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Cytotoxicity of Gold(I) N-Heterocyclic Carbene Complexes Assessed by Using Human Tumor Cell Lines

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The discovery of cisplatin in 1965 and its subsequent approval as an anticancer drug in 1978, paved the way for advances in anticancer platinum-based drugs.[1] However, cisplatin is only effective for a narrow spectrum of tumor cells and is associated with various side effects such as neurotoxicity or nephrotoxicity.[1b,2] Like many other cytotoxic drugs, tumor cells acquire resistance to cisplatin treatment that further limits its effectiveness. Cisplatin causes cell death by interacting with the cells DNA, which results in activation of several cell cycle pathways and triggers apoptosis and hence is not cancer-cell specific.[3] For this reason, transition-metal complexes are still under investigation to improve cytotoxicity and selectivity toward the cancer cells.[4] Among the transition metals, organogold complexes have emerged as interesting antitumoral agents.[5] The first generation came with Auranofin and some other phosphane gold(I) complexes.[6] Since the turn of the millennium, gold N-heterocyclic carbenes (Au–NHCs) complexes have been investigated as alternatives to gold tertiary phosphane (Au–PR_3) compounds.[7] [Au^I(NHC)L] (where L is a two-electron donor) have shown promising results with the compounds targeting the mitochondrial function leading to apoptosis.[5,6a,7b,8] These complexes have been demonstrated to induce mitochondrial membrane permeabilization, which plays an essential role in mitochondria-induced apoptosis and that this behavior is directly linked to the lipophilicity of the complexes.[1b,5,8b] Furthermore, a family of lipophilic cationic gold(I) complexes of NHC was found to specifically target cancer cells derived from established breast cancer cell lines.[7c] Auranofin, a gold complex drug used for the treatment of rheumatoid arthritis,[9] induces apoptosis by a mechanism involving inhibition of the mitochondrial enzyme thioredoxin reductase.[5,8b] Au^I–NHC complexes have been shown to cause cancer-specific cell death in a similar way.[7c] The compounds accumulate in mitochondria of cancer cells, to cause cell death through a mitochondrial apoptotic pathway and to inhibit the activity of thioredoxin reductase (TrxR).[7c]

Recently, Nolan and co-workers have reported the synthesis of novel Au^I–NHC complexes.[10] Taking advantage of these developments and the associate synthetic routes facilitated by a versatile gold–NHC synthon, we chose to investigate the selectivity of the synthon and related complexes toward cancer cells. A selection of Au–NHC complexes were tested by using established breast, prostate, and bladder tumor cell lines and novel normal and tumor cell lines derived from the same patient from the breast and prostate.[11] Herein, results on the cytotoxicity of these Au^I–(NHC) complexes on these cell lines are presented.

Amoung complexes available in our laboratory, two different series were selected in addition to the two synthetic precursors, [Au(IPr)OH][10e] (**1**) and [Au(IPr)Cl][12] (**2**) (IPr = *N*,*N*′-bis(2,6-diisopropylphenyl)imidazol-2-ylidene). The first series chosen comprised a number of Au–IPr bearing biocompatible moieties (complexes **3**–**9**). 1-Thio-β-D-glucose tetraacetate (**3**), amino acids (such as L-proline (**4**), L-cysteine (**6**), D-alanine (**7**), L-DOPA (L-3,4-dihydroxyphenylalanine) (**8**)), β-estradiol (**5**), and saccharin (**9**) were selected as gold drug candidates for the present study (Scheme 1).

Complexes **2**–**9** will permit the evaluation of the impact of the presence of biocompatible entities such as a thiosugar, an amino acid, a steroid, or a fragment related to the sugar moiety, namely saccharin.

