License: Article 25fa pilot End User Agreement

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with explicit consent by the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) ‘Article 25fa implementation’ pilot project. In this pilot research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and/or copyrights owner(s) of this work. Any use of the publication other than authorised under this licence or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please contact the Library through email: OpenAccess@library.leidenuniv.nl

Article details
Zhou X.-Q., Busemann A., Meijer M.S., Siegler M.A., Bonnet S. (2019), The two isomers of a cyclometallated palladium sensitizer show different photodynamic properties in cancer cells, Chemical Communications 55: 4695-4698.
Doi: 10.1039/C8CC10134E
The two isomers of a cyclometallated palladium sensitizer show different photodynamic properties in cancer cells†

Xue-Quan Zhou, A. Anja Busemann, Michael S. Meijer, Maxime A. Siegler and Sylvestre Bonnet†

This report demonstrates that changing the position of the carbon–metal bond in a polypyridyl cyclometalated complex, i.e. going from PdL1 (N–N–N–C) to PdL2 (N–N–N–C), dramatically influences the photodynamic properties of the complex in cancer cells. This effect is primarily attributed to the significantly different in absorbance and singlet oxygen quantum yields between the two isomers.

The success of cisplatin, a milestone drug in the treatment of cancers, stimulated the generation of many platinum-based anticancer drugs, of which three (carboplatin, oxaliplatin and nedaplatin) are approved worldwide. However, the unsselective covalent binding of cisplatin with DNA in cancer cells and healthy cells results in serious side effects and drug resistances, which has encouraged the development of anticancer drugs based on alternative metals.1–4 In this regard, palladium(II) complexes have been proposed as potential metal-based anticancer drugs for their similar d8 coordination sphere and square-planar structure, compared to platinum(II) complexes.5,6 One of them, called padeliporfin or WST11, was recently approved for photodynamic therapy (PDT) of prostate cancer.7–12 PDT is a form of light-activated anticancer treatment. It emerges as a more patient-friendly approach due to the controlled toxicity effect and low invasiveness of light irradiation.13–17 In PDT, a photosensitizing agent (PS) is irradiated by visible light at the tumor site, where it generates cytotoxic reactive oxygen species (ROS), which induces cancer cell death.18 Poly(pyridyl) metal complexes typically form excellent PDT sensitizers, provided they strongly absorb visible light.19,20 The light absorption properties of such complexes can be tuned by changing the metal or the ligands. Critically, good photosensitizers should be photoslabile, which can be achieved using multidentate ligands. The single coordination bonds in multidentate complexes are no stronger than those of monodentate ligands, they are simply less likely to all decoordinate at once.

Recently, bioactive pincer palladium complexes with tridentate N-heterocyclic carbene ligands have been shown to possess stable metal–carbon bonds and tuneable physicochemical properties.21–24 However, intracellular substitution of the remaining monodentate ligands makes their speciation in biological media and mode-of-action complicated to understand. In addition, due to the smaller ionic radius of Pd2+ ions, Pd-ligand bonds are longer and more labile than their Pt-ligand analogues,25 so that anticancer drugs based on palladium(n) are still comparatively rare.6 To overcome these drawbacks, we investigated the design and properties of palladium(n) PDT sensitizers built from single tetradeutate cyclometallating ligands, which are expected to be more stable in biological media compared with the tridentate N-heterocyclic carbene ligands. Cyclometallation was considered for different reasons. Firstly, the strong Pd–C bond can stabilize these compounds in biological media. Secondly, the lower charge density of the cyclometallated ligand can improve the lipophilicity and cellular uptake of the metal complexes.26 Finally, the presence of a Pd–C bond should in principle lead to a bathochromic shift of the visible absorption bands of the metal complex, which is key for PDT applications.27

