ChemComm

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Journal:	*ChemComm*
Manuscript ID	CC-COM-03-2020-002174.R1
Article Type:	Communication

Journal Name

COMMUNICATION

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Resistance to the platinum-based chemotherapy drug, cisplatin, is significant setback in ovarian cancer. We engineered fatty acid-like Pt(IV) prodrugs that harness the fatty acid transporter CD36 to facilitate their entry to ovarian cancer cells. We show that these novel constructs effectively kill cisplatin-resistant ovarian cancer cells.

CD36 is emerging as a new therapeutic target in cancer therapy.[1, 2] CD36 is a member of the class B scavenger receptor family of cell surface proteins that facilitates the uptake of free fatty acids for lipid metabolism.[3] CD36 promotes tumour growth by fueling cancer metastasis, supporting drug resistance, and modulating tumor immunity.[1, 4] Recent studies indicate that CD36 is upregulated in ovarian tumors.[5, 6] Interaction with adipocytes in the tumor microenvironment causes upregulation of CD36 which enhances ovarian tumor metastasis.[2] CD36-based therapy, including monoclonal antibodies and polypeptides, has been proven effective at inhibiting cancer metastasis.[1] However, with respect to drug resistance in ovarian cancer, the role of CD36 is unclear and there are no reports about harnessing CD36 to shuttle therapeutics to target drug resistant ovarian cancer cells.

Accumulating evidence suggest that mitochondria play a critical role in drug resistance in ovarian cancer cells.[7-9] A recent study indicates that drug resistant ovarian cancer cells have increased mitochondrial oxidative phosphorylation.[10] Mitochondrial-targeted agents, such as salinomycin and niclosamide, have shown activity toward overcoming drug resistance by impairing oxidative phosphorylation.[11-13] However, systematic toxicity limits the use of these agents in clinics, since they can also damage mitochondria of non-cancerous cells.[14, 15] Designing metallodrugs to target mitochondria enhances their efficacy against drug resistant cancer cells via attacking mitochondrial DNA and damaging oxidative phosphorylation, and this tactic bypasses the drug resistance mechanism caused by nuclear DNA damage repair.[8, 9, 16-28] A need still exists to generate mitochondria-damaging metallodrugs with high specificity against ovarian cancer cells.

Here, we present the first demonstration that harnessing CD36 could empower mitochondria-damaging fatty acid-like Pt(IV) prodrugs (FALPs) for treatment of cisplatin resistant ovarian cancer (Fig 1). We have engineered Pt(IV) prodrugs (Fig 2 and 3) that mimic the fatty acid structure and utilize them as a "Trojan horse" to exploit upregulated CD36 receptors to facilitate their cell entry (Fig 2). Notably, we found that CD36 is upregulated in A2780cis cisplatin-resistant ovarian cancer cells (Fig 2). Unlike fatty acids that fuel lipid metabolism, these Pt(IV) prodrugs are able to trigger mitochondrial damage (Fig 3). Thus, FALPs can readily eliminate drug resistant ovarian cancer cells.

First, we evaluated CD36-dependent cell entry and cytotoxicity profiles of FALPs by using a cisplatin-sensitive ovarian cancer cell line (A2780) and its cisplatin-resistant line (A2780cis) along with a non-cancerous cell line (HEK293). The evidence from qPCR analysis (Fig 2B), flow cytometric studies

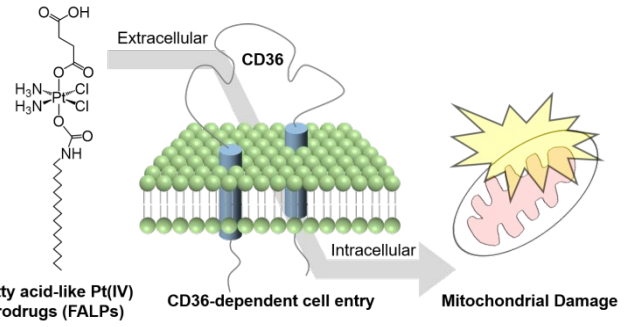

Figure 1. Graphical representation of FALPs harnessing CD36-dependent cell entry and mitochondrial damage toward overcoming drug resistance in ovarian cancer.