For the second series, cationic bis-(NHC), $[Au(NHC)_2]^+$ and NHC(phosphane)gold(I) complexes,[10f] $[Au(PR_3)(NHC)]^+$ (**10**–**20**) were selected because they are known to induce selectively apoptosis in the cancer cell line MDA MB231.[7c] For these cationic gold complexes **10**–**20**, three N-arylated, three N-alkylated NHCs, and three tertiary phosphanes were selected (Scheme 2). This selection was made based on the different complex lipophilicity properties found, which are important parameters for the accumulation in the mitochondria.[8]

The cytotoxicity of complexes **1**–**20** was evaluated toward human tumor cell lines. The IC_{50} and IC_{10} concentrations

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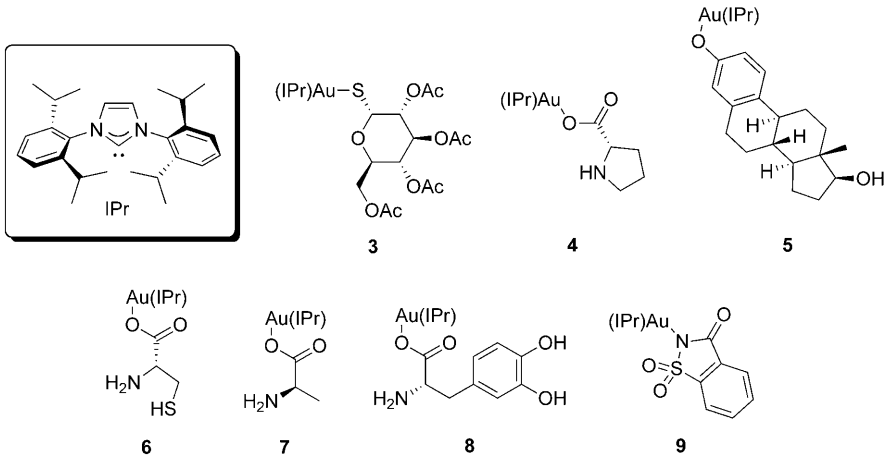
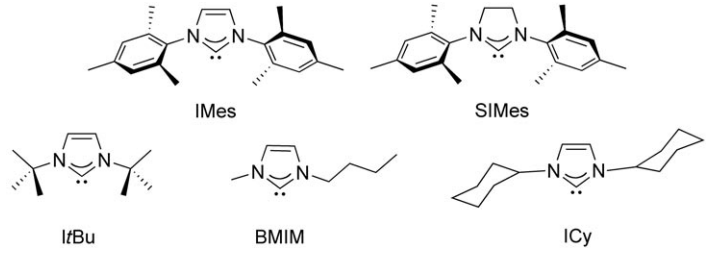


Scheme 1. Selected [Au(IPr)L] complexes bound to biocompatible moieties **3**–**9** used in this study.



[Au(IPr)(ICy)][BF_4] **10**
[Au(IPr)(BMIM)][Cl] **11**
[Au(IPr)(BMIM)][PF_6] **12**
[Au(IPr)(BMIM)][BF_4] **13**
[Au(IPr)(I*t*Bu)][BF_4] **14**
[Au(IPr)(IMes)][BF_4] **15**
[Au(IPr)(SIMes)][BF_4] **16**
[Au(IPr)(IPr)][BF_4] **17**
[Au(IPr)(PPh_3)][BF_4] **18**
[Au(IPr)(P*t*Bu_3)][BF_4] **19**
[Au(IPr)(P*n*Bu_3)][BF_4] **20**

Scheme 2. Selected $[Au(NHC)_2]^+$ and $[Au(PR_3)(NHC)]^+$ complexes **10**–**20** used in this study.

varied for the different compounds tested using the LNCaP (prostate carcinoma) and MDA MB231 (breast carcinoma) cell lines (Table 1). For the LNCaP and MDA MB231 tumor cell lines, compounds **1**–**3** and **10**–**20** were more effective than compounds **4**–**9**. The dose response relationships for each compound were similar for each of the cell lines. There was a marked difference in cytotoxicity for compounds **3** and **5** for example (Figure 1), **5** exhibited no cytotoxicity at 3 μM compared to **3** in which survival was only a few percent. Similarly compounds **4**, **6**, **7**, **8**, and **9** were not active at concentrations below 2.5 μM (Figure 1). Thus based on the responses of the prostate and breast cancer lines, compounds **1**, **2**, **3**, and **10** as active compounds and **4**–**9** as control inactive compounds were selected for further studies. The normal human urothelial cell line SV-HUC-1 and the bladder carcinoma cell line MGH-U1 also exhibited a similar sensitivity to compounds **1**–**3** and **10** with little difference in sensitivity between the two cell lines (Table 2). The breast cancer cell line B42 CL16 also showed similar patterns with compounds **1**–**3** being more cytotoxic than **4**–**9** (Table 3).

