Palladacyclopentadienyl complexes bearing purine-based NHCs: a new class of promising antiproliferative agents against human ovarian cancer.

Thomas Scattolin[1], Sonia Giust[1], Paola Bergamini[2], Isabella Caligiuri[3], Luciano Canovese[1], Nicola Demitri[4], Roberto Gambari[5], Ilaria Lampronti[5], Flavio Rizzolio[1,3] and Fabiano Visentin*[1].

[1]Dipartimento di Scienze Molecolari e Nanosistemi, Università Ca' Foscari, Campus Scientifico Via Torino 155, 30174 Venezia-Mestre, Italy. E-mail: fvise@unive.it;

[2]Dipartimento di Scienze Chimiche e Farmaceutiche, Università degli Studi di Ferrara, Via Fossato di Mortara 74, 44121 Ferrara, Italy.

[3]Pathology Unit, Department of Molecular Biology and Translational Research, National Cancer Institute and Center for Molecular Biomedicine - CRO, Aviano, Italy.

[4]Elettra – Sincrotrone Trieste, S.S. 14 Km 163.5 in Area Science Park, 34149 Basovizza, Trieste, Italy,

[5]Dipartimento di Scienze della Vita e Biotecnologie, Università degli Studi di Ferrara, Via Fossato di Mortara 74, 44121 Ferrara, Italy.

ABSTRACT

A complete protocol for the synthesis of new palladacyclopentadienyl complexes with purine-based carbenes as supporting ligands is described.

The new organometallic compounds were exhaustively characterised by NMR, IR spectroscopy and elemental analysis. The single crystal X-ray structure of complex **2b** coordinating also a triphenylphosphine was resolved.

Some of these complexes showed a comparable or better antiproliferative activity than cisplatin on two human ovarian cancer A2780 (cisplatin-sensitive) and A2780cis (cisplatin-resistant) lines. Moreover, for the complexes **2** and **3** (coordinating one purine-based NHC ligand and one phosphine) the cytotoxicity is associated with an evident induction of apoptosis. Finally, complexes **3,** bearing one purine-based NHC ligand and one PTA (1,3,5-triaza-7-phosphaadamantane), proved practically inactive on non-tumor fibroblast cells (MRC-5).

1 INTRODUCTION

In the recent years a number of different platinum complexes have been synthesized with the aim of reproducing, or possibly enhancing, the therapeutic properties of cisplatin and its analogues of second and third generation.[1,2] It is worth noting that such compounds still represent the most widely used chemotherapeutic metallo-drugs active in the battle against neoplastic pathologies despite the resistance mechanism developed by some typology of neoplasia[3], the remarkable toxicity and their reduced versatility toward several tumors. On the other hand, the development of

synthetic analogues might be of great interest in lowering the toxicity or implementing the multi-drug approach to cancer therapy. Moreover, the multiplicity of the possible cellular targets and/or the difficulty to predict the mechanism of interaction with biomolecules render very demanding to plane on paper the structure and features of a new metal chemotherapeutic drug.

Therefore, no impressive improvements have been obtained so far.[4] One alternative approach to the problem is based on testing systematically unexplored classes of metal compounds. The possibility to choose ancillary ligands and organometallic fragments offers many variables from the combination of which might emerge a new effective chemotherapeutic drug.[5, 6]

In this respect, palladium might represent a valid alternative to platinum thanks to the similarity among the structures of their complexes. On the other hand, the palladium derivatives show a better solubility in water and a remarkably enhanced reactivity with respect to those of platinum.[7, 8] In this context, it was recently shown that palladium complexes stabilized by strong ligands often display a higher anticancer activity than the usual platinum agents.[9]

On the basis of such observation, we have recently carried out a study describing the synthesis of several allyl palladium complexes stabilized by different NHC spectator ligands derived from three appropriately functionalized methylxanthines [10] which show an antiproliferative activity in many cases remarkably higher than that of the reference cisplatin.[11a] We have planned this sort of investigation accepting some literature suggestions. Firstly, we supposed that the easier dissociation path in bloodstream and/or cellular environment of palladium complexes compared to platinum derivatives[8b,c] could be reduced by the strong stabilizing NHC ligands.[12] In this respect it is worth noting that some NHC-Palladium derivatives described in the literature display reduced cytotoxicity [9a,e,13] and a noticeable cancer growth suppression *in vivo*.[9e,12c] Secondly, the natural origin of the xanthines suggested a possible increased compatibility and therefore an enhanced interaction of the related derivatives with the biological environment, as already shown elsewhere.[14]

In the present paper and at variance with the previous work focused on the palladium-allyl moiety[11a], we opted for the palladacyclopentadienyl group since it was shown that palladacycles can be very effective against different cancer cell lines.[9e] Moreover, the experience we developed on these substrates[15] suggests that this organometallic fragment should remain stable in a biological environment.

As supporting ligands, in addition to purine-based NHCs, we choose triphenyl phosphine (PPh_3), 2,6-dimethylphenyl isocyanide (DIC) and 1,3,5-triaza-7-phosphadamantane (PTA), with the aim at obtaining complexes with different steric and electronic features. In particular, it is well-known that PTA can increases the water solubility of its derivatives.[16]

The palladacyclopentadienyl derivatives synthesized and tested toward the A2780 (cisplatin sensitive) and A2780*cis* (cisplatin resistant) cancer lines are reported in the following Scheme 1.

Scheme 1: Starting imidazolium salts (**a**-**c**) and palladacyclopentadienyl derivatives (**1**-**4**).

2 RESULTS AND DISCUSSION

2.1 Synthesis of purine-based imidazolium salts

Compounds **a**-**c** were obtained pure and in quantitative yields by metathesis of the tetrafluoborate ions with tetraphenylarsonium chloride of the alkylated xanthines, synthesized according to methods published elsewhere[11] (see Fig. S1a, S.I.).

The chloride derivatives were characterized by ^{1}H and ^{13}C NMR spectroscopy and the spectra of compounds **a**-**c** display slight modification with respect to those of the original tetrafluoroborate derivatives. (See Figs. S1b-g, S.I.) In particular, the marked downfield shift ($\Delta\delta \sim 1.5$ ppm) of the barely significant imidazolic proton is worth noting.

2.2 Synthesis of silver purine-based NHC complexes

The reaction between compounds **a**-**c** with Ag_2O carried out in CH_3CN for four hours in the dark, yields the silver complexes **Aga**-**c**.

Scheme 2: Synthesis of silver purine-based NHC complexes

The comparison between the NMR spectra of the starting alkylated xanthines and the ensuing silver complexes confirms that the reaction summarized in Scheme 3 took place. As can be seen (Figs. S2a-d and S2g-j, S.I.), the peak of the imidazole proton disappears and all the signals belonging to alkyl and aryl groups undergo shift upon coordination with the metal. The complete assignment of the signals has been carried out on the basis of the bi-dimensional HSQC and HMBC NMR spectra. However, the true molecular structure of complexes **Aga**-**c** was assigned by means of the ESI-MS spectra which indicate that the carbene silver complexes, according to Nolan and co-workers suggestion,[17] are better described as [(NHC)-Ag-(NHC)][$AgCl_2$] rather than the simpler [(NHC)-Ag-(NHC)]Cl (see Figs. S2e-f, S.I.).

2.3 Synthesis of bis-NHC palladacyclopentadienyl complexes

The synthesis of the title complexes was carried out according to Scheme 3.

Scheme 3: Synthesis of type **1** complexes

Type 1 complexes were synthesized by reacting the compounds **Aga-c** and the polymeric precursor $[PdC_4(COOCH_3)]_n$ which were prepared according to published methods.[11,18]

Despite the difficulty we have hitherto faced when bulky carbenes are used for the preparation of bis-carbene complexes,[15e] the synthesis of derivatives **1** proceeded without particular problems and with a reasonable yield.

The NMR spectra, however, are characterized by the doubling of all the signals ascribable to the synthesized complexes, as can be noticed in Fig.1 and Figs. S3 (S.I.).

Fig. 1: ^1H NMR spectrum of complex **1c** ($CDCl_3$, T= 298K)

In the case of complexes **1c** (Fig.1) and **1b**, an AB system, due to the presence of two non-equivalent $\underline{CH_2}Ph$ groups, together with the already cited doubling of all the other signals, strongly suggest the concomitant and essentially equimolecular formation of two atropoisomers in which the benzyl fragments lie on the same or opposite side of the main plane of the complexes owing to the hindered rotation about the Pd-C bond of the non-symmetric carbene (Fig. 2).

Fig. 2: DFT representation[19] of the atropoisomers of complex **1b** (for the sake of optimization of computer time, group COOMe was substituted with the less disordered CN)

The detailed ^1H and ^{13}C NMR investigations, supported by HSQC and HMBC spectra (See Figs. S3 in S.I.), confirm the structure in solution of type **1** complexes.

In particular:

i) the carbenic NCH_3 groups of any atropoisomer resonate at different chemical shift than those of the silver complexes.

ii) Two different couple of signals for the OCH_3 groups in both the ^1H and ^{13}C NMR spectra are detected.

iii) The already cited AB signal ascribable to the CH_2Ph benzyl protons in the case of complexes **1b** and **1c** is observed.

iv) Two different carbene carbon signals are observed at ca. 188 ppm.

2.4 Synthesis of mixed NHC-PPh_3 palladacyclopentadienyl complexes

As a consequence of a detailed study entailing theoretical approaches and experimental controls,[20] we were able to prepare pure type **2** complexes by the non-conventional synthetic methodology reported in Scheme 4.

Scheme 4: Synthesis of type **2** complexes

This methodology does not always warrant the formation of the mixed complexes since well-defined thermodynamic and kinetic conditions must be obeyed in order to avoid the formation of the bis-carbene and bis-phosphino complexes only, or a mixture of all the possible derivatives. A preliminary DFT study on the thermodynamic stability of the involved species[19] (See Fig. S4, S.I.) and the consequent experimental check show that in this one and in other cases (*vide infra*) the pre-conditions elsewhere identified[20] have been satisfied.