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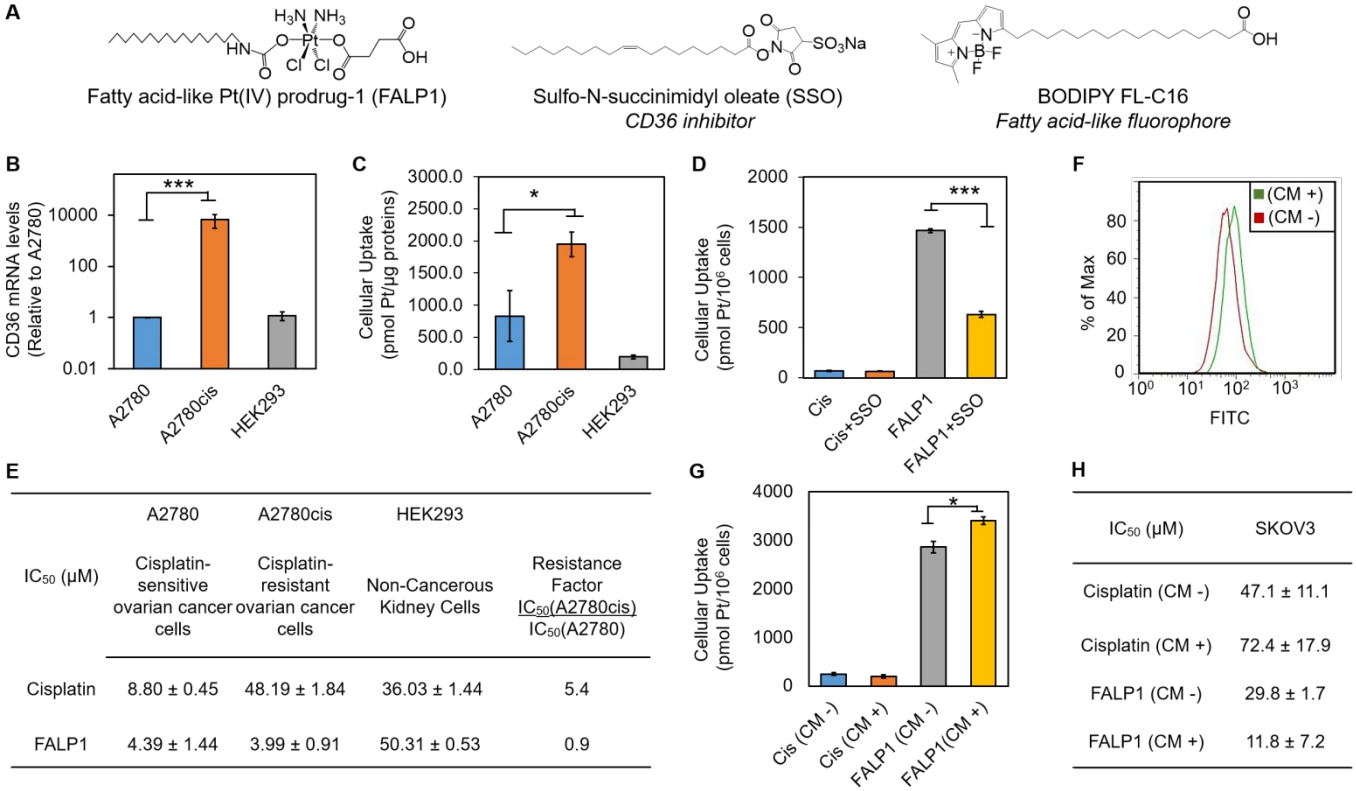
Electronic Supplementary Information (ESI) available: Experimental details regarding synthesis and characterization, cell culture, cellular uptake, cell viability assays, and flow cytometric analysis are presented in the ESI. See DOI: 10.1039/x0xx00000x

