Transporter and protease mediated delivery of platinum complexes for precision oncology

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Abstract: Precision approaches are rapidly becoming the norm in the treatment of cancer and this is already impacting on the way platinum complexes are used. In this commentary, we will argue that there is the potential for platinum complexes to make a much greater contribution to precision oncology, one that is complementary to many of the other approaches being used and developed. Our focus will be on two methods for targeting anticancer agents: ligand-targeted drug delivery and protease activation of prodrugs. We will describe work done to date and discuss the directions that appear to be showing most promise. We will also discuss the challenges involved in the testing of targeted prodrugs in biological models and the possible consequences of these difficulties.

Background

Platinum complexes have been a mainstay of chemotherapeutic treatments for malignant tumours for more than four decades and their use continues to grow [1-3]. Over the past decade platinum complexes have increasingly been used in conjunction with molecularly targeted agents such as kinase inhibitors [4] and recently carboplatin has been coupled with immune checkpoint inhibitors in a new and highly promising frontline treatment for non-small cell lung cancer [5]. The reliance on platinum-based drugs means that oncologists must manage the toxicity of the current generation of platinum complexes and while this is generally possible, it provides strong motivation for the search for less toxic and more effective compounds.

The use of platinum complexes in the treatment of tumours is increasingly being stratified using precision oncology approaches which match the treatment to the genomic, proteomic and metabolomic changes evident in the individual cancer, generally on the basis of a molecular level understanding of these changes. For example, the subset of breast cancers classified as triple negative are frequently responsive to the platinums and they are now standard treatment for such cancers [6]. Other markers, including the level of CTR1 expression, can guide the use of the platinums [7-10]. However, achieving the hoped-for growth and improvement in precision oncology approaches would be facilitated by having platinum complexes that are selectively delivered or activated based on the chemical and biological features of a cancer.

One of the most exciting recent developments has been the use of albumin to deliver platinum(IV) complexes to tumours [11, 12] and clinical trials are anticipated. Albumin attachment has been shown to be an effective means for delivering paclitaxel (Abraxane)

[13] and trials are underway of albumin delivery of other active drugs such as doxorubicin [14, 15]. It is possible that the enhanced permeability and retention (EPR) effect contributes to the effectiveness of albumin as a delivery vehicle, but EPR has been shown to modest and variable in the clinical setting [16] and the observation that an albumin targeted platinum(IV) complex is active against leukemias where the EPR effect is not expected to be operational [17] suggests that other mechanisms may contribute. The high demand of cancer cells for nutrients is expected to result in the upregulation of albumin receptors and this is likely to contribute to the effectiveness of albumin coupling of anticancer agents [18]. If so, it is a case in which the "ligand"[1] (albumin) is delivering the agent in a targeted fashion to the cancer cells, an example of a strategy with enormous potential. A complementary approach is to use the unique chemistry and biochemistry of the extracellular microenvironments of tumours to selectively activate prodrugs. In this paper, we review the work done to date on the selective delivery and uptake of platinum(II) and platinum(IV) agents via each of these strategies. We also consider the features required for a successful targeted compound and the approaches to testing compounds and deciding which of them should be taken forward for further development.

Approaches to precision oncology

Precision medicine as applied to the treatment of cancers involves a matching of the treatment regime to the features of an individual patient's cancer type [19] and its application to the individual is often referred to as personalised medicine. The

[1] In this paper, ligand is used to refer to both molecules and ions coordinated to a metal and to moieties that target uptake mechanisms. While this duality is undesirable, it is unavoidable because the use of the term in each of these contexts is entrenched.

differentiation of cancers can be based on the overexpression of enzymes such as kinases and molecularly targeted inhibitors of these kinases provide selective treatment or targeted therapy. However, such approaches, while a huge step forward, are rarely curative and mutations involving a number of pathways frequently result in relapse with a form of the cancer that is unresponsive to the inhibitor [20, 21].

Ligand-targeted drug delivery (LTDD) and protease-based activation of prodrugs (PBA) (Fig. 1) offer alternative and complementary approaches to precision oncology. LTDD is based on the levels of expression of a variety of proteins that selectively transport molecules across the cellular membrane and PBA depends on the level and activity of the extracellular proteases. Cancer cells, by virtue of both their rapid growth and aberrant biology (such as their dependence on aerobic glycolysis for ATP production, the Warburg effect) upregulate numerous transporters such as those for folate, glucose, and neutral amino acids, thereby providing both the molecules and the raw materials needed to sustain their growth [22]. For example, the folate receptor (FR) is overexpressed in more than 90% of some classes of ovarian cancer [23]. The expression patterns will identify which targeting mechanism can be most effectively exploited and therefore which compounds, or indeed combinations of compounds, would be most effective for that individual's cancer. Similarly, the levels of expression of proteases in the extracellular environment of a tumour could be used to determine which protease activated prodrugs are most likely to be successful, providing another means of personalising the treatment. Thus, using these mechanisms to deliver known or new platinum agents in a targeted fashion should increase their efficacy and substantially increase the contributions they can make to precision oncology.

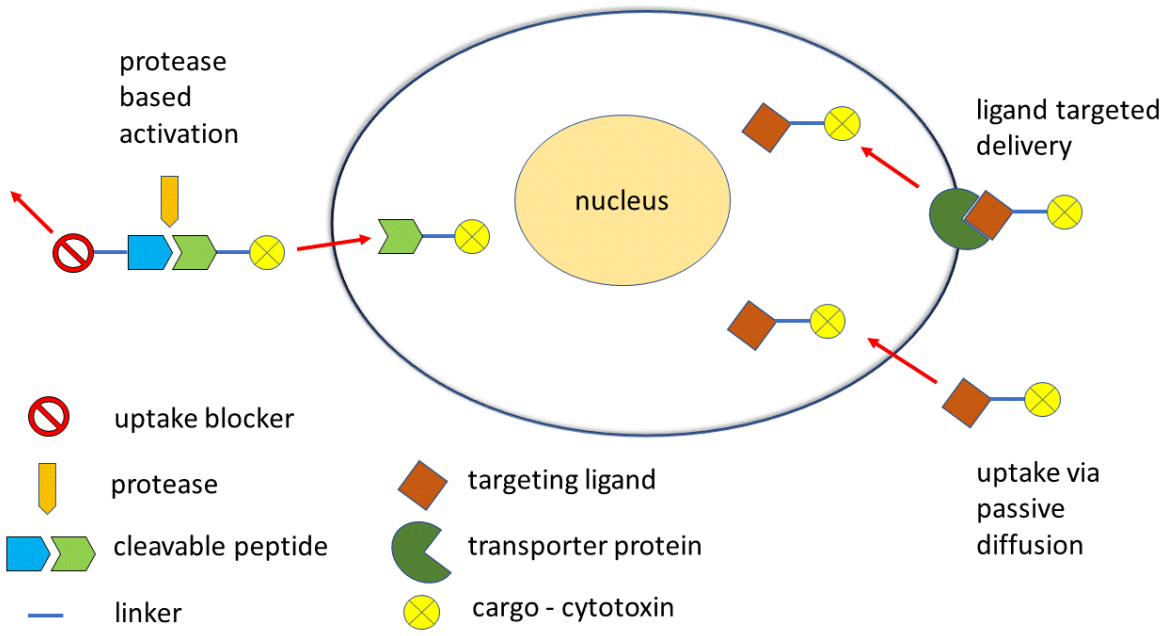

Fig. 1 The primary cellular uptake mechanisms discussed in this paper.