The characterization of type **2** complexes was carried out by NMR, IR (Figs. S5, S.I.) and in the case of complex **2b** by the diffractometric resolution of its solid-state structure.

The $^{31}P\{^1H\}$NMR of the complexes shows a single signal at ca. 25-26 ppm at lower field than that of free PPh_3 ($\Delta\delta \approx 30$ ppm) (see Fig. S5a, S.I.). Due to the asymmetry induced by the different spectator ligands, the ^1H and ^{13}C NMR spectra of all the complexes show four signals related to the COOMe groups. (Figs. S5b-c and S5g-j S.I.)

In the ^1H NMR spectra all the $NC\underline{H}_3$ protons are clearly detectable whereas those belonging to the $C\underline{H}_2Ph$ groups in the case of complexes **2b** and **2c** resonate as AB systems owing to their magnetic non-equivalence induced by the hindered rotation of the carbene about the Pd-C bond. Finally, the carbene carbon resonates as a doublet at ca. 190 ppm owing to the coupling with vicinal phosphorus.

As a definitive confirmation of the steric nature of this sort of complexes we report in Fig. 3 the resolved solid state structure of complex **2b** which will be discussed further on. Other ORTEP[21] representations of complex **2b** are reported in Fig. S8 (S.I.).

Fig. 3: Ellipsoid representation of **2b** crystals ASU contents (50% probability)

2.5 Synthesis of mixed NHC-PTA palladacyclopentadienyl complexes

As can be seen in Scheme 5, also in this case the thermodynamic and kinetic conditions yielding these complexes by direct synthesis were fulfilled.

Scheme 5: Synthesis of type **3** complexes

The NMR investigations confirm the nature of the title derivatives and the related NMR and IR spectra are reported in Figs. S6 (S.I.).

Thus, in the ^{1}H NMR investigation, the singlets ascribable to the NCH_3 groups belonging to all the complexes and the AB systems of the NCH_2 fragments of complexes **3b** and **3c** are detected in the range 3.40 ÷ 4.20 and 5.00 ÷ 6.50 ppm, respectively. The NCH_2N and NCH_2P protons of the coordinated PTA resonate in the range 4.30 ÷ 4.50 and 3.50 ÷ 4.00 ppm, respectively, whereas the four OCH_3 groups are observed between 3.2 and 3.8 ppm.

In the $^{13}C\{^{1}H\}$NMR spectra, the carbene carbon resonates in the range 180 ÷ 190 ppm as a doublet ($J_{C\text{-}P} \approx$ 17-18 Hz) whereas NCH_2P and NCH_2N are detected at ca. 50 ppm (doublet, $J_{C\text{-}P} \approx$ 10Hz) and 73 ppm (doublet, $J_{C\text{-}P} \approx$ 6 Hz), respectively.

2.6 Synthesis of mixed NHC-DIC complexes (4)

Despite the electronic difference between the good π-acceptor 2,6-dimethylphenyl isocyanide (DIC) and the other ligands used in this research, which are all σ-donating molecules although to a different degree, the synthetic protocol yielding type **4** complexes remains almost the same as those previously described (see Scheme 6). As a matter of fact, the most relevant difference between protocols is the reaction rate, which in this case is considerably lower (48 h) than those related to the formation of type **1**-**3** complexes (1h). Moreover, soon after the mixing of the reagents three complexes, namely the bis-substituted isocyanide, bis-substituted carbene and the mixed palladacyclopentadienyl derivative **4** are detected in solution. These kinds of reaction are not unprecedented and have been recently studied in detail.[20] Eventually, the thermodynamically stable mixed species **4** was exhaustively formed and the long time necessary for the completion of the reaction is mainly due to the steric retard induced by the bulky homogeneously substituted bis-carbene and bi-isocyanide palladacyclopentadienyl complexes reacting with each other.[22]

Scheme 6: Synthesis of type **4** complexes

The characterization of complexes **4a-c** was carried out by NMR and IR spectrometry and the related spectra are reported in S.I. (Figs. S7a-i).

In particular, in the ^{1}H NMR spectra, the purinic NCH_3 groups are observed as singlets in the range 3.35 ÷ 4.35 ppm, whereas in the case of complexes **4b** and **4c** the AB systems of the benzyl NCH_2 protons are detected in the range 5.10 ÷ 6.00 ppm. The four OCH_3 fragments resonate in the range 3.29 ÷ 3.81 ppm whereas the isocyanide methyl groups, at variance with those of the free moiety, are observed as singlets in the range 2.00 ÷ 2.50 ppm.

In the $^{13}C\{^{1}H\}$NMR spectra the isocyanide and the carbene carbons are easily observed at ca. 149 and in the range 180 ÷190 ppm, respectively.

2.6 X-ray diffraction analysis - Structural characterization of complex 2b

The crystalline form of **2b** contains one crystallographically independent palladium complex (Fig. 3). Palladium centers adopt square planar coordination spheres with bond lengths and angles (Table 1) in agreement with literature structural data of complexes with similar ligands. CSD database (version 5.39 – November 2017) reports only one alternative Pd(II)-Xanthine complex showing compatible Pd-C bond length (2.008(14) Å).[23] Furthermore, the molecular model of **2b** is well superimposable with the related (2,6-dimethylphenylisocyanide)-(1,2,3,4-tetrakis(methoxycarbonyl) buta-1,3-diene-1,4-diyl)-(triphenylphosphine)-palladium structure (green sticks - CCDC Number: 825712) complex[24] (CCDC Number: 714135); R.M.S.D. of common atoms shared by the two models is 0.81 Å) (Fig. S9, S.I.).

The structure of **2b** shows that the xanthine minimizes steric repulsions in the solid state, adopting a roughly perpendicular orientation with respect to the palladium coordination plane (69.48° in **2b**). Neighbor ligand sidechains (a phenyl from the phosphine and one carbomethoxy from the buta-1,3-diene-1,4-diyl ligand) adopt conformations parallel to the xanthine, with average plane distances of 3.1(5) Å suggesting that π···π interligand contacts might stabilize the complex.

Crystal packing shows hydrophobic contacts among neighbor molecules, involving several CH···π and minor π···π interactions, while water molecules are trapped in polar cavities where hydrogen bonds link carbomethoxy groups of neighbor palladium complexes.

Table 1: Selected bond distances and angles (Å and degrees) for the palladium coordination of **2b** sphere. Naming schemes are reported in Fig. 3.

2.7 Anti-proliferative activity

The complexes containing PTA **3a-c** are very soluble in water. All other complexes are scarcely soluble in water and for this reason the stock solutions (50 mM) for biological tests were prepared in dmso and the working solutions were then obtained by dilution with ethanol.

The stability of complexes was preliminarily checked in D_2O (**3**) or dmso-d6 (**1**, **2** and **4**): after 48 hours at room temperature no ligand replacement and no degradation was observed. The ability of all the synthesized complexes in inhibiting the cell proliferation of ovarian cancer cells A2780 and A2780*cis* was analyzed after 72 hours treatment, in order to explore their biological activity in comparison with cisplatin, a known antineoplastic agent. The two treated cell lines exhibited a

sharply different response to cisplatin exposure. One (A2780) was sensitive to cisplatin, while the other (A2780*cis*) was cisplatin resistant. In Table 2, the results obtained from three independent experiments are reported. The strong anti-proliferative activity of cisplatin (IC_{50} = 0.6 ± 0.1 μM) on the A2780 cell line was confirmed;[11, 31] in addition, the obtained data confirmed the cisplatin-resistance of the A2780*cis* cell line (IC_{50} = 6 ± 1 μM). For all the tested complexes, with the exception of **2c**, the IC_{50} values against the A2780 and A2780*cis* cell lines were similar, indicating that they were active also on the resistant cell line. Remarkably, compound **3a** exhibited higher activity on A2780*cis* cells with respect to A2780 cell. The compounds exhibiting the highest *in vitro* antiproliferative activity ($IC_{50} \leq 1$ μM) were **3a**, **3b**, **3c** and **4c**. All these compounds displayed a very interesting ability in inducing cytotoxicity on both the cell lines, featuring higher activity than cisplatin on the resistant cells.

Table 2: Effects of the Pd-complexes on the proliferation of A2780 and A2780*cis* cells. The inhibition of cell growth is represented as IC_{50}. DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

In order to preliminarily evaluate a potential selectivity of the palladacyclopentadienyl complexes *versus* cancer cells, normal human fibroblasts (MRC-5) were treated for 72 hours with the complexes **2b** and the most effective **3c**. As shown in Table 3, the compounds are poorly active (**2b**) or inactive (**3c**) on this non-tumor cell line, suggesting a preferential activity of the analysed compounds on cancer cells.