(Fig S6), and immunostaining (Fig S7) revealed that CD36 was overexpressed in A2780cis cells, but not in A2780 or HEK293 cells. FALP1 was synthesized according to the reported procedure.[29] Consistent with CD36 expression levels, the graphite furnace atomic absorption spectroscopy (GFAAS) results (Fig 2C) revealed that FALP1 preferentially accumulated in (1.95±0.19 nmol Pt/μg proteins) A2780cis cells compared to those in A2780 (0.83±0.39 nmol Pt/μg proteins) and HEK293 (0.19±0.03 nmol Pt/μg proteins) cells. Importantly, cisplatin did not show any favorable uptake across these cell lines (Fig S8). The cellular uptake studies using SSO (a CD36 inhibitor shown in Fig 2A) further supports the CD36-dependent cell entry of FALP1 in A2780cis cells. As shown in Fig 2D, cellular uptake of FALP1 in A2780cis is significantly impeded upon the treatment of SSO (1.47±0.02 nmol Pt/10^6 cells in (SSO -) and 0.63±0.03 nmol Pt/10^6 cells in (SSO +)), and in the control experiments, cellular uptake of cisplatin was not affected by SSO. Notably, upon inhibition by SSO (Fig 2D), uptake of FALP1 still excels that of cisplatin, and that is attributed to its high lipophilicity. Next, we evaluated the cytotoxicity profiles of FALP1 and cisplatin across these three cell lines (Fig 2E). These cells were treated with cisplatin or FALP1 for 24 h. As shown in Fig 2E, in the A2780cis platinum-resistant ovarian cancer cell line, the IC_{50} value of FALP1 (3.99±0.91 μM) is 12 times lower than that of cisplatin (48.2±1.84 μM). Interestingly, as for A2780 platinum-sensitive ovarian cancer cells, the IC_{50} value of FALP1 (4.39±1.44 μM) is just slightly lower than that of cisplatin (8.80±0.45 μM). Thus, the RF(cisplatin) and RF(FALP1) is 5.4 and 0.9, respectively. Notably, FALP1 exhibits lower toxicity (IC_{50} = 50.31±0.53 μM) against non-cancerous HEK293 cells comparing to cisplatin (IC_{50} = 36.03±1.44 μM). In sum, these results support that FALPs harness CD36 to facilitate their cell entry toward killing A2780cis cisplatin-resistant CD36-overexpressing ovarian cancer cells.

To further validate CD36-dependent cell entry and cytotoxicity profiles of FALPs, we modulated CD36 expression levels in SKOV3 ovarian cancer cells. It has been shown that interaction with adipocytes can increase CD36 expression in ovarian tumors.[3] Thus, we used the conditioned medium of 3T3-L1 derived adipocytes to increase expression of CD36 in SKOV3



E

IC_{50} (μM)	A2780 Cisplatin-sensitive ovarian cancer cells	A2780cis Cisplatin-resistant ovarian cancer cells	HEK293 Non-Cancerous Kidney Cells	Resistance Factor $\frac{IC_{50}(A2780cis)}{IC_{50}(A2780)}$
Cisplatin	8.80 ± 0.45	48.19 ± 1.84	36.03 ± 1.44	5.4
FALP1	4.39 ± 1.44	3.99 ± 0.91	50.31 ± 0.53	0.9

H

IC_{50} (μM)	SKOV3
Cisplatin (CM -)	47.1 ± 11.1
Cisplatin (CM +)	72.4 ± 17.9
FALP1 (CM -)	29.8 ± 1.7
FALP1 (CM +)	11.8 ± 7.2

Figure 2. CD36-dependent cellular entry and cytotoxicity profiles of the fatty acid-like Pt(IV) prodrug: **A**. Structures of fatty acid-like Pt(IV) prodrug-1 (FALP1), the CD36 inhibitor (SSO), and the fatty acid-like fluorophore (BODIPY FL-C16); **B.** qPCR analysis mRNA levels of CD36 in A2780 (cisplatin-sensitive) and A2780cis (cisplatin-resistant) ovarian cancer cells and HEK293 non-cancerous human kidney cells; **C.** GFAAS measurement of cellular uptake of FALP1 against A2780, A2780cis, and HEK293 cells (10 μM, 4 h); **D.** Cellular uptake of cisplatin and FALP1 ([Pt] = 10 μM, 4 h) against the A2780cis cells with (SSO +) and without (SSO -) the pre-treatment of SSO (200 μM, 0.5 h). **E.** The table of IC_{50} values determined by MTT assays for cisplatin and FALP1 against A2780, A2780cis, and HEK293 cells with 24-h incubation; **F.** Flow cytometric analysis of uptake of BODIPY FL-C16 in SKOV3 cells treated with or without the conditioned medium (CM +) or (CM -) of the adipocytes differentiated from 3T3-L1 (72 h); **G.** GFAAS measurements of cellular uptake of cisplatin (Cis) and FALP1 against SKOV3 with and without the pre-treatment of the conditioned medium (CM +) or (CM -) of the 3T3-L1-derived adipocytes; **H.** The table of IC_{50} values determined by MTT assays for cisplatin and FALP1 against SKOV3 ovarian cancer cells treated with or without the conditioned medium (CM +) or (CM -) of the adipocytes differentiated from 3T3-L1 (72 h). *P<0.05. ***P<0.0005.by t test (n=3, mean ± SEM).

