Dual-targeting dual-action platinum(IV) platform for enhanced anticancer activity and reduced nephrotoxicity

Babak, Maria V.; Zhi, Yang; Czarny, Bertrand; Toh, Tan Boon; Hooi, Lissa; Chow, Edward Kai-Hua; Ang, Wee Han; Gibson, Dan; Pastorin, Giorgia

2019

Babak, M. V., Zhi, Y., Czarny, B., Toh, T. B., Hooi, L., Chow, E. K.-H., . . . Pastorin, G. (2019). Dual-targeting dual-action platinum(IV) platform for enhanced anticancer activity and reduced nephrotoxicity. *Angewandte Chemie International Edition*, 58(24), 8109-8114. doi:10.1002/anie.201903112

<https://hdl.handle.net/10356/144107>

<https://doi.org/10.1002/anie.201903112>

This is the accepted version of the following article: Babak, M. V., Zhi, Y., Czarny, B., Toh, T. B., Hooi, L., Chow, E. K.-H., . . . Pastorin, G. (2019). Dual-targeting dual-action platinum(IV) platform for enhanced anticancer activity and reduced nephrotoxicity. *Angewandte Chemie International Edition*, 58(24), 8109-8114. doi:10.1002/anie.201903112, which has been published in final form at <http://doi.org/10.1002/anie.201903112>. This article may be used for non-commercial purposes in accordance with the Wiley Self-Archiving Policy [<https://authorservices.wiley.com/authorresources/Journal-Authors/licensing/self-archiving.html>].

COMMUNICATION

Dual-targeting Dual-action Platinum(IV) Platform for Enhanced Anticancer Activity and Reduced Nephrotoxicity

Maria V. Babak,^{[a][b]} Yang Zhi,^[a] Bertrand Czarny,^[c] Tan Boon Toh,^[d] Lissa Hooi,^[d] Edward Kai-Hua Chow,^{[d][e]} Wee Han Ang,^{[b][f]*} Dan Gibson,^{[g]*} and Giorgia Pastorin^{[a][f][h]*}

Abstract: A novel and highly efficient dual-targeting platform was designed to ensure targeted *in vivo* delivery of dual-action Pt(IV) prodrugs. The dual targeting was established by liposomal encapsulation of Pt(IV) complexes, thereby utilizing the enhanced permeability and retention (EPR) effect as the first stage of targeting to attain high accumulation of the drug-loaded liposomes in the tumor. After the release of the Pt(IV) prodrug inside cancer cells, a second stage of targeting directed a portion of the Pt(IV) prodrugs to the mitochondria. Upon intracellular reduction, these Pt(IV) prodrugs released two bioactive molecules, acting both on the mitochondrial and on the nuclear DNA. Our Pt(IV) system showed excellent activity *in vitro* and *in vivo*, characterized by a cytotoxicity in a low micromolar range and complete tumor remission, respectively. Notably, marked *in vivo* activity was accompanied by reduced kidney toxicity, highlighting the unique therapeutic potential of our novel dual-targeting dual-action platform.

Although Pt anticancer drugs are extensively used to treat cancer, their efficacy is limited by toxicity and resistance.^[1] To ameliorate side-effects and increase bioavailability, various targeting and delivery strategies have been developed, while the issue of cancer resistance has been tackled by means of chemotherapy combination regimens. In particular, combination treatments of Pt anticancer drugs with mitochondrial sensitizers, such as

dichloroacetate (DCA) and phenylbutyrate (PHB), were reported to be more effective than respective monotherapies.^[2]

Scheme 1. Chemical structures of mitochondria-targeting dual-action Pt(IV) complexes used in this study.

