MedChemComm

View Online
Dynamic Article Links

Cite this: *Med. Chem. Commun.*, 2011, **2**, 274

www.rsc.org/medchemcomm

**CONCISE ARTICLE**

# Platinum-oxazoline complexes as anti-cancer agents: syntheses, characterisation and initial biological studies†‡

**Paras N. Yadav,§[a] Ramsay E. Beveridge,¶[a] Jonathan Blay,[b] Alaina R. Boyd,¶[a] Maja W. Chojnacka,¶[c] Andreas Decken,‖[d] Ankur A. Deshpande,¶[a] Michael G. Gardiner,**[e] Trevor W. Hambley,[f] Michael J. Hughes,¶[a] Leslie Jolly,¶[e] Jacquelyn A. Lavangie,¶[a] Timothy D. MacInnis,¶[a] Sherri A. McFarland,*[a] Elizabeth J. New[f] and Robert A. Gossage*[ac]**

***Received 10th November 2010, Accepted 14th December 2010***
**DOI: 10.1039/c0md00211a**



The syntheses, characterisation and biological activities ($IC_{50}$; DNA binding) of four mononuclear Pt oxazoline complexes are reported. These materials are the compounds *cis*-[$PtCl_2(NH_3)$(Etox-$\kappa^1N$)] (**1**: Etox = 2-ethyl-2-oxazoline), [$PtCl_2$(anilox-$\kappa^2N,N'$)] (**2**: anilox = 4,4-dimethyl-2-[*o*-anilinyl]-2-oxazoline), *cis*-[$PtCl_2$(Etox-$\kappa^1N)_2$] (**3**) and [PtCl(pyox-$\kappa^3N,N',N'$)] (**4**: pyoxH = pyridine-2-carboxyanil-[*o*-{4,4-dimethyl-2-oxazolinyl}-ide]) and all four are shown to have slightly lower cytotoxicities *in vitro* when compared to cisplatin against the A2780 ovarian cancer cell line. These new materials all appear to be bio-active *via* the formation of DNA adducts. Complexes **1** and **2** have been further characterised in the solid-state by X-ray diffraction methods.

## Introduction

Platinum compounds containing *N*-donor ligands have played a unique role in the development of both coordination and medicinal chemistry. The serendipitous discovery (*circa* 1965) and later clinical application of the simple inorganic complex *cisplatin* (*i.e.*, *cis*-[$PtCl_2(NH_3)_2$]: Fig. 1) as an anti-cancer agent marks[1] an important milestone in the development of inorganic chemotherapy agents.[1,2] Since that time, a number of 2nd and 3rd generation Pt-containing reagents have been designed, tested and/or entered clinical use.[3,4] Modifications of the original cisplatin scaffold have been undertaken to increase clinical efficacy and/or to reduce the (toxic) side-effect profile. Synthetically, this has focused on three main areas: (*i*) application of Pt complexes with higher formal oxidation state(s) at the metal centre (primarily $Pt^{4+}$) such as *Satraplatin* (a.k.a. **JM216**: Fig. 1);[5] (*ii*) the use of non-halide formal anions within the metal coordination sphere (*e.g.*, *Carboplatin*; Fig. 1)[3,4] and/or (*iii*) replacement of one or more ammine ligands with other *N*-donor fragments, such as the substituted pyridine ligand found in *Picoplatin* (*i.e.*, **ZD0473**: Fig. 1).[3] These modifications come from the very early establishment of the observed Structure–Activity Relationship (SAR) within the general class of bio-active Pt species.[1,4]

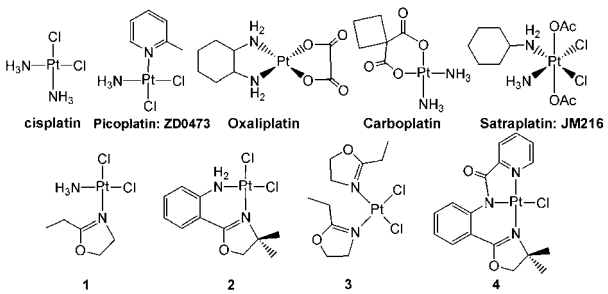


**Fig. 1** Some current clinically used Pt-based drugs and the compounds (**1–4**) used in this study.

[a]*Department of Chemistry, Acadia University, Wolfville, NS B4P 2R6, Canada. E-mail: sherri.mcfarland@acadiau.ca; Fax: +1-902-5851114*
[b]*Department of Pharmacology, Sir Charles Tupper Medical Building, Dalhousie University, 5850 College Street, Halifax, NS B3H 1X5, Canada*
[c]*Ryerson University, Department of Chemistry & Biology, 350 Victoria Street, Toronto, ON M5K 2B3, Canada. E-mail: gossage@ryerson.ca; Fax: +1-416-9795044*
[d]*Department of Chemistry, University of New Brunswick, Fredericton, NB E4L 1G8, Canada*
[e]*School of Chemistry, The University of Tasmania, Hobart, TAS 7001, Australia*
[f]*School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia*

