Article

Cycloaurated Gold(III) Complexes - Possible Alternatives to Cisplatin?

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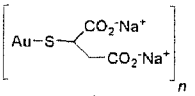
Gold in Medicine – A Brief Introduction

The serendipitous discovery of the anti-tumour activity of *cisplatin* [*cis*-$PtCl_2(NH_3)_2$] in 1969 has led to increased interest in the development of new metal-based anti-cancer drugs. However, regardless of the large numbers of new metal-containing compounds generated, many of which demonstrate anti-tumour activity, *cisplatin* still remains one of the most widely used anti-tumour drugs in the western world.

The similarities between gold(III) and platinum(II) (both are d^8 and form four-coordinate square planar complexes) give rise to the possibility of developing gold(III) analogues of *cisplatin*. The use of gold and its compounds in medicine is not a novel concept – ancient Arabic, Indian and Chinese physicians used gold preparations for the treatment of a wide variety of ailments. In 1890, Koch demonstrated that $[Au(CN)_2]^-$ has bacteriostatic effects, and in the 1920's gold compounds were used for the treatment of tuberculosis but later shown to be ineffective. Today gold(I) thiolates, namely Myocrisin® (sodium aurothiomalate) **1** and auranofin (aurothioglucose) **2**, are used for the treatment of rheumatoid arthritis (*chrysotherapy*). In addition, gold complexes continue to be screened for activity as anti-HIV, anti-microbial and anti-malarial agents. Several comprehensive reviews are dedicated to the medicinal applications of gold compounds and readers are directed towards these for a full and interesting history of the topic.[1] A large number of gold complexes also have been screened previously for anti-tumour activity, the details of which can be found in selected references.[2-4] However, despite promising results, no gold complexes have made it into clinical use as anti-tumour agents yet, although there continues to be a large quantity of research devoted to the development of new gold drugs. This article summarizes work conducted with neutral cycloaurated complexes and details some that has been conducted recently with gold(III) complexes in our Department.



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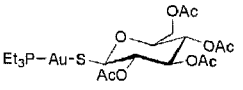

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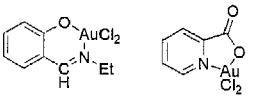
Cycloaurated Compounds as Anti-tumour Agents

The major set-back in the use of gold(III) in medicine is that it has a large reduction potential and is relatively easily reduced to gold(I) or gold(0). This is a potential problem in the body because of the presence of reducing thiol moieties, *e.g.* in the amino acid cysteine $HSCH_2CH(NH_2)CO_2H$. This problem seems to be largely overcome by incorporating the gold into a metallacyclic ring. If a chelating monoanionic ligand (L) is used, the resulting complex will have the general formula $LAuX_2$, where X is typically a unidentate anion such as Cl^- (examples of bidentate dianionic ligands are known – see below). Thus, the use of these types of systems provides a complex that remains neutral with four-coordinate square planar geometry as is seen in *cisplatin*. In addition, the presence of the chelating ligand forces the two anionic (Cl^-) ligands into a cis arrangement that further increases the similarity to *cisplatin*.

Initial studies on cycloaurated systems centred on those containing bidentate *N,O* donor ligands – either salicylaldimine-derived Schiff bases or pyridine-2-carboxylate as in **3** and **4**, respectively. However, the results were not promising as the complexes underwent immediate reduction to elemental gold in the presence of biological media that, presumably, was accompanied by oxidation of cellular material giving a disastrous toxicity.[5] Later, independent studies demonstrated that these compounds did display some level of cytotoxicity,[6] possibly from binding to DNA,[7] although other mechanisms could not be excluded - see below.