Table 3: Effects of the Pd-complexes on the proliferation of MRC-5 human fibroblasts. The inhibition of cell growth is represented as IC_{50}. DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

2.8 Pro-apoptotic effects

In order to verify whether the anti-proliferative activity of the newly synthesized compounds is associated with the activation of the apoptotic pathway (as is known when cisplatin is administered to tumor cells of different origin),[32-35] the complexes of each series demonstrating the best anti-proliferative activity (**1b**, **2b**, **3c**, **4c**) were tested. The Annexin V test and a MUSE cell analyzer were employed to this purpose. Representative results are shown in Figures 4 and 5, and the

summary of all the obtained data displayed in Table 4. All the selected derivatives, with the exception of the compound **4c**, induced evident apoptosis on both cell lines. These assays have been performed using previously identified concentrations liable to determine expected anti-proliferative effects, i.e. the IC_{50} and IC_{75} values on A2780 and A2780*cis* cell lines. Cisplatin was added to the cultured cells, demonstrating its evident and already reported pro-apoptotic effects on the A2780 cell line, when used at 1.0 μM concentration (55.9% of total apoptosis), while on the resistant A2780*cis* cells it was inactive at the same concentration. When the compounds were assayed on the A2780 cell line, at the used concentrations, the best pro-apoptotic activity was obtained with the complexes **1b**, **2b** and **3c** which were able to induce a very high percentage of apoptosis, exhibiting a better pro-apoptotic activity than cisplatin (Figure 4 and Table 3). Fully in agreement with the data shown in Table 2, derivatives **2b** and **3c** induced pro-apoptotic effects also on the resistant A2780*cis* cells. The compound **1b** displayed low pro-apoptotic effects on A2780*cis* cells, whereas it is active on A2780, suggesting a different mechanism of action with respect to compounds **2b** and **3c.**

Fig. 4: Representative apoptosis profiles of A2780 cells untreated (C-), treated for 72 hours with cisplatin (C+, 0.5 and 1.0 μM) and treated with complexes **1b**, **2b**, **3c** and **4c** at different concentrations (IC_{50} and IC_{75} values). DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

Fig. 5: Representative apoptosis profiles of A2780*cis* cells untreated (C-), treated for 72 hours with cisplatin (C+, 1.0 and 5.0 μM) and treated with complexes **1b**, **2b**, **3c** and **4c** at different concentrations (IC_{50} and IC_{75} values). DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

Table 4: Pro-apoptotic effects of the Pd-complexes on A2780 and A2780*cis* cell lines detected at two different concentrations. (C-: untreated cells). DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

3 CONCLUSIONS

We have synthesized twelve new palladacyclopentadienyl complexes bearing two *N*-heterocyclic carbenes or one NHC and one phosphine or isocyanide. In order to enhance the biological affinity

of the complexes, three different compounds derived from modified natural xanthines have been used as NHC ligands.

The cytotoxicity of the complexes toward the ovarian cancer cellular lines A2780 (cisplatin sensitive) and A2780*cis* (cisplatin resistant) was determined. Remarkably, with the exception of the biscarbene derivative **1c**, all the synthesized complexes show an antiproliferative activity comparable to cisplatin.

A correlation between the antiproliferative activity of the derivatives and their structure has been observed. In particular, the less hindered complexes of the type NHC-PTA or NHC-DIC show a higher efficiency with respect to the bis-NHC and the NHC-PPh_3 derivatives. Complexes **3** proved particularly promising since they are able to associate a significant antiproliferative activity, especially on A2780*cis* line, with the ability to induce apoptosis. Moreover, complex **3c**, which is the most active species, is almost inactive on the non-tumor cells (MRC-5 fibroblasts).

This observation might be important in view of possible application of the studied compounds in anticancer treatments, suggesting that pre-clinical tests on different primary tumor cell isolates should be considered before proposing these compounds for clinical trials; in addition, it will be necessary in the future to test the activity on *in vivo* model systems, in order to clarify whether the described cytotoxicity on tumor cell lines involves negligible side effects on normal cells and networks.

Finally, the presence of PTA makes complexes **3** very soluble in water, a requisite that is very important for drug administration.

The natural development of this work will be to define the main cellular target and the mechanism of action of the most promising complexes.

4 EXPERIMENTAL

4.1 Solvents and Reagents

The solvents CH_2Cl_2 and CH_3CN were distilled over CaH_2, acetone was refluxed over 4Å molecular sieves and distilled. The precursor $[PdC_4(COOCH_3)_4]_n$ has been synthesized according to published procedures.[18] All other solvents and chemicals were commercial grade products and used as purchased.

4.2 IR, NMR Measurements and Elemental Analysis

The IR, ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Perkin-Elmer Spectrum One spectrophotometer and on Bruker Avance 300 or Ascend 400 spectrometers, respectively. The

elemental analysis of the synthesized complexes was carried out using an Elementar CHN "CUBO micro Vario" analyzer.
ESI-MS analyses were carried out by the group of Prof. Sgarbossa (Padua University).

4.3 Crystal Structure Determinations

The crystal data of compound **2b** were collected at 100K at the XRD1 beamline of the Elettra Synchrotron, Trieste (Italy).[25] The data sets were integrated, scaled and corrected for Lorentz and polarization effects with the XDS package.[26] The structure was solved by direct methods using SHELXT program[27] and refined using full−matrix least−squares with all non−hydrogen atoms anisotropically and hydrogens included on calculated positions, riding on their carrier atoms. Disordered water molecules were modeled in cavities, located on crystallographic inversion centers. The electron content per void area has been estimated to be 14 e^- (~70 $Å^3$, PLATON[28] 'SQUEEZE' routine), in agreement with refined water occupancies. Hydrogen atoms for water molecules were not included in the refined models since it was not possible to locate them unambiguously in electron-density peaks of Fourier difference maps (contributions of these missing H atoms are still included in the properties reported in Table S1, S.I.). All calculations were performed using SHELXL−2018/3.[29] The Coot program was used for structure building.[30] The crystal data are given in Table S1 (S.I.). Pictures were prepared using Ortep3[21] software.
Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 1881566 These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures.

4.4 Synthesis of purine-based imidazolium salts (a-c)

These compounds were synthesized by chloride-BF_4 metathesis of the tetrafluoroborate imidazolium salt obtained according to published procedures.[11]

Compound a

To 356.2 mg (1.203 mmol) of the tetrafluoroborate salt of tetramethyl caffeine, dissolved in 100 mL of water, 503.9 mg (1.203 mmol) of $AsPh_4Cl$ was added. The immediate formation of $AsPh_4BF_4$ as a white fluffy precipitate was observed. The reaction mixture was stirred for ca. 30 min and the $AsPh_4BF_4$ was filtered off by a millipore apparatus. The clear solution was dried under vacuum at low temperature (< 50 °C) and the residue ground in 20 mL of CH_2Cl_2, filtered off on a gooch, washed with diethyl ether and dried under vacuum. 293.3 mg (yield 99%) of compound **a** was obtained as a white solid.

^{1}H-NMR (400 MHz, CD_3CN, T = 298K, ppm) δ: 3.34 (s, 3H, NCH_3), 3.75 (s, 3H, NCH_3), 4.13 (s, 3H, NCH_3), 4.21 (s, 3H, NCH_3), 10.03 (s, 1H, NCHN).

^{13}C{^{1}H}-NMR (CD_3CN, T = 298K, ppm) δ: 28.6 (CH_3, NCH_3), 31.8 (CH_3, NCH_3), 36.2 (CH_3, NCH_3), 37.5 (CH_3, NCH_3), 108.8 (C, C^5), 140.1 (C, C^4), 140.4(CH, N-CH-N), 151.1 (C, C=O), 154.2 (C, C=O).

IR (KBr): $\nu_{C=O}$ = 1719, 1675 cm^{-1}, ν_{C-O} = 1304, 1264 cm^{-1}.

Compounds **b** and **c** were prepared in a similar way from the appropriate reactants.

Compound b

White solid; Yield 98%.

^{1}H-NMR (400 MHz, CD_3CN, T = 298K, ppm) δ: 3.33 (s, 3H, NCH_3), 3.73 (s, 3H, NCH_3), 4.10 (s, 3H, NCH_3), 5.72 (s, 2H, NCH_2), 7.44-7.50 (m, 5H, Ph), 8.92 (s, 1H, NCHN).

^{13}C{^{1}H}-NMR (CD_3CN, T = 298K, ppm) δ: 28.2 (CH_3, NCH_3), 31.2 (CH_3, NCH_3), 37.3 (CH_3, NCH_3), 52.0 (CH_2, NCH_2), 107.8 (C, C^5), 128.6-133.4 (Ph), 138.6 (CH, NCHN), 140.0 (C, C^4), 150.4 (C, C=O), 153.4 (C, C=O).

IR (KBr): $\nu_{C=O}$ = 1717, 1671 cm^{-1}, ν_{C-O} = 1267 cm^{-1}

Compound c

White solid; Yield 99%.

^{1}H-NMR (400 MHz, CD_3CN, T = 298K, ppm) δ: 3.73 (s, 3H, NCH_3), 4.09 (s, 3H, NCH_3), 4.10 (s, 3H, NCH_3), 5.16 (s, 2H, NCH_2), 7.33-7.39 (m, 5H, Ph), 8.82 (s, 1H, NCHN).

^{13}C{^{1}H}-NMR (CD_3CN, T = 298K, ppm) δ: 31.3 (CH_3, NCH_3), 35.8 (CH_3, NCH_3), 37.0 (CH_3, NCH_3), 45.0 (CH_2, NCH_2), 108.6 (C, C^5), 127.7-136.5 (Ph), 139.0 (CH, NCHN), 139.8 (C, C^4), 150.4 (C, C=O), 153.5 (C, C=O).

IR (KBr): $\nu_{C=O}$ = 1722, 1674 cm^{-1}.

4.5 Synthesis of silver purine-based NHC complexes (Aga-c)

Complex Aga

To 142.5 mg (0.5824 mmol) of compound **a**, dissolved in 35 mL of anhydrous CH_3CN in a two necked flask under inert atmosphere (Ar), 74.2 mg (0.320 mmol) of Ag_2O were added. The mixture was vigorously stirred in the dark for 4h and a fluffy white solid, which was filtered off on a gooch, was progressively formed. The white solid was dissolved in 200 mL of CH_2Cl_2 and from the

resulting mixture the Ag_2O in excess removed by filtration in a millipore apparatus. The clear solution was concentrated under vacuum and the complex **Aga** precipitated as white solid by addition of diethylether, filtered off on a gooch and dried under vacuum. 121.4 mg of the title complex was obtained (yield 60%).

^{1}H-NMR (400 MHz, CD_2Cl_2, T = 298K, ppm) δ: 3.41 (s, 6H, $2NCH_3$), 3.84 (s, 6H, $2NCH_3$), 4.17 (s, 6H, $2NCH_3$), 4.24 (s, 6H, $2NCH_3$).

^{13}C{^{1}H}-NMR (d^6-DMSO, T = 298K, ppm) δ: 28.7 (CH_3, NCH_3), 31.2 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 38.3 (CH_3, NCH_3), 109.3 (C, C^5), 140.9 (C, C^4), 151.0 (C, C=O), 153.7 (C, C=O), 207.0 (C, carbene).

