In vitro and in vivo studies of neutral cyclometallated complexes against murine leukæmias[1]

Gavin L. Edwards, David St.C. Black, Glen B. Deacon, and Laurence P.G. Wakelin

Abstract: Cyclometallated μ-halogeno dimers derived from nitrogen donor ligands (1-phenylpyrazoles, 2-phenylpyridine, and 1-(2′-pyridyl)indole) were treated with unidentate nitrogen and phosphorus donor ligands to give a series of neutral monomeric palladium(II) and platinum(II) complexes. An initial prescreen of the complexes against the mouse lymphoid leukæmia cell line L1210 indicated that the complexes exhibited growth inhibitory activity over a relatively wide concentration range. Two factors that gave rise to increased activity were steric hindrance about the metal centre resulting from hindered ligands such as 2,6-dimethylpyridine, or the presence of a phosphorus donor ligand. Little correlation between palladium and platinum complexes was noted. Four complexes were selected for further in vivo study and, while none of the palladium complexes showed more than marginal activity against P388 leukæmia at doses below toxic levels, one platinum complex with a hindered metal centre did display significant antitumour activity against this model.

Key words: cyclometallation, palladium, platinum, cytotoxicity, anticancer.

Résumé : Des dimères μ-halogéno cyclométallés dérivés de ligands donneurs d'azote (1-phénylpyrazoles, 2-phénylpyridine, 1-(2′-pyridyl)indole) ont été traités avec des ligands donneurs unidentates d'azote et de phosphore pour conduire à la formation d'une série de complexes monomères neutres du palladium(II) et du platine(II). Un préfiltrate de l'activité de ces complexes contre la lignée L1210 de cellules de leucémie lymphoïde de la souris ont montré qu'ils présentent une activité inhibitrice de la croissance sur une grande plage de concentration. Il y a deux facteurs qui provoquent une augmentation de l'activité, l'empêchement stérique autour du centre métallique résultant de la présence de ligands encombrés comme la 2,6-diméthylpyridine et la présence d'un ligand donneur de phosphore. On n'a pas noté de corrélation entre le palladium et le platine. Quatre complexes ont été sélectionnés pour des études in vivo ultérieures et, même si aucun des complexes du palladium n'a montré plus qu'une activité marginale contre la leucémie P388 à des doses inférieures aux degrés toxiques, un des complexes du platine avec un centre métallique encombré a présenté une activité antitumorale significative contre ce modèle.

Mots clés : cyclométallation, palladium, platine, cytotoxicité, anticancéreux.

[Traduit par la Rédaction]

Introduction

Throughout history heavy metals have been used as therapeutic agents (1); mercury was once used as a treatment for syphilis (2), and arsenic compounds have been used for over 2000 years (3). In modern times, heavy metals fell out of favour and until relatively recently they have been largely ignored as starting points for rational drug design. Rosenberg et al. (4) changed the face of modern chemotherapy with their observation of the antiproliferative activity of *cis*-diamminedichloroplatinum(II) (cisplatin) and their subsequent discovery of the anticancer properties of a range of

Received 17 December 2004. Published on the NRC Research Press Web site at http://canjchem.nrc.ca on 25 August 2005.

It is a pleasure to honour Howard Alper's very significant contributions both to chemistry and to the chemical profession.

There once was a Canuck called Howard,
Over other chemists he towered,
His group in fine fettle,
Used many a metal,
Leaving the science empowered.

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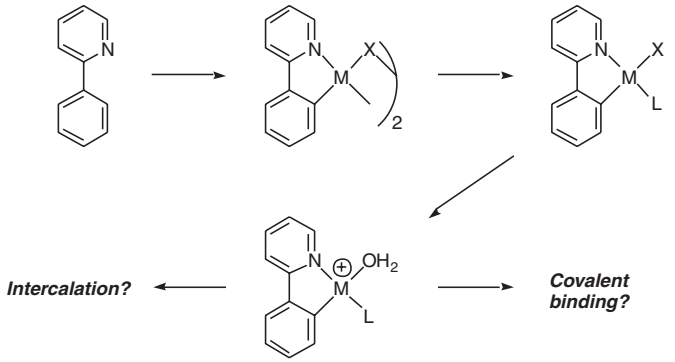
 doi: 10.1139/V05-109

Fig. 1. Potential mechanisms of action.



platinum complexes (5), resulting in the introduction of cisplatin into the clinic in 1978 (6).

The development of second and third generation platinum drugs has followed in an attempt to broaden the spectrum of activity, overcome resistance, and obviate toxic side effects. Some general principles emerged as a guide to the design of new bioactive platinum containing therapeutics, and several useful platinum based anticancer drugs were developed as a result (6, 7). Active agents are neutral platinum(II) or (IV) complexes, normally containing two kinetically inert ligands that should be primary amines or ammonia, and also containing two labile anionic ligands in a cis configuration to enable bidentate binding to the heterocyclic bases of the target DNA (7). Complexes containing aromatic amines such as pyridine, monohalo complexes ("monofunctional" complexes), and those with trans stereochemistry, were generally inactive, although there have been some notable exceptions (7–9), and there remains significant scope for the development of more varied platinum containing structures (10) as drugs.

The formation of bifunctional lesions upon the binding of cisplatin to DNA has been linked to its activity, and studies on the relatively small number of monofunctional platinum complexes — those with a single labile ligand — have reinforced the notion that bifunctional binding is necessary for activity. Interesting exceptions are found in a new class of organoamidoplatinum(II) complexes that are monofunctional and have *trans*-amine ligands, yet possess excellent activity across in vitro, cellular, and animal studies (11). However, many monofunctional platinum complexes studied to date have been charged and therefore issues such as membrane permeability may influence the biological activity of these complexes adversely (7). As a part of a programme investigating nitrogen directed metallations (12, 13), we sought to develop a series of neutral cyclometallated complexes as potential monofunctional DNA binding agents (Fig. 1). Cyclometallation of ligands such as 2-phenylpyridine gives μ-halogeno dimers, which, upon cleavage with appropriate unidentate ligands, provide neutral monomeric complexes. These complexes could act as monofunctional DNA binding agents where, in the low chloride concentration of intracellular fluid, aquation would lead to a reactive cationic species. While the aquated complex could bind covalently to DNA, another possible interaction would be intercalation of the planar chromophore into the DNA double helix, in a similar manner to metallointercalation reagents studied by Lippard (14), Barton and co-workers (15), and others (16, 17). Recent reports on highly cytotoxic cyclometallated complexes by Kodaka and co-workers (18), Navarro-Ranninger and co-workers (19), and Ma and Che (20) have prompted us to publish our findings on a range of mononuclear platinum(II) and palladium(II) complexes that exhibit significant activity against murine leukæmia L1210 in vitro with a notable effect shown by complexes containing bulky ligands. This correlates with the recently discovered activity of the crowded complex *cis*-amminedichloro(2-methylpyridine)platinum(II) AMD473 (ZD0473) (21), which has progressed to phase III clinical trials (10). In the preceding paper (22), we have also reported our results on the synthesis and biological activity of a series of cationic metallointercalation complexes, many of which incorporate a cyclometallated chromophore.