† Oxazoles XXVI

‡ CCDC reference numbers 800341 and 800342. For crystallographic data in CIF or other electronic format see DOI: 10.1039/c0md00211a

§ Current address: School of Chemistry, Institute of Science & Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
¶ Undergraduate research participant; these authors contributed equally to this research.
‖ Author to whom correspondence should be addressed concerning the crystallographic characterisation of complex **1**. E-mail: adecken@unb.ca
** Author to whom correspondence should be addressed concerning the crystallographic characterisation of complex **2**. E-mail: michael.gardiner@chem.utas.au

Our particular interests lie in the arena of oxazole (and 4,5-dihydro-1,3-oxazole: *i.e*, 2-oxazoline)[6,7] coordination chemistry and in ligand design strategies involving these heterocyclic frameworks. Oxazoles (ox) are found in a wide variety of both natural and synthetic products and have found a number of diverse applications (*e.g.*, coordination chemistry, catalysis, *etc*.).[6] Despite these advances, relatively few transition metal oxazol(in)e compounds have been investigated as anti-cancer agents and even fewer contain Pt as the metal centre.[8] The work described herein details the synthesis of representatives of four classes of Pt-ox compounds: (*a*) ox analogues (**1**) of **ZD0473** (*i.e*., *cis*-[$PtCl_2(NH_3)$(2-picoline)]: Fig. 1); (*b*) relatives of *Oxaliplatin* which contain an *N*,*N*′-chelating ox ligand (**2**); (*c*) *bis*-ox Pt-compounds devoid of an N–H bond (**3**) and finally (*d*) a Pt complex containing a tridentate ligand of our own design which incorporates both a pyridine and an ox fragment (**4**).[9] These four compounds are shown schematically in Fig. 1. Hence, the work described herein addresses the latter of the three main areas of Pt chemotherapy design, namely the replacement of one or more ammine ligands (*vide supra*). These four model compounds will serve as indicators as to the efficacy of the four general molecular designs (Fig. 1).

## Results and discussion

### Syntheses and structural characterisation

Compound **1** is obtained by employing previously established protocols[8] in which cisplatin is first converted *in situ* to $Pt(NH_3)_2(NO_3)_2$, followed by ligand metathesis with 2-ethyl-2-oxazoline (Etox) to give $Pt(Etox)(NH_3)(NO_3)_2$, and finally treatment with excess KCl to yield **1**.[10,11] Compound **2** can be obtained by the addition of solid 4,4-dimethyl-2-(2′-anilinyl)-2-oxazoline[7b,9] to a stoichiometric aq. solution of $K_2PtCl_4$ containing a trace of *c*. $H_2SO_4$ (as ligand transfer agent). Subsequent stirring yields **2** in the form of a yellow-coloured powder.[12,13] The treatment of a methanolic suspension of the inorganic polymer $[PtCl_2]_n$ with excess Etox and stirring overnight at room temperature (RT) yielded grey-coloured **3**,[14,15] and **4** was prepared as reported previously.[9]

Compounds **1** and **2** have been further characterised in the solid-state by single crystal X-ray diffraction methods.[11,13,15] Both materials are found to be mononuclear Pt complexes as expected and exhibit the desired cisoidal oriented chlorido ligands. Molecular representations of a molecule of **1** or **2** that is found in the unit cell can be found below in Fig. 2 and 3, respectively.

These two materials contain a Pt atom that is in essentially a square planar coordination environment.[11,13] The Pt–Cl and Pt–N bond lengths are typical for a formally Pt(II) metal centre and are otherwise unsurprising.[17]

### Biological screening

The results of the biological screening of complexes **1–4** can be found in Table 1; the results for cisplatin are also given.[18] The latter four materials show slightly lower cytotoxicity values when compared with cisplatin. However, all are active within an order of magnitude. These results suggest that the four representatives of the potential classes of 2nd generation cisplatin analogues

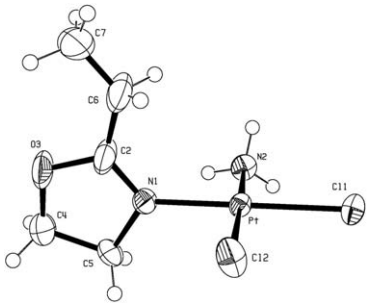


**Fig. 2** ORTEP[16] representation of a molecule of complex **1** showing the atomic numbering scheme (thermal ellipsoids are shown at the 50% probability level).