Targeting requirements

Targeting based on ligand uptake is limited by the number of the transporter proteins expressed at the cell surface and the rate at which the cargo is internalised and activated. Similarly, protease activation is limited by the concentration and activity of the protease. Consequently, not all transporters and proteases will be plausible mechanisms for the selective delivery of anticancer agents.

Given the potential limitations of transport or activation mechanisms, the delivery of highly potent anticancer agents is more likely to be successful and an analysis of the rate of uptake suggested that the cargo should ideally have a cytotoxicity of 1-10 nanomolar [22, 24]. The non-targeted platinum complexes currently in use are not considered to be particularly cytotoxic, having IC_{50} values that are typically in the low micromolar range. However, it has been known for many years that increasing their cellular uptake by the addition of highly lipophilic groups can lead to IC_{50} values in the low- to mid-nanomolar range [25]. This

reveals that the apparent potency is influenced by low or slow uptake and/or accumulation. Thus, the inherent potency of the established platinum anticancer agents is higher than the cytotoxicity values suggest and consequently targeted delivery has the potential to be more effective than might have been expected. In order to determine whether this is the case, it is essential to have biological models that accurately replicate the amount and activity of the transporter proteins found in real tumours or the level of extracellular proteases found in tumour environments so that the optimal compounds can be selected for taking forward.

Ligand-Targeted Drug Delivery

Ligand-targeted drug delivery (LTDD) provides a mechanism for the selective delivery of cytotoxic agents to cancer cells (Fig. 2) and is showing substantial promise in the delivery of agents such as Ifosfamide, Paclitaxel, Tubulysin, and Desacetylvinblastine hydrazide [22]. Here, ligand refers to a moiety that is preferentially transported into cancer cells and carries the active agent with it. To date, the most promising ligands in this regard have been folate, glucose, and compounds that bind to the prostate specific membrane antigen (PSMA) [22].

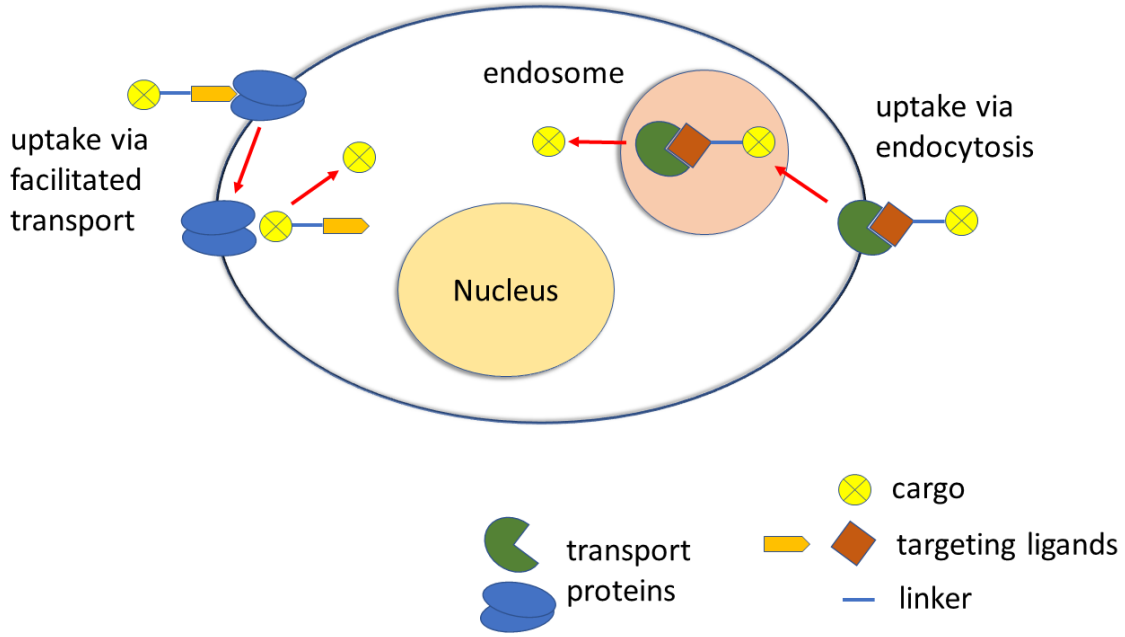

Fig. 2 Selective uptake pathways for ligand-targeted prodrugs.

For LTDD to work most effectively the prodrug must have a number of features:

1. it must be stable and non-toxic in the blood stream,
2. it must be minimally taken up by non-targeted cells including red blood cells,
3. it must be taken up rapidly and extensively by the tumour cells reaching a concentratioin sufficient to kill the cell,
4. it must be rapidly activated within those cells,
5. and the released active agent must be able to reach the regions of the cell where it can effect its activity.

Achieving all of these features is highly demanding and while substantial progress has been made to date, activation only where desired (points 1 and 4) and release from endosomes where these are involved in the uptake pathway (point 5) have recently been identified as the two key issues remaining for the effective delivery of the agents listed above [22]. For platinum complexes, achieving the necessary concentration of drug (point 3) is also a key

issue because, as discussed above, their relatively lower potency raises the question as to whether sufficient concentrations of the targeted drug can be reached to effect its cytotoxic action.

Platinum complexes could be delivered by LTDD in either their 2+ or 4+ oxidation states and there are examples of both in the literature. We briefly discuss below the advantages and disadvantages of each and then describe work done to date in the context of each of the transport pathways that has been exploited.

Platinum(II)

All platinum complexes currently used in the clinic are neutral platinum(II) agents with two anionic donors and two am(m)ine donors (Fig. 3), each in cis arrangements. Such platinum(II) complexes can be delivered using LTDD by attaching the targeting ligand to either an anionic leaving group (X) or a non-leaving amine group (NH_2R). The relatively rapid reactions of such platinum(II) complexes and their consequent trapping within the cell as charged intermediates are formed is a distinct advantage. However, the platinum(II) complexes used need to have sufficient stability to survive largely unchanged in the blood stream for long periods because uptake via transporters is likely to be relatively slow. More inert platinum(II) complexes may not be taken up at the levels required to kill the cells and may not be activated rapidly enough once inside the cell. Also, attachment of a targeting ligand to an amine group may interfere with DNA and other biomolecule binding, reducing the efficacy. Consequently, the focus of recent efforts has been on the delivery of platinum(IV) complexes.