Fig. 2. Ligands and abbreviations with positions of metallation indicated.

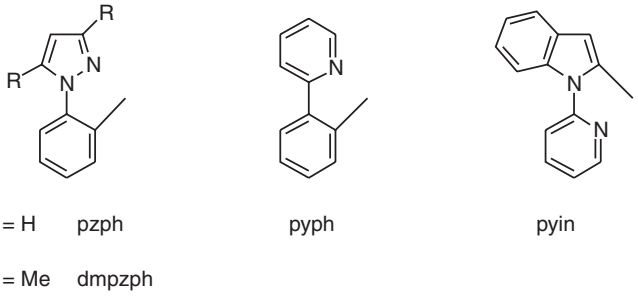


Table 1. Cyclometallated dimers.

Compound	Aromatic ligand N–C	Metal	Halogen	Yield (%)
1	pyph	Pd	Cl	89
2	pyph	Pd	Br	73
3	pyph	Pd	I	71
4	pyph	Pt	Cl	65
5	pyph	Pt	Br	62
6	pzph	Pd	Cl	99
7	pzph	Pd	Br	79
8	pzph	Pd	I	76
9	dmpzph	Pd	Cl	45
10	pyin	Pd	Cl	97

Results and discussion

Synthesis of metal complexes

Preparation of the required ligands (Fig. 2) and the subsequent cyclometallation reactions to provide μ-chloro dimers **1** (23), **4** (12), **6** (24), **9** (22), and **10** (22) (Table 1) have been described previously. While the nature of the halide was of little importance for the cationic metallointercalators where the halide would merely act as a counterion, variation between coordinated chloride, bromide, and iodide could be crucial for increased activity in the series of neutral complexes, and therefore several μ-chloro dimers were converted to the corresponding bromo and iodo derivatives **2** (12), **3** (12), **5** (12), **7** (24), and **8** (12) (Table 1) by metathesis reactions with the appropriate lithium halide as described previously (12). The structures were confirmed by elemental analysis, far-IR, and ^{1}H NMR spectroscopy. ^{1}H NMR analy-

Table 2. Monomeric complexes and growth inhibition of L1210 cells in vitro.

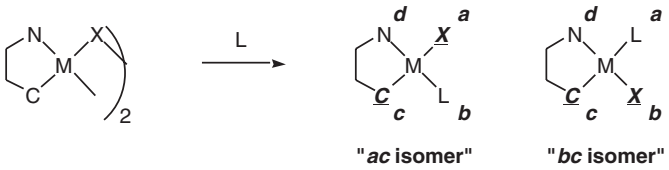
Complex	Aromatic ligand	Metal	"a" Ligand[a] (trans to C)	"b" Ligand[a] (trans to N)	IC_{50} (μmol/L)
11 (12)	pyph	Pd	Cl	py-d_5	11
12 (12)	pyph	Pd	Br	py-d_5	12
13 (12)	pyph	Pd	I	py-d_5	12
14	pyph	Pd	Cl	blut	11
15 (12)	pyph	Pd	Cl	alut	1.2
16	pyph	Pd	Cl	NH_3	12
17	pyph	Pd	Cl	$MeNH_2$	10
18	pyph	Pd	Cl	*t*-$BuNH_2$	14
19	pyph	Pd	Cl	PPh_3	1.8
20	pyph	Pd	Cl	$P(OMe)_3$	1.1
21	pyph	Pt	Cl	py	5.0
22 (12)	pyph	Pt	Cl	py-d_5	5.8
23 (12)	pyph	Pt	Cl	2phpy	3.2
24 (12)	pyph	Pt	Cl	alut	1.5
25 (12)	pyph	Pt	Cl	*S*-dmso	15
26	pyph	Pt	Cl	PPh_3	10
27 (12)	pyph	Pt	alut	Cl	19
28 (12)	pyph	Pt	alut	Br	11
29 (24)	pzph	Pd	Cl	py	52
30 (12)	pzph	Pd	Br	py-d_5	61
31 (12)	pzph	Pd	I	py-d_5	45
32	pzph	Pd	Cl	pic	34
33 (12)	pzph	Pd	Cl	alut	6.4
34	dmpzph	Pd	Cl	alut	3.0
35	pyin	Pd	Cl	py-d_5	7.7
36	pyin	Pd	Cl	$P(OMe)_3$	1.2

[a]Key to unidentate ligands: pyridine (py), perdeuteropyridine (py-d_5), β-lutidine (3,5-dimethylpyridine) (blut), α-lutidine (2,6-dimethylpyridine) (alut), 2-phenylpyridine (2phpy), dimethylsulfoxide coordinated through sulfur (*S*-dmso), α-picoline (2-methylpyridine) (pic).

sis of the dimers proved difficult because of their extremely low solubility in common solvents, and therefore the μ-halogeno dimers were converted to the monomers by the addition of a drop of ($^2H_5$)pyridine (py-d_5) to a suspension in $CDCl_3$ providing the monomeric complexes in solution; loss of one proton confirmed that cyclometallation had taken place, and observation of a single set of resonances demonstrated that metathesis was complete (12).