The improved efficacy stems from Pt-induced DNA damage in combination with “mitochondrial reprogramming” of cancer cells by DCA or PHB.^{[3] [4] [2b, 5]} However, sufficient anticancer effects could only be achieved when DCA and PHB were used in prohibitively high doses due to their poor cellular uptake, resulting in high toxicity.^[6] To further improve anticancer effects, DCA and PHB were chemically conjugated to Pt(II) pharmacophores, yielding Pt(IV) prodrugs, which simultaneously released different moieties once inside cancer cells, resulting in the synergistic action of their active components.^{[7] [8] [9]} However, despite promising anticancer properties, these Pt(IV) complexes lack targeting moieties that facilitate the delivery of mitochondrial sensitizers to their intended target.^[10] When DCA and PHB ligands are released outside mitochondria, these small molecules compete with pyruvate for the mitochondrial entry *via* mitochondrial pyruvate transporter, thereby abrogating their anticancer efficacy.^[10a] Additionally, poor intramitochondrial uptake results in insufficient targeting of mitochondrial DNA (mtDNA) by Pt(II) species. Conversely, as mitochondria lack the nucleotide excision repair (NER) pathway required for removal of Pt-DNA adducts,^[11] enhanced mtDNA targeting could attenuate cancer cell resistance to Pt agents.^[11]

In order to unlock the full therapeutic potential of Pt(IV) complexes with mitochondrial sensitizers, we designed Pt(IV) prodrugs to accumulate within the mitochondrion to achieve a targeted effect at their intended site of action and facilitate the access of Pt(II) pharmacophores to mtDNA.^[12] Therefore, we developed a dual-targeting dual-action platform based on the simultaneous tumor- and mitochondria-targeting approach through liposomally encapsulated dual-action Pt(IV) complexes, where the DNA-damaging Pt(II) pharmacophores were simultaneously conjugated to mitochondrial sensitizers (DCA or PHB) as well as lipophilic mitochondria-directing TPP cation.

[a] Dr. M.V. Babak, Dr. Y. Zhi, Prof. G. Pastorin
Department of Pharmacy, National University of Singapore
3 Science Drive 2, 117543 Singapore
E-mail: phapq@nus.edu.sg

[b] Dr. M.V. Babak, Prof. W.H. Ang
Department of Chemistry, National University of Singapore
3 Science Drive 2, 117543 Singapore
E-mail: ang.weehan@nus.edu.sg

[c] Prof. B. Czarny
School of Materials, Science and Engineering & Lee Kong Chian School of Medicine (LKCmedicine)
Nanyang Technological University, 50 Nanyang Avenue, 639798 Singapore

[d] L. Hooi, Dr. T.B. Toh, Prof. E. K.-H. Chow
Cancer Science Institute of Singapore, National University of Singapore, 14 Medical Drive, 117599 Singapore

[e] Prof. E. K.-H. Chow
Department of Pharmacology, Yong Loo Lin School of Medicine
National University of Singapore

[f] Prof. W.H. Ang, Prof. G. Pastorin
NUS Graduate School for Integrative Sciences and Engineering

[g] Prof. D. Gibson
Institute for Drug Research, School of Pharmacy
The Hebrew University, Jerusalem, 91120 Israel
E-mail: dang@ekmd.huji.ac.il

[h] Prof. G. Pastorin
NUS Nanoscience & Nanotechnology Initiative (NUSNNI)
National University of Singapore, 2 Engineering Drive 3, 117411 Singapore

Supporting information for this article is given via a link at the end of the document.

COMMUNICATION

Figure 1. Pt content in A2780 cells and organelles treated with 5 μ M of compounds **1-4**, CDP and OXP for 24 h at 37 $^{\circ}$ C and analysed by ICP-MS. (A) Intracellular Pt accumulation, (B) intracellular distribution of Pt in nuclei, mitochondria and cytosol, (C) Pt nuclear content, (D) Pt mitochondrial content. Statistical differences between nuclear and mitochondrial Pt content (B) and between compounds of interest (C and D) were analysed by two-tailed *t*-test with *p* < 0.05 considered as significant.

We prepared two series of non-symmetric *cis,cis,trans*-Pt(IV) complexes, each containing an axial TPP ligand with either cisplatin (CDP) or oxaliplatin (OXP) as pharmacophores, differing only on the nature of the other axial ligand (**1cis-4cis** and **1oxa-4oxa**, respectively; Scheme 1). Complexes **1cis** and **2cis** were prepared in accordance with literature procedures, and applied to **1oxa** and **2oxa**.^[13] Complexes **3cis/oxa** and **4cis/oxa** with DCA or PHB ligands were obtained by stirring mono-TPP **1** with DCA anhydride or PHB anhydride, respectively, in DMF for 24-48 h. Detailed synthesis and characterization of Pt(IV) complexes, as well as their purity assessment are presented in the Supporting Information (Figures S1-S18).