There was, for example, a marked difference in cytotoxicity between compound **3** and **4** and **5** (Figure 2), **4** and **5** exhibiting weak cytotoxicity at concentrations below 1 μM, whereas **3** was markedly cytotoxic at 0.5 μM with cell survival below 1%. A normal breast epithelial cell line B42 from the same patient showed a similar sensitivity to the tumor line (data not shown).

The responses of the two prostate cell lines derived from the same patient were compared, a normal epithelial cell line P21TZ and a tumor cell line P21PZ. The pattern of activity was similar to that reported for the other cell lines with compounds **1**–**3** and **10** exhibiting greater cytotoxicity than compounds **4**–**9** (Table 4). The sensitivity of the normal prostate epithelial cells and tumor cells were similar, for example showing marked responses to

Table 1. Comparison of the cytotoxicity of a series of Au–NHC compounds (**1**–**20**) using a prostate carcinoma cell line (LNCaP) and a breast carcinoma cell line (MDA MB231).[a]

Compound	IC_{50} [μM] LNCaP	IC_{10} [μM] LNCaP	IC_{50} [μM] MDA MB231	IC_{10} [μM] MDA MB231
1	1.40	2.30	0.90	1.42
2	1.90	2.60	0.92	>1.5
3	1.25	2.40	0.82	2.00
4	>4.5	>4.5	>3.0	>3.0
5	>4.5	>4.5	>3.0	>3.0
6	>4.5	>4.5	>3.0	>3.0
7	>4.5	>4.5	>3.0	>3.0
8	>4.5	>4.5	>3.0	>3.0
9	>4.5	>4.5	>3.0	>3.0
10	0.75	2.75	0.21	1.90
11	0.41	2.85	0.48	2.75
12	0.43	2.75	1.00	2.80
13	0.63	2.90	0.82	3.00
14	0.48	2.90	0.46	2.40
15	0.42	2.40	0.38	1.45
16	0.38	2.80	0.36	1.90
17	0.37	2.00	0.37	1.70
18	0.73	1.20	0.38	0.94
19	0.41	2.00	0.40	1.30
20	0.38	2.80	0.35	2.50

[a] Average of 3–5 experiments. Experimental errors are between 10 and 15%.

Table 2. Comparison of the cytotoxicity of a series of Au–NHC compounds (**1**, **2**, **3**, and **10**) using a human urothelial cell line (SV-HUC-1) and a bladder carcinoma cell line (MGH-U1).[a]

Compound	IC_{50} [μM] SV-HUC-1	IC_{10} [μM] SV-HUC-1	IC_{50} [μM] MGH-U1	IC_{10} [μM] MGH-U1
1	0.10	0.35	0.18	0.45
2	0.10	0.24	0.18	>0.5
3	0.20	0.47	0.23	>0.5
10	0.30	0.55	0.18	0.90

[a] Average of 3–5 experiments. Experimental errors are between 10 and 15%.