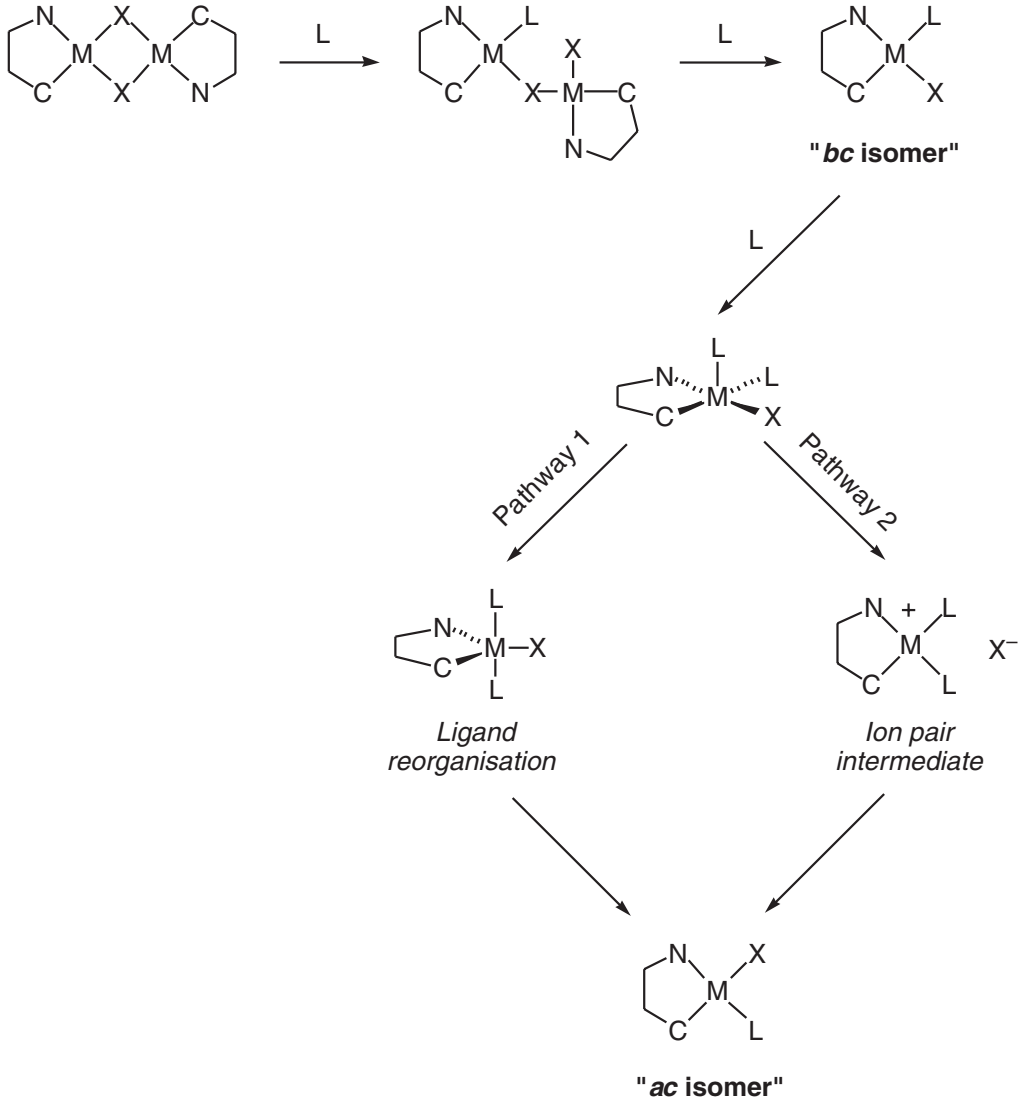
Synthesis of the monomeric complexes **11**–**36** (Table 2) was effected by treatment of a suspension of a μ-halogeno dimer in dichloromethane or acetone with a unidentate ligand (L) to give clear solutions; the products usually separated as microcrystalline solids upon the addition of hexane or pentane. The palladium dimers reacted quite rapidly; by comparison, cleavage of the platinum–halogen bridge appeared to be much slower. The complexes were stable at room temperature and the platinum complexes could be recrystallized from hot solvents, however attempts to recrystallize the analogous palladium complexes (where L = nitrogen donor ligands) at elevated temperatures led to dissociation of the ligand L and reformation of the μ-halogeno dimers. Thus, these palladium complexes were purified by dissolution in an appropriate solvent at room temperature and reprecipitation upon the addition of hexane or pentane. Far-IR and ^1H NMR spectroscopy as well as microanalysis established the identity and purity of our products. Any attempts to heat the palladium complexes — to speed drying, measure melting points, or volatilize the complexes for mass spectrometric analysis — led to quantitative loss of the unidentate ligand. This may explain the discrepancy between the observed (mp 155 °C (dec.)) and reported (24) (mp 200 °C (dec.)) melting points for compound **29**: a significant difference in the rate of heating could mean that a larger proportion of one sample may have degraded before reaching the melting (decomposition) point. Pérez et al. (25) have recently determined the activation energy for the decomposition of this class of complexes by this pathway as 90–100 kJ mol^{-1}.

Fig. 3. Possible products of bridge cleavage.



While the bridge cleavage reactions can, in principle, produce two isomeric products (Fig. 3) — one, with the halogen

Fig. 4. Mechanism for isomerization of the *bc* isomer to the *ac* isomer (adapted from ref. 12).



ligand trans to the carbon donor (the *ac* isomer)[3] and the other with the unidentate ligand L trans to the carbon donor ligand (the *bc* isomer) — the palladium complexes and many of the platinum complexes were isolated as the *ac* isomers exclusively. In these reactions, the strong kinetic trans effect of the σ-carbon ligand means that the first bond in the bridge to be cleaved is that trans to the aryl substituent. Thus, the initial (kinetic) product from bridge cleavage should be the *bc* isomer (Fig. 4). Subsequent rearrangement of this kinetic product could occur either via formation of a five-coordinate transition state with accompanying ligand reorganisation (Pathway 1), or formal formation of a square-planar ion pair as an intermediate (Pathway 2). The subsequent conversion of this ionic intermediate to the neutral complex would be governed by the strong kinetic trans effect of the σ-carbon ligand, leading to selective cleavage of the C—L bond trans to the aryl group. In all cases, the palladium complexes are sufficiently reactive and only the thermodynamically favoured *ac* isomers are observed. Platinum(II) complexes are less labile and, if there is sufficient steric hindrance around the metal centre (2,6-dimethylpyridine ligands introduce hindrance above and below the metal), the kinetic *bc* product can be isolated. Other platinum complexes are isolated as the *ac* products. A fuller mechanistic discussion and supporting experimental evidence has been published previously (12).

In all cases, the structures and stereochemistry of the monomeric products were confirmed by spectroscopic analysis. Comparison between the far-IR spectra of analogous complexes below 300 cm^{-1} often allowed assignment of the palladium–halogen stretching frequencies and these values were used as a guide to the assignment of ν(Pd–X) for other neutral monomeric species. The relatively low frequencies observed for most ν(Pd–X) (for example, palladium–chlorine stretching frequencies were observed in the range 230 to ≈260 cm^{-1}, while ν(Pd–Br) was approximately 160 cm^{-1}, and ν(Pd–I) was in the range 120–140 cm^{-1}) (12) were indicative that the halogen ligand is trans to an σ-carbon donor

[3] The *ac*/*bc* nomenclature system was adopted in our previous report (12) to simplify the discussion of stereochemistry: all *ac* complexes have the halide trans to the carbon donor ligand and all *bc* complexes have the halide cis to the carbon donor ligand.

of strong trans influence (26); this confirmed that the complexes had *ac* stereochemistry. Structural assignments for the platinum complexes were discussed in the mechanistic report (12).

The ^{1}H NMR spectra of the complexes likewise confirmed the assigned structures, where several features were noteworthy. Coordination of unidentate pyridine ligands results in deshielding of the α-pyridyl protons (27) or the α substituents; for example, 2,6-dimethyl substituents move downfield by about 0.7 ppm on coordination. Presumably, this effect is a combination of the inductive nature of the metal as well as the through-space paramagnetic anisotropy of the metal atom (28). As described previously (12), strong shielding of the proton ortho to the site of metallation (H_{ortho}) was observed where L is a pyridine ligand, owing to the proximity of the proton to the adjacent orthogonal pyridine ring. Greater shielding with increased α substitution on the pyridine nucleus indicates that the α-methyl groups restrict rotation about the Pd—N bond and thus prevent rotation of the ring current associated with the unidentate ligand away from H_{ortho}. While ammine and primary amine ligands (complexes **16**–**18**) produced no noticeable effect on H_{ortho} (the proton ortho to the C—M bond), deshielding of the pyridyl H6 in the cyclometallated ligand of 2-pyridylphenyl complexes was observed, consistent with coordination to the metal and also with the through-space paramagnetic anisotropy of the adjacent halide ligand.