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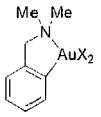
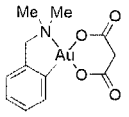
Most work conducted with cycloaurated organometallic complexes has centred around (damp)$AuCl_2$ [**5**; damp = 2-(*N,N*-dimethylaminomethyl)phenyl]. As in *cisplatin*, the metal atom is square-planar with two labile chloride ligands. Additionally, gold(III) is a relatively soft metal centre so that the softer (relative to *N,O* above) *C,N* ligand system stabilizes the metal towards reduction. Parish, Fricker and co-workers[8-10] initially investigated the *in vitro* anti-tumour activity of **5** against a range of human tumour cell lines and found that it was comparable to *cisplatin*, a control in the experiment. Like *cisplatin*, **5** showed greatest toxicity against breast (ZR-75-1), bladder (HT1376) and ovarian (SK-OV-3) cell lines. Because **5** showed good activity *in vitro* towards the breast tumour line it was also evaluated against a solid form of the tumour, grown as a xenograft in nude mice. The results indicated that **5** possessed modest anti-tumour activity as it reduced the size of the tumour, but there was also evidence of cytotoxicity; several mice died before the conclusion of the experiment. Overall, the results were not as promising as the initial *in vitro* screening predicted, possibly because the low solubility of the compound in aqueous media hindered its transport from the injection site to the tumour.[8]

In light of these results, it seemed likely that analogues of **5** with enhanced aqueous solubility characteristics would show increased activity. Replacement of the chloride



5, X = Cl
6, X = OAc



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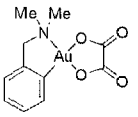

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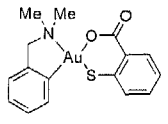
ligands by acetate, malonate or oxalate groups gave complexes **6-8**, respectively, which have better solubility. An added attractive feature of the acetato **6** is that, like *cisplatin*, the two labile ligands are hydrolysed in aqueous solutions.[9] Thus, **5-8** were evaluated *in vitro* against a panel of human solid tumours and, consequently, the bladder carcinoma (HT1376) was consistently the most sensitive to the new gold(III) complexes. In a similar manner to that described above, the complexes were evaluated *in vivo* against a bladder cell (HT1376) xenograft. Diacetato-**6** and malonato-**7** complexes showed activity similar to that of *cisplatin*; dichloride precursor **5** showed reduced activity and the oxalato derivative **8** was inactive, again possibly because of reduced solubility. In addition, acetato-**6** was also found to be active against the *cisplatin*-sensitive PXN/109/TC tumour albeit less so.[10]

As gold(III) is considerably more labile than platinum(II), it was suggested that compounds containing ligands bound fairly tightly to the gold centre may exhibit enhanced activity.[11] Therefore, Henderson and co-workers synthesized a range of bis-metallacyclic compounds that, in addition to the original damp metallacycle, contained a second metallacyclic ring produced upon substitution of the two chloride ligands with dianionic thiosalicylate,[12] salicylate,[12] aurathietane dioxide,[11] ureylene,[13] catecholate[14] or amidate[15] ligands. The anti-tumour activity was evaluated *in vitro* against the P388 murine leukemia cell line and the results are presented in Table 1. The activity is reported as an IC_{50} value, which is the concentration (μM) required to reduce the cell growth by 50%; smaller concentrations equate to a higher activity. In particular, thiosalicylate-**10** and catecholate-**14** show good anti-tumour activity, and derivatives containing these ligands could benefit from further study. In addition, the complexes carrying a methoxy substituent on the phenyl ring of the damp ligand show better activity than their unsubstituted analogues, *cf.* **10** *vs* **9**; this is possibly because of increased solubility.



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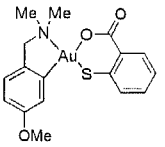

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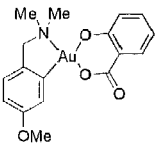

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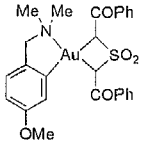

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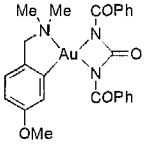

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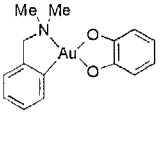

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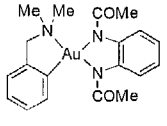

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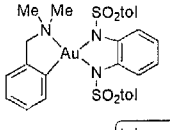

16 (tol = *p*-MeC$_6$H$_4$)