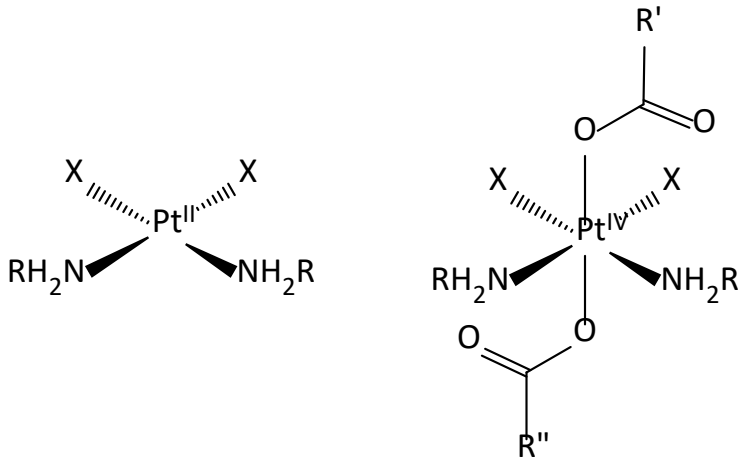

Fig. 3 Structures of classical platinum(II) and platinum(IV) anticancer active complexes

Platinum(IV)

Platinum(IV) complexes have been the subject of intense and increasing interest over the past two decades because of their greater stability and the increased options for modifying them without interfering with the ultimate mode of action. In particular, platinum(IV) complexes have the advantage of more straightforward modification via the R' and R" groups in the axial sites (Fig. 3), these being the sites that become available on oxidation from platinum(II) to platinum(IV). Activation of a platinum(IV) prodrug by reduction can be rapid, can be controlled by modification of the coordination sphere, and potentially releases an unmodified platinum(II) agent of known activity such as cisplatin, carboplatin, or oxaliplatin. However, reduction must first occur, and this must happen at a rate that is competitive with efflux. Also, the prodrug must reach regions of the cell where reduction can occur and it is not currently known whether there are regions of the cell where is this more or less likely. The advantages and disadvantages of platinum(IV) complexes have been extensively reviewed [2, 26, 27] and substantial progress has been made on synthetic methodologies and on the understanding of the features that provide the required

combination of stability and reactivity in the extracellular and intracellular environments [28-34].

Glucose and other sugars

Glucose transporters are highly overexpressed in most cancers because of the Warburg effect, the abnormal metabolism in which aerobic glycolysis is used to generate ATP resulting in a much higher demand for glucose [35]. Glucose is transported into cells via a set of facilitative uptake proteins known as the GLUTs each of which has a different set of substrate preferences. The dominant transporter, GLUT1, is the major route for glucose uptake and is the most frequently overexpressed in cancer [36], but a number of other GLUTs also play a role. Glucose can be attached to a linker via any one of its five hydroxyl groups (Fig. 4) and which is used will influence how it interacts with transporters and enzymes.

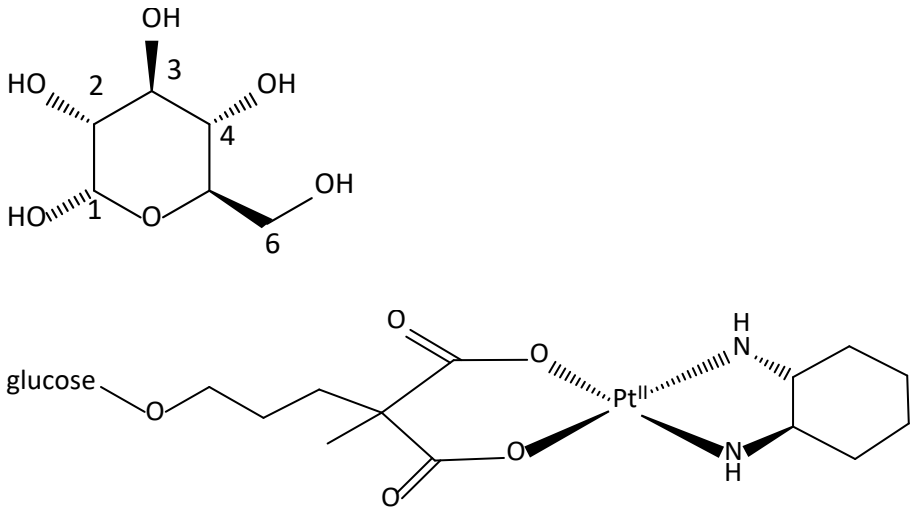

Fig. 4 The structure of glucose showing the numbering used for each of the possible sites for connection to a linker (top) and the platinum(II) complex attached at each of these sites by Lippard and colleagues [37] (bottom).

The first use of an unprotected glucose bound to a platinum(II) complex was by reported Chen, Wang, and colleagues who attached it to a diamine group with encouraging results in the cell lines tested [38]. Subsequently, Gao and colleagues coupled the glucose to the 2-position of malonate [39-41]. The results obtained with the platinum(IV) complex generated with *trans-R,R*-cyclohexane-1,2-diamine were encouraging, with a six-fold greater cytotoxicity than oxaliplatin against the L1210 cell line and encouraging *in-vivo* results. Particularly notable was the lower toxicity seen in mice, suggesting a higher therapeutic index which is a key success indicator for such complexes. In a follow up study, mannose and galactose were also investigated as targeting ligands and both generated encouraging results [42]. The galactose targeted complex was generally less active than the glucose complex against a panel of cell lines, but it was similarly active against the HT29 line, while the mannose complex was notably more active against the SKOV-3 line. Both observations demonstrate the selectivity that might be achieved using targeting agents, and thereby demonstrate the potential for their use in precision oncology approaches. GLUT1 knockdown versions of the HT29 cell line were substantially less sensitive to all three complexes, demonstrating that the GLUT1 transporter is likely to play a role in the uptake of all three complexes [42]. *In vivo* studies of the glucose targeted complex and oxaliplatin against the HT29 line showed that at equitoxic doses, the glucose complex was significantly more active [42].

In studies of a closely related set of complexes, but lacking a fluorine in the 2-position of the malonate, it was shown that quercetin, a GLUT1 transport inhibitor, decreased the

cytotoxicity of the three complexes, and all three complexes inhibited the uptake of 2-NBDG, a fluorescent glucose analogue [43].