ESI-MS (CH_3CN): m/z 525.04 $[Ag(NHC)_2]^+$.

IR (KBr): $\nu_{C=O}$ = 1709, 1669 cm^{-1}.

Derivatives **Agb** and **Agc** were prepared in a similar way from the appropriate reactants.

Complex Agb

White solid; Yield 67%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 3.31 (s, 6H, $2NCH_3$), 3.73 (s, 6H, $2NCH_3$), 4.16 (s, 6H, $2NCH_3$), 5.62 (s, 4H, $2NCH_2$), 7.20-7.41 (m, 10H, 2Ph).

^{13}C{^{1}H}-NMR ($CDCl_3$, T = 298K, ppm) δ: 28.8 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 40.1 (CH_3, NCH_3), 54.1 (CH_2, NCH_2), 109.1 (C, C^5), 128.3-135.5 (Ph), 140.3 (C, C^4), 150.6 (C, C=O), 153.1 (C, C=O), 188.1 (C, carbene).

ESI-MS (CH_3CN): m/z 677.11 $[Ag(NHC)_2]^+$.

IR (KBr): $\nu_{C=O}$ = 1713, 1673 cm^{-1}.

Complex Agc.

White solid; Yield 70%

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 3.83 (s, 6H, $2NCH_3$), 4.15 (s, 6H, $2NCH_3$), 4.19 (s, 6H, $2NCH_3$), 5.19 (s, 4H, $2NCH_2$), 7.29-7.50 (m, 10H, 2Ph).

^{13}C{^{1}H}-NMR (d^6-DMSO, T = 298K, ppm) δ: 31.2 (CH_3, NCH_3), 32.1 (CH_3, NCH_3), 38.3 (CH_2, NCH_2), 109.3 (C, C^5), 127.7-137.3 (Ph), 141.3 (C, C^4), 150.9 (C, C=O), 153.5 (C, C=O), 206.9 (C, carbene).

ESI-MS (CH_3CN): m/z 677.08 $[Ag(NHC)_2]^+$.

IR (KBr): $\nu_{C=O}$ = 1711, 1674 cm^{-1}.

4.6 Synthesis of bis-NHC palladacyclopentadienyl complexes (1)

Complex 1a

In a 50 mL two necked flask, 18.2 mg (0.0466 mmol) of $[PdC_4(COOCH_3)_4]_n$ was dissolved in 5 mL of anhydrous CH_2Cl_2 under inert atmosphere (Ar). 27.3 mg (0.0388 mmol) of complex **Aga**, suspended in 25 mL of anhydrous CH_2Cl_2, was added to the solution of $[PdC_4(COOCH_3)_4]_n$ and precipitation of AgCl was immediately observed. The mixture was reacted for 1h at R.T. and then filtered by a millipore apparatus. The clear yellow solution was concentrated under vacuum and the title complex precipitated by addition of diethylether. The yellow microcrystalline precipitate was filtered off on a gooch, washed with diethylether and dried under vacuum. 21.3 mg (70% yield) of complex **1a** was obtained.

^{1}H-NMR (400 MHz, CD_2Cl_2, T = 298K, ppm) δ: 3.30 (s, 12H, 4 OCH_3), 3.36 (s, 12H, 4NCH_3), 3.61 (s, 12H, 4 OCH_3), 3.78 (s, 6H, 2NCH_3), 3.79 (s, 6H, 2NCH_3), 4.11 (s, 6H, 2NCH_3), 4.15 (s, 6H, 2NCH_3), 4.32 (s, 6H, 2NCH_3), 4.34 (s, 6H, 2NCH_3).

^{13}C{^{1}H}-NMR (CD_2Cl_2, T = 298K, ppm) δ: 28.3 (CH_3, NCH_3), 31.8 (CH_3, NCH_3), 37.2 (CH_3, NCH_3), 37.4 (CH_3, NCH_3), 39.2 (CH_3, NCH_3), 39.3 (CH_3, NCH_3), 50.6 (CH_3, OCH_3), 51.0 (CH_3, OCH_3), 109.8 (C, C^5), 140.5 (C, C^4), 144.6 (C, C-$COOCH_3$), 150.6 (C, C=O), 153.0 (C, C=O), 164.8 (C, C$OOCH_3$), 164.9 (C, C$OOCH_3$), 167.1 (C, C-$COOCH_3$), 167.2 (C, C-$COOCH_3$), 175.7 (C, C$OOCH_3$), 175.9 (C, C$OOCH_3$), 188.1 (C, carbene), 188.4 (C, carbene).

IR (KBr): $\nu_{C=O}$ = 1710, 1672 cm^{-1}, ν_{C-O} = 1209 cm^{-1}.

Anal. Calcd. for $C_{30}H_{36}N_8O_{12}Pd$: C 44.65, H 4.50, N 13.88. Found: C 44.77, H 4.38, N 13.80.

Derivatives **1b** and **1c** were prepared in a similar way from the appropriate reactants.

Complex 1b

Yellow microcrystals; Yield 91%.

^{1}H-NMR (400 MHz, CD_2Cl_2, T = 298K, ppm) δ: 3.27 (s, 12H, 4 OCH_3), 3.29 (s, 12H, 4NCH_3), 3.43 (s, 6H, 2NCH_3), 3.61 (s, 6H, 2 OCH_3), 3.63 (s, 6H, 2 OCH_3), 3.75 (s, 6H, 2NCH_3), 3.79 (s, 6H, 2NCH_3), 4.32 (s, 6H, 2NCH_3), 4.98 and 5.40 (AB system, 4H, *J* = 15.5 Hz, 2NCH_2), 5.77 and 5.85 (AB system, 4H, *J* = 16.3 Hz, 2NCH_2), 6.90-7.33 (m, 20H, 4Ph).

^{13}C{^{1}H}-NMR (CD_2Cl_2, T = 298K, ppm) δ: 28.3 (CH_3, NCH_3), 31.5 (CH_3, NCH_3), 31.9 (CH_3, NCH_3), 38.4 (CH_3, NCH_3), 39.4 (CH_3, NCH_3), 50.6 (CH_3, OCH_3), 51.0 (CH_3, OCH_3), 52.7 (CH_2, NCH_2), 109.0 (C, C^5), 109.5 (C, C^5), 125.1-136.7 (Ph), 140.7 (C, C^4), 140.8 (C, C^4), 145.1 (C, C-$COOCH_3$), 145.2 (C, C-$COOCH_3$), 150.4 (C, C=O), 150.5 (C, C=O), 152.3 (C, C=O), 164.8 (C,

$\underline{C}OOCH_3$), 165.1 (C, $\underline{C}OOCH_3$), 166.7 (C, $\underline{C}$-$COOCH_3$), 167.1 (C, $\underline{C}$-$COOCH_3$), 175.4 (C, $\underline{C}OOCH_3$), 188.6 (C, carbene), 189.1 (C, carbene).

IR (KBr): $\nu_{C=O}$ = 1710, 1668 cm^{-1}, ν_{C-O} = 1206 cm^{-1}.

Anal. Calcd. for $C_{42}H_{44}N_8O_{12}Pd$: C 52.59, H 4.62, N 11.68. Found: C 52.74, H 4.79, N 11.57.

Complex 1c

Yellow microcrystals; Yield 80%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 3.28 (s, 6H, 2 OCH_3), 3.31 (s, 6H, 2 OCH_3), 3.62 (s, 6H, 2 OCH_3), 3.64 (s, 6H, 2 OCH_3), 3.74 (s, 6H, 2NCH_3), 3.78 (s, 6H, 2NCH_3), 4.09 (s, 6H, 2NCH_3), 4.13 (s, 6H, 2NCH_3), 4.35 (s, 6H, 2NCH_3), 4.36 (s, 6H, 2NCH_3), 5.14 (m, 8H, 4NCH_2), 7.27-7.40 (m, 20H, 4Ph).

$^{13}C\{^1H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 31.9 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 37.4 (CH_3, NCH_3), 37.6 (CH_3, NCH_3), 39.4 (CH_3, NCH_3), 39.6 (CH_3, NCH_3), 45.1 (CH_2, NCH_2), 50.8 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 109.9 (C, C^5), 127.9-136.3 (Ph), 140.7 (C, C^4), 140.8 (C, C^4), 145.0 (C, $\underline{C}$-$COOCH_3$), 145.3 (C, $\underline{C}$-$COOCH_3$), 150.4 (C, C=O), 152.9 (C, C=O), 165.0 (C, $\underline{C}OOCH_3$), 165.2 (C, $\underline{C}OOCH_3$), 166.6 (C, $\underline{C}$-$COOCH_3$), 166.9 (C, $\underline{C}$-$COOCH_3$), 176.1 (C, $\underline{C}OOCH_3$), 176.2 (C, $\underline{C}OOCH_3$), 188.3 (C, carbene), 189.1 (C, carbene).

IR (KBr): $\nu_{C=O}$ = 1709, 1671 cm^{-1}, ν_{C-O} = 1208 cm^{-1}.

Anal. Calcd. for $C_{42}H_{44}N_8O_{12}Pd$: C 52.59, H 4.62, N 11.68. Found: C 52.74, H 4.56, N 11.67.

4.7 Synthesis of mixed NHC-PPh_3 and NHC-PTA palladacyclopentadienyl complexes (2-3)

Complex 2a

In a 50 mL two necked flask, 27.9 mg (0.0713 mmol) of $[PdC_4(COOCH_3)_4]_n$ was dissolved in 5 mL of anhydrous CH_2Cl_2 under inert atmosphere (Ar). 21.8 mg (0.0310 mmol) of complex **Aga** and 17.1 mg (0.0651 mmol) of PPh_3, suspended in 30 mL of anhydrous CH_2Cl_2, were added to the solution of $[PdC_4(COOCH_3)_4]_n$ and precipitation of AgCl was immediately observed. The mixture was reacted for 1h at R.T. and then filtered by a millipore apparatus. The clear yellow solution was concentrated under vacuum and the title complex precipitated by addition of diethylether. The yellow microcrystalline precipitate was filtered off on a gooch, washed with diethylether and dried in desiccator. 509 mg (83% yield) of complex **2a** was obtained.