(*i.e*., Fig. 1) are all worthy of further investigations. Hence, the syntheses, screening and establishment of more detailed Structure–Activity Relationships for a larger series of each class represented by **1–4** is warranted.

### DNA interaction studies

The cytotoxicity associated with *cisplatin* and its analogues is generally believed to arise from direct interactions with DNA. Intracellular formation of monoaquated platinum species leads to monofunctional adducts with DNA primarily through attachment to guanine *N*7 residues. Upon further coordination to DNA, such adducts cyclise to form guanine-guanine and adenine-guanine cross-links. This class of modification of the

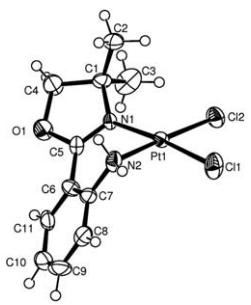


**Fig. 3** ORTEP[16] representation of a molecule of complex **2** showing the atomic numbering scheme (thermal ellipsoids are shown at the 50% probability level).

**Table 1** Cytotoxicity of **1–4** and cisplatin *vs*. the A2780 cancer cell line (72 h incubation) and $r_b$ values with pUC19 plasmid DNA

| Compounds | Inhibition $IC_{50}$, μM[a] | Reference | $r_b$ values |
|---|---|---|---|
| **cisplatin** | 1.3 (± 0.1) | this work | 0.076 |
| **1** | 4.1 (± 0.3) | this work | 0.083 |
| **2** | 16.9 (± 0.5) | this work | 0.41 |
| **3** | 9.9 (± 1.0) | this work | 0.20 |
| **4** | 20.2 (± 1.7) | this work | 0.81 |
| **$[PtCl_2(NH_3)(Meox)]$**[b,c] | 0.65 (± 0.1) | 8 | — |
| **cisplatin**[c] | 0.2 ± (0.05) | 8 | — |

[a] Values are mean for data from at least three independent experiments with quadruplicate readings in each experiment. [b] *cis*-isomer; Meox = 2-methyl-2-oxazoline-κ[1]*N*. [c] *n.b*., 96 h exposure.

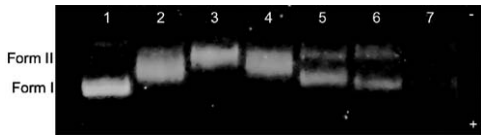


**Fig. 4** DNA unwinding assay for **1** with 800 ng pUC19 plasmid (electrophoresed for 50 min on a 1% agarose gel in 1X TAE at 80 V). Lane 1, DNA only; Lane 2, 5 μM **1**; Lane 3, 10 μM **1**; Lane 4, 50 μM **1**; Lane 5, 100 μM **1**; Lane 6, 200 μM **1**; and Lane 7, 400 μM **1**.

DNA tertiary structure by Pt compounds can be followed by an electrophoretic mobility shift assay, whereby the unwinding of covalently-closed, supercoiled plasmid DNA is monitored.[20] It has been shown previously that *cisplatin* unwinds supercoiled (Form I) DNA by 13° with a bound drug-to-nucleotide ratio ($r_b$) of 0.076, where $r_b$ corresponds to the amount of platinum complex that is necessary for complete removal of all supercoils from DNA.[21] Experimentally, $r_b$ is measured as the coalescence point in a titration of plasmid DNA with a platinum complex, where the rate of migration of Form I decreases until it comigrates with relaxed, nicked DNA (Form II). Fig. 4 shows the DNA unwinding assay for **1** with pUC19 plasmid DNA. The coalescence point in a titration of 120 μM pUC19 occurs with 10 μM **1**, corresponding to an $r_b$ value of 0.083, which is only slightly larger than that measured for *cisplatin*. The degree of distortion in the DNA helix induced by **1** is especially evident at higher concentrations (Lane 7), where the gel bands disappear because the intercalating dye used for imaging can no longer penetrate the helix. In general, the magnitude of $r_b$ for **1–4** parallels the cytotoxicity observed toward the A2780 cell line, with smaller values of $r_b$ translating to smaller $IC_{50}$ values. As expected, platinum complexes that interact more strongly with DNA are the most cytotoxic. An exception to this trend occurs in the case of **3**, underscoring the importance of additional factors in predicting anticancer activity. Together these results indicate that the Pt-oxazoline compounds interact with DNA much like *cisplatin* but may offer certain advantages, including reduced toxicity toward normal cells or higher selectivity toward cancer cells.