Palladium complexes **19**, **20**, and **36** containing triphenylphosphine and trimethyl phosphite ligands were more stable than those with nitrogen donor ligands. They could be recrystallized from hot solvents without reverting to the dimeric precursors, and mass spectra could also be measured. Numerous absorptions because of the triphenylphosphine ligand were observed in the far-IR spectra of complexes **19** and **26**; the most distinctive of these were several bands in the regions 400–460 cm^{-1} and 490–550 cm^{-1} (29). In the ^{1}H NMR spectra of the complexes, several ^{31}P–^{1}H and ^{195}Pt–^{1}H couplings were observed. In the triphenylphosphine complexes **19** and **26**, the phenyl protons H5 and H6 were both shielded by the ring currents of the adjacent P–Ph groups, confirming the *ac* stereochemistry. In the low-field region of the ^{1}H NMR spectrum of complex **26**, the signal owing to the pyridyl H6 proton is observed as a distorted triplet with fine coupling and ^{195}Pt satellites. Although exact coupling constants could not be assigned, it is apparent that $J_{5,6} \approx J_{P,H6} \approx$ 4.5–5.5 Hz. The estimated $J_{Pt,H6}$ of 27 Hz is lower than observed for other platinum complexes (27) as a result of the strong trans influence of the *trans*-phosphine ligand. Platinum-195 coupling to phenyl H6 (56 Hz) is comparable with literature values (24), and ^{31}P–^{1}H coupling to pyridyl H6 is also similar to published results (30).

In vitro and in vivo testing

Most metal complexes with anticancer activity are based on platinum; while the coordination chemistry of platinum(II) and palladium(II) are similar, many palladium complexes show moderate in vivo activity at best (7). Although some palladium complexes have shown promising activity in vitro (31) or in animal models (22), to date they have not made a successful transition to clinical trials (7). However, the possibility of covalent or intercalative mechanisms of action for this class of compounds suggested that the monofunctional palladium complexes were worthy of study.

The complexes were subjected to an in vitro prescreen for growth inhibitory activity against the murine leukæmia cell line L1210, as described in the preceding paper (22). As the complexes are neutral, water proved to be an unsuitable solvent for preparation of stock solutions or for serial dilutions, and therefore ethanol, ethanol – dimethyl sulfoxide, or dimethyl sulfoxide were used. Serial dilutions of stock solutions were made using ethanol without precipitation. It is desirable that the complexes retain their structures in solution prior to the addition of the drug to the cell culture, and ^{1}H NMR spectroscopy was employed to examine the behaviour of selected complexes in solution. The spectrum of the 2,6-dimethylpyridine complex **15** in perdeuteromethanol was unchanged after the solution was allowed to stand at room temperature for 3 days, and the spectrum was well-resolved with the exception of a broadened signal corresponding to a single proton at low field that could be assigned as pyridyl H6 for the cyclometallated ligand. The observed broadening of this signal could result from weak axial association and exchange of CD_3OD ligands, or from reversible displacement of the chloride ligand; the chemical shift of this pyridyl H6 correlates with a coordinated ligand and therefore the cyclometallated ligand is essentially unaffected. The 2,6-dimethylpyridine ligand also remains complexed to the metal centre. The ^{1}H NMR spectra of monomeric py-d_5 complexes **11**–**13** in perdeuterodimethylsulfoxide ($(CD_3)_2SO$) were broadened by comparison with the spectra recorded in $CDCl_3$ (12), however the spectra of the three complexes in $(CD_3)_2SO$ were clearly different from one another, and the observation of signals at low field (above 9 ppm, pyridyl H6) and at high field (below 6.1 ppm, phenyl H6) indicates that a significant concentration of unchanged complex exists in solution and the clear differences indicated that the halogen had not been displaced.

The results of growth inhibition experiments against L1210 cells in vitro, expressed as drug concentrations that caused a 50% inhibition of cell growth (IC_{50}), are given in Table 2. For comparison, cisplatin has an IC_{50} of 0.9 μmol/L under similar conditions.

Antitumour screening of simple analogues of cisplatin ($Pt(NH_3)_2X_2$) has indicated that there is a strong relationship between the halide leaving group X and biological activity, where antitumour activity generally decreases with larger halogens (32). In this study, results for complexes **11**–**13** and **29**–**31** suggest that changing halogen has no significant effect on the biological activity of the organopalladium complexes in vitro. Either aquation of the labile palladium complexes (or displacement of the halogen by other ligands) occurs and therefore the drugs are converted to the same active intermediates, or reaction of the Pd—X bond is not crucial for activity. Two platinum complexes with *bc* stereochemistry were also evaluated and the bromo complex **28** was almost twice as active as the chloro complex **27**; while a definitive statement concerning trends cannot be made based on two results, it does appear as though the bulkier halides lead to more active complexes.

The unidentate ligand (L) is observed to play an important role in the biological activity of these drugs. Small amine ligands such as NH_3 (complex **16**), $MeNH_2$ (**17**), and *t*-

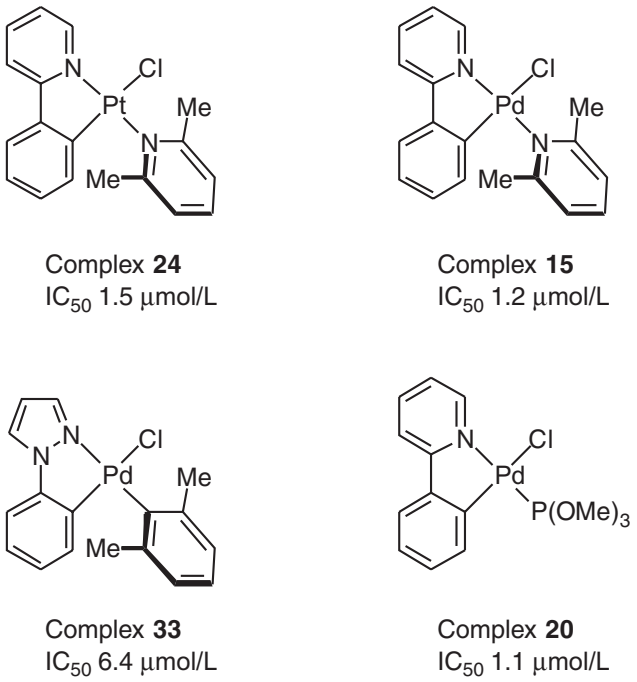
$BuNH_2$ (**18**), and unsubstituted pyridine ligands (**11** and **29**) cause 50% growth inhibition at similar concentrations. Use of pyridine (platinum complex **21**) or py-d_5 (**22**) has no significant effect on activity, suggesting that reaction of the coordinated unidentate ligand is not involved in the modes of action of the drugs. A pyridine ligand that is substituted away from the metal–nitrogen bond (the 3,5-dimethylpyridine ligand (blut), complex **14**) does not affect the biological activity compared to the unsubstituted analogue. However and notably, introduction of steric crowding around the metal ion with successive α substituents on the pyridine rings (compare complexes **29**, **32**, and **33**, or **11** and **15**) leads to greatly enhanced activity. The mechanism of action of this class of compounds is not clear, but the poor activity of free 2,6-dimethylpyridine (IC_{50} > 200 μmol/L) indicates that the enhanced biological activity is not due to the unidentate ligands following release from the metal. In fact, for good activity it appears as though the neutral ligand L must remain coordinated to the metal.