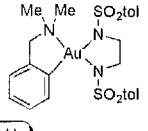

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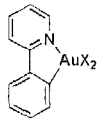
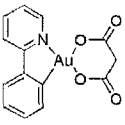
Table 1. The anti-tumour activity (P388) of selected gold(III) metallacycles

	IC_{50} (μM)		IC_{50} (μM)
9	4.01	**14**	0.46
10	0.59	**15**	14.5
11	10.2	**16**	6.68
12	0.70	**17**	1.09
13	12.9		

Other cycloaurated complexes that have been evaluated for anti-tumour activity *in vitro* are **18-22**. These contain the related *C,N* co-ordinated 2-phenylpyridine ligand. The activity against MOLT-4 (human leukemia) and C2C12 (mouse tumour) cell lines indicated that the gold complexes were more active than *cisplatin* against the MOLT-4 cell line. However, with the exception of **22** they were inactive towards the C2C12 cell line.[16] Gold(III) complexes that contain monoanionic *N,N'*-chelating ligands, *e.g.* picolinamide **23**, or two bridging pyrazolide ions **24** and **25** have also been evaluated against the MOLT-4 and C2C12 cell lines; the results indicate that the complexes have activity comparable to *cisplatin*.[17]



18, X = Cl
19, X = OAc
20, X = PhCO$_2$-



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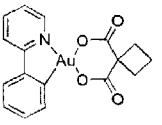

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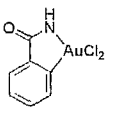

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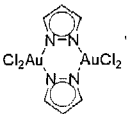

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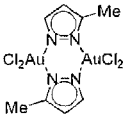

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Mechanistic Considerations

It is well established that the molecular target of *cisplatin* is DNA. After *in vivo* hydrolysis of the chloride ligands (to form a species such as $[PtCl(NH_3)_2(H_2O)]^+$), the complex binds to adjacent nitrogen atoms (N-7) of guanine residues forming intra-strand crosslinks. Hydrogen bonding between *cisplatin* and phosphate groups on the DNA backbone may aid the interactions. It seems unlikely that the gold(III) complexes will interact with DNA in the same fashion as some show activity against *cisplatin* resistant cell lines. In a series of experiments, it was shown that reaction of the damp acetate-**6** with *N*-donor nucleosides, such as guanosine, gave non-quantitative binding and, in addition, the coordination was to alternative nitrogen atoms, *viz.* not N-7.[9] Later, a series of detailed experiments conducted by Parish and Fricker (also with **6**) demonstrated that this complex, and by inference other damp analogues, reacts by way of a different mechanism from that for *cisplatin*, and that DNA may not be the target molecule.[10]

An alternative molecular target for gold(III) complexes may be cathepsin B, a cysteine protease that is implicated in the pathophysiology of a variety of diseases including cancer. Although the exact role of cathepsin B in solid tumours is not fully known, it is thought to be involved

in tumour metathesis, angiogenesis, and tumour progression. As gold drugs are known to react with thiols, the interaction of gold(III) complexes with cathepsin B was investigated and, indeed, the gold(III) damp complexes - and related six-membered ring analogues - were shown to be moderate inhibitors of the enzyme. Binding of the gold complexes to the enzyme was tight, but reversible and this is preferable in a drug candidate. Recently, it has been proposed that gold(III) complexes have anti-microbial effects, possibly by inhibiting the enzyme thioredoxin reductase.[3]

Current Research at Waikato

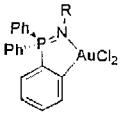
We have become interested in developing new metallacyclic systems that contain a gold(III) metal centre because of the promising anti-tumour activity displayed by certain of the gold(III) complexes described above. We have prepared a series of new compounds and subjected them to preliminary *in-vitro* screening against the P388 murine leukemia cell line.

Cycloaurated Phosphorimine Systems

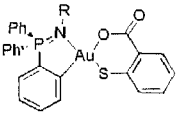
Phosphorimines are ligands that, like damp, coordinate to the gold centre through carbon and nitrogen. Because of the similarity of these complexes to the damp systems described above, a selection of gold(III) iminophosphorane complexes, along with their thiosalicylate and catecholate derivatives, were screened for anti-tumour activity against the P388 murine leukemia cell line.[18,19] The results appear in Table 2.