Another example of successful delivery of a platinum(II) moiety using glucose as the targeting ligand has recently been reported by Patra, Lippard, and colleagues [37, 44]. They addressed a number of critical issues that have impacted on previous attempts to exploit GLUT transporters, using modelling to show that attachment via the 6-position of glucose should not interfere with binding to the GLUT1 transporter and established that attachment to platinum via a malonate was successful in achieving GLUT1 dependent uptake. They undertook extensive studies to show that the bulk of the transport was via glucose dependent pathways and GLUT1 in particular. This is an important step because attachment of a complex to glucose has the potential to facilitate uptake by other pathways such as passive diffusion, thereby limiting the degree of tumour cell targeting, a critical but under-appreciated aspect of ligand-targeted drug delivery. The results they obtained suggest that GLUT transport is able to achieve sufficiently high levels of platinum inside the cell for effective treatment, consistent with the hypothesis outlined above that platinum complexes are sufficiently potent to be effective when delivered by such transporters.

In a subsequent study, they investigated the impact of substitution at each of the various attachment sites on glucose (Fig. 4) on the degree of targeting and accumulation, and showed that attachment via the 2-position was most effective [37].

Researchers in our group have independently investigated attachment via the 2-position for the delivery of a fluorophore [45, 46]. Our rationale was that attachment via this position allows for phosphorylation of the glucose by hexokinase following transport by a GLUT transporter. Phosphorylation adds a charge and prevents escape of the adduct from the cell,

increasing accumulation. We also confirmed in that study the importance of limiting uptake via off-target mechanisms.

There have also been studies of the use of glucose in the axial site of platinum(IV) complexes to target tumours. In one case the ligand in the other axial position was a long chain fatty acid [47] and it is likely that this group increased the degree of passive uptake, complicating the interpretation of the accumulation and cytotoxicity results. In a follow up study, in which the second axial site was occupied by a hydroxide ligand, very high levels of sensitivity to GLUT1 inhibitors were demonstrated for the lead complex, which had a glucose attached via the 6-position [48].

Folate

Tetrahydrofolate is essential for pyrimidine and therefore DNA synthesis, and is taken up by all cells via a facilitated transport pathway. Most of the tetrahydrofolate in the body is derived from folic acid (Fig. 5) in the diet. It is reduced by dihydrofolate reductase within cells in the liver, where the folic acid is taken up by the folate transporter, a membrane bound protein that is endocytosed following folate binding [49]. Cancer cells frequently express high levels of the folate receptor and dihydrofolate reductase because of the demands their rapid growth places on the nutrients needed for DNA synthesis [50]. Since the folate transporter is not expressed at significant levels by most cells in the body, it is an excellent target for the selective treatment of cancer cells [24, 51].

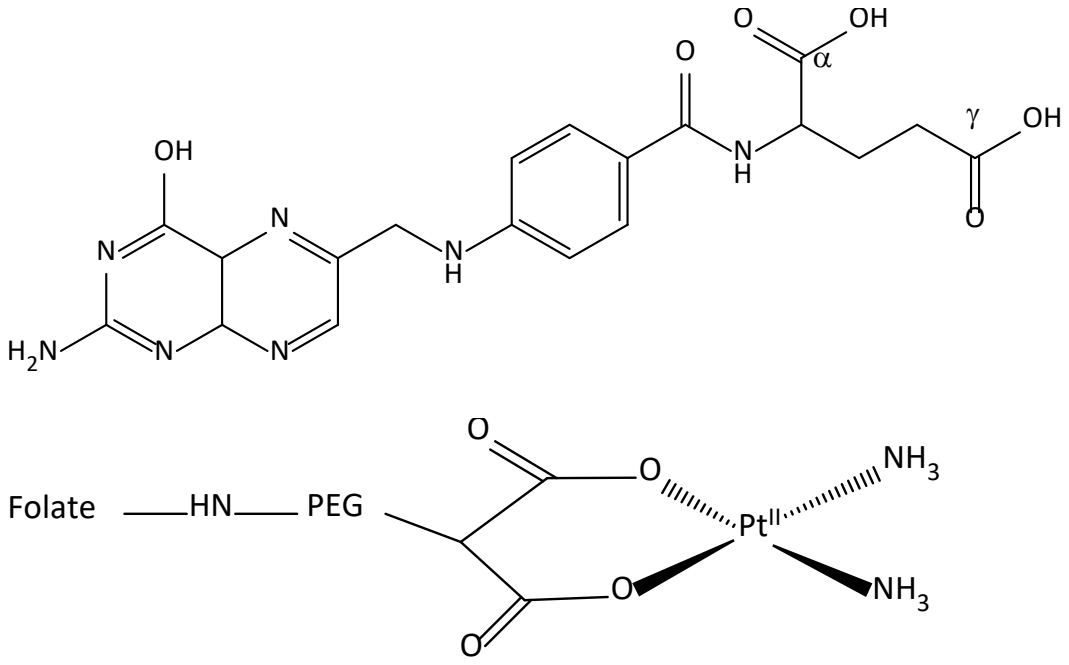

Fig. 5 The structure of folic acid (top) and a platinum(II) folate adduct (bottom) reported by Gibson and colleagues [52]. Attachment is predominantly via the γ carboxylate of the glutamic acid.

One of the earliest investigations of LTDD to deliver a platinum(II) complex was reported by Gibson and colleagues in 2003 [52]. They attached a folate to a malonate ligand via a PEG linker that was included to increase solubility. As expected, a control complex with the PEG linker alone showed lower levels of accumulation than either carboplatin or the folate coupled complex, but surprisingly it was the most cytotoxic and exhibited the highest levels of DNA binding, perhaps indicating that the PEG linker modified the sub-cellular distribution. The folate coupled complex exhibited the lowest cytotoxicity, but this was only 50% lower than that of carboplatin and given that only the 65% of the complex coupled via the gamma carboxylate is expected to be biologically active, the results are encouraging. This result also points to the need for other measures of efficacy of targeted complexes. If the targeting is

effective, then lower toxicity to healthy cells can be expected and higher doses can be used. Equally, the expression of FR may be significantly higher in tumour regions that are starved of nutrients. Thus, in assessing the efficacy we need to take account of both toxicity and activity, and it may be that doing is so is achievable only through studies of real tumours.

Lippard and colleagues prepared a platinum(IV) complex with folate as a targeting ligand attached via a PEG linker to a succinate in the axial site. They also prepared the complex with a carbon nanotube attached via a succinate in the other axial site [53]. The latter complex was substantially more cytotoxic than cisplatin in cells that expressed the folate receptor, and only similarly active in cells that did not. The complex without the nanotubes attached was surprisingly lower in activity, being less active than cisplatin even in cell lines that did express folate. This is a surprising result and again may reflect issues with escape of this complex from the endosomes involved in uptake, indicating that this is an aspect that needs further investigation.