cells and measure cellular uptake of FALP1 in SKOV3 cells with and without the conditioned medium. Flow cytometric analysis (Fig S9) indicated that protein expression of CD36 was increased in SKOV3 cells treated with the conditioned medium of adipocytes (CM +) compared to untreated control cells (CM -). We also performed flow cytometric analysis to measure the cellular uptake of BODIPY FL-C16 (a fatty acid-like fluorophore shown in Fig 2A) under these conditions. As shown in Fig 2F, (CM +) SKOV3 cells accumulated more BODIPY FL-C16 than (CM -) cells. Further analysis using GFAAS showed higher accumulation of FALP1 (3.40±0.08 nmol Pt/10^6 cells in the treated SKOV3 cells (CM +)) compared to the control (2.86±0.12 nmol Pt/10^6 cells in (CM -)) (Fig 2G). Importantly, the uptake of cisplatin was not depending on CD36 expression levels and was not affected by the addition of the conditioned medium (Fig 2G). Consisted with the uptake assay results, MTT assays (Fig 2H) show that FALP1 significantly suppressed growth of SKOV3 cells in the presence of the conditioned medium (IC_{50} = 11.8±7.2 μM in (CM +) and IC_{50} = 29.8±1.7 μM in (CM -)). In contrast, cisplatin was less effective in the growth suppression of SKOV3 cells and the effect was independent of the conditioned medium (IC_{50} = 72.4 ± 17.9 μM in (CM +) and IC_{50} = 47.1 ± 11.1 μM in (CM -)). Based on these results, we conclude that FALPs can harness CD36 to facilitate their cell entry toward killing SKOV3 ovarian cancer cells that overexpress CD36.

Finally, we explored the mechanism of how FALPs kills ovarian cancer cells. In addition to FALP1, FALP2-3 were designed to provide additional insight into the mitochondrial damaging effects. As shown in Fig 3A, FALP2 and FALP3 are the cationic derivatives designed to promote further mitochondrial accumulation of the compounds which would enhance the efficacy of FALP1. Synthesis of FALP2-3 can be found in Supporting Information. First, we verified mitochondrial accumulation of FALPs by using GFAAS and fluorescence imaging. Cisplatin was used as a control. For the A2780cis cells treated with cisplatin (Fig 3B), a negligible amount of Pt (Pt_{mito} = 31.5±8.44 pmol Pt/10^6 cells) was found in the mitochondria, while the majority (Pt_{cyt} = 231±25.5 pmol Pt/10^6 cells) was detected in the cytosol. In contrast, FALP1-3 carried significant amounts of Pt content (FALP1: Pt_{mito} = 307±61.3 pmol Pt/10^6 cells; FALP2: Pt_{mito} = 1458±218 pmol Pt/10^6 cells; FALP3: Pt_{mito} = 1423±266 pmol Pt/10^6 cells) to the mitochondria and is comparable to the amount (FALP1: Pt_{cyto} = 2575±578 pmol Pt/10^6 cells; FALP2: Pt_{cyto} = 2748±715 pmol Pt/10^6 cells; FALP3: Pt_{cyto} = 2239±322 pmol Pt/10^6 cells) delivered to the cytosol. Next, we determined the mitochondrial damage that had been triggered by FALPs. As shown in Fig 3C (left panel), the treatment of FALP1 (10 μM, 24 h) increased the mitochondrial ROS levels compared to the control. Again, the treatment of cisplatin at an even higher concentration (60 μM, 24 h) resulted in an insignificant change with respect to mitochondrial ROS