^{1}H-NMR (400 MHz, $CDCl_3$, T=298K, ppm) δ: 2.53 (s, 3H, OCH_3), 3.25 (s, 3H, OCH_3), 3.36 (s, 3H, NCH_3), 3.48 (s, 3H, NCH_3), 3.63 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.76 (s, 3H, NCH_3), 3.82 (s, 3H, NCH_3), 7.32-7.51 (m, 15H, 3Ph).

$^{13}C\{^{1}H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 28.6 (CH_3, NCH_3), 31.5 (CH_3, NCH_3), 36.6 (CH_3, NCH_3), 38.2 (CH_3, NCH_3), 50.0 (CH_3, OCH_3), 50.8 (CH_3, OCH_3), 51.2 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 109.7 (C, C^5), 128.3-134.1 (Ph), 140.2 (C, C^4), 145.9 (d, C, J_{C-P} = 5.4 Hz, C-$COOCH_3$), 148.8 (d, C, J_{C-P} = 7.0 Hz, C-$COOCH_3$), 150.2 (C, C=O), 152.7 (C, C=O), 164.0-166.4 (C, $COOCH_3$), 168.1 (C, C-$COOCH_3$), 169.6 (C, C-$COOCH_3$), 174.9 (d, C, J_{C-P} = 4.9 Hz, $COOCH_3$), 175.6 (d, C, J_{C-P} = 5.3 Hz, $COOCH_3$), 189.1 (d, C, J_{C-P} = 15.8 Hz, carbene).

$^{31}P\{^{1}H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 26.1.

IR (KBr): $\nu_{C=O}$ = 1730, 1692 cm^{-1}, ν_{C-O} = 1212 cm^{-1}.

Anal. Calcd. for $C_{39}H_{39}N_4O_{10}PPd$: C 54.39, H 4.56, N 6.51. Found: C 54.49, H 4.51, N 6.62.

Derivatives **2b, 2c, 3a**, **3b** and **3c** were prepared in a similar way from the appropriate reactants.

Complex 2b

Yellow microcrystals; Yield 86%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 2.56 (s, 3H, OCH_3), 3.04 (s, 3H, OCH_3), 3.38 (s, 3H, NCH_3), 3.46 (s, 3H, NCH_3), 3.63 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.85 (s, 3H, NCH_3), 5.40 and 5.50 (AB system, 2H, *J* = 14.2 Hz, NCH_2), 7.12-7.52 (m, 20H, 4Ph).

$^{13}C\{^{1}H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 28.7 (CH_3, NCH_3), 31.8 (CH_3, NCH_3), 39.0 (CH_3, NCH_3), 50.0 (CH_3, OCH_3), 50.9 (CH_3, OCH_3), 51.2 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 53.6 (CH_2, NCH_2), 109.5 (C, C^5), 128.2-134.7 (Ph), 140.9 (C, C^4), 148.8 (d, C, J_{C-P} = 5.6 Hz, C-$COOCH_3$), 149.0 (d, C, J_{C-P} = 7.3 Hz, C-$COOCH_3$), 150.3 (C, C=O), 152.7 (C, C=O), 164.9-165.1 (C, $COOCH_3$), 166.0 (C, C-$COOCH_3$), 166.4 (C, C-$COOCH_3$), 174.5 (d, C, J_{C-P} = 5.2 Hz, $COOCH_3$), 174.9 (d, C, J_{C-P} = 6.4 Hz, $COOCH_3$), 189.4 (d, C, J_{C-P} = 15.5 Hz, carbene).

$^{31}P\{^{1}H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 25.1.

IR (KBr): $\nu_{C=O}$ = 1710, 1670 cm^{-1}, ν_{C-O} = 1208 cm^{-1}.

Anal. Calcd. for $C_{45}H_{43}N_4O_{10}PPd$: C 57.67, H 4.62, N 5.98. Found: C 57.81, H 4.73, N 5.87.

Complex 2c

Yellow microcrystals; Yield 85%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 2.53 (s, 3H, OCH_3), 3.24 (s, 3H, OCH_3), 3.48 (s, 3H, NCH_3), 3.63 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.80 (s, 6H, $2NCH_3$), 5.08 and 5.13 (AB system, 2H, *J* = 14.5 Hz, NCH_2), 7.24-7.50 (m, 20H, 4Ph).

$^{13}C\{^{1}H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 31.6 (CH_3, NCH_3), 36.6 (CH_3, NCH_3), 38.3 (CH_3, NCH_3), 45.0 (CH_2, NCH_2), 49.9 (CH_3, OCH_3), 50.8 (CH_3, OCH_3), 51.2 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 109.9 (C, C^5), 128.0-136.5 (Ph), 140.1 (C, C^4), 145.9 (d, C, J_{C-P} = 5.5 Hz, C-$COOCH_3$),

148.8 (d, C, J_{C-P} = 7.1 Hz, C-$COOCH_3$), 150.1 (C, C=O), 152.5 (C, C=O), 164.1-166.3 (C, $COOCH_3$), 168.1 (C, C-$COOCH_3$), 169.2 (C, C-$COOCH_3$), 174.3 (d, C, J_{C-P} = 5.5 Hz, $COOCH_3$), 175.6 (d, C, J_{C-P} = 5.5 Hz, $COOCH_3$), 189.4 (d, C, J_{C-P} = 16.0 Hz, carbene).

$^{31}P\{^1H\}$-NMR ($CDCl_3$, T=298K, ppm) δ: 26.2.

IR (KBr): $\nu_{C=O}$ = 1699, 1674 cm^{-1}, ν_{C-O} = 1205 cm^{-1}.

Anal. Calcd. for $C_{45}H_{43}N_4O_{10}PPd$: C 57.67, H 4.62, N 5.98. Found: C 57.78, H 4.85, N 6.02.

Complex 3a

Brown microcrystals; Yield 91%.

^1H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 3.28 (s, 3H, OCH_3), 3.42 (s, 3H, NCH_3), 3.61 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, NCH_3), 4.00 (d, 6H, J = 2.1 Hz, $3NCH_2P$), 4.04 (s, 3H, NCH_3), 4.20 (s, 3H, NCH_3), 4.49 (s, 6H, $3NCH_2N$).

$^{13}C\{^1H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 28.7 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 37.0 (CH_3, NCH_3), 38.9 (CH_3, NCH_3), 50.9 (CH_3, OCH_3), 51.1 (d, CH_2, J_{C-P} = 10.2 Hz, NCH_2P), 51.2 (CH_3, OCH_3), 51.4 (CH_3, OCH_3), 51.6 (CH_3, OCH_3), 73.1 (d, CH_2, J_{C-P} = 6.4 Hz, NCH_2N), 110.1 (C, C^5), 140.6 (C, C^4), 164.3-165.0 (C, $COOCH_3$), 165.4 (C, C-$COOCH_3$), 168.7 (C, C-$COOCH_3$), 175.1 (d, C, J_{C-P} = 5.7 Hz, $COOCH_3$), 177.3 (d, C, J_{C-P} = 5.8 Hz, $COOCH_3$), 186.9 (d, C, J_{C-P} = 18.4 Hz, carbene).

$^{31}P\{^1H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: -64.8.

IR (KBr): $\nu_{C=O}$ = 1711, 1672 cm^{-1}, ν_{C-O} = 1206 cm^{-1}.

Anal. Calcd. for $C_{27}H_{36}N_7O_{10}PPd$: C 42.89, H 4.80, N 12.97. Found: C 42.72, H 4.93, N 12.82.

Complex 3b

Brown microcrystals; Yield 90%.

^1H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 3.23 (s, 3H, OCH_3), 3.40 (s, 9H, $3NCH_2P$+NCH_3), 3.53 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.78 (s, 3H, NCH_3), 4.14 (s, 3H, NCH_3), 4.06 and 4.20 (AB system, 6H, *J* = 14.8 Hz, $3NCH_2N$), 5.19 and 6.02 (AB system, 2H, *J* = 13.4 Hz, NCH_2), 7.34-7.44 (m, 5H, Ph).

$^{13}C\{^1H\}$-NMR ($CDCl_3$, T = 298K, ppm) δ: 28.8 (CH_3, NCH_3), 32.2 (CH_3, NCH_3), 39.3 (CH_3, NCH_3), 50.1 (d, CH_2, J_{C-P} = 10.0 Hz, NCH_2P), 51.0 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 51.4 (CH_3, OCH_3), 51.6 (CH_3, OCH_3), 52.8 (CH_2, NCH_2), 72.7 (d, CH_2, J_{C-P} = 6.5 Hz, NCH_2N), 110.1 (C, C^5), 128.6-136.5 (Ph), 140.6 (C, C^4), 164.7-165.2 (C, $COOCH_3$), 166.6 (C, C-$COOCH_3$), 167.7 (C, C-$COOCH_3$), 175.1 (d, C, J_{C-P} = 5.5 Hz, $COOCH_3$), 177.3 (d, C, J_{C-P} = 5.9 Hz, $COOCH_3$), 188.0 (d, C, J_{C-P} = 17.5 Hz, carbene).

$^{31}P\{^1H\}$-NMR ($CDCl_3$, T=298K, ppm) δ: -66.7.

IR (KBr): $\nu_{C=O}$ = 1709, 1670 cm^{-1}, ν_{C-O} = 1242 cm^{-1}, 1207cm^{-1}.

Anal. Calcd. for $C_{33}H_{40}N_7O_{10}PPd$: C 47.63, H 4.85, N 11.78. Found: C 47.75, H 4.82, N 11.82.

Complex 3c

Brown microcrystals; Yield 92%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 3.24 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, NCH_3), 4.00 (d, 6H, J = 2.0 Hz, $3NCH_2P$), 4.04 (s, 3H, NCH_3), 4.20 (s, 3H, NCH_3), 4.48 (s, 6H, $3NCH_2N$), 5.18 (s, 2H, NCH_2), 7.32-7.50 (m, 5H, Ph).