In polypyridyl metal complexes, introducing a metal–carbon bond usually generates a series of isomers that might have different properties. Herein we investigated two novel cyclometalated isomers PdL1 (H2L1 = N(3-(pyridin-2-yl)phenyl)-[2,2'-bipyridin]6-amine) and PdL2 (H2L2 = N(6-phenylpyridin-2-yl)2,2'-bipyridin]6-amine) (Scheme 1 a). In PdL1, the Pd–C bond was introduced in a pyridyl group that is adjacent to the non-bonded nitrogen bridge of the ligand, while in PdL2 it is introduced in one of the terminal aromatic rings. The ligands H2L1 and H2L2 were synthesized by Buchwald–Hartwig coupling reactions (Scheme S1 and Fig. S1–S4, ESI†).28–30 Palladation was achieved in more than 90% yield by reacting the corresponding ligand with palladium(n) acetate in acetic acid (Scheme S1 and Fig. S5–S8, ESI†). Neither 1H NMR (Fig. S5 and S7, ESI†) nor infrared spectroscopy (Fig. S9, ESI†) showed any sign of a protonated...
secondary amine bridge, which altogether suggested that these complexes were much more acidic than expected. According to $^{13}$C-APT NMR (Fig. S2, S4, S6 and S8, ESI†), the ligands $\text{H}_2\text{L}_1$ and $\text{H}_2\text{L}_2$ have six quaternary carbon peaks, while their palladium complexes have seven, thus demonstrating that cyclometallation did occur. Altogether $\text{PdL}_1$ and $\text{PdL}_2$ appear to be neutral complexes; their identical HRMS data also demonstrated they are coordination isomers.

Vapor diffusion of diethyl ether into a methanol solution of $\text{PdL}_2$ yielded red rectangular crystals suitable for X-ray structure determination (Table S1, ESI† and Scheme 1b). $\text{PdL}_2$ crystallized in the centrosymmetric $\text{P}2_1/\text{n}$ monoclinic space group. Three nitrogen and one carbon atom were coordinated to the palladium(n) cation, with bond lengths in the range 1.988(3)-2.028(4) Å for the three $\text{Pd-N}$ bonds, and a $\text{Pd-C}$ bond distance of 2.017(4) Å. The coordination sphere was slightly distorted, with a dihedral angle $\angle \text{N1-Pd1-N2} = 172.2(2)^\circ$ and $\angle \text{N4-Pd1-N2} = 181.99(15)^\circ$. Also, unlike for $[\text{Fe(Hbbpya)(NCS)}_2]$,32 no residual electron density was found near the bridging N atom in the structure of $\text{PdL}_2$, which is typical of an essentially square planar complex. Deprotonation of the nitrogen bridge was evident from the $\Delta$H$_{\text{f}}$ value between the two coordination isomers was quite intriguing. In this solvent the molar absorptivity at 455 nm for $\text{PdL}_1$ and $\text{PdL}_2$ was still a decent $\text{O}_2$ generator. In methanol, the absorbance spectra of both complexes (Fig. 2) were similar in the 270–300 nm region; however, $\text{PdL}_2$ showed a much higher absorption in the near-UV region ($\lambda_{\text{abs}}$ = 347 nm), compared to $\text{PdL}_1$ that absorbed in the near-UV region ($\lambda_{\text{abs}}$ = 422 nm). In this solvent the molar absorptivity at 455 nm for $\text{PdL}_1$ and $\text{PdL}_2$ towards A549 and A431 human cancer cell lines. 95% confidence interval (CI in µM) and photoindex (PI = $\text{EC}_{50}$, dark/$\text{EC}_{50}$, light) are also indicated.

![Scheme 1](image)

**Table 1.** The cell growing inhibition effective concentrations ($\text{EC}_{50}$ in µM) of $\text{PdL}_1$ and $\text{PdL}_2$ towards A549 and A431 human cancer cell lines. 95% confidence interval (CI in µM) and photoindex (PI = $\text{EC}_{50}$, dark/$\text{EC}_{50}$, light) are also indicated.

| Complexes | $\text{EC}_{50}$ (µM) | A549 | CI | A431 | CI |
|-----------|----------------------|------|----|------|----|
| $\text{PdL}_1$ | Dark | 12 | +3.0 | 20 | +4.0 |
| | Light | 0.9 | +0.8 | 5.0 | +2.0 |
| | PI | 13 | 4.0 | |
| $\text{PdL}_2$ | Dark | 8.0 | +2.0 | 14 | +2.0 |
| | Light | 6.0 | +0.8 | 10 | +1.0 |
| | PI | 1.3 | 1.4 | |

Irradiation condition: 455 nm blue light, 5 min, 10.5 mW cm$^{-2}$, 3.2 J cm$^{-2}$. Data is the mean over three independent experiments.