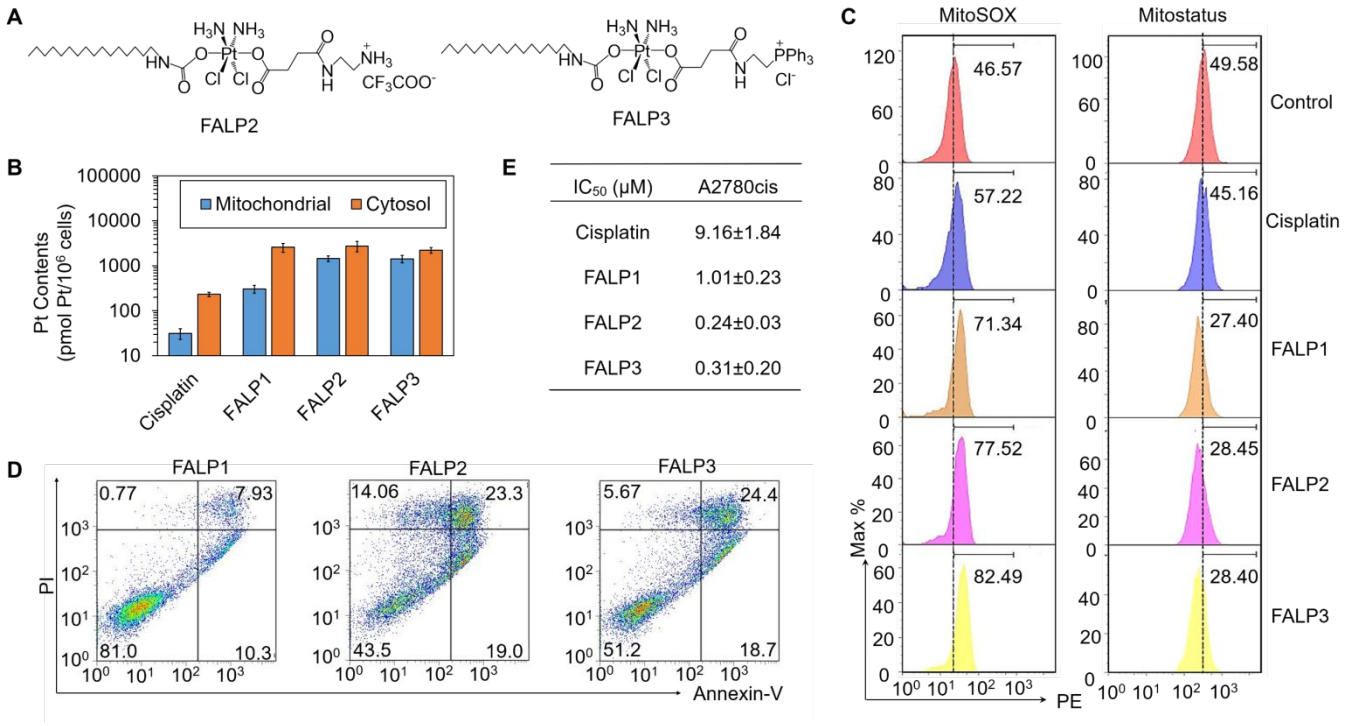


Figure 3. Mitochondrial damage triggered by the fatty acid-like Pt(IV) prodrugs: **A.** Structures of cationic derivatives of FALPs (FALP2 and FALP3); **B.** GFAAS analysis of mitochondrial and cytosolic Pt contents in the A2780cis cells treated with cisplatin (200 μM), FALP1 (10 μM), FALP2 (10 μM), and FALP3 (10 μM) for 6 h at 37 °C, 5% CO_2; **C.** Flow cytometric analysis of mitochondrial ROS level (*left*, MitoSOX assays) and membrane potentials (*right*, MitoStatus assays) of the A2780cis cells treated with cisplatin (60 μM), FALP1 (10 μM), FALP2 (1 μM), and FALP3 (1 μM) for 24 h at 37 °C, 5% CO_2; **D**. Flow cytometric analysis of apoptosis of the A2780cis cells treated with FALP1 (10 μM), FALP2 (1 μM), and FALP3 (1 μM) for 72 h at 37 °C, 5% CO_2; **E**. The table of IC_{50} values determined by MTT assays of cisplatin, FALP1, FALP2, and FALP3 against A2780cis cells with 72-h incubaiton at 37 °C, 5% CO_2.