The introduction of α substituents on the unidentate pyridine ligands (L) gives increased steric hindrance around the metal, and interactions between the α-methyl groups and the bidentate organometallic ligand would cause the ligand L to lie approximately orthogonal to the coordination plane of the metal. These α-methyl substituents would hinder the formation of a trigonal bipyramidal intermediate, and therefore impede substitution by an associative mechanism. This effect is similar to that observed for the sterically hindered α-picoline platinum complex, AMD473 (ZD0473) (8, 21), however, in the case of the palladium complexes reported herein two α-methyl substituents are required before significant enhancement of activity is noted. Whether, in the case of drugs reported in this study, the enhanced activity with extra α substituents on L is due to slower displacement of the halide ligand (X) or of the unidentate ligand (L), is unclear.

Complexes with unidentate phosphorus donor ligands in general effect growth inhibition at relatively low concentrations with some complexes showing IC_{50} values approaching that of cisplatin. The phosphorus donor ligands L give stable complexes where L is not readily displaced, and the trimethyl phosphite ligands (complexes **20** and **36**) would introduce negligible steric hindrance around the metal and therefore $P(OCH_3)_3$ would not impede substitution at the metal centre by an associative mechanism. Thus, if the mechanism of action of these complexes is similar to that for the nitrogen donor ligands, then slow or negligible displacement of the unidentate ligand is indicated as an important event for activity. This can be achieved by either steric or coordinative stabilization of the complexes with respect to substitution. Another alternative is that the antiproliferative effects could arise from localized release of the phosphine/phosphite ligands within the cells; the lower activity observed for platinum complex **26** (IC_{50} = 10 μmol/L) compared to its palladium analogue **19** (IC_{50} = 1.8 μmol/L) could reflect the greater lability of the palladium complex and the faster release of bioactive phosphine ligands (33).

The nature of the cyclometallated ligand also appears to be crucial for activity. If an intercalative mode of binding is implicated in the mechanism of action, increased surface area should result in higher activity. Pyrazolylphenyl ligands gave complexes that generally caused growth inhibition at relatively high concentrations, but addition of methyl groups produced a more active drug (compare pzph complex **33** (IC_{50} = 6.4 μmol/L) with dmpzph complex **34** (IC_{50} = 3.0 μmol/L)). Likewise, fusion of an extra benzene ring (compare pyin complex **35** (IC_{50} = 7.7 μmol/L) with pzph complex **29** (IC_{50} = 52 μmol/L)) resulted in enhanced activity. These factors are consistent with an intercalative mechanism.

Fig. 5. Complexes selected for in vivo testing against P388 leukæmia (in vitro IC_{50} vs. L1210 given for comparison).



Insufficient platinum complexes have been prepared in this study for clear trends to become evident, however, a comparison of the *ac* (complexes **22**–**24**, for instance) and *bc* (**27** and **28**) complexes suggests that the thermodynamically favoured *ac* complexes are likely to be more active. Further work is needed to confirm this observation. Additionally, it is apparent that there is not necessarily any firm correlation between the palladium and the platinum complexes; in some cases, the platinum complexes are more active than the palladium analogues (compare **22** (Pt) with **11** (Pd)), while in others the palladium complex was more active (compare **26** (Pt) with **19** (Pd)), and the key 2,6-dimethylpyridine complexes (compare **24** (Pt) with **15** (Pd)) have similar activities.

Four of the more active complexes (Fig. 5) were selected for evaluation of potential antitumour activity against P388 leukæmia in mice. The drugs were tested over a range of dose levels with results expressed as a ratio of mean survival times of treated animals (T) relative to controls (C). Significant antitumour activity is indicated by a %T/C greater than 125%. A %T/C of 0% indicates that none of the treated animals survived as a result of the toxicity of the drug at that dose level. Results are shown in Tables 3*a* and 3*b*; the anticancer agent 5-fluorouracil (5-FU) is used for comparison. Platinum complex **24** showed significant activity (%T/C 138%) against P388 leukæmia at 80 mg/kg. Complex **15**, its

Table 3. Antitumour testing against P388 leukæmia.

(*a*)

Dose (mg/kg/injection)	%T/C Complex **24**	Dose (mg/kg/injection)	%T/C Complex **20**
0 (Control)	100	0 (Control)	100
10	114	2	99
20	108	4	101
40	105	8	108
80	138	16	84
160	58	32	0
50 (5-FU: standard)	168	50 (5-FU: standard)	158

(*b*)

	%T/C	
Dose (mg/kg/injection)	Complex **15**	Complex **33**
0	100	100
25	110	116
50	116	116
100	118	120
200	116	97
400	126	0
50 (5-FU: standard)	162	162

palladium analogue, demonstrated only marginal activity (%T/C 126%) at the highest dose level of 400 mg/kg despite showing almost identical activity against L1210 in vitro. Pyrazolylphenyl complex **33** achieved a %T/C of 120% at doses of 100 mg/kg before toxicity then became evident. Finally, trimethyl phosphite complex **20**, while being highly active in vitro, showed no antitumour activity in vivo, and was toxic at relatively low doses with no treated animals surviving doses of 16 mg/kg or higher. Among the palladium complexes chosen for study, only marginal antitumour activity was observed at best. The very high dose levels required to demonstrate activity, and the possibility that complexes such as **15** may be approaching limiting toxicity, indicated that further testing of those complexes was not warranted.

On the other hand, platinum complex **24** has steric bulk around the metal centre in a similar manner to the clinically active 2-methylpyridine containing complex AMD473 (21), and the results obtained in this study for this complex **24** are encouraging (%T/C 138% at 80 mg/kg). In light of Kodaka and co-workers' recent reports (18, 34) of the promising activity against a cisplatin resistant mouse sarcoma 180 cell line of platinum complex **23** previously prepared in our laboratories (12), extension into the platinum series focussing on systems with bulky ligands is being investigated further.

Experimental

General procedure

General experimental details, including instrumental specifications and in vitro and in vivo biological testing protocols, have been described previously (22). Mass spectra were obtained on a VG Micromass 7070F spectrometer at 70 eV, samples being introduced by direct evaporation. Where relevant, the listed value is the most intense peak (^{195}Pt, ^{231}(PtCl), ^{106}Pd, ^{143}(PdCl)) of a cluster with the correct isotope pattern. Cyclometallated dimers **1** (23), **2**–**5** (12), **6** and **7** (24), **8** (12), and **9** and **10** (22) were prepared by published procedures, and monomeric complexes **11**–**13**, **15**, **22**–**25**, **27**–**31**, and **33** were prepared by the procedures referred to in Table 2. Unidentate ligands (2-methylpyridine, 2-phenylpyridine, 2,6-dimethylpyridine, 3,5-dimethylpyridine, triphenylphosphine, and trimethyl phosphite) were obtained from the Aldrich Chemical Company and were recrystallized or distilled prior to use. ($^2H_5$)Pyridine (py-d_5) (Aldrich Gold Label) was used without further purification. Solvents and organic reagents were purified by established methods.