![Fig. 1](image)
PdL\textsuperscript{2} was 2004 M\textsuperscript{-1} cm\textsuperscript{-1} and 133 M\textsuperscript{-1} cm\textsuperscript{-1}, respectively, indicating a 15-fold enhanced absorption of PdL\textsuperscript{1} in the blue region, compared with PdL\textsuperscript{2}. Considering their similar lifetime (0.271 vs. 0.333 ns for the main component of their biexponential decay, Table S3 and Fig. S13, ESI\textsuperscript{†}), the difference in ¹O\textsubscript{2} generation efficiency is probably a consequence of the higher phosphorescence quantum yield for PdL\textsuperscript{1} (0.0017) vs. PdL\textsuperscript{2} (0.00084, Table S3, ESI\textsuperscript{†}), which points to the slower non-radiative decay pathways for the former, compared to the latter. Altogether, the dramatically higher phototoxicity of PdL\textsuperscript{1}, compared to PdL\textsuperscript{2}, seems to result from the much better absorption of blue light of PdL\textsuperscript{1}, coupled to its higher phosphorescence quantum yield, which leads to higher ¹O\textsubscript{2} generation efficiency. Although different log \(P_{\text{sol}}\) values may lead to different cell uptake and sub-cellular localization for both isomers as well, the better photobiological properties of PdL\textsuperscript{1} depend, at least in part, on the much better photophysical properties of PdL\textsuperscript{1} (\(\epsilon_{\text{exc}} = 55\) s, \(\varphi_{\text{a}} = 3\)), compared to its isomer PdL\textsuperscript{2}.

Density functional theory (DFT) calculations were performed to understand why PdL\textsuperscript{1} exhibited higher absorption in the blue domain than its isomer PdL\textsuperscript{2}. The nature of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbitals (LUMO) is highly relevant to predict the photophysical properties of metal complexes.\textsuperscript{34–36} As showed in Fig. 3, the HOMO and LUMO orbitals of both isomers PdL\textsuperscript{1} and PdL\textsuperscript{2} had \(\pi\) symmetry and were centered on the ligand, with a negligible contribution of the palladium(II) center. The bridged secondary amine is the major contributor to the HOMO of both palladium complexes, attributing for 20.4 (PdL\textsuperscript{1}) and 21.8\% (PdL\textsuperscript{2}) of the electron density. The rest of the HOMO orbital density was located in the aromatic rings directly connected to the nitrogen bridge. By contrast, the LUMO orbitals for both complexes were centered on the bipyridyl fragment. This suggested that the lowest energy absorption band of both palladium complexes should be of ligand-to-ligand charge transfer character, from the amine to the bipyridyl.

The calculated energies of HOMOs, LUMOs and energy gaps are listed in Table S4 (ESI\textsuperscript{†}). The HOMO of PdL\textsuperscript{1} was significantly higher in energy than that of PdL\textsuperscript{2}, indicating the higher electron-donating effect of the negatively charged carbon atom of PdL\textsuperscript{1}, compared with that of PdL\textsuperscript{2} which is further away from the nitrogen bridge. By contrast, the LUMO energy levels of both palladium complexes were similar, because LUMO orbitals are located on the almost equivalent bipyridyl fragments. Such lower energy gap of PdL\textsuperscript{1} suggested better absorption of low-energy light, which explains the observed differences in the UV-vis spectra of the two isomers. These results were confirmed by time-dependent density functional theory calculations (TDDFT) for both complexes in methanol, using COSMO to simulate solvent effects (Fig. S14, ESI,\textsuperscript{†} left). The calculated spectrum of PdL\textsuperscript{1} (Fig. S14, ESI,\textsuperscript{†} left) showed a lower energy (515 nm) for the HOMO–LUMO transition, compared to PdL\textsuperscript{2} (449 nm). These transition energies were increased (404 and 367 nm, respectively) by protonation of the nitrogen bridge (Fig. S14, ESI,\textsuperscript{†} right), which may happen in the slightly acidic environment of cancer cells; however, the trend between [Pd(HL\textsuperscript{1})\textsuperscript{+}] and [Pd(HL\textsuperscript{2})\textsuperscript{+}] was identical to that seen for PdL\textsuperscript{1} and PdL\textsuperscript{2}. Overall, calculations clearly demonstrated that a change of the position of the carbon–metal bond had a strong influence on the HOMO–LUMO energy gaps of these cyclopalladated palladium complexes.