levels, in line with its mechanism of action. Likewise, as shown in Fig 3C (right panel), the treatment of FALP1 (10 μM, 24 h) induced the loss of $\Delta\psi_m$ of the mitochondria of A2780cis cells. In addition, FALP2-3 (1 μM, 24 h) were able to trigger similar mitochondrial damage as FALP1 with a much lower dosage, and this is attributed to higher mitochondrial accumulation. Consistent with these results, our flow cytometric analysis (Fig 3D) showed that a larger population of cells were in late (23.3% for FALP2 or 24.4% for FALP3) and early (19.0% for FALP2 or 18.7% for FALP3) stages of apoptosis compared to the effect of FALP1, which only induced 7.93% and 10.3% of cells to undergo late and early stages of apoptosis, respectively (Fig 3D and Fig S10). Finally, the results of cytotoxicity analysis using MTT assays (Fig 3E) and cell confluence assays (Fig S11) further support the higher efficacy of FALP2-3 in killing cisplatin-resistant ovarian cancer cells than that of FALP1. Taken together, these results indicate that FALPs readily accumulate in mitochondria and trigger mitochondrial damage and apoptosis, thus leading to elimination of ovarian cancer cells.

In conclusion, we present for the first-time platinum-based fatty acid derivatives (FALPs) that act like a "Trojan horse" exploiting the upregulated CD36 to facilitate cell entry in ovarian cancer. Unlike fatty acids which provide energy for lipid metabolism, these Pt compounds accumulate in mitochondria and induce mitochondrial damage. As a result, FALPs exhibit high potency against ovarian cancer cells with cisplatin resistance. Success of this work not only delivers new knowledge about the interplay between metallodrugs and CD36, but also paves a new method toward overcoming drug resistance in ovarian cancer.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Y.-R. Z thanks the financial support provided by the startup fund, Farris Family Innovation Fellowship, and LaunchPad Award provided by Kent State University. S.M. and Y.-R. Z wish to acknowledge the National Science Foundation REU program (CHE-1659571) for support. Y.-R. Z thanks the support from The Research Council of Kent State University. M. K. thanks the support from the NIH (grant# CA208384) and the Mary Kay Foundation.