All tested Pt complexes displayed antiproliferative activities in the low micromolar concentration range, comparable to other structurally similar Pt complexes reported in the literature,^[7] whereas uncoordinated DCA and PHB did not show any cytotoxicity up to 100 μ M (Table S1). Comparison of the cytotoxicity in A2780 cell line and its resistant analogue A2780/CPR revealed that all Pt(IV) complexes (except **4cis**) demonstrated low cross-resistance with CDP. In general, the cytotoxicity increased in the following order: **1cis/oxa** $\leq$ **2cis/oxa** < CDP < OXP $\leq$ **3cis/oxa** $\approx$ **4cis/oxa**. The incorporation of DCA

and PHB ligands into a Pt(IV) scaffold improved cytotoxicity up to 5-fold, indicating the beneficial effects of mitochondrial sensitizers on the overall activity of Pt(IV) complexes. Subsequently, we measured intracellular accumulation and distribution in A2780 cancer cells (Figure 1A & S19 and Table S2). The results demonstrated that the total intracellular accumulation (excluding cellular membrane) of Pt complexes increased in the following order **1cis/oxa** $\approx$ **2cis/oxa** < CDP $\approx$ OXP $\leq$ **3cis/oxa** $\leq$ **4cis/oxa**, which correlates with the cytotoxicity of these compounds. As expected, CDP and OXP preferentially accumulated in the nucleus ($\approx$ 50-70% of Pt-nuclear fraction and $\approx$ 8-15% of Pt-mitochondrial fraction from total intracellular content), whereas Pt(IV) complexes with TPP ligands preferentially accumulated in the mitochondria (31-56% of Pt-mitochondrial fraction from total intracellular content), thus validating our design strategy (Figure 1B). Intriguingly, the most cytotoxic complexes **3cis/oxa** and **4cis/oxa** demonstrated up to $\approx$ 1.8 times higher mitochondrial accumulation in comparison with **1cis/oxa** and **2cis/oxa** and $\approx$ 5 times higher mitochondrial accumulation than CDP and OXP (Figures 1C & 1D and S19). Further quantification of Pt-bound nDNA and mtDNA revealed significantly higher levels of Pt bound mtDNA, compared to nDNA, in A2780 cells treated with **1cis/oxa**-

COMMUNICATION

4cis/oxa (Figure S19). Pt-mtDNA levels increased in the order **1cis/oxa** $\approx$ **2cis/oxa** $\ll$ **3cis/oxa** $\approx$ **4cis/oxa**, in agreement with the differential accumulation of Pt(IV) complexes in mitochondria. In contrast, the reverse was observed in CDP-treated A2780 cells, as reported in the literature.^[12]

To determine the impact of Pt complexes on mitochondrial function, we performed JC-1 assay to investigate mitochondria depolarization ($\Delta\psi_m$), using equimolar concentration of tested compounds for 24 h (Figures 2A, S20 and S21). DCA and PHB ligands did not affect the mitochondrial membrane but all Pt complexes, including CDP and OXP, reduced the mitochondrial membrane potential. The strongest effects were exhibited by **3cis/oxa** and **4cis/oxa**. Since mitochondrial membrane potential is directly related to the mitochondrial respiratory chain activity, we analysed whether Pt(IV) complexes altered cellular mitochondrial bioenergetic processes by measuring oxygen consumption rate (OCR) (Figures 2C and S22). Complexes **3cis/oxa** and **4cis/oxa** with DCA and PHB ligands markedly deregulated different states of mitochondrial respiration including basal respiration, ATP-linked respiration and mitochondria-specific respiration, indicating complete inhibition of all mitochondrial respiratory processes. These results suggest mitochondrion as a primary target of **3cis/oxa** and **4cis/oxa**. Other tested complexes demonstrated only minor changes in the OCR profiles, in agreement with the literature.^[13]

Figure 2. (A) JC-1 analysis of mitochondrial membrane potential; (B) Flow cytometric analysis using double Annexin V/PI staining; (C) mitochondrial respiration characterized by oxygen consumption rate (OCR). Respiration modulators were added in sequence; (D) immunofluorescence staining of AIF expression. A2780 cells were treated with 5 μ M of compounds **3cis**, CDP and DCA for 24 h at 37 °C.