In summary, the new cyclopalladated complex PdL\textsuperscript{1} showed good absorbance in the blue region of the spectrum, low phosphorescence, and excellent singlet oxygen quantum yield (0.89), which altogether translated into high phototoxicity in human cancer cells. By contrast, its isomer PdL\textsuperscript{2} had low absorption and low singlet oxygen quantum yield (0.38), resulting in negligible activation by blue light \textit{in vitro}. DFT calculation showed that the higher absorption in the blue region of PdL\textsuperscript{1}, and thus its lower HOMO–LUMO energy gap, was due to the closer proximity between the electron-rich cyclometallated aromatic cycle and the nitrogen bridge of the ligand, while in PdL\textsuperscript{2} both aromatic rings adjacent to the N bridge are electron-poor pyridine rings, which lowers the HOMO energy level. To the best of our knowledge, this study is the first report that two isomers of organometallic prodrugs have different photobiological properties. These results demonstrate that changing the position of the carbon–metal bond in the coordination sphere of photactive organometallic prodrugs can be used to tune the energy gap between their frontier orbitals, and hence their absorption in the visible region of the spectrum.
X. Zhou gratefully acknowledges the China Scholarship Council (CSC) for a personal grant (No. 201606200045). This work is supported by an ERC Starting Grant to S. Bonnet.