Notes and references

1. G. Pascual, A. Avgustinova, S. Mejetta, M. Martin, A. Castellanos, C. Attolini, A. Berenguer, N. Prats, A. Toll, J. Hueto, C. Bescos, L. Di Croce and S. Benitah, *Nature*, 2017, **541**, 41-45.
2. A. Ladanyi, A. Mukherjee, H. Kenny, A. Johnson, A. Mitra, S. Sundaresan, K. Nieman, G. Pascual, S. Benitah, A. Montag, S. Yamada, N. Abumrad and E. Lengyel, *Oncogene*, 2018, **37**, 2285-2301.
3. Y. Park, *Exp. Mol. Med.*, 2014, **46**. e99
4. W. W. Feng, O. Wilkins, S. Bang, M. Ung, J. Li, J. An, C. Del Genio, K. Canfield, J. DiRenzo, W. Wells, A. Gaur, R. B. Robey, J. Y. Guo, R. L. Powles, C. Sotiriou, L. Pusztai, M. Febbraio, C. Cheng, W. B. Kinlaw and M. Kurokawa, *Cell Rep.*, 2019, **29**, 3405-3420.e3405.
5. S. Wang, A. Blois, T. El Rayes, J. Liu, M. Hirsch, K. Gravdal, S. Palakurthi, D. Bielenberg, L. Akslen, R. Drapkin, V. Mittal and R. Watnick, *Sci. Trans. Med.*, 2016, **8**. 329ra34
6. J. Zhao, Z. Zhi, C. Wang, H. Xing, G. Song, X. Yu, Y. Zhu, X. Wang, X. Zhang and Y. Di, *Oncol. Rep.*, 2017, **38**, 2105-2115.
7. S. Weinberg and N. Chandel, *Nat. Chem. Biol.*, 2015, **11**, 9-15.
8. S. Wisnovsky, J. Wilson, R. Radford, M. Pereira, M. Chan, R. Laposa, S. Lippard and S. Kelley, *Chem. Biol.*, 2013, **20**, 1323-1328.
9. S. Marrache, R. Pathak and S. Dhar, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, 10444-10449.
10. S. Dar, J. Chhina, I. Mert, D. Chitale, T. Buekers, H. Kaur, S. Giri, A. Munkarah and R. Rattan, *Sci. Rep.*, 2017, **7**.
11. Y. Wang, T. Chao, C. Chang, Y. Yo, M. Yu and H. Lai, *Plos One*, 2013, **8**. e74538.
12. Y. Yo, Y. Lin, Y. Wang, C. Balch, R. Huang, M. Chan, H. Sytwu, C. Chen, C. Chang, K. Nephew, T. Huang, M. Yu and H. Lai, *Mol. Cancer Ther.*, 2012, **11**, 1703-1712.
13. P. Gupta, T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R. Weinberg and E. Lander, *Cell*, 2009, **138**, 645-659.
14. J. Pan, K. Ding and C. Wang, *Chin. J. Cancer*, 2012, **31**, 178-184.
15. S. Zhou, F. Wang, E. Wong, E. Fonkem, T. Hsieh, J. Wu and E. Wu, *Curr. Med. Chem.*, 2013, **20**, 4095-4101.
16. R. G. Kenny and C. J. Marmion, *Chem. Rev.*, 2019.
17. M. Stilgenbauer, A. M. D. S. Jayawardhana, P. Datta, Z. Yue, M. Gray, F. Nielsen, D. J. Bowers, H. Xiao and Y. R. Zheng, *Chem. Commun.*, 2019, **55**, 6106-6109.
18. K. Laws, G. Bineva-Todd, A. Eskandari, C. Lu, N. O'Reilly and K. Suntharalingam, *Angew. Chem. Int. Ed. Engl.*, 2018, **57**, 287-291.
19. Z. Wang, Z. Deng and G. Zhu, *Dalton Trans*, 2019, **48**, 2536-2544.
20. C. C. Konkankit, S. C. Marker, K. M. Knopf and J. J. Wilson, *Dalton Trans.*, 2018, **47**, 9934-9974.
21. D. Gibson, *Dalton Trans.*, 2016, **45**, 12983-12991.
22. T. C. Johnstone, K. Suntharalingam and S. J. Lippard, *Chem Rev*, 2016, **116**, 3436-3486.
23. U. Basu, B. Banik, R. Wen, R. K. Pathak and S. Dhar, *Dalton Trans*, 2016, **45**, 12992-13004.
24. Z. Wang, Z. Xu and G. Zhu, *Angew. Chem. Int. Ed. Engl.*, 2016, **55**, 15564-15568.
25. J. Mayr, P. Heffeter, D. Groza, L. Galvez, G. Koellensperger, A. Roller, B. Alte, M. Haider, W. Berger, C. R. Kowol and B. K. Keppler, *Chem Sci*, 2017, **8**, 2241-2250.
26. X. Wang, S. Jin, N. Muhammad and Z. Guo, *Chem. Rev.*, 2019, **119**, 1138-1192.
27. A. Lasorsa, O. Stuchlíková, V. Brabec, G. Natile and F. Arnesano, *Mol. Pharm.*, 2016, **13**, 3216-3223.
28. A. M. D. S. Jayawardhana, Z. Qiu, S. Kempf, H. Wang, M. Miterko, D. J. Bowers and Y. R. Zheng, *Dalton Trans.*, 2019, **48**, 12451-12458.
29. Y.-R. Zheng, K. Suntharalingam, T. C. Johnstone, H. Yoo, W. Lin, J. G. Brooks and S. J. Lippard, *J. Am. Chem. Soc.*, 2014, **136**, 8790-8798.