“Mitochondrial reprogramming” of cancer cells can be achieved by inhibition of pyruvate dehydrogenase kinase (PDK) enzyme.^[3] In order to ascertain whether altered metabolism in Pt-treated A2780 cells was related to pyruvate dehydrogenase kinase (PDK) inhibition, we determined pyruvate dehydrogenase (PDH) activity levels, which are suppressed when PDK enzyme is active (Figure S23). All Pt complexes, including CDP and OXP, activated PDH to varying extent but the most pronounced effects were observed in cells treated with **3cis** and **4cis**, in agreement with other experiments. DCA and PHB did not activate PDH, since their effects only manifest at high concentrations. The PDK inhibition has important implications for the nature of Pt-induced cellular damage. It is known that mitochondria in cancer cells involve PDK activity to counteract chemotherapy-induced ROS damage.^[14] Thus, PDK inhibition leads to excessive mitochondrial ROS induction and increased mtDNA damage.

We therefore investigated the ability of Pt(IV) complexes to induce acute ROS insult using DCFDA assay (Figure S24) after short exposure. The most active **3cis/oxa** and **4cis/oxa** caused marked ROS induction already after 3 h treatment, suggesting that the observed ROS stress occurred before induction of cell death processes. Similar effects have been previously observed for CDP and other Pt(IV) complexes and were shown to occur independently of nDNA damage.^[15] All other compounds did not cause ROS induction even at longer time points. We further validated that occurrence of the ROS insult by demonstrating activation of cellular antioxidant defence mechanisms in A2780 cells treated with **3cis/oxa** and **4cis/oxa** via the induction of *nrf2* regulator and its downstream related genes, namely detoxification enzyme *gclc* and the antioxidant enzyme *nqo1* (Figures S25 and S26).

Figure 3. Proposed mechanism of action of **3cis**.

COMMUNICATION

Figure 4. (A) Growth curves of CT26 tumors starting from day 8 when tumors became palpable. On day 10 tumors reached a volume of $\approx 50 \text{ mm}^3$ and mice were subsequently treated with compounds of interest at the indicated doses *via i.p.* route once a week (at days 10, 17 and 24) (depicted by blue arrows). The injected dose was normalized by Pt content. Statistical analysis was carried out by the two-tail ANOVA test with Bonferroni post-test (PBS vs DCA, CDP vs PBS, **3cis** (0.78) vs. **3cis** (1.95), **Lipo-3cis** vs **3cis** (1.95)); (B) Pt accumulation in mouse tumors obtained from mice treated with compounds of interest and sacrificed at the endpoint on day 32 and quantified by ICP-MS; (C) Pt accumulation in mouse tissues normalized by Pt content in the tumor. Statistical differences between drug-treated groups were identified by two-tailed t-test. (D) mRNA expression of inflammatory markers TNF- α and IL-1 β and the major superoxide generating enzyme NOX4 in the kidneys after vehicle or drug treatment.

Changes in the membrane potential are characterized by the opening of the mitochondrial permeability transition pore, allowing release of pro-apoptotic factors, such as cytochrome C and apoptosis inducing factor (AIF), thereby facilitating apoptosis.^[16] We demonstrated that all Pt complexes induced apoptosis in A2780 cells after 24 h treatment, although the highest effects were observed when cells were treated with **3cis/oxa**, **4cis/oxa** and CDP (Figures 2B, S27 and S28). Intriguingly, **4cis/oxa** also induced necrotic cell death. Apoptotic cell death was accompanied by AIF release from the permeable mitochondrial membrane, which was detected by immunofluorescence staining and was in a good correlation with the results of JC-1 assay (Figure 2D, S29 and S30). Furthermore, **3cis/oxa** and **4cis/oxa** induced significant upregulation of p53 protein and its downstream pro-apoptotic genes (Bax, Noxa and Puma), similar to CDP, indicating p53-regulated apoptosis (Figures S31-S37). To further establish the nature of induced apoptosis, we monitored the expression of caspase 3 and compared the cytotoxicity of Pt(IV) complexes in the presence or absence of

poly-caspase inhibitor Z-VAD-FMK (Figures S38). The cytotoxicity of all Pt complexes significantly decreased when A2780 cells were co-treated with Z-VAD-FMK. Together with the increased caspase 3 expression (Figures S36 and S37), these results indicated the occurrence of p53-mediated mitochondrial caspase 3-dependent apoptosis. The proposed mechanism of action of **3cis** is illustrated in Figure 3.

