.19, 134.19, 134.04, 130.95, 130.11, 128.99, 128.88, 126.57, 125.94, 123.34, 118.24, 71.57, 59.6, 59.3, 42.73, 29.06, 28.12, 26.36, 22.51 ppm. MS (HR-ESI): calcd. for $C_{37}H_{34}AuN_2OPH^+$ ($M + H^+$) 751.2147 (100), 752.2180 (40), 753.2211 (10); found 751.2135 (100), 752.2162 (40), 753.2192 (10).

***Synthesis of 10,11-Didehydroquincoridine-Gold(I)-PPh_3 complex* (7)**

To a suspension of 100 mg of 10,11-Didehydroquincoridine-gold(I) polymer (**3**) (0.28 mmol) in CH_2Cl_2 (15 mL) was added 72 mg of PPh_3 (0.28 mmol). The reaction mixture was stirred for 60 minutes at room temperature and filtered through anhydrous $MgSO_4$. The solution was concentrated under vacuum to 1 mL and then hexane (10 mL) was added. After stirring for 15 hours, the suspension was filtered and the solid was air dried to give a white solid. Yield*:* 68% (118 mg, 0.19 mmol).

^1H NMR (300 MHz): δ = 7.54-7.36 (m, 15H, PPh_3), 3.78-3.67 (m, 1H, H-9), 3.51-3.42 (m, 1H, H-9), 3.09–2.79 (m, 5H, H-7, H-7, H-6, H-6, H-2), 2.72–2.64 (m, 1H, H-5), 1.98-1.93 (m, 1H, H-3), 1.71–1.42 (m, 4H, H-4, H-8, H-8, H-3), 1.23(s, 1H, OH) ppm. ^{13}C NMR (125 MHz): δ = 142.98, 142.82, 133.51, 133.38, 132.45, 132.33, 131.98, 131.94, 126.75, 126.61, 125.33, 124.52, 83.14 (C-10), 71.55 (C-11), 67.93 (C-9), 54.27 (C-2), 40.68 (C-6), 30.27 (C-7), 26.03 (C-5), 25.87 (C-4), 23.35(C-8), 22.77 (C-3) ppm. ^{31}P NMR (121 MHz): δ = 41.61 ppm.

***Synthesis of 10,11-Didehydroquincorine-gold(I)-PPh_3 complex* (8)**

To a suspension of 100 mg of 10,11-Didehydroquincorine-gold(I) polymer (**4**) (0.28 mmol) in CH_2Cl_2 (15 mL) was added 72 mg of PPh_3 (0.28 mmol). The reaction mixture was stirred for 1h and filtered through anhydrous $MgSO_4$. The solution was concentrated under vacuum to 1 mL and then hexane (10 mL) was added. After stirring for 15 hours, the suspension was filtered and the solid was air dried to give a white solid. Yield*:* 55% (96 mg, 0.154 mmol).

^1H NMR (200 MHz): δ = 7.31-7.22 (m, 15H, PPh_3), 3.53-3.48 (m, 2H, H-9, H-9), 3.33-3.24 (m, 1H, H-6), 3.19–3.09 (m, 1H, H-2), 2.95–2.86 (m, 1H, H-3), 2.62-2.51 (m, 1H, H-5), 2.19–2.05 (m, 1H, H-3), 1.96–1.90 (m, 1H, H-4), 1.55–1.24 (m, 3H, H-8, H-8, OH), 0.92–0.78 (m, 1H, H-3) ppm. ^{13}C NMR (75 MHz): 87.75 (C-10), 68.74 (C-11), 62.77 (C-9), 57.0 (C-2), 56.73 (C-6), 39.65 (C-7), 27.76 (C-5), 26.50 (C-4), 26.29 (C-8), 24.81 (C-3) ppm. ^{31}P NMR (81 MHz): 20.72.

In vitro antitumor activity towards human tumor cell lines

Antitumor activity of the compounds was tested in a monolayer cell survival and proliferation assay using human tumor cell lines.

Ten out of the twelve cell lines as tested were established at Oncotest from patient-derived human tumor xenografts passaged subcutaneously in nude mice.[39] The origin of the donor xenografts was described.[40,41] The cell line 22RV1 was supplied by ATCC ((Rockville, MD), HT-29 was kindly provided by the National Cancer Institute (Bethesda, MA, USA). Cells were cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum and 0.1 mg/mL gentamicin under standard conditions (37 °C, 5% CO2). Authenticity of all cell lines was proven by STR analysis at the DSMZ (Braunschweig, Germany).

A modified propidium iodide assay was used to assess the compounds' activity toward human tumor cell lines.[42] Briefly, cells were harvested from exponential phase cultures by trypsinization, counted and plated in 96-well flat-bottom microtiter plates at a cell density dependent on the cell line (4.000–20.000 cells/well). After 24 h recovery period to allow the cells to adhere and resume exponential growth, compounds were added at 10 concentrations in half-log increments and left for further 4 days. The inhibition of proliferation was determined by measuring the DNA content using an aqueous propidium iodide solution (7 μg/mL). Fluorescence was measured using the Enspire Multimode-Plate Reader (excitation λ = 530 nm, emission λ = 620 nm), providing a direct relationship to the total viable cell number. In each experiment, all data points were determined in duplicates. Anti-tumor activity was reported as the absolute IC_{50} value, which

reflects the concentration of the test compound that achieves test/control values of 50%. Calculation was done by 4 parameter non-linear curve fit (Oncotest Data Warehouse Software). The overall potency of a compound was reflected by the geometric mean IC_{50} values of all individual IC_{50} values.

CONCLUSIONS

Molecular Gold(I) complexes **5 - 8** related to *Cinchona* alkaloids were synthesized starting from the alkynes 10,11-Didehydrocinchonine, 10,11-Didehydrocinchonidine, 10,11-Didehydroquincorine and 10,11-Didehydroquincoridine in two steps. The polymeric Gold(I)-intermediates **1 - 4** were isolated and characterized by IR spectroscopy. Compounds **5 - 8** were tested for anti-tumor activity *in vitro* towards a panel of 12 cell lines using a monolayer cell survival and proliferation assay. With an IC_{50} value of 0.78 μM, compound **5** was the most active and most selective towards the cell lines PAX 1657, MAXF 401, MEXF 462 and OVXF 899. It has been shown that the conformation at the Quinuclidinic core has a huge influence in the activity.

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