^{13}C{^{1}H}-NMR ($CDCl_3$, T = 298K, ppm) δ: 32.0 (CH_3, NCH_3), 37.0 (CH_3, NCH_3), 39.0 (CH_3, NCH_3), 45.3 (CH_2, NCH_2), 50.9 (CH_3, OCH_3), 51.1 (d, CH_2, J_{C-P} = 10.3 Hz, NCH_2P), 51.3 (CH_3, OCH_3), 51.4 (CH_3, OCH_3), 51.6 (CH_3, OCH_3), 73.1 (d, CH_2, J_{C-P} = 6.4 Hz, NCH_2N), 110.1 (C, C^5), 128.0-136.2 (Ph), 140.8 (C, C^4), 163.6-164.3 (C, $\underline{C}OOCH_3$), 165.4 (C, $\underline{C}$-$COOCH_3$), 168.9 (C, $\underline{C}$-$COOCH_3$), 175.1 (d, C, J_{C-P} = 5.5 Hz, $\underline{C}OOCH_3$), 177.3 (d, C, J_{C-P} = 5.7 Hz, $\underline{C}OOCH_3$), 186.0 (d, C, J_{C-P} = 18.7 Hz, carbene).

^{31}P{^{1}H}-NMR ($CDCl_3$, T = 298K, ppm) δ: -64.8.

IR (KBr): $\nu_{C=O}$ = 1710, 1672 cm^{-1}, ν_{C-O} = 1243, 1207 cm^{-1}.

Anal. Calcd. for $C_{33}H_{40}N_7O_{10}PPd$: C 47.63, H 4.85, N 11.78. Found: C 47.78, H 4.92, N 11.86.

4.8 Synthesis of mixed NHC-DIC palladacyclopentadienyl complexes (4)

Complex 4a

In a 50 mL two necked flask, 43.8 mg (0.112 mmol) of $[PdC_4(COOCH_3)_4]_n$ was dissolved in 5 mL of anhydrous CH_2Cl_2 under inert atmosphere (Ar). 39.4 mg (0.0560 mmol) of complex **Aga** and 14.7 mg (0.112 mmol) of DIC, suspended in 25 mL of anhydrous CH_2Cl_2, were added to the solution of $[PdC_4(COOCH_3)_4]_n$ and precipitation of AgCl was immediately observed. The mixture was reacted for 48h at R.T. and then filtered by a millipore apparatus. The clear brownish solution was concentrated under vacuum and the title complex precipitated by addition of diethylether. The brownish microcrystalline precipitate was filtered off on a gooch, washed with diethylether and dried under vacuum. 73.1 mg (89% yield) of complex **4a** was obtained.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 2.35 (s, 6H, $2CH_3^{DIC}$), 3.34 (s, 3H, OCH_3), 3.41 (s, 3H, NCH_3), 3.58 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.83 (s, 3H, NCH_3), 4.14 (s, 3H, NCH_3), 4.30 (s, 3H, NCH_3), 7.12 (d, 2H, J = 8.1 Hz, $2H^{meta}$), 7.23 (t, 1H, J= 8.1 Hz, H^{para}).

^{13}C{^{1}H}-NMR ($CDCl_3$, T = 298K, ppm) δ: 18.5 (CH_3, CH_3^{DIC}), 28.6 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 37.1 (CH_3, NCH_3), 39.0 (CH_3, NCH_3), 51.0 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 110.1 (C, C^5), 128.2-135.1 (Ph^{DIC}), 140.6 (C, C^4), 145.9 (C, $\underline{C}$-$COOCH_3$), 147.7 (C, $\underline{C}$-$COOCH_3$), 149.0 (C,

CN), 150.5 (C, C=O), 153.1 (C, C=O), 164.3 (C, $\underline{C}OOCH_3$), 164.5 (C, $\underline{C}OOCH_3$), 164.8 (C, $\underline{C}$-$COOCH_3$), 166.5 (C, $\underline{C}$-$COOCH_3$), 174.9 (C, $\underline{C}OOCH_3$), 175.6 (C, $\underline{C}OOCH_3$), 185.8 (C, carbene).

IR (KBr): $\nu_{C=N}$ = 2176 cm^{-1}, $\nu_{C=O}$ = 1710, 1670 cm^{-1}, ν_{C-O} = 1207 cm^{-1}.

Anal. Calcd. for $C_{30}H_{33}N_5O_{10}Pd$: C 49.36, H 4.56, N 9.59. Found: C 49.52, H 4.68, N 9.47.

Derivatives **4b,** and **4c** were prepared in a similar way from the appropriate reactants.

Complex 4b

Yiellow microcrystals; Yield 84%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 2.11 (s, 6H, $2CH_3^{DIC}$), 3.29 (s, 3H, OCH_3), 3.35 (s, 3H, NCH_3), 3.56 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.84 (s, 3H, NCH_3), 4.33 (s, 3H, NCH_3), 5.60 and 5.94 (AB system, 2H, *J* = 14.7 Hz, NCH_2), 7.04-7.44 (m, 8H, Ph^{DIC}, Ph^{Bn}).

^{13}C{^{1}H}-NMR ($CDCl_3$, T = 298K, ppm) δ: 18.4 (CH_3, CH_3^{DIC}), 28.7 (CH_3, NCH_3), 32.0 (CH_3, NCH_3), 39.3 (CH_3, NCH_3), 51.0 (CH_3, OCH_3), 51.1 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 51.4 (CH_3, OCH_3), 53.1 (CH_2, NCH_2), 109.7 (C, C^5), 128.0-135.9 (Ph), 140.7 (C, C^4), 145.7 (C, $\underline{C}$-$COOCH_3$), 148.2 (C, $\underline{C}$-$COOCH_3$), 149.1 (C, CN), 150.5 (C, C=O), 152.8 (C, C=O), 163.7 (C, $\underline{C}OOCH_3$), 164.6 (C, $\underline{C}OOCH_3$), 164.8 (C, $\underline{C}$-$COOCH_3$), 166.9 (C, $\underline{C}$-$COOCH_3$), 174.9 (C, $\underline{C}OOCH_3$), 175.5 (C, $\underline{C}OOCH_3$), 187.4 (C, carbene).

IR (KBr): $\nu_{C=N}$ = 2177 cm^{-1}, $\nu_{C=O}$ = 1710, 1670 cm^{-1}, ν_{C-O} = 1208 cm^{-1}.

Anal. Calcd. for $C_{36}H_{37}N_5O_{10}Pd$: C 53.64, H 4.63, N 8.69. Found: C 53.73, H 4.78, N 8.61.

Complex 4c

Yiellow microcrystals; Yield 87%.

^{1}H-NMR (400 MHz, $CDCl_3$, T = 298K, ppm) δ: 2.35 (s, 6H, $2CH_3^{DIC}$), 3.32 (s, 3H, OCH_3), 3.57 (s, 3H, NCH_3), 3.65 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.15 (s, 3H, NCH_3), 4.28 (s, 3H, NCH_3), 5.14 and 5.20 (AB system, 2H, *J* = 13.8 Hz, NCH_2), 7.10-7.48 (m, 8H, Ph^{DIC}, Ph^{Bn}).

^{13}C{^{1}H}-NMR ($CDCl_3$, T = 298K, ppm) δ: 18.6 (CH_3, CH_3^{DIC}), 32.0 (CH_3, NCH_3), 37.1 (CH_3, NCH_3), 39.0 (CH_3, NCH_3), 45.2 (CH_2, NCH_2), 51.0 (CH_3, OCH_3), 51.3 (CH_3, OCH_3), 51.4 (CH_3, OCH_3), 110.2 (C, C^5), 128.0-136.3 (Ph), 140.7 (C, C^4), 145.8 (C, $\underline{C}$-$COOCH_3$), 147.7 (C, $\underline{C}$-$COOCH_3$), 149.0 (C, CN), 150.4 (C, C=O), 153.0 (C, C=O), 164.2 (C, $\underline{C}OOCH_3$), 164.5 (C, $\underline{C}OOCH_3$), 164.8 (C, $\underline{C}$-$COOCH_3$), 166.4 (C, $\underline{C}$-$COOCH_3$), 174.9 (C, $\underline{C}OOCH_3$), 175.6 (C, $\underline{C}OOCH_3$), 186.0 (C, carbene).

IR (KBr): $\nu_{C=N}$ = 2178 cm^{-1}, $\nu_{C=O}$ = 1710, 1675 cm^{-1}, ν_{C-O} = 1205 cm^{-1}.

Anal. Calcd. for $C_{36}H_{37}N_5O_{10}Pd$: C 53.64, H 4.63, N 8.69. Found: C 53.51, H 4.69, N 8.72.

4.9 Cell growth evaluation

Cell viability inhibition assays were carried out using human ovarian A2780 (cisplatin-sensitive), A2780*cis* (cisplatin-resistant) cancer cells and human lung fibroblast MRC-5 normal cells. The cells were obtained from ATCC (Manassas, VA). The A2780 cell lines were maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin (100 Units mL^{-1}) and glutamine (2 mM) (complete medium); to maintain the resistance, 1 μM cisplatin was routinely added to the A2780*cis* cells. The pH of the medium was 7.2 and the incubation was performed at 37°C in a 5% CO_2 atmosphere. Adherent cells were routinely used at 70% of confluence and passaged every 3 days by treatment with 0.25% trypsin-EDTA (Sigma-Aldrich, St. Louis, MO, US).

The MRC-5 were maintained in Eagle's Minimum Essential Medium, non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate and 1500 mg/L sodium bicarbonate. To make the complete growth medium, it has been added the following components to the base medium: fetal bovine serum to a final concentration of 10% and penicillin/streptomycin (100 Units mL^{-1}).