## Acknowledgements

This work has been generously supported by funding from Research Corporation (RAG: Cottrell College Science Award). The authors are also indebted to the support of The Canadian Breast Cancer Foundation (RAG: Research Project Grant), NSERC Canada (Discovery Grants: JB, SAM and RAG; USRA: MJH, JAL and MWC), in addition to the support of Acadia and Ryerson Universities. RAG was the recipient of a 2006 J. W. T. Jones Travelling Fellowship, generously supplied by the Royal Society of Chemistry, which enabled this author to carry out some of this research during a sabbatical leave. The technical assistance of Annette Hooper (SAM) is also acknowledged.

## References

1 (*a*) N. J. Wheate, S. Walker, G. E. Craig and R. Oun, *Dalton Trans.*, 2010, **39**, 8113; (*b*) B. Lippert (Ed.), *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug*, Wiley-VCH: Oxford, 1999.

2 (*a*) T. W. Hambley, *Dalton Trans.*, 2007, 4929; (*b*) R. A. Alderden, M. D. Hall and T. W. Hambley, *J. Chem. Educ.*, 2006, **83**, 728.

3 M. Gielen and E. R. T. Tiekink (ed.), *Metallotherapeutic Drugs and Metal-Based Diagnostic Agents: the Use of Metals in Medicine*, Wiley-VCH: Toronto, 2005.

4 (*a*) A. M. Montaña and C. Batalla, *Curr. Med. Chem.*, 2009, **16**, 2235; (*b*) E. Wong and C. M. Giandomenico, *Chem. Rev.*, 1999, **99**, 2451; (*c*) B. W. Harper, A. M. Krause-Heuer, M. P. Grant, M. Manohar, K. B. Garbutcheon-Singh and J. R. Aldrich-Wright, *Chem. Eur. J.*, 2010, **16**, 7064.

5 M. D. Hall, H. R. Mellor, R. Callaghan and T. W. Hambley, *J. Med. Chem.*, 2007, **50**, 3403.

6 T. Eicher and S. Hauptmann, *The Chemistry of Heterocycles, 2nd Ed.*, Wiley-VCH: Weinheim, 2003, Ch. 5.23-5.25.

7 (*a*) For example: R. A. Gossage, P. N. Yadav, T. D. MacInnis, J. W. Quail and A. Decken, *Can. J. Chem.*, 2009, **87**, 368; (*b*) F. J. Baerlocher, R. Bucur, A. Decken, C. R. Eisnor, R. A. Gossage, S. M. Jackson, L. Jolly, S. L. Wheaton and R. S. Wylie, *Aust. J. Chem.*, 2010, **63**, 47.

8 F. P. Intini, A. Boccarelli, V. C. Francia, C. Pacifico, M. F. Sivo, G. Natile, D. Giordano, P. De Rinaldis and M. Coluccia, *J. Biol. Inorg. Chem.*, 2004, **9**, 768.

9 A. Decken, R. A. Gossage and P. N. Yadav, *Can. J. Chem.*, 2005, **83**, 1185.

10 Synthesis of **1**. An analogous procedure was used as previously reported[8] with the substitution of Etox *in lieu* of Meox (Yield: 36%). Mp 194 °C (decomp.). $^1$H NMR (dmso-$d_6$): $\delta$ = 1.20 (t, 3H, $J$ = 7.6 Hz, –C $H_3$), 2.83 (q, 2H, $J$ = 7.6 Hz, –CC $H_2$), 3.78 (t, 2H, $J$ = 9.6 Hz, –NC $H_2$), 4.14 (s [br], 3H, N $H_3$), 4.45 (t, 2H, $J$ = 9.6 Hz, –OC $H_2$). Anal. Calc. (Found): C 15.71 (15.73); H 3.16 (3.08); N 7.33 (7.22)%. MS (EI, 70 eV): $m/z$ = 382.0 ($M^+$).