There have been a number of studies in which folate or other targeting ligands and platinum(II) complexes have been added separately to nanoparticles of various types. These have been reviewed by Butler and Sadler [54] and are not discussed further here.

PSMA substrates

Glutamate carboxypeptidase II (GCPII) is found in the brain, the small intestine, the kidneys, and in the prostate and it performs different tasks in each location. It was identified as an important antigen and biomarker on the surface of prostate cancer cells and in that context

is referred to as prostate specific membrane antigen (PSMA) [55]. Its role is to cleave peptides that incorporate negatively charged amino-acids and it can deliver them into the cell via an endocytic process. PSMA is showing enormous promise for the delivery of radiopharmaceuticals for diagnosis and therapy of prostate cancer [56, 57]. There have been no reported studies of PSMA substrates bound directly to platinum, but Lippard and colleagues have reported on platinum(IV) complexes encapsulated in nanoparticles that had such substrates bound [58, 59]. Co-delivery of docetaxel and the same platinum(IV) complex in the PSMA targeted nanoparticles showed even more promising results in animal studies [60]. Thus, PSMA mediated uptake appears to be able to deliver sufficient platinum to be effective and is therefore a strategy worthy of further investigation.

Neutral amino acids

Neutral amino acid transporters are highly overexpressed in many cancers [61], often because of a dependence on a very high uptake of glutamine for survival, particularly in breast [62] and prostate cancer [63]. These transporters have been an area of increased focus in recent years and there have been a small number of attempts to use the neutral amino acid uptake pathway for the selective delivery of imaging [64] or bioactive agents [65] including a platinum(IV) complex [66]. The *in vitro* results for the platinum(IV) complex showed promise with glutamine dependent uptake and activity observed, but about 50% of the uptake and activity appeared to be independent of glutamine concentration [66]. This result is consistent with uptake by passive diffusion making a significant contribution and highlights the challenges in using targeting ligands that do not strongly inhibit uptake by other pathways.

Vitamin B12

Vitamin B12 is an important nutrient with a selective uptake pathway and again uptake is increased in cancer cells. It has been shown to be suitable for the delivery of diagnostic radionuclides [67] and has been investigated for the delivery of platinum(II) complexes [68, 69]. It was shown that the presence of the platinum complex did not interfere with the cobalamin uptake or processing [69, 70] and therefore it is a suitable delivery vehicle. The cytotoxicity of conjugates with platinum(II) moieties were substantially lower than that of cisplatin, but as the authors correctly identify, this is not the only indicator of whether they should be considered for further investigation [69]. However, whether vitamin B12 is likely to be abe to deliver sufficient platinum in a clinical setting has yet to be established.

Protease-Based Activation of Drug Delivery

An alternative approach to ligand-based targeting of tumour cells is to exploit the chemistry and biochemistry of the extracellular environment. This can be based on chemical factors such as the low pH that derives in part from the reliance on anerobic glycolysis and the subsequent production of very high levels of lactic acid [71-73], or the hypoxia that derives from the inadequate blood supply found in most tumours [74]. However, an approach that is likely to be more selective is to exploit the overexpression of protease enzymes that typifies many tumours [75, 76]. For example, matrix metalloproteases (MMPs) are overexpressed in most tumours, in part at least because of the role they play in remodelling the extracellular environment as the tumour undergoes rapid growth [77]. Another example is the protease human kallikrein-3 (prostate specific antigen, PSA) which is overexpressed in most prostate cancers [75]. These enzymes can be used to cleave appropriately designed

peptides, which when attached to a payload, block cellular uptake until that cleavage occurs as shown in Fig. 1 [77, 78].

We have investigated both MMP and PSA cleavage as activators of prodrugs using model fluorescent systems in the first instance to track cleavage and/or uptake in the presence and absence of the enzyme being targeted. For example, we have used an MMP-2/9 targeted sequence with two fluorophores to establish that cleavage by added or endogenous MMP-2/9 did modify the cellular uptake properties [79]. We also observed very substantial impacts on the penetration and uptake of the fluorescent groups in spheroids of the DLD colon cancer cell line, consistent with MMPs being generated in these 3D cell culture models and cleaving and activating the peptides [79]. Using a fluorescent tagged PSA substrate we showed that negatively charged amino acids were effective at preventing cellular uptake and that cleavage by PSA enabled uptake in cells that produced PSA and more rapid and extensive uptake in cells where PSA was added [80].

To investigate the potential of MMPs for activating peptide coupled platinum complexes members of our research group have prepared molecules of the type shown in Fig. 6 and undertaken preliminary studies of their activity [81]. Complexes without the negatively charged uptake blocking group showed similar or better activity to a close analogue of the platinum(IV) complex being delivered, but the complex with the blocking group was inactive. This may reflect low levels of MMP activity in the cell culture model, an issue that is discussed further below.

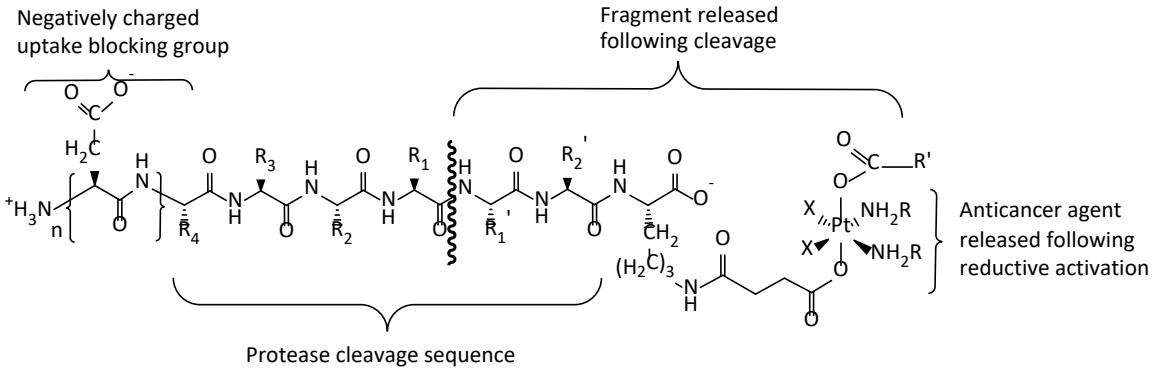

Fig. 6 Diagram showing the general structure protease activated platinum drug.

Biological Models for Testing

Since the activity of targeted platinum complexes should be entirely dependent on the expression and activity of the transporters and proteases being targeted, the choice of biological models for testing, refining, and selecting compounds to go forward is critically important.