All the derivatives were added at serial dilutions and incubated in cell cultures: 50 mM stock solutions of each Pd-complex were prepared using pure DMSO, and the working solutions (5 mM, 500 μM and 50 μM) were obtained by successive dilution in EtOH. The water-soluble derivatives **3a-c** were assayed also using aqueous solutions, showing IC_{50} values comparable to those obtained with DMSO/EtOH solutions. After 72h of treatment, cells were washed with PBS 1X and detached with trypsin. Cells were then suspended in physiological solution and counted with a Z2 Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The cell number/mL was determined as IC_{50} after 3 days of culture, when untreated cells are in log phase of cell growth.[11,31]. Cisplatin, solubilized in sterile water, was employed as positive control and untreated cells were placed in every plate as negative control. The cells were exposed to the compounds in 1000 μL total volume.

4.10 Apoptosis assays

Annexin V and Dead Cell assays on A2780 and A2780*cis* cells, untreated and treated for 72 h with increasing doses of analyzed derivatives, were performed with a Muse cell analyzer (Millipore, Billerica, MA, USA), according to the instructions supplied by the manufacturer. This procedure utilizes Annexin V to detect PS (PhosphatidylSerine) on the external membrane of apoptotic cells. Moreover, a dead cell marker is also utilized in the same kit as indicator of cell membrane structural integrity. Cells were washed with sterile PBS 1X, tripsinized, resuspended in the original medium and diluted (1:1) with one step addition of the Muse Annexin V & Dead Cell reagent. After 20 min of incubation at room temperature in the dark samples were analyzed.[11] Data from prepared

samples were acquired and recorded utilizing the Annexin V and Dead Cell Software Module (Millipore, Billerica, MA, USA).

ACKNOWLEDGEMENTS

This work was supported by University of Ferrara (Grant FAR 2017 to IL and RG). RG is funded by AIRC (Associazione Italiana Ricerca sul Cancro) and by CIB (Consorzio Interuniversitario per le Biotecnologie).

REFERENCES

[1] a) B. Rosenberg, L. van Camp, T. Krigas, *Nature* **1965**, *205*, 698; b) B. Rosenberg in *Cisplatin, Chemistry and Biochemistry of a Leading Anticancer Drug*, ed S.J. Lippert, Wiley-VCH, Weinheim, 1999.

[2] a) M.A. Fuentes, C. Alonso, J.M. Perez, *Chem. Rev.* **2003**, *103*, 645; b) Y. Jung, S. J. Lippard, *Chem. Rev*. **2007**, *107*, 1387; c) J. Reedijk, *Eur. J. Inorg. Chem*. **2009**, 1303.

[3] N.J. Wheate, S. Walker, G.E. Craig, R. Oun, *Dalton Trans.* **2010**, *39*, 8113.

[4] a) Z.J. Guo, P.J. Sadler, *Adv. Inorg Chem.* **2000**, *49*, 183; b) E. Alessio in *Bioinorganic Medicinal Chemistry*, ed. WILEY-VCH, Weinheim, 2008; c) M. A. Jakupec, M. Galanski, V.B. Arion, C.G. Hartinger, B.K. Keppler, *Dalton Trans.* **2008**, 183; d) F. Arnesano, G. Natile, *Pure Appl. Chem.* **2008**, *80*, 2715; e) J.C. Dabrowiak, *Metals in Medicine*, John Wiley & Sons, Ltd, Chichester, 2009; f) T. Gianferrara, I. Bratsos, E. Alessio, *Dalton Trans.* **2009**, 7588; g) A. Casini, C.G. Hartinger, A.A. Nazarov, P.J. Dyson, *Top Organomet Chem* **2010**, *32* , 57; h) P. Zhang, P.J. Sadler, J. *Organomet. Chem.* **2017**, *839*, 5.

[5] (a) A.R. Kapdi, I.J.S. Fairlamb, *Chem. Soc. Rev.* **2014**, *43*, 4751; b) M.A. Jakupec, M.Galanski, V.B. Arion, C.G. Hartinger, B.K. Keppler, *Dalton Trans.* **2008**, 183; c) C. Sanchez-Cano, M. J. Hannon, *Dalton Trans.* **2009**, 10702; d) I. Ott, *Coord. Chem. Rev.* **2009**, *253*, 1670; e) C. Gabbiani, L. Messori, *Anti-Cancer Agents Med. Chem.* **2011**, *11*, 929; f) A. Bindoli, M.P. Rigobello, G. Scutari, C. Gabbiani, A. Casini, L. Messori, *Coord. Chem. Rev.* **2009**, *253*, 1692; g) A. Casini, L. Messori, *Curr. Top. Med. Chem.* **2011**, *11*, 2647.

[6] E. Alessio, *Bioinorganic Medicinal Chemistry*, Wiley-VCH, 2011.

[7] M.D. Coskun, F. Ari, A.Y. Oral, M. Sarimahmut, H.M. Kutlu, V.T. Yilmaz, E. Ulukaya, *Bioorg. Med. Chem.* **2013**, *21*, 4698.

[8] a) M.M. Shoukry, A.A. Shoukry, M.N. Hafez, *J. Coord. Chem.* **2010**, *63*, 652; b) H. Hohamann, R. Van Eldik, *Inorg. Chim. Acta* **1990**, *174*, 87; c) H. Hohamann, H. Suvachittanont, R. van Eldik, *Inorg. Chim. Acta* **1990**, *177*, 5.

[9] a) S. Ray, R. Mohan, J. K. Singh, M. K. Samantaray, M. M. Shaikh, D. Panda, P. Ghosh, *J. Am. Chem. Soc.* **2007**, *129*, 15042; b) N. Cutillas, G. S. Yellol, C. de Haro, C. Vicente, V. Rodríguez, J. Ruiz, *Coord. Chem. Rev*. **2013**, *257*, 2784; c) S. Medici, M. Peana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi, M. A. Zoroddua, *Coord. Chem. Rev*. **2015**, *284*, 329; d) S. Y. Hussaini, R. A. Haque, M. R. Razali, *J. Organomet. Chem.* **2019**, *17*, 91.

[10] a) A. Kascatan-Nebioglu, M. J. Panzner, J. C. Garrison, C. A. Tessier, W. J. Youngs, *Organometallics* **2004**, *23*, 1928; b) J. Schütz, W. A. Herrmann, J. *Organomet. Chem*. **2004**, *689*, 2995; c) A. Kascatan-Nebioglu, A. Melaiye, K. Hindi, S. Durmus, M. J. Panzner, L. A. Hogue, R. J. Mallett, C. E. Hovis, M. Coughenour, S. D. Crosby, A. Milsted, D. L. Ely, C. A. Tessier, C. L. Cannon, W. J. Youngs, *J. Med. Chem.* **2006**, *49*, 6811; d) F.-T. Luo, H.-K. Lo, *J. Organomet. Chem.* **2011**, *696*, 1262; e) A. Makhloufi, W. Frank, C. Ganter, *Organometallics* **2012**, *31*, 7272; f) A. Monney, M. Albrecht, *Coord. Chem. Rev.* **2013**, *257*, 2420; g) S. E. Flowers, B. M. Cossairt, *Organometallics* **2014**, *33*, 4341; h) J. Zhang, J. K. Muenzner, M.A. Abu el Maaty, B. Karge, R. Schobert, S. Wölf, I. Ott, *Dalton Trans.* **2016**, *45*, 13161; i) E. Mohammadi, B. Movassagh, *J. Organomet. Chem*. **2016**, *822*, 62; l) A. Szadkowska, S. Staszko, E. Zaorska , R. Pawlowski, *RSC Adv*. **2016**, *6*, 44248.

[11] a) T. Scattolin, I. Caligiuri, L. Canovese, N. Demitri, R. Gambari, I. Lampronti, F. Rizzolio, C. Santo, F. Visentin, *Dalton Trans.* **2018**, *47*, 13616; b) T. Scattolin, L. Canovese, F. Visentin, S. Paganelli, P. Canton, N. Demitri, *Appl. Organomet. Chem.* **2018**, *32*, e4034 .

[12] a) W.A. Hermann, *Angew. Chem. Int. Ed.* **2002**, *41*, 1290; b) L. Cavallo, A. Correa, C. Costabile, H. Jacobsen, *J. Organomet. Chem.* **2005**, *690*, 5407; c) S. Díez-González, S. P. Nolan, *Coord. Chem. Rev.* **2007**, *251*, 874; d) P. de Frémont, N. Marion, S.P. Nolan, *Coord. Chem. Rev.* **2009**, *253*, 862; c) T.-H. Fong, C.-N. Lok, C. Y.-S. Chung,Y.-M. E. Fong, P.-K. Chow,P.-K. Wan, C.-M. Che, *Angew. Chem. Int. Ed.* **2016**, *55*, 11935.

[13] a) J.G. Cropp, *Am. J. Med*. **1996**, *100* (1A), 195; b) M. Teyssot, A. Jarrousse, M. Manin, A. Chevry, S. Roche, F. Norre, C. Beaudoin, L. Morel, D. Boyer, R. Mahioue, A. Gautier, *Dalton Trans.* **2009**, 6894; c) A. John, P. Ghosh, *Dalton Trans.* **2010**, *39*, 7183; d) S. B. Aher, P. N. Muskawar , K. Thenmozhi , P. R- Bhagat , *Eur. J. Med. Chem.* **2014**, *81*, 408.

[14] a) T. A. K. Al-Allaf and L. J. Rashan, *Eur. J. Med. Chem.* **1998**, *33*, 817, b) A. Valentini, F. Conforti, A. Crispini, A. De Martino, R. Condello, C. Stellitano, G. Rotillo, M. Ghedini, G. Federici, S. Bernardini, D. Pucci, *J. Med. Chem.* **2009**, *52*, 484; c) A. Monney, M. Albrecht,

Coord. Chem. Rev. **2013**, *257*, 2420; d) M. Tanaka, H. Kataoka, S. Yano, H. Ohi, K. Kawamoto, T. Shibahara, T. Mizoshita, Y. Mori, S. Tanida, T. Kamiya, T. Joh, *BMC Cancer* **2013**, *13*, 327.