Subsequently, we selected **3cis** as the lead compound for *in vivo* studies in female Balb/C mice using murine colon CT26 adenocarcinoma tumor model. Briefly, CT26 cells were injected subcutaneously and when tumors became palpable, mice were treated intraperitoneally (*i.p.*) with PBS, DCA (3 mg/kg), CDP (3 mg/kg = 1.95 mg/kg of Pt) and **3cis** (3 mg/kg = 0.78 mg/kg of Pt and 8.35 mg/kg = 1.95 mg/kg of Pt) (Figure 4A) on day 10, 17 and 24. Body weight changes were monitored and recorded every other day for 32 days and shown in Figure S39. The chosen CDP regimen is a commonly used low toxic regimen aimed at minimizing renal toxicity.^[17] Compound **3cis** was administered at

COMMUNICATION

two doses and the highest dose was normalized to CDP group according to the Pt content. As expected, CDP-treated group already demonstrated significant tumor reduction (*** $p < 0.001$) 4 d after the 1st injection while the DCA-treated group was characterized by rapid tumor progression similar to the control (PBS) group. Satisfyingly, **3cis** demonstrated 30% increase in activity as compared to CDP at the low dose (equimolar concentration to CDP). At higher dose of **3cis** (equivalent Pt concentration to CDP), the tumor size at the endpoint was ≈ 5 times smaller than in the CDP-treated group and ≈ 8 times smaller than in the control group. To determine if such drastic differences in *in vivo* activity were related to the differential tumor uptake of Pt complexes, the mice were euthanized at endpoint and the intratumoral Pt content in groups treated with CDP and the high dose of **3cis** was compared. Strikingly, Pt content displayed no significant differences between these two groups ($p > 0.05$), indicating that the enhanced anticancer effects of **3cis** were not due to increased Pt accumulation (Figure 4B). Next, we compared the Pt biodistribution in tumor-bearing mice treated with CDP and **3cis** (Figure 4C). Both groups were characterized by similar biodistribution patterns and the highest Pt accumulation was observed in liver and kidneys, followed by spleen, tumor and other organs. High drug accumulation in kidneys is expected but undesirable, since it is related to the severe CDP-induced nephrotoxicity in patients.^[18]

With the aim to further improve the anticancer effect of **3cis** and to limit its off-target reactivity, we prepared a long-circulating PEGylated liposomal formulation with the encapsulated **3cis** complex (**lipo-3cis**). This type of liposomal formulation was previously used to reduce CDP toxicity.^[19] The preparation and the physicochemical characteristics of empty and loaded liposomes are presented in Supplementary Information. While empty liposomes were not cytotoxic (Figure S40), the activity of **lipo-3cis** in A2780 cells was similar to the free drug (Table S1). When **lipo-3cis** was subjected to the *in vivo* study, the dose was normalized by the amount of encapsulated **3cis** (1.95 mg/kg of Pt). The **lipo-3cis** treatment group was characterized by the continuous decrease of the tumor size (2 d after the first injection) and demonstrated complete tumor remission by the end of treatment (tumors not palpable).

To assess whether the marked *in vivo* activity of CDP, **3cis** and **lipo-3cis** was associated with nephrotoxicity, we analyzed renal oxidative stress and inflammatory markers in mice kidneys at the endpoint. The mRNA levels of NOX4, TNF- α and IL-1 β were determined after 32 d of drug treatment. Our results showed that IL-1 β levels were not elevated in all drug-treated groups; however, NOX4 and TNF- α mRNA levels were markedly different. Mice treatment with sub-nephrotoxic concentration of CDP^[17] resulted in the significant elevation of TNF- α , which is a potent pro-inflammatory cytokine and mediator of inflammatory tissue damage, and NOX4, the key NAD(P)H oxidase isoform in the kidneys, in agreement with its known nephrotoxic effects.^[20] **Lipo-3cis** and **3cis**, however, demonstrated significant attenuation of NOX4 and TNF- α level as compared to the CDP-treated group. Previously, it was reported that DCA was able to preserve the renal functions when used in conjunction with CDP, thereby supporting our observations.^[21]