Protease targeting

The expression and activation of the extracellular proteases used to cleave the peptide targeted compounds described above are highly dependent on the environment, and cells grown in 2-D culture are unlikely to replicate all aspects of the environment found in a tumour. Also, measurement of protease levels can be misleading because most methods don't distinguish between active and inactive enzyme. For instance, it has been estimated that most or all of the PSA in the extracellular environment of LnCaP cells is inactive whereas up to 90% of that in human derived tumours is active [82]. To overcome this in our studies of activation of prodrugs by PSA, we have added known amounts of active enzyme to the colon cancer DLD-1 cell line since it expresses no PSA and thereby have been able to establish that the active enzyme at relevant concentrations is able to activate our prodrug

model [80]. Studies of cleavage by the PSA produced by LnCaP cells showed much lower levels of uptake than seen when PSA was added even though the concentration of added enzyme was substantially lower than that found in tumours [80].

Since MMPs are involved in the remodelling of the extracellular matrix, their expression and activation is highly dependent on the environment the cells are in and particularly, the cell-cell contacts they make. 3-D cell cultures such as tumour cell spheroids are able to replicate some of these features and, for example, we have observed that DLD-1 cells which in 2-D cell culture do not express significant levels of MMP, do so in spheroids and in that environment, but not in 2D cell culture, are able to activate our model prodrugs [79].

Ligand-targeted compounds

For preliminary studies of prodrugs exploiting transporter-based targeting mechanisms, 2-D cell culture provides an excellent platform for investigating uptake and accumulation. Measurement of accumulation is straightforward in the case of platinum complexes because the intracellular platinum levels can be measured using ICP-MS. However, relevance to the clinical environment depends on the level and the activity of the transporter. The levels of transporter expression can be measured using mRNA levels or Western blots and this is more informative than it is for the proteases discussed above. However, these levels do not always indicate the amount of transporter available at the cell surface nor its activity. It is highly likely that one or both will vary during the cell cycle, and they may also be affected by the environment the cell is in. For example, cells in hypoxic environments express much higher levels of some GLUT transporters [83, 84].

In studying the behaviour of targeted complexes in biological systems, there is a risk that the effectiveness of a pathway will be overestimated, but the risk of it being underestimated

would appear to be much greater and that could lead to an effective prodrug being abandoned. Genetic modification of cell lines so that they constitutively express the transporter being targeted can give reliable information on the ability of the prodrug to exploit the pathway [61]. This will be most effective if a cell line is chosen that expresses very low levels of the transporter in normal circumstances. However, it will not enable the determination of the levels of accumulation that can be expected in a tumour. Xenografts can provide additional information, but these rarely replicate all of the critical features of a real tumour. Tumour cell spheroids replicate some of the features and, for example, we have observed selective accumulation of a GLUT transport targeting compound in the hypoxic regions of spheroids [46]. Human tumour explants are almost certainly the best laboratory models of the true tumour environment currently available, but achieving reproducibility is likely to be a challenge and therefore using explants to select compounds for further developments again risks promising compounds being rejected. Therefore, for this field to progress better models are required and the risk of abandoning promising compounds because of inadequate models needs to be kept in mind.

Off target accumulation

It is particularly important to distinguish between target and off-target based accumulation. Off-target accumulation such as passive diffusion will decrease the selectivity and increase side-effects. It also has the potential to give the impression of higher levels of targeting derived activity than is actually the case. Measuring accumulation at 4^{o} C and 37^{o} C can provide insights into the relative proportions of compounds taken up via active or facilitated

pathways and passive pathways because the former are energy dependent and therefore greatly diminished in activity at the lower temperature.

Time scales and dependencies

Finally, account needs to be taken of the time scales associated with uptake and the recovery of pathways that involve endocytosis of the transport protein. In a clinical setting, slow accumulation may be acceptable if the lifetime of the prodrug is long and the uptake is selective enough to allow high concentrations to be used, but it is difficult to replicate this process in laboratory-based models.

The level of activity of transporter proteins in particular is likely to vary substantially through the cell cycle and this could impact on the estimations of the effectiveness of a targeted complex. Single cell studies would enable temporal variations to be investigated and reveal with such variations are significant enough to impact on the conclusions.

Other metals

While the reductive activation of platinum(IV) complexes and the potency of the platinum(II) products that result gives it unique advantages, there is every reason to investigate similar targeting strategies for other metals, particularly since some of these are potentially more potent. Albumin targeted delivery of an iridium complex has recently been reported demonstrated promising light activation dependent cytotoxicity [85] while glucose transporter targeted delivery of ruthenium complexes was less promising [86]. Peptide conjugates of a wide variety of organometallic agents have been described [87] and some of

these have potential to be transporter mediated agents with selectivity against cells that overexpress the relevant transporter. Also, highly potent osmium complexes have been reported [88] and some have been shown be effective against cancer stem cells [89]. Since the role of the targeting mechanisms discussed is to keep the complexes out of cells unless they are taken up via selective mechanisms, high toxicity becomes less of a concern. More important is that complexes are stable while attached to the targeting vector and moving around in the blood stream and that they are active once inside cells. Establishing stability and selectivity of activation is probably the greatest challenge in moving beyond platinum.

Conclusions

Despite the limited number of studies of ligand-targeted delivery or protease activation of prodrug forms of platinum anticancer agents, the results are encouraging and suggest that this is an area that merits further attention. Of particular significance is that each of the pathways discussed above appears to have the potential to deliver sufficient of the standard platinum(II) entities to be effective. However, it is clear that further investigation of the effectiveness of these existing drugs when delivered by targeted mechanisms is needed in models that are closer to the clinical situation in order to determine which of them should go forward. Also, using these and other targeting strategies for delivering more potent agents is an area that would appear to be particularly promising.

While delivery of platinum(II) complexes is possible, the benefits of the more inert higher oxidation state and the greater ease and opportunities for modification lead us to conclude that platinum(IV) complexes hold the greatest promise for targeted delivery using the methods discussed here.

Success in developing targeted platinum agents would not only result in more potent and less toxic agents, but also has the potential to make a substantial contribution to precision oncology.