Preparations

General method

The appropriate unidentate ligand was added to a stirred suspension of the μ-halogeno dimer in dichloromethane or acetone. The mixture was stirred at room temperature until the reaction was complete (this was indicated when the solid had dissolved completely). Hexane or pentane was added to precipitate the crude product, which was filtered off and washed with hexane or pentane. The solid was dissolved in a polar solvent (dichloromethane or acetone) at room temperature, and the solution was filtered. Slow addition of hexane or pentane gave the monomeric complex as a microcrystalline solid, which was dried at room temperature.

a-*Chloro*-b-*(3″,5″-dimethylpyridine*-N″)-dc-*[2-(2′-pyridyl)-phenyl*-N′,C]*palladium (14)*

This compound was prepared from μ-chloro dimer **1** (0.359 g, 0.61 mmol) and 3,5-dimethylpyridine (10 drops) in acetone (15 mL). Recrystallization of the crude product from acetone–hexane at room temperature gave the title compound **14** as a pale yellow microcrystalline solid (0.399 g, 82%); mp 204 to 205 °C. IR (cm^{-1}): 1606, 1595, 1578, 1568, 1437, 1422, 1275, 1157, 857, 854, 790, 766, 756, 731,

699, 410, 323, 300, 238 ν(Pd–Cl). ^{1}H NMR (90 MHz, $CDCl_3$) δ: 9.45 (d, J = 5.6 Hz, 1H, pyridyl H6′), 8.59 (s br, 2H, pyridyl H3″ and H5″), 7.90–6.85 (m, 7H, aryl), 6.18 (dd, J = 7.3, 1.5 Hz, 1H, phenyl H6), 2.35 (s, 6H, 2 × Me). Elemental anal. calcd. for $C_{18}H_{17}ClN_2Pd$ (%): C 53.6, H 4.25, N 6.95; found: C 54.0, H 4.3, N 6.8.

b-*Ammine*-a-*chloro*-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]palladium (16)

A solution of anhydrous ammonia in methanol was added dropwise to a stirred suspension of **1** (0.116 g, 0.20 mmol) in dichloromethane (5 mL) until a colourless solution resulted. Hexane was added to give a white solid, which was filtered off and washed with hexane. The crude product gradually became yellow on standing in air. Recrystallization twice from dichloromethane–ether at room temperature gave the title compound **16** (35) as small, bright yellow needles (0.090 g, 73%); mp > 300 °C. IR (cm^{-1}): 3315 (br), 3210 (br), 3045, 3040, 1606, 1579, 1437, 1421, 1204, 1182, 1166, 1156, 1022, 738, 722, 404, 230 ν(Pd–Cl). ^{1}H NMR (90 MHz, $CDCl_3$) δ: 9.05 (d, J = 5.0 Hz, 1H, pyridyl H6), 7.68–6.71 (m, 7H, aryl), 2.86 (s br, 3H, NH_3). Elemental anal. calcd. for $C_{11}H_{11}ClN_2Pd$ (%): C 42.2, H 3.5, N 8.95; found: C 42.3, H 3.5, N 8.8.

a-*Chloro*-b-*(methylamine)*-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]-palladium (17)

This compound was prepared by the general method by the addition of anhydrous methylamine (as a solution in dichloromethane) to **1** (0.153 g, 0.26 mmol) in dichloromethane (2 mL). Recrystallization of the crude product from dichloromethane–hexane at room temperature gave the title compound **17** as fine yellow needles (0.139 g, 82%); mp 205 °C (dec.). IR (cm^{-1}): 3295 (br), 3220 (br), 3150, 1605, 1588, 1582, 1437, 1424, 1161, 1156, 1030, 748, 727, 406, 243 ν(Pd–Cl) or 215 ν(Pd–Cl) (either assignment possible). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.98 (dd, J = 5.7, 1.2 Hz, 1H, pyridyl H6), 7.62 (td, J = 7.8, 1.6 Hz, 1H, aryl), 7.36 (d, J = 8.0 Hz, 1H, pyridyl H3), 7.21–7.18 (m, 1H, aryl), 7.01–6.38 (m, 4H, aryl), 3.34 (m br exch, 2H, NH_2), 2.82 (t (s after exch), J = 6.6 Hz, 3H, Me). Elemental anal. calcd. for $C_{12}H_{13}ClN_2Pd$ (%): C 44.1, H 4.0, N 8.6; found: C 44.2, H 4.0, N 8.6.

b-*(*tert*-Butylamine)*-a-*chloro*-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]-palladium (18)

This compound was prepared from **1** (0.255 g, 0.43 mmol) and *tert*-butylamine (10 drops) in dichloromethane (4 mL). Recrystallization of the crude product from dichloromethane–hexane at room temperature gave the title compound **18** as pale yellow microcrystals (0.256 g, 81%); mp > 290 °C. IR (cm^{-1}): 3275 (br), 3220 (br), 3140, 1606, 1588, 1578, 1439, 1426, 1160, 1149, 1030, 749, 733, 413, 227 ν(Pd–Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.31 (dd, J = 5.7, 1.0 Hz, 1H, pyridyl H6), 7.74 (td, J = 7.8, 1.6 Hz, 1H, aryl), 7.54 (d, J = 7.9 Hz, 1H, pyridyl H3), 7.36 (dd, J = 7.2, 1.8 Hz, 1H, aryl), 7.09 (ddd, J = 7.4, 5.7, 1.3 Hz, 1H, pyridyl H5), 7.06–6.92 (m, 3H, aryl), 3.41 (s br exch, 2H, NH_2), 1.51 (s, 9H, CMe_3). Elemental anal. calcd. for $C_{15}H_{19}ClN_2Pd$ (%): C 48.8, H 5.2, N 7.6; found: C 48.7, H 5.1, N 7.4.

a-*Chloro*-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]-b-*(triphenylphosphine)palladium (19)

This compound was prepared from **1** (0.156 g, 0.26 mmol) and triphenylphosphine (0.140 g, 0.53 mmol) in dichloromethane (16 mL). Recrystallization of the crude product from dichloromethane–hexane gave the title compound **19** (34) as small pale yellow prisms (0.242 g, 82%); mp 211–214 °C (dec.). IR (cm^{-1}): 1602, 1579, 1566, 1439, 1434, 1426, 1423, 1313, 1100, 1093, 1018, 750, 745, 731, 705, 695, 689, 628, 531, 513, 501, 498, 310, 244 ν(Pd–Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.64 (dddd, J = 5.4, 1.0, 0.6 Hz, $J_{P,H}$ = 3.7 Hz, 1H, pyridyl H6), 7.86–7.73 (m, 8H, aryl), 7.52 (d, J = 7.9 Hz, 1H, aryl), 7.47–7.33 (m, 9H, aryl), 7.26–7.21 (m, 1H, aryl), 6.98–6.92 (m, 1H, aryl), 6.57–6.48 (m, 2H, H5 and H6). Elemental anal. calcd. for $C_{29}H_{23}ClNPPd$ (%): C 62.4, H 4.15, N 2.5; found: C 62.2, H 3.9, N 2.8.

a-*Chloro*-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]-b-*(trimethyl phosphite)palladium (20)

