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)-PPh3 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 antiarrhythmnic).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-receptors4-12 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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Although the antitumor activity of Cinchona alkaloids is not impressive (IC\textsubscript{50} of quinine and quinidine for MCF-7 line \textit{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.\textsuperscript{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.\textsuperscript{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\textsubscript{50} values in the range between 0.72-1.70 µM.\textsuperscript{19}

Taking into consideration the general biological activity of Cinchona alkaloids\textsuperscript{20-25} 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. 1 – Main representatives of Cinchona alkaloids family.

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

Fig. 3 – The Cinch bases Cinchonidine and Cinchonine.
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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)-PPh3 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(SMe2) 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 explode33-37 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 ν(C≡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.

[Diagrams and schemes are not transcribed here, but are referenced in the text.]
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)-PPh₃ 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).
ANTITUMOR 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.
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<th>compound</th>
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<th>CXF HT-29</th>
<th>GXF 251</th>
<th>LXFA 629</th>
<th>LXFL 529</th>
<th>MAXF 401</th>
<th>MDF 462</th>
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<th>PRXF 22Rv1</th>
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<th>RXF 486</th>
<th>UXF 1138</th>
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Fig. 7 – Heatmap presentation of absolute IC50 values for compounds 5, 6, 7 and 8 in a panel of 12 human tumor cell lines.

![Heatmap Image]

Fig. 8 – Selectivity profile of compound 5.

**EXPERIMENTAL**

Cinchonine, Cinchonidine, Quinchorine and Quincondine 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. 1H-NMR, 13C-NMR and 31P-NMR spectra were recorded at room temperature on a Bruker Avance 200 operating at 200 MHz for 1H and 50 MHz for 13C. 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 CH2Cl2 (20 mL) were successively added triethylamine (1 mL) and 519 mg...
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AuCl(SMe₂) (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 Et₂O (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(I) polymer (2)

To a solution of 257 mg of 10,11-Didehydrocinchonine, (0.88 mmol) in CH₂Cl₂ (10 mL) were successively added triethylamine (0.5 mL) and 260 mg AuCl(SMe₂), (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 Et₂O (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 CH₂Cl₂ (10 mL) were successively added triethylamine (0.5 mL) and 295 mg AuCl(SMe₂), (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 Et₂O (2 x 5 mL) and dried. Yield: 92% (332 mg, 0.92 mmol).

IR (ATR): 1/λ = 3335, 2929, 2871, 1979, 1643, 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, CDCl₃): 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. ¹³C NMR (75.47 MHz, CDCl₃): 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. ³¹P NMR (121.49 MHz): δ = 42.78 ppm. MS (HR-ESI): calcd. for C₃₇H₃₄AuN₂OPH⁺ (M⁺H⁺) 751.2147 (100), 752.2180 (40), 753.2214 (10); found 751.2147 (100), 752.2180 (40), 753.2214 (10); found 751.2146 (100), 752.2175 (40), 753.2234 (10).

Synthesis of 10,11-Didehydrocinchonidine-Gold(I)-PPh₃ complex (5)

To a suspension of 100 mg of 10,11-Didehydrocinchonidine-gold(I) polymer (1) (0.2 mmol) in CH₂Cl₂ (15 mL) was added 54 mg of PPh₃ (0.2 mmol). The reaction mixture was stirred for 60 minutes at room temperature and filtered through anhydrous MgSO₄. 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, CDCl₃): 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. ¹³C NMR (75.47 MHz, CDCl₃): 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. ³¹P NMR (121.49 MHz): δ = 42.78 ppm. MS (HR-ESI): calcd. for C₃₇H₃₄AuN₂OPH⁺ (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-Didehydrocinchonidine-Gold(I)-PPh₃ complex (6)

To a suspension of 100 mg of 10,11-Didehydrocinchonidine-gold(I) polymer (2) (0.2 mmol) in CH₂Cl₂ (15 mL) was added 54 mg of PPh₃ (0.2 mmol). The reaction mixture was stirred for 60 minutes at room temperature and filtered through anhydrous MgSO₄. 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): $\frac{1}{\lambda} = 3220, 3052, 2943, 2800, 2750, 2522, 1591, 1572, 1510, 1480, 1326, 1237, 1130, 1027, 997, 958, 862, 838, 750, 693, 617, 537$ cm$^{-1}$. $^1$H NMR (CDCl$_3$): $\delta = 8.83$ (d, $J = 4.6$ Hz, 1H), 8.12-7.99 (m, 2H), 7.62-7.58 (m, 2H), 7.49-7.36 (m, 1H), 7.49-7.36 (m, 1H), 7.49-7.36 (m, 1H), 2.54 (m, 1H), 2.25 (m, 1H), 1.65 (m, 1H), 1.41 (m, 1H) ppm. $^{13}$C NMR (CDCl$_3$): $\delta = 150.12, 149.09, 148.19, 134.19, 134.04, 130.95, 130.11, 128.99, 128.88, 126.57, 125.94, 123.34, 118.24, 71.57, 59.6, 59.3, 42.73, 29.06, 28.12, 26.36, 22.51 ppm. MS (HR-ESI): calcd. for C$_{37}$H$_{34}$AuN$_2$OPH$^+$ (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$_2$Cl$_2$ (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).

$^1$H NMR (300 MHz): $\delta = 7.54-7.36$ (m, 1H, 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, OH), 0.92–0.78 (m, 1H, H-3) ppm. $^{13}$C NMR (125 MHz): 87.75 (C-10), 68.74 (C-11), 62.77 (C-9), 57.0 (C-2), 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. The origin of the donor xenografts was described. 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. 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 $\lambda = 530$ nm, emission $\lambda = 620$ 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-Didehydroquinocorine and 10,11-Didehydroquinocoridine in two steps. The polymeric Gold(I)-intermediates 1 - 4 were isolated and characterized by IR spectroscopy. Compounds MAXF 401, MEXF 462 and OVXF 899. It has been most selective towards the cell lines PAX 1657, Cinchona the alkynes 10,11-Didehydrocinchonine, 10,11-

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

**REFERENCES**