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References

1 L. R. Kelland (2007) Nat Rev Cancer 7:573-584
2 T. C. Johnstone, K. Suntharalingam and S. J. Lippard (2016) Chemical Reviews 116:3436-3486
3 N. J. Wheate, S. Walker, G. E. Craig and R. Oun (2010) Dalton Transactions 39:8113-8127
4 G. Aravantinos and D. Pectasides (2014) J Ovarian Res 7:13
5 M. Mathew, T. Enzler, C. A. Shu and N. A. Rizvi (2018) Pharmacol Ther 186:130-137
6 F. Poggio, M. Bruzzone, M. Ceppi, N. F. Ponde, G. La Valle, L. Del Mastro, E. de Azambuja and M. Lambertini (2018) Annals of Oncology 29:1497-1508
7 S. Sun, J. Cai, Q. Yang, S. M. Zhao and Z. H. Wang (2017) Oncotarget 8:16036-16051
8 S. R. Martens-de Kemp, A. Brink, I. H. van der Meulen, R. X. de Menezes, D. E. te Beest, C. R. Leemans, V. W. van Beusechem, B. J. M. Braakhuis and R. H. Brakenhoff (2017) Mol Cancer Ther 16:540-550
9 N. Ogane, M. Yasuda, Y. Kameda, T. Yokose, H. Kato, A. Itoh, S. Nishino, Y. Hashimoto and S. Kamoshida (2013) Biomed Res 34:143-151
10 D. Kilari, K. A. Iczkowski, C. Pandya, A. J. Robin, E. M. Messing, E. Guancial and E. S. Kim (2016) Anticancer Research 36:495-501
11 Y. R. Zheng, K. Suntharalingam, T. C. Johnstone, H. Yoo, W. Lin, J. G. Brooks and S. J. Lippard (2014) Journal of the American Chemical Society 136:8790-8798
12 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 (2017) Chem Sci 8:2241-2250
13 A. M. Sofias, M. Dunne, G. Storm and C. Allen (2017) Adv Drug Deliv Rev 122:20-30

14 J. Gong, J. Yan, C. Forscher and A. Hendifar (2018) Drug Des Dev Ther 12:777-786
15 M. Seetharam, K. R. Kolla and K. N. Ganjoo (2018) Future Oncol 14:2323-2333
16 Y. S. Youn and Y. H. Bae (2018) Adv Drug Deliv Rev 130:3-11
17 C. R. Kowol, P. Heffeter, W. Berger, B. K. Keppler, J. Mayr and V. Pichler (2018) In: W. I. P. Organisation (ed). Austria,
18 K. Wosikowski, E. Biedermann, B. Rattel, N. Breiter, P. Jank, R. Loser, G. Jansen and G. J. Peters (2003) Clinical Cancer Research 9:1917-1926
19 S. E. Jackson and J. D. Chester (2015) International Journal of Cancer 137:262-266
20 S. A. Rosenzweig (2018) In: K. D. Tew and P. B. Fisher (eds) Advances in cancer research, vol 138. Elsevier Academic Press Inc, San Diego, pp. 71-98
21 S. Y. Lim, A. M. Menzies and H. Rizos (2017) Cancer 123:2118-2129
22 M. Srinivasarao and P. S. Low (2017) Chemical Reviews 117:12133-12164
23 W. S. Graybill and R. L. Coleman (2016) Drugs of the Future 41:137-143
24 I. R. Vlahov and C. P. Leamon (2012) Bioconjugate Chem 23:1357-1369
25 L. R. Kelland, B. A. Murrer, G. Abel, C. M. Giandomenico, P. Mistry and K. R. Harrap (1992) Cancer Research 52:822-828
26 M. D. Hall and T. W. Hambley (2002) Coord Chem Rev 232:49-67
27 M. D. Hall, H. R. Mellor, R. Callaghan and T. W. Hambley (2007) J Med Chem 50:3403-3411
28 M. D. Hall, C. T. Dillon, M. Zhang, P. Beale, Z. Cai, B. Lai, A. P. J. Stampfl and T. W. Hambley (2003) J Biol Inorg Chem 8:726-732
29 H. R. Mellor, S. Snelling, M. D. Hall, S. Modok, M. Jaffar, T. W. Hambley and R. Callaghan (2005) Biochem Pharmacol 70:1137-1146
30 M. D. Hall, R. A. Alderden, M. Zhang, P. Beale, Z. Cai, B. Lai, A. P. J. Stampfl and T. W. Hambley (2006) J Struct Biol 155:38-44
31 J. Z. Zhang, E. Wexselblatt, T. W. Hambley and D. Gibson (2012) Chem Commun 48:847-849
32 J. Z. Zhang, P. Bonnitcha, E. Wexselblatt, A. Klein, Y. Najajreh, D. Gibson and T. W. Hambley (2013) Chem - Eur J 19:1672-1676
33 C. K. J. Chen, J. Z. Zhang, J. B. Aitken and T. W. Hambley (2013) Journal of Medicinal Chemistry 56:8757-8764
34 E. Wexselblatt and D. Gibson (2012) J Inorg Biochem 117:220-229
35 O. Warburg (1956) Science 124:269-270
36 L. Szablewski (2013) Biochim Biophys Acta-Rev Cancer 1835:164-169
37 M. Patra, S. G. Awuah and S. J. Lippard (2016) Journal of the American Chemical Society 138:12541-12551
38 Y. Chen, M. J. Heeg, P. G. Braunschweiger, W. Xie and P. G. Wang (1999) *Angew Chem Int Ed* 38:1768-1769
39 H. Li, X. Q. Gao, R. Liu, Y. Wang, M. H. Zhang, Z. Fu, Y. Mi, Y. Q. Wang, Z. Yao and Q. Z. Gao (2015) European Journal of Medicinal Chemistry 101:400-408
40 P. X. Liu, Y. H. Lu, X. Q. Gao, R. Liu, D. Zhang-Negrerie, Y. Shi, Y. Q. Wang, S. Q. Wang and Q. Z. Gao (2013) Chemical Communications 49:2421-2423
41 Q. Mi, Y. Ma, X. Q. Gao, R. Liu, P. X. Liu, Y. Mi, X. G. Fu and Q. Z. Gao (2016) Journal of Biomolecular Structure & Dynamics 34:2339-2350
42 R. Liu, Z. Fu, M. Zhao, X. Q. Gao, H. Li, Q. Mi, P. X. Liu, J. N. Yang, Z. Yao and Q. Z. Gao (2017) Oncotarget 8:39476-39496
43 T. L. Li, X. Q. Gao, L. Yang, Y. L. Shi and Q. Z. Gao (2016) ChemMedChem 11:1069-1077
44 M. Patra, T. C. Johnstone, K. Suntharalingam and S. J. Lippard (2016) Angewandte Chemie-International Edition 55:2550-2554
45 A. Glenister (2017) School of Chemistry. The University of Sydney,
46 A. Glenister, M. I. Simone and T. W. Hambley (2019) Submitted,
47 J. Ma, Q. P. Wang, Z. L. Huang, X. D. Yang, Q. D. Nie, W. P. Hao, P. G. Wang and X. Wang (2017) Journal of Medicinal Chemistry 60:5736-5748