This compound was prepared from **1** (0.116 g, 0.20 mmol) and trimethyl phosphite (0.049 g, 0.40 mmol) in dichloromethane (6 mL). Recrystallization of the crude product from cyclohexane gave the title compound **20** as fine, pale yellow needles (0.121 g, 74%), mp 139 to 140 °C. IR (cm^{-1}): 1604, 1578, 1436, 1427, 1012, 810, 801, 770, 764, 740, 544, 285, 238 ν(Pd–Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.48 (dddd, J = 5.7, 1.0, 0.7 Hz, $J_{P,H}$ = 4.9 Hz, 1H, pyridyl H6), 7.85 (td, J = 7.7, 1.6 Hz, 1H, aryl), 7.74 (d, J = 8.2 Hz, 1H, pyridyl H3), 7.62 (td, J = 7.2, 1.5 Hz, 1H, aryl), 7.58 (d, J = 8.0 Hz, 1H, aryl), 7.28–7.09 (m, 3H, aryl), 3.89 (d, $J_{P,H}$ = 12.8 Hz, 9H, $P(OMe)_3$). Mass spectrum m/z (%): 421 (M^+, 1.1), 384 ($C_{14}H_{17}NO_3PPd^+$, 0.1), 369 ($C_{13}H_{14}NO_3PPd^+$, 0.2), 297 ($C_{11}H_8ClNPd^+$, 0.2), 278 ($C_{11}H_{10}NOPd^+$, 22), 260 ($C_{11}H_8NPd^+$, 2.3). Elemental anal. calcd. for $C_{14}H_{17}ClNO_3PPd$ (%): C 40.0, H 4.1, N 3.3; found: C 40.1, H 4.1, N 3.3.

a-*Chloro*-b-*(pyridine*-N″)-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]-platinum (21)

This compound was prepared by the general method by the addition of pyridine (5 drops) to the μ-chloro dimer **4** (0.21 g, 0.27 mmol) in dichloromethane (15 mL). Recrystallization of the crude product from dichloromethane–pentane gave the title compound **21** as small yellow needles (0.25 g, 99%). ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.70 (dd with satellites, J = 5.1, 1.6 Hz, $J_{Pt,H}$ = 39 Hz, 1H, pyridyl H6′), 9.01 (dt with satellites, J = 5.1, 1.4 Hz, $J_{Pt,H}$ = 46 Hz, 2H, pyridyl H2″ and H6″), 7.95–7.79 (m, 2H, aryl), 7.66 (d with satellites, J = 8 Hz, $J_{Pt,H}$ = 9 Hz, 1H, pyridyl H3′), 7.51–7.41 (m, 3H, aryl), 7.19–7.07 (m, 2H, aryl), 6.98 (td with unresolved satellites, J = 7.4, 1.4 Hz, 1H, phenyl H5), 6.36 (dd with satellites, J = 7.5, 0.9 Hz, $J_{Pt,H}$ = 48 Hz, 1H, phenyl H6). Mass spectrum (%): m/z 464 (M^+, 6), 428 ($C_{16}H_{13}N_2Pt^+$, 1), 385 ($C_{11}H_8ClNPt^+$, 8), 348 ($C_{11}H_7NPt^+$, 9), 321 ($C_{10}H_6Pt^+$, 2). This compound is the py analogue of py-d_5 complex **22** and full analytical data for **22** has been previously reported (12).

a-*Chloro*-dc-*[2-(2′-pyridyl)phenyl*-N′,C*]-b-*(triphenylphosphine)platinum (26)

This compound was prepared from **4** (0.232 g, 0.30 mmol) and triphenylphosphine (0.200 g, 0.76 mmol) in dichloromethane (50 mL). Recrystallization of the crude product

from dichloromethane–pentane gave the title compound **26** as yellow needles (0.195 g, 50%); mp 305–307 °C (dec.). IR (cm^{-1}): 1605, 1583, 1439, 1436, 1425, 1100, 1096, 750, 746, 731, 706, 702, 695, 690, 542, 519, 505, 300, 284 ν(Pt–Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.90–9.87 (m with satellites, $J_{Pt,H} \approx 27$ Hz, 1H, pyridyl H6), 7.89–7.76 (m, 8H, aryl), 7.52 (J = 7.8, 1.4 Hz, 1H, aryl), 7.46–7.33 (m, 9H, aryl), 7.29 (qt, J = 7.1, 6.0, 1.5 Hz, $J_{P,H}$ = 1.5 Hz, 1H, pyridyl H5), 6.95 (td, J = 7.5, 1.2 Hz, 1H, aryl), 6.67 (ddd with satellites, J = 7.9, 0.9 Hz, $J_{P,H}$ = 3.3 Hz, $J_{Pt,H}$ = 56 Hz, 1H, phenyl H6), 6.52 (td with unresolved satellites, J = 7.6, 1.4 Hz, 1H, phenyl H5). Mass spectrum *m/z* (%): 647 (M^+, 14), 611 ($C_{29}H_{23}NPPt^+$, 3). Elemental anal. calcd. for $C_{29}H_{23}ClNPPt$ (%): C 53.8, H 3.6, N 2.2; found: C 53.6, H 3.5, N 2.1.

a-*Chloro*-dc-*[2-(1′-pyrazolyl)phenyl*-N′,C*]*-b-*(pyridine*-N″)-*palladium–dichloromethane (2/1) (29)*

This compound was prepared from the μ-chloro dimer **6** (0.110 g, 0.19 mmol) and pyridine (0.033 g, 0.42 mmol) in dichloromethane (2 mL). Recrystallization of the crude product from dichloromethane–hexane at room temperature gave the title compound **29** (24) as colourless plates (0.131 g, 83%); mp 155 °C (dec.) (lit. value (24) mp 200 °C (dec.)). IR (cm^{-1}): 3095, 3085, 1446, 1416, 1408, 1398, 1345, 1205, 1080, 757, 734, 692, 252 ν(Pd–Cl) (lit. value (24) ν(Pd–Cl) 254 cm^{-1}). ^{1}H NMR (90 MHz, $CDCl_3$) δ: 8.95 (d, J = 5.0 Hz, 2H, pyridyl H2 and H6), 8.19 (d, J = 2.0 Hz, 1H, pyrazolyl H3), 7.95–7.82 (m, 2H, aryl), 7.47 (t, J_{av} = 6.9 Hz, 2H, pyridyl H3 and H5), 7.14–6.81 (m, 3H, aryl), 6.46 (t, J = 2.5 Hz, 1H, pyrazolyl H4), 6.30 (d, J = 7.6 Hz, 1H, phenyl H6), 5.29 (s, 1H, 0.5 CH_2Cl_2). Elemental anal. calcd. for $C_{14.5}H_{13}Cl_2N_3Pd$ (%): C 42.8, H 3.2, N 10.3; found: C 42.95, H 3.0, N 10.4.

a-*Chloro*-b-*(2″-methylpyridine*-N″)-dc-*[2-(1′-pyrazolyl)-phenyl*-N′,C*]palladium (32)*