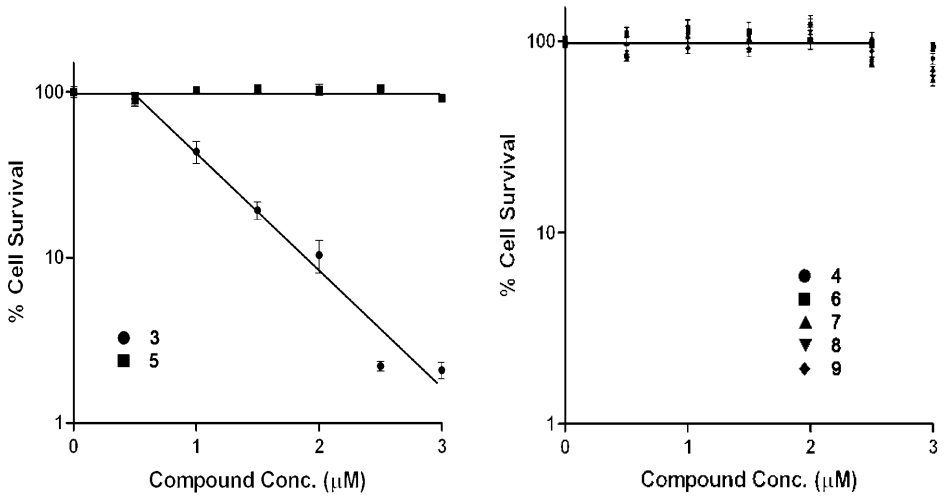


Figure 1. Comparison of the cytotoxicity of compounds **3** and **5** (left) and **4**, **6**, **7**, **8**,and **9** (right) using the human prostate carcinoma cell line (LNCaP).

While the patterns of cytotoxicity for the different compounds were the same, the sensitivity of the different cell lines varied. The well-established prostate and breast cancer lines (LNCaP and MDA MB231) were less sensitive than the bladder cell lines (SV-HUC-1 and MGH-U1), which in turn had a similar sensitivity to the prostate and breast cell lines (B42 CL16 and P21TZ and P21PZ). There were only small differences between the sensitivity of the normal epithelial cells and tumor cells derived from the same patient. The active compounds are

Table 3. Comparison of the cytotoxicity of a series of Au-NHC compounds (**1**-**10**) using a human breast carcinoma cell line (B42 CL16).[a]

Compound	IC_{50} [μM] B42 CL16	IC_{10} [μM] B42 CL16
1	0.18	0.41
2	0.20	0.70
3	0.20	0.40
4	0.76	>1
5	0.67	>1
6	0.72	>1
7	0.58	>1
8	0.52	>1
9	0.42	>1
10	0.04	0.06

[a] Average of 3–5 experiments. Experimental errors are between 10 and 15%.

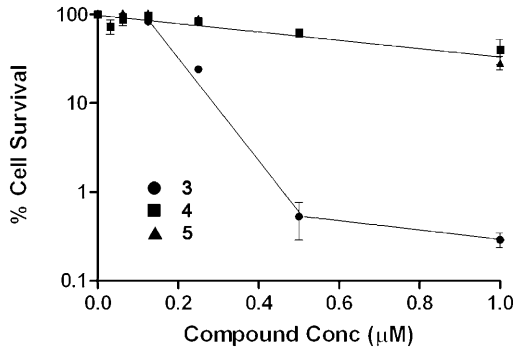


Figure 2. Comparison of the cytotoxicity of compounds **3**, **4**, and **5** using the human breast cancer cell line (B42 CL16).

3 compared to **5** (Figure 3). The prostate (LNCaP) and breast (MDA MB231) cancer cell lines have IC_{50}values of 18 and 28 μM, respectively, and the urothelial cell line an IC_{50} of 25 μM in response to cisplatin (Table 5). For our active compounds, the IC_{50}values were much lower than those found for cisplatin with the same cell lines by a factor of 20 to 100.

Table 4. Comparison of the cytotoxicity of a series of Au–NHC compounds (**1**–**10**) using a human prostate epithelial cell line P21TZ and a prostate carcinoma cell line P21PZ derived from the same patient.[a]

Compound	IC_{50} [μM] P21TZ	IC_{10} [μM] P21TZ	IC_{50} [μM] P21PZ	IC_{10} [μM] P21PZ
1	0.43	0.82	0.35	0.50
2	0.43	0.80	0.40	0.83
3	0.32	0.48	0.30	0.47
4	>1	>1	>1	>1
5	0.92	>1	0.90	>1
6	0.88	>1	0.94	>1
7	>1	>1	>1	>1
8	>1	>1	>1	>1
9	0.82	>1	0.92	>1
10	0.24	0.48	0.12	0.80

[a] Average of 3–5 experiments. Experimental errors are between 10 and 15%.