48 J. Ma, H. F. Liu, Z. Q. Xi, J. Z. Hou, Y. G. Li, J. Niu, T. Liu, S. N. Bi, X. Wang, C. J. Wang, J. J. Wang, S. Q. Xie and P. G. Wang (2018) Front Chem 6:15
49 P. Mc Carron, A. Crowley, D. O'Shea, M. McCann, O. Howe, M. Hunt and M. Devereux (2018) Current Medicinal Chemistry 25:2675-2708
50 H. Elnakat and M. Ratnam (2004) Adv Drug Deliv Rev 56:1067-1084
51 C. P. Leamon (2008) Curr Opin Investig Drugs 9:1277-1286
52 O. Aronov, A. T. Horowitz, A. Gabizon and D. Gibson (2003) Bioconjugate Chemistry 14:563-574
53 S. Dhar, Z. Liu, J. Thomale, H. J. Dai and S. J. Lippard (2008) Journal of the American Chemical Society 130:11467-11476
54 J. S. Butler and P. J. Sadler (2013) Curr Opin Chem Biol 17:175-188
55 W. R. Fair, R. S. Israeli and W. D. W. Heston (1997) Prostate 32:140-148
56 Y. Chen, C. A. Foss, Y. Byun, S. Nimmagadda, M. Pullambahatla, J. J. Fox, M. Castanares, S. E. Lupold, J. W. Babich, R. C. Mease and M. G. Pomper (2008) J Med Chem 51:7933-7943
57 S. A. Kularatne, K. Wang, H. K. R. Santhapuram and P. S. Low (2009) Mol Pharmaceut 6:780-789
58 S. Dhar, N. Kolishetti, S. J. Lippard and O. C. Farokhzad (2011) Proc Nat Acad Sci USA 108:1850-1855
59 S. Dhar, F. X. Gu, R. Langer, O. C. Farokhzad and S. J. Lippard (2008) Proceedings of the National Academy of Sciences of the United States of America 105:17356-17361
60 N. Kolishetti, S. Dhar, P. M. Valencia, L. Q. Lin, R. Karnik, S. J. Lippard, R. Langer and O. C. Farokhzad (2010) Proceedings of the National Academy of Sciences of the United States of America 107:17939-17944
61 A. J. Scopelliti, J. Font, R. J. Vandenberg, O. Boudker and R. M. Ryan (2018) Nat Commun 9:12
62 M. van Geldermalsen, Q. Wang, R. Nagarajah, A. D. Marshall, A. Thoeng, D. Gao, W. Ritchie, Y. Feng, C. G. Bailey, N. Deng, K. Harvey, J. M. Beith, C. I. Selinger, S. A. O'Toole, J. E. J. Rasko and J. Holst (2016) Oncogene 35:3201-3208
63 J. Twum-Ampofo, D. X. Fu, A. Passaniti, A. Hussain and M. M. Siddiqui (2016) Curr Opin Oncol 28:241-247
64 L. Lattuada, S. Demattio, V. Vincenzi, C. Cabella, M. Visigalli, S. Aime, S. G. Crich and E. Gianolio (2006) Bioorganic & Medicinal Chemistry Letters 16:4111-4114
65 C. F. Wang, J. M. Wu, Z. J. Wang, Z. P. Yang, Z. Li, H. H. Deng, L. Li, X. Peng and M. Feng (2018) Biomaterials 183:77-92
66 M. Ravera, E. Gabano, S. Tinello, I. Zanellato and D. Osella (2017) Journal of Inorganic Biochemistry 167:27-35
67 S. Kunze, F. Zobi, P. Kurz, B. Spingler and R. Alberto (2004) Angewandte Chemie-International Edition 43:5025-5029
68 S. Mundwiler, B. Spingler, P. Kurz, S. Kunze and R. Alberto (2005) Chemistry-a European Journal 11:4089-4095
69 P. Ruiz-Sanchez, C. Konig, S. Ferrari and R. Alberto (2011) Journal of Biological Inorganic Chemistry 16:33-44
70 P. Ruiz-Sanchez, S. Mundwiler, B. Spingler, N. R. Buan, J. C. Escalante-Semerena and R. Alberto (2008) Journal of Biological Inorganic Chemistry 13:335-347
71 T. W. Hambley (2007) Dalton Trans:4929-4937
72 N. Yamamoto, S. Danos, P. D. Bonnitcha, T. W. Failes, E. J. New and T. W. Hambley (2008) J Biol Inorg Chem 13:861-871
73 N. Yamamoto, A. K. Renfrew, B. J. Kim, N. S. Bryce and T. W. Hambley (2012) J Med Chem 55:11013-11021
74 W. A. Denny (2004) Aust J Chem 57:821-828
75 A. M. LeBeau, P. Singh, J. T. Isaacs and S. R. Denmeade (2009) Biochemistry 48:3490-3496

76 J. M. Atkinson, C. S. Siller and J. H. Gill (2008) Brit J Pharmacol 153:1344-1352
77 T. Jiang, E. S. Olson, Q. T. Nguyen, M. Roy, P. A. Jennings and R. Y. Tsien (2004) Proceedings of the National Academy of Sciences of the United States of America 101:17867-17872
78 S. R. Denmeade and J. T. Isaacs (2012) Oncotarget 3:666-667
79 N. Yamamoto, N. S. Bryce, N. Metzler-Nolte and T. W. Hambley (2012) Bioconjugate Chem 23:1110-1118
80 L. Di Marco, J. Z. Zhang, J. Doan, B. J. Kim, N. Yamamoto, N. S. Bryce and T. W. Hambley (2019) Bioconjugate Chemistry 30:124-133
81 J. Norman (2013) School of Chemistry. The University of Sydney,
82 S. R. Denmeade, L. J. Sokoll, D. W. Chan, S. R. Khan and J. T. Isaacs (2001) Prostate 48:1-6
83 Y. Shao, T. L. Wellman, K. M. Lounsbury and F. Q. Zhao (2014) Am J Physiol-Regul Integr Comp Physiol 307:R237-R247
84 E. C. Nakajima, C. Laymon, M. Oborski, W. Z. Hou, L. Wang, J. R. Grandis, R. L. Ferris, J. M. Mountz and B. Van Houten (2014) PLoS One 9:12
85 P. Y. Zhang, H. Y. Huang, S. Banerjee, G. J. Clarkson, C. Ge, C. Imberti and P. J. Sadler (2019) Angewandte Chemie-International Edition 58:2350-2354
86 M. Prohl, T. Bus, J. A. Czaplewska, A. Traeger, M. Deicke, H. Weiss, W. Weigand, U. S. Schubert and M. Gottschaldt (2016) European Journal of Inorganic Chemistry, doi 10.1002/ejic.201600801:5197-5204
87 B. Albada and N. Metzler-Nolte (2016) Chemical Reviews 116:11797-11839
88 S. H. van Rijt, I. Romero-Canelon, Y. Fu, S. D. Shnyder and P. J. Sadler (2014) Metallomics 6:1014-1022
89 K. Suntharalingam, W. Lin, T. C. Johnstone, P. M. Bruno, Y. R. Zheng, M. T. Hemann and S. J. Lippard (2014) Journal of the American Chemical Society 136:14413-14416