In summary, we prepared a novel series of mitochondria-targeted dual-action Pt(IV) complexes with DCA or PHB ligands, designed to enhance accumulation within the mitochondria to maximize the effect of the PDK inhibitors. *In vitro* data demonstrated that Pt complexes accumulated both in the mitochondria and the nucleus leading to the synergistic action of Pt center of **3cis** and organic mitochondrial sensitizers. This resulted in a marked improvement of *in vivo* activity, characterized by more than 5-fold decrease of tumor burden in comparison with CDP. The additional level of tumor targeting was attained by the development of a liposomal formulation of **3cis**, which revealed further improvement of the anticancer activity, characterized by the complete tumor remission. Furthermore, we demonstrated that chemical conjugation of DCA to a Pt(IV) center resulted in a prevention of Pt-induced renal damage.

Acknowledgements

G.P. acknowledges the National University of Singapore (NUS) for financial support (grant C-141-000-097-001). W.H.A. acknowledges Ministry of Education Singapore (MOE) for financial support (grant R143000680114). D.G. acknowledges the support of the Israel Science Foundation (grant 1611/14) and the support of the Alex Grass Center for Drug Design and Synthesis. Y.Z. acknowledges the Department of Pharmacy (NUS) & Hebrew University of Jerusalem (HUJ) for the research scholarship for a joint PhD degree. M.V.B. acknowledges E. Fasolko and T. Hajsz for drawing artworks.

Conflict of interest

The authors declare no conflict of interest.

Keywords: drug discovery • metabolism • platinum • prodrugs • sensitizers

- [1] E. Wong, C. M. Giandomenico, *Chem. Rev. (Washington, D. C.)* **1999**, 99, 2451-2466.
- [2] aU. Olszewski, T. T. Poulsen, E. Ulsperger, H. S. Poulsen, K. Geissler, G. Hamilton, *Clin. Pharmacol.: Adv. Appl.* **2010**, 2, 177-183; bR. Ferriero, C. Iannuzzi, G. Manco, N. Brunetti-Pierri, *J. Inherited Metab. Dis.* **2015**, 38, 895-904; cW. Fiebigler, U. Olszewski, E. Ulsperger, K. Geissler, G. Hamilton, *Clin. Transl. Oncol.* **2011**, 13, 43-49.
- [3] G. Sutendra, E. D. Michelakis, *Front Oncol* **2013**, 3, 38.
- [4] aJ. Xie, B.-S. Wang, D.-H. Yu, Q. Lu, J. Ma, H. Qi, C. Fang, H.-Z. Chen, *Int. J. Oncol.* **2011**, 38, 409-417; bE. D. Michelakis, L. Webster, J. R. Mackey, *Br. J. Cancer* **2008**, 99, 989-994.
- [5] R. Ferriero, G. Manco, E. Lamantea, E. Nusco, M. I. Ferrante, P. Sordino, P. W. Stacpoole, B. Lee, M. Zeviani, N. Brunetti-Pierri, *Sci. Transl. Med.* **2013**, 5, 175ra131, 112 pp.
- [6] aM. O. James, S. C. Jahn, G. Zhong, M. G. Smeltz, Z. Hu, P. W. Stacpoole, *Pharmacol. Ther.* **2017**, 170, 166-180; bL. H. Stockwin, S. X. Yu, S. Borgel, C. Hancock, T. L. Wolfe, L. R. Phillips, M. G. Hollingshead, D. L. Newton, *Int. J. Cancer* **2010**, 127, 2510-2519.
- [7] aS. Dhar, S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, 106, 22199-22204, S22199/22191-S22199/22111; bR. Raveendran, J. P. Braude, E. Wesselblatt, V.