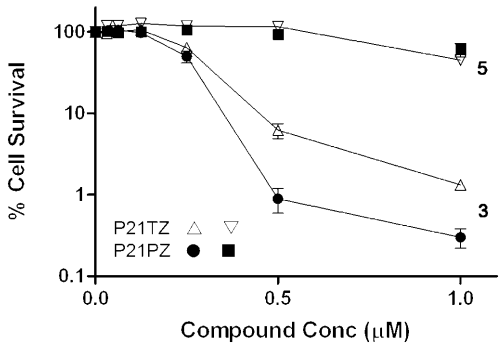


Figure 3. Comparison of the cytotoxicity of compounds **3** and **5** using the human prostate epithelial cell line P21TZ and the prostate cancer cell line P21PZ derived from the same patient.

[Au(OH)(IPr)] (**1**), [AuCl(IPr)] (**2**), and complexes **3** and **10**, whereas the inactive compounds are the amino acid, the steroid, and the saccharin gold(I) complexes **4**–**9**.

Table 5. Comparison of the cytotoxicity of Cisplatin using a human urothelial cell line (SV-HUC-1), a prostate carcinoma cell line (LNCaP) and a breast carcinoma cell line (MDA MB231).[a]

Cell Line	SV-HUC-1	LNCaP	MDA MB231
IC_{50} [μM]	25	18	28

[a] Average of two experiments. Experimental error is $\leq 10\%$.

Thus, the cytotoxicity is dependent on the group attached to the gold moiety. The IC_{50} for the active compounds were <1 μM, which is more active than the benzimidazole NHC compounds with IC_{50} values in the range 4.6–14.9 μM. No tumor-selective behavior was observed in our study using breast, prostate, and bladder cells similar to that reported for fibroblasts and kidney cells.[13] While this is of interest, the tissues that are likely to limit therapeutic use are the haematopoietic and lymphoid tissues.

In summary, the cationic complexes **10**–**20** were more effective than the neutral congeners, **1**–**9**. These active compounds are lipophilic, an important parameter associated with mitochondrial accumulation. Compounds of this type have previously been shown to cause cell death by an apoptotic pathway in cancer cell lines.[7c] While there were differences in the responses of the different cell lines investigated, compounds **4**–**9** consistently were less active than the other compounds tested in each cell line studied. The two neutral complexes (**1** and **2**) and the 1-thio-β-D-glucose complex were more active than the other complexes with associated biocompatible ligands (**4**–**9**). This probably reflects the processing of these complexes within the cells resulting in minimal cytotoxicity. The cationic NHC complexes (**10**–**20**) were also in general more potent than the neutral complexes (**1**–**3**) that were active. This may reflect the importance of the mitochondrial apoptotic pathway in the action of the effective compounds. Future work will exploit xenograft models to investigate the in vivo efficacy of the active compounds.

Experimental Section

Cytotoxicity assay:[11a] The compounds were dissolved in DMSO and diluted in DMSO. The final dilution was in the respective culture medium and the final concentration of DMSO was always below 0.01%. Cells were pipetted into microtitre plates (NUNC) at 4000 cells per well and incubated at 37 °C in 5% CO_2 in air for 24 h. Varying concentrations of the compounds were applied to the cells in 10 μL volumes. The plates were incubated for three days at 37 °C 5% CO_2 in air. The viability of the cells was measured by using the Dojindo kit CCK-8 (cell counting kit 8, Dojindo technologies USA, CK04–11) method after incubation for 3 h in the absence of light. The plates were read on an ELISA plate reader at a wavelength of 450 nm.

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Keywords: cytotoxicity · gold complexes · human tumor cells · N-heterocyclic carbenes

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