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 T. C. Johnstone, K. Suntharalingam and S. J. Lippard, Chem. Rev., 2016, 116, 3436–3486.
2 L. Kelland, Nat. Rev. Cancer, 2007, 7, 573–584.
3 D. Wang and S. J. Lippard, Nat. Rev. Drug Discovery, 2005, 4, 307–320.
4 L. Ma, N. Wang, R. Ma, C. Li, Z. Xu, M. K. Tse and G. Zhu, Angew. Chem., Int. Ed., 2018, 57, 1–6.
5 S. Medici, M. Peana, V. M. Nurchi, J. I. Lachowicz, G. Crisponi and M. A. Zoroddu, Coord. Chem. Rev., 2015, 284, 329–350.
6 N. Cutillas, G. S. Yellol, C. de Haro, C. Vicente, V. Rodriguez and J. Ruiz, Coord. Chem. Rev., 2013, 257, 2784–2797.
7 L. Zeng, P. Gupta, Y. Chen, E. Wang, L. Ji, H. Chao and Z. S. Chen, Chem. Soc. Rev., 2017, 46, 5771–5804.
8 F. E. Poynton, S. A. Bright, S. Blasco, D. C. Williams, J. M. Kelly and T. Gunnlaugsson, Chem. Soc. Rev., 2017, 46, 7706–7756.
9 H. Huang, P. Zhang, H. Chen, L. Ji and H. Chao, Chem. – Eur. J., 2015, 21, 715–725.
10 T. T. Fong, C. N. Lok, C. Y. Chung, Y. M. Fung, P. K. Chow, P. K. Wan and C. M. Che, Angew. Chem., Int. Ed., 2016, 55, 11935–11939.
11 M. Fanelli, M. Formica, V. Fusi, L. Giorgi, M. Micheloni and P. Paoli, Coord. Chem. Rev., 2016, 310, 41–79.
12 A.-R. Azzouzi, S. Vincendeau, E. Barret, A. Cicco, F. Kleinclauss, H. G. van der Poel, C. G. Stief, J. Rassweiler, G. Salomon, E. Solsona, A. Alcaraz, T. T. Tammela, D. J. Rosario, F. Gomez-Vega, G. Ahlgren, F. Bengaughou, B. Gaillac, B. Amzal, F. M. J. Debruyne, G. Fromont, C. Gratzke and M. Emberton, Lancet Oncol., 2017, 18, 181–191.
13 H. Cao, L. Wang, Y. Yang, J. Li, Y. Qi, Y. Li, Y. Li, H. Wang and J. Li, Angew. Chem., Int. Ed., 2018, 57, 7759–7763.
14 Y. Ma, X. Li, A. Li, P. Yang, C. Zhang and B. Tang, Angew. Chem., Int. Ed., 2017, 56, 13752–13756.
15 S. H. Askes, A. Bahreman and S. Bonnet, Angew. Chem., Int. Ed., 2014, 53, 1029–1033.
16 S. L. Higgins and K. J. Brewer, Angew. Chem., Int. Ed., 2012, 51, 11420–11422.
17 H. Bi, Y. Dai, P. Yang, J. Xu, D. Yang, S. Gai, F. He, B. Liu, C. Zhong, G. An and J. Lin, Small, 2018, 14, e1703809.
18 H. Bi, Y. Dai, P. Yang, J. Xu, D. Yang, S. Gai, F. He, G. An, C. Zhong and J. Lin, Chem. Eng. J., 2019, 356, 543–553.
19 J. D. Knoll and C. Turro, Coord. Chem. Rev., 2015, 282–283, 110–126.
20 F. Heinemann, J. Karges and G. Gasser, Acc. Chem. Res., 2017, 50, 2727–2736.
21 J.-Y. Lee, J.-Y. Lee, Y.-Y. Chang, C.-H. Hu, N. M. Wang and H. M. Lee, OrganoMetallics, 2015, 34, 4359–4368.
22 S. Ray, R. Mohan, J. K. Singh, M. K. Samantaray, M. Maikhi, D. Panda and P. Ghosh, J. Am. Chem. Soc., 2007, 129, 15042–15053.
23 S. G. Churusova, D. V. Aleksanyan, E. Y. Bybalkina, O. Y. Susowa, V. V. Brunova, R. R. Aysin, Y. V. Nelyubina, A. S. Peregu dov, E. I. Gutsul, Z. S. Klemenkova and V. A. Kozlov, Inorg. Chem., 2017, 56, 9834–9850.
24 W. Liu and R. Gust, Chem. Soc. Rev., 2013, 42, 755–773.
25 J. Ruiz, V. Rodriguez, C. de Haro, A. Espinosa, J. Perez and C. Janiak, Dalton Trans., 2010, 39, 3290–3301.
26 G. Gasser, I. Ott and N. Metzler-Nolte, J. Med. Chem., 2013, 57, 5771–5804.
27 M. A. Siegler and S. Bonnet, Chem. Sci., 2016, 7, 4922–4929.
28 E. M. Hernández, S. Zheng, H. J. Shepherd, D. S. Yufit, K. Ridier, S. Bedoui, W. Nicolazzi, V. Velázquez, S. Bonnet, G. Molnár and A. Bousseksou, J. Phys. Chem. C, 2016, 120, 27680–27681.
29 I. Yang, D. R. Powell and R. P. Houser, Dalton Trans., 2007, 955–964.
30 S. Zheng, N. R. Reintjens, M. A. Siegler, O. Rouboue, E. Bouwman, A. Rudavskyi, R. W. Havenith and S. Bonnet, Chem. – Eur. J., 2016, 22, 331–339.
31 S. L. Hopkins, B. Siewert, S. H. Askes, P. Veldhuizen, R. Zwier, M. Heger and S. Bonnet, Photochem. Photobiol. Sci., 2016, 15, 644–653.
32 X. Li, J. Zhang, Z. Zhao, L. Wang, H. Yang, Q. Chang, N. Jiang, Z. Liu, Z. Bian, W. Liu, Z. Lu and C. Huang, Adv. Mater., 2018, 30, e1705005.
33 F. F. Hung, S. X. Wu, W. P. To, W. L. Kwong, X. Guan, W. Lu, K. H. Low and C. M. Che, Chem. – Asian J., 2017, 12, 145–158.
34 J. Fernandez-Cestau, B. T. Bertrand, A. Pintus and M. Bochmann, Organometallics, 2017, 36, 3304–3312.