[15] a) L. Canovese, F. Visentin, T. Scattolin, C. Santo, V. Bertolasi, J. *Organomet. Chem.* **2016**, *808*, 48; b) L. Canovese, F. Visentin, T. Scattolin, C. Santo, V. Bertolasi, *Polyhedron* **2016**, *113*, 25; c) T. Scattolin, F. Visentin, C. Santo, V. Bertolasi, L. Canovese, *Dalton Trans.* **2016**, *45*,11560; d) L. Canovese, F. Visentin, T. Scattolin, C. Santo, V. Bertolasi, *Dalton Trans.* **2015**, *44*, 15049; e) L. Canovese, C. Santo, T. Scattolin, F. Visentin, V. Bertolasi, J. *Organomet. Chem.* **2015**, *794*, 288; f) F. Visentin, C. Santo, T. Scattolin, N. Demitri, L. Canovese, *Dalton Trans.* **2017**, *46*,10399.

[16] a) D. A. Krogstad, J. Cho , A. J. DeBoer , J. A. Klitzke , W. R. Sanow , H. A. Williams , J. A. Halfen, *Inorg. Chim. Acta* **2006**, *359*, 136; b) E. Vergara, S. Miranda, F. Mohr, E. Cerrada, E R. T. Tiekink, P. Romero, A. Mendía, M. Laguna, *Eur. J. Inorg. Chem.* **2007**, 2926; c) J. Spencer, A. Casini, O. Zava, R. P. Rathnam, S. K. Velhanda, M. Pfeffer, S. K. Callear, M. B. Hursthouseand P. Dyson, *Dalton Trans.* **2009**, 10731; d) J. Lasri, M. J. Fernández Rodríguez, M. F. C. Guedes da Silva, P. Smolenski, M. N. Kopylovich, J.J.R. Fraústo da Silva, A. J.L. Pombeiro, *J. Organomet. Chem.* **2011**, *696*, 3513; e) M. Carreira, R. Calvo-Sanjuan, M. Sanauí, I. Marzo, M. Contel, *Organometallics* **2012**, *31*, 5772; f) E. Guerrero, S. Miranda, S. Luüttenberg, N. Froöhlich, J.Koenen, F. Mohr, E. Cerrada, M. Laguna, A. Mendía, *Inorg. Chem*. **2013**, *52*, 6635; g) J. Braddock-Wilking , S. Acharya, N. P. Rath, *Polyhedron* **2014**, *79***,** 16; h) V. Ferretti, M. Fogagnolo, A. Marchi, L. Marvelli, F. Sforza, P. Bergamini, *Inorg. Chem.* **2014**, *53 (10)*, 4881.

[17] P. de Fremont, N.M. Scott, E.D. Stevens, T. Ramnial, O.C. Lightbody, C.L.B. Macdonald, J.A.C. Clyburne, C.D. Abernethy, S.P. Nolan, *Organometallics* **2005**, *24*, 6301.

[18] K. Moseley, P.M. Maitlis, *J. Chem. Soc. Dalton Trans.* **1974**, 169.

[19] Gaussian 09, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Jr. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski,

R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador,J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford, CT, 2009.

[20] L. Canovese, F. Visentin, T. Scattolin, C. Santo, V. Bertolasi, *Polyhedron* **2018,** *144***,** 131.

[21] L. Farrugia, *Journal of Applied Crystallography* **2012**, *45(4)*, 849.

[22] Among the four reactions hypothesized when the synthesis of the mixed complexes **4** is taken into consideration, the formation of the bis-substituted isocyanide species should be the fastest one (reaction 1). Afterwards, the bis-isocyanide species reacts with the silver carbene complex to give the mixed species **4** which was detected in solution at the very beginning of the reaction (reaction 2). The unreacted silver carbene complex concomitantly attacks **PdCy** to give the bis-carbene palladium species (reaction 3). In order to have all the species in solution, reaction 2 and 3 might be almost isochronous whereas reaction 4 (the combination of the bis-carbene and the bis-isocyanine palladium complexes) giving the thermodynamically stable complexes **4** must be considerably slower than all the others.

[23] A. Romerosa, J. Suarez-Varela, M.A. Hidalgo, J.C. Avila-Roson, E. Colacio, *Inorg. Chem*. **1997**, *36*, 3784.

[24] L. Canovese, F. Visentin, C. Levi, C. Santo, V. Bertolasi, *Inorg. Chim. Acta* **2011**, *378*, 239.

[25] A. Lausi, M. Polentarutti, S. Onesti, J.R. Plaisier, E. Busetto, G. Bais, L. Barba, A. Cassetta, G. Campi, D. Lamba, A. Pifferi, S. C. Mande, D. D. Sharma, S. M. Sharma, G. Paolucci, *The European Physical Journal Plus* **2015**, *130 (43)*, 1.

[26] W. Kabsch, XDS. *Acta Crystallographica Section D* **2010**, *66(2)*, 125.

[27] G. M. Sheldrick, *Acta Crystallographica Section A* **2015**, *71*, 3.

[28] A. Spek, *Acta Crystallographica Section D* **2009**, *65(2)*, 148.

[29] G. M. Sheldrick, *Acta Crystallographica Section C* **2015**, *71*, 3.

[30] P. Emsley, B. Lohkamp, W. Scott, K. Cowtan, *Acta Crystallographica Section D* **2010**, *66*, 486.

[31] P. Bergamini, L. Marvelli, V. Ferretti, C. Gemmo, R. Gambari, Y. Hushcha, I. Lampronti, *Dalton Trans*. **2016**, *45*, 10752.

[32] K.M. Henkels, J.J. Turchi, *Cancer research* **1999**, *59*, 3077.

[33] S. Dasari, P. B. Tchounwou, *Eur J Pharmacol.* **2014**, *740*, 364.

[34] C. Blanc, Q. L. Deveraux, S. Krajewsk, R.U. Jänicke, A. G. Porter, J. C. Reed, R. Jaggi, A. Marti, *Cancer Research* **2000**, *60*, 4386.

[35] Z. Ai, Y. Lu, S. Qiu, Z. Fan, *Cancer Lett.* **2016**, *373*, 36.

Table 1: Selected bond distances and angles (Å and degrees) for the palladium coordination of **2b** sphere. Naming schemes are reported in Fig. 3.

2b			
Distances	**(Å)**	**Angles**	**(°)**
Pd_1-C1_3	2.0757(17)	C1_3-Pd_1-C4_3	80.08(7)
Pd_1-C4_3	2.0647(18)	C1_3-Pd_1-P_2	95.81(5)
Pd_1-P_2	2.3367(8)	P_2-Pd_1-C8_4	92.83(5)
Pd_1-C8_4	2.0379(17)	C8_4-Pd_1-C4_3	91.21(7)

Table 2: Effects of the Pd-complexes on the proliferation of A2780 and A2780*cis* cells. The inhibition of cell growth is represented as IC_{50}. DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

	IC_{50} (μM)	
Complex	**A2780 (IC_{50})**	**A2780*cis* (IC_{50})**
Cisplatin	0.6 ± 0.1	6 ± 1
$[PdC_4(COOCH_3)_4]_n$	>100	>100
1a	9.0 ± 0.7	7.03 ± 0.01
1b	6.5 ± 0.5	5.53 ± 0.06
1c	81 ± 4	60 ± 3
2a	5.3 ± 0.7	6.7 ± 0.8
2b	5.0 ± 0.2	5.25 ± 0.05
2c	6.66 ± 0.03	16 ± 1
3a	4.3 ± 0.7	0.65 ± 0.2
3b	0.9 ± 0.1	1.0 ± 0.3
3c	0.56 ± 0.08	0.62 ± 0.07
4a	3.3 ± 0.6	2.1 ± 0.4
4b	1.6 ± 0.3	1.8 ± 0.9
4c	0.7 ± 0.05	0.87 ± 0.09

Table 3: Effects of the Pd-complexes on the proliferation of MRC-5 human fibroblasts. The inhibition of cell growth is represented as IC_{50}. DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

Complex	**IC_{50} (μM)**
Cisplatin	14 ± 1
2b	30 ± 15
3c	>100

Table 4: Pro-apoptotic effects of the Pd-complexes on A2780 and A2780*cis* cell lines detected at two different concentrations. (C-: untreated cells). DMSO stock solutions (50 mM) of the Pd-complexes were diluted with EtOH to obtain relative working solutions. Cisplatin was dissolved in water.

Complex	**Total apoptosis (%)**	**Dead cells (%)**	**Total apoptosis (%)**	**Dead cells (%)**
	A2780	**A2780**	**A2780*cis***	**A2780*cis***
C-	3.14	4.03	8.53	4.12
Cisplatin	26.7 (0.5 μM) 55.9 (1.0 μM)	0.95 (0.6 μM) 0.55 (1.0 μM)	13.50 (1.0 μM) 27.40 (5.0 μM)	0.95 (1.0 μM) 0.20 (6.0 μM)
1b	88.38 (6.5 μM) 87.59 (9.0 μM)	0.58 (6.5 μM) 0.56 (9.0 μM)	11.46 (5.5 μM) 21.87 (8.5 μM)	8.13 (5.5 μM) 2.95 (8.5 μM)
2b	88.38 (5.0 μM) 84.96 (7.5 μM)	0.47 (5.0 μM) 0.33 (7.5 μM)	77.38 (5 μM) 77.96 (8 μM)	0.50 (5 μM) 0.40 (8 μM)
3c	47.99 (0.6 μM) 76.15 (0.8 μM)	0.78 (0.6 μM) 0.85 (0.8 μM)	41.87 (0.6 μM) 52.47 (0.9 μM)	3.10 (0.6 μM) 5.60 (0.9 μM)
4c	6.21 (0.7 μM) 14.22 (1.0 μM)	1.67 (0.7 μM) 6.23 (1.0 μM)	15.30 (0.9 μM) 10.91 (1.3 μM)	3.02 (0.9 μM) 1.99 (1.3 μM)