COMMUNICATION

- Novohradsky, O. Stuchlikova, V. Brabec, V. Gandin, D. Gibson, *Chem. Sci.* **2016**, 7, 2381-2391.
- [8] E. Petruzzella, R. Sirota, I. Solazzo, V. Gandin, D. Gibson, *Chem. Sci.* **2018**, 9, 4299-4307.
- [9] E. Petruzzella, J. P. Braude, J. R. Aldrich-Wright, V. Gandin, D. Gibson, *Angew. Chem., Int. Ed.* **2017**, 56, 11539-11544.
- [10] aR. K. Pathak, S. Marrache, D. A. Harn, S. Dhar, *ACS Chem. Biol.* **2014**, 9, 1178-1187; bS. Marrache, S. Dhar, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, 109, 16288-16293, S16288/16281-S16288/16222.
- [11] G. Damia, L. Imperatori, M. Stefanini, M. D'Incalci, *Int. J. Cancer* **1996**, 66, 779-783.
- [12] S. Marrache, R. K. Pathak, S. Dhar, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, 111, 10444-10449.
- [13] S. Jin, Y. Hao, Z. Zhu, N. Muhammad, Z. Zhang, K. Wang, Y. Guo, Z. Guo, X. Wang, *Inorg. Chem.* **2018**, 57, 11135-11145.
- [14] aK. Kirito, Y. Hu, N. Komatsu, *Cell Cycle* **2009**, 8, 2844-2849; bJ. T. Newington, T. Rappon, S. Albers, D. Y. Wong, R. J. Rylett, R. C. Cumming, *J. Biol. Chem.* **2012**, 287, 37245-37258.
- [15] aM. Berndtsson, M. Hagg, T. Panaretakis, A. M. Havelka, M. C. Shoshan, S. Linder, *Int J Cancer* **2007**, 120, 175-180; bV. Pichler, S. Goeschl, E. Schreiber-Brynzak, M. A. Jakupec, M. Galanski, B. K. Keppler, *Metallomics* **2015**, 7, 1078-1090.
- [16] J. D. Ly, D. R. Grubb, A. Lawen, *Apoptosis* **2003**, 8, 115-128.
- [17] L. Vicente-Vicente, F. Sanchez-Juanes, O. Garcia-Sanchez, V. Blanco-Gozalo, M. Pescador, M. A. Sevilla, J. M. Gonzalez-Buitrago, F. J. Lopez-Hernandez, J. M. Lopez-Novoa, A. I. Morales, *Toxicol. Lett.* **2015**, 234, 99-109.
- [18] R. P. Miller, R. K. Tadagavadi, G. Ramesh, W. B. Reeves, *Toxins* **2010**, 2, 2490-2518.
- [19] M. S. Newman, G. T. Colbern, P. K. Working, C. Engbers, M. A. Amantea, *Cancer Chemother. Pharmacol.* **1999**, 43, 1-7.
- [20] aB. Zhang, G. Ramesh, C. C. Norbury, W. B. Reeves, *Kidney Int.* **2007**, 72, 37-44; bH. Pan, P. Mukhopadhyay, M. Rajesh, V. Patel, B. Mukhopadhyay, B. Gao, G. Hasko, P. Pacher, *J. Pharmacol. Exp. Ther.* **2009**, 328, 708-714.
- [21] R. Galgamuwa, K. Hardy, J. E. Dahlstrom, A. C. Blackburn, E. Wium, M. Rooke, J. Y. Cappello, P. Tummala, H. R. Patel, A. Chuah, L. Tian, L. McMorro, P. G. Board, A. Theodoratos, *J. Am. Soc. Nephrol.* **2016**, 27, 3331-3344.

COMMUNICATION

Entry for the Table of Contents

COMMUNICATION

All in one!: We developed a novel strategy for targeted delivery of dual-action Pt(IV) prodrugs into cancer cells through simultaneous tumor- and mitochondria-targeting. The novel highly active and non-toxic system demonstrates unique therapeutic potential for cancer treatment.

Maria V. Babak, Yang Zhi, Bertrand Czarny, Tan Boon Toh, Lissa Hooi, Edward Kai-Hua Chow, Wee Han Ang,* Dan Gibson,* and Giorgia Pastorin*

Page No. – Page No.

Dual-targeting Dual-action Platinum(IV) Platform for Enhanced Anticancer Activity and Reduced Nephrotoxicity