This compound was prepared from **6** (0.130 g, 0.23 mmol) and 2-methylpyridine (5 drops) in dichloromethane (5 mL). Recrystallization of the crude product from dichloromethane–hexane at room temperature gave the title compound **32** as colourless microcrystals (0.105 g, 61%); mp 210 to 211 °C (dec.). IR (cm^{-1}): 3130, 1605, 1512, 1441, 1424, 1410, 1396, 1341, 1288, 1105, 1072, 1052, 1025, 775, 752, 746, 310, 258 ν(Pd–Cl). ^{1}H NMR (90 MHz, $CDCl_3$) δ: 8.97 (d, J = 5.5 Hz, 1H, pyridyl H6), 8.18 (d, J = 2.1 Hz, 1H, pyrazolyl H3), 7.94 (d, J = 2.9 Hz, 1H, pyrazolyl H5), 7.79 (td, J = 7.6, 1.4 Hz, 1H, aryl), 7.48–6.70 (m, 5H, aryl), 6.48 (t, J = 2.5 Hz, 1H, pyrazolyl H4), 5.98 (d, J = 7.3 Hz, 1H, phenyl H6), 3.12 (s, 3H, Me). Elemental anal. calcd. for $C_{15}H_{14}ClN_3Pd$ (%): C 47.6, H 3.7, N 11.1; found: C 47.4, H 3.7, N 11.2.

a-*Chloro*-b-*(2″,6″-dimethylpyridine*-N″)-dc-*[2-(3′,5′-dimethyl-1′-pyrazolyl)phenyl*-N′,C*]-palladium (34)*

This compound was prepared from the μ-chloro dimer **9** (0.180 g, 0.29 mmol) and 2,6-dimethylpyridine (5 drops) in dichloromethane (10 mL). Recrystallization of the crude product from dichloromethane–hexane at room temperature gave the title compound **34** as fine, colourless needles (0.114 g, 47%); mp > 300 °C. IR (cm^{-1}): 1603, 1580, 1568, 1551, 1440, 1422, 1400, 1392, 1028, 795, 786, 761, 749, 299, 263 ν(Pd–Cl). ^{1}H NMR (90 MHz, $CDCl_3$) δ: 7.67 (J = 7.1 Hz, 1H, pyridyl H4), 7.26–7.17 (m, 3H, aryl), 7.03 (td, J = 7.5, 1.5 Hz, 1H, aryl), 6.66 (td, J = 7.4, 1.5 Hz, 1H, aryl), 5.98 (s, 1H, pyrazolyl H4), 5.70 (d, J = 7.6 Hz, 1H, phenyl H6), 3.20 (s, 6H, 2″- and 6″-Me), 2.76 (s, 3H, pyrazolyl Me), 2.64 (s, 3H, pyrazolyl Me). Elemental anal. calcd. for $C_{18}H_{20}ClN_3Pd$ (%): C 51.45, H 4.8, N 10.0; found: C 51.2, H 4.9, N 9.8.

a-*Chloro*-b-*[($^2H_5$)pyridine*-N″]-dc-*[1-(2′-pyridyl)indol-2-yl*-N′,C*]palladium (35)*

This compound was prepared from the μ-chloro dimer **10** (0.281 g, 0.42 mmol) and ($^2H_5$)pyridine (10 drops) in dichloromethane (5 mL). The crude product was dissolved in dichloromethane and the resulting solution was filtered and diluted with ether. On addition of hexane, the title compound **35** separated as small yellow prisms (0.232 g, 66%); mp 236–238 °C (dec.). IR (cm^{-1}): 2290 ν(C–D), 1612, 1598, 1563, 1510, 1496, 1448, 1322, 1318, 1290, 1258, 1189, 1147, 1085, 777, 756, 742, 740, 728, 616, 538, 530, 304, 240 ν(Pd–Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.25 (dd, J = 5.7, 1.7 Hz, 1H, pyridyl H6), 7.86 (ddd, J = 8.5, 7.3, 1.7 Hz, 1H, aryl), 7.69–7.65 (m, 2H, aryl), 7.37–7.34 (m, 1H, aryl), 7.16 (td, J = 7.3, 1.6 Hz, 1H, aryl), 7.12 (td, J = 7.2, 1.2 Hz, 1H, aryl), 6.95 (ddd, J = 7.2, 5.7, 1.1 Hz, 1H, pyridyl H5), 5.61 (d, J = 0.7 Hz, 1H, indolyl H3). Elemental anal. calcd. for $C_{18}H_9(^2H_5)ClN_3Pd$ (%): C 51.6, H 3.5, N 10.0; found: C 51.2, H 3.2, N 9.7.

a-*Chloro*-dc-*[1-(2′-pyridyl)indol-2-yl*-N′,C*]*-b-*(trimethyl phosphite)palladium (36)*

This compound was prepared from **10** (0.209 g, 0.31 mmol) and trimethyl phosphite (0.087 g, 0.70 mmol) in dichloromethane (8 mL). Recrystallization of the crude product from cyclohexane gave the title compound **36** as lustrous yellow plates (0.222 g, 78%); mp 168 to 169 °C. IR (cm^{-1}): 1607, 1596, 1506, 1323, 1293, 1261, 1181, 1166, 1151, 1071, 1065, 1004, 826, 808, 768, 763, 746, 740, 730, 613, 546, 537, 274, 236 ν(Pd–Cl). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.28 (dddd, J = 5.8, 1.7, 0.7 Hz, $J_{P,H}$ = 5.1 Hz, 1H, pyridyl H6), 7.94 (ddd, J = 8.6, 7.1, 1.8 Hz, 1H, aryl), 7.85 (dt, J = 7.5, 1.0 Hz, 1H, pyridyl H3), 7.76–7.73 (m, 1H, aryl), 7.53–7.50 (m, 1H, aryl), 7.23–7.14 (m, 2H, aryl), 7.08 (dddd, J = 7.1, 5.8, 1.3 Hz, $J_{P,H}$ = 2.0 Hz, 1H, pyridyl H5), 6.68 (d, $J_{P,H}$ = 2.3 Hz, 1H, indolyl H3), 3.93 (d, $J_{P,H}$ = 13.1 Hz, 9H, $P(OMe)_3$). Mass spectrum *m/z* (%): 460 (M^+, 7.5), 423 ($C_{16}H_{18}N_2O_3PPd^+$, 0.5), 408 ($C_{15}H_{15}N_2O_3PPd^+$, 0.4), 364 ($C_{14}H_{14}N_2O_3Pd^+$, 0.5), 336 ($C_{13}H_9ClN_2Pd^+$, 3.4), 317 ($C_{13}H_{11}N_2OPd^+$, 57), 299 ($C_{13}H_9N_2Pd^+$, 3). Elemental anal. calcd. for $C_{16}H_{18}ClN_2O_3PPd$ (%): C 41.85, H 3.95, N 6.1; found: C 41.9, H 4.0, N 6.3.

Acknowledgments

This work was initiated during the tenure of a grant from the Anti-Cancer Council of Victoria, and was supported by the Australian Research Council. The authors would also like to thank Johnson Matthey Chemicals Ltd., UK, for the generous loan of platinum and palladium precursors. We would also like to thank Dr. Ian A.G. Roos for helpful discussions, and Virginia A. Leopold and Dr. Lorraine Webster,

Experimental Chemotherapy and Pharmacology Unit, Cancer Institute, Melbourne, Victoria Australia, for assistance with the biological testing.

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