Table 2. The anti-tumour activity (P388) of selected gold(III) iminophosphoranes

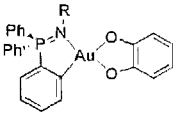
	IC_{50} (μM)		IC_{50} (μM)
26	10.7	**29**	0.97
27	33.4	**30**	<0.74
28	<0.69	**31**	1.03



26, R = Ph
27, R = Bu^t



28, R = Ph
29, R = Bu^t

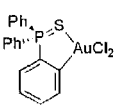


30, R = Ph
31, R = Bu^t

The series of complexes that contain a phenyl substituted iminophosphorane ligand, namely **26**, **28** and **30**, are consistently more active than the analogues **27**, **29** and **31** that carry a *t*-Bu substituent. In addition, the thiosalicylate or catecholate derivatives **28-31** show a *ca.* ten-fold increase in activity over the dichloride precursors. The IC_{50} values of **30** (<0.74 μM) and **28** (<0.69 μM) are comparable to the analogous damp complexes **14** (0.46 μM) and **10** (0.59 μM), respectively. This indicates that changing the cycloaurated precursor and introducing a phosphorus into the metallacyclic ring, whilst slightly altering the electronic and steric properties, does not significantly change the activity of the complexes.

Cycloaurated Phosphine Sulfide Systems

Complexes **32-34** are a unique class of cycloaurated complexes in that the neutral donor ligand is ***not*** nitrogen; they also contain a ligand coordinated to the gold centre that is potentially reducing. These complexes were screened to see if changing the neutral donor ligand had any significant effect on the anti-tumour activity. Compound **32**, which contains a cycloaurated triphenylphosphine sulfide moiety, is essentially inactive against the P388 murine leukemia cell line (IC_{50} >22 μM). Compound **33**, in which the two free phenyl rings of **32** are replaced by NEt_2 groups, was much more active (IC_{50} = 6.31 μM), possibly because of increased solubility. As with the above, thiosalicylate-**34** again is somewhat more active (IC_{50} = 2.4 μM).[19]



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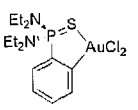

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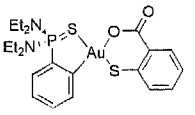

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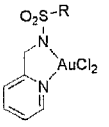
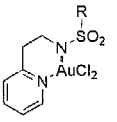
Cycloaurated Pyridylsulfonamide Systems

The *N,N*-coordinated system no longer contains a carbon-gold σ bond but instead a σ (pyridyl)nitrogen-gold bond, analogous to cycloaurated picolinamide **23**. Although **23** had promising activity against MOLT-4 and C2C12 cell lines, the anti-tumour activity of complexes **35-39** was poor as all had IC_{50} values > 22 μM. These complexes are not as stable as the *C,N* counterparts as they undergo reduction with mild reducing agents such as phosphines; this may well be a factor in the poor biological activity of these complexes.[20]



35, R=tol
36, R=Ph
37, R=Me



38, R=tol
39, R=Ph

Concluding Remarks

The structural similarities between gold(III) and platinum(II) suggest that there is a possibility to develop gold analogues of the prominent anti-cancer drug *cisplatin*. Despite promising results from early preliminary screenings, no gold drugs have made it into clinical use. The gold(III) complexes synthesized in our laboratories contain a carbon-gold bond and show promising anti-tumour activity against the P388 murine leukemia cell line. This is especially so when the dichloride ligands are replaced with chelating dianionic ligands such as thiosalicylate or catecholate. In addition, the iminophosphorane ligand is easily tuned to increase desirable properties in the final complex, and work is currently underway to investigate more water soluble derivatives. Further studies on these compounds, especially the more water soluble variants and their thiosalicylate and catecholate derivatives, will include testing against other cell lines. When the neutral donor ligand is changed from nitrogen to sulphur the activity decreases and if the complexes contain no carbon-gold bond, *e.g.* with *N,N* cycloaurated ligands, complete inactivity is recorded.

Acknowledgements

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