11 X-Ray diffraction data of **1**. Formula: $C_5H_{12}N_2OCl_2Pt$, $M_r$: 382.16 g mol$^{-1}$, crystal size: 0.425 × 0.10 × 0.025 mm$^3$, space group: $C2/c$, $a$ = 18.924(2), $b$ = 10.8634(7), $c$ = 11.9925(10) Å, $\beta$ = 127.540(2)°, $V$ = 1954.8(3) Å$^3$, $T$ = 173(1) K, $Z$ = 8, $\rho_c$ = 2.597 g mL$^{-1}$, Mo-K$\alpha$ = 0.71073 Å, $F$(000): 1408, *hkl* range: –23/+22, –13/+14, –15/+14, refl. measured: 6568, refl. unique: 2194, $R_{int}$ = 0.0591, parameters refined: 102, $R$(F)/w $R$($F^2$) (all refl.): 0.0484/0.1109, $GoF$ ($F^2$): 1.024, $\Delta\rho_{fin}$ (max./min.): 2.709/-2.530 eÅ$^{-3}$. Selected bond lengths (Å) and angles (°): Pt–Cl1 2.305(1), Pt–Cl2 2.299(2), Pt–N1 1.995(4), Pt–N2 2.042(7), N1–C2 1.282(9); Cl1–Pt–Cl2 91.79(6), Cl1–Pt–N1 179.0(2), Cl1–Pt–N2 90.3(2), N1–Pt–N2 88.9(2) Cl1–N2–C7 121.1(6).

12 Synthesis of **2**. A sample of $K_2PtCl_4$ (0.3760 g: 0.9058 mmol) was dissolved in 7 mL of water in the presence of 8 drops of c. $H_2SO_4$. To this mixture was added a sample of 2-(2′anilinyl)-4,4-dimethyl-2-oxazoline[7b,9, 22] (0.1733 g: 0.9109 mmol) in the form of a solid. The mixture was then stirred rapidly at RT (overnight). The resulting precipitate was isolated by filtration and washed with water (2 × 2 mL), 95% aq. EtOH (1 mL) and $Et_2O$ (2 × 2 mL). The yield of yellow-orange powder was 0.2111 g (52%). Mp >200 °C. $^1$H NMR (acetone-$d_6$): $\delta$ = 1.47 (s, 6H, –C $H_3$), 4.42 (s, 2H, –OC $H_2$), 6.65 (m, 1H, Ar *H*), 6.82 (m, 1H, Ar *H*), 7.28 (m, 1H, Ar *H*), 7.63 (m, 1H, Ar *H*). Anal. Calc. (Found): C 28.96 (28.44); H 3.09 (2.97); N 6.14 (6.13)%. Crystals suitable for X-ray diffraction work were obtained by recrystallisation of **2** from aq. 95% EtOH at RT.

13 X-ray diffraction data of **2**. Formula: $C_{11}H_{14}N_2OCl_2Pt$, $M_r$ = 456.22 g mol$^{-1}$, space group $P2_1/n$, $a$ = 6.525(2), $b$ = 11.816(3), $c$ = 17.129(4) Å, $\beta$ = 99.99(4)°, $V$ = 1300.6(6) Å$^3$, $T$ = 193(2) K, $Z$ = 4, $\rho_c$ = 2.330 g mL$^{-1}$, Mo-K$\alpha$ = 0.71073 Å, $F$(000): 856, *hkl* range: ±7, ±14, ±20, refl. unique = 2230, $R_{int}$ = 0.0454, parameters refined = 160, $R$(F)/w $R$($F^2$) (all refl.) = 0.0454/0.1335, $\Delta\rho_{fin}$ (max./min.) = 0.428/0.313 eÅ$^{-3}$. Selected bond lengths (Å) and angles (°): Pt–Cl1 2.294(3), Pt–Cl2 2.324(3), Pt–N1 2.043(7), Pt–N2 2.06(1), N1–C5 1.28(1); Cl1–Pt–Cl2 88.3(1), Cl1–Pt–N1 174.1(2), Cl1–Pt–N2 90.6(3), N1–Pt–N2 84.3(3) Pt1–N2–C7 110.0(7).

14 Synthesis of **3**. A sample of $PtCl_2$ (0.12 g: 0.45 mmol) was suspended in 7 mL of MeOH. To this mixture was added 2-ethyl-2-oxazoline (0.22 g: 2.2 mmol) and the mixture stirred rapidly at RT overnight. The resulting precipitate was isolated by filtration and washed with $Et_2O$ (3 × 5 mL). The yield of cream coloured powder was 0.15 g (72%). Mp >200 °C. $^1$H NMR (MeOH-$d_4$): $\delta$ = 1.29 (t, 3H, $J$ = 7.6 Hz, –C $H_3$), 3.00 (q, 2H, $J$ = 7.6 Hz, –CC $H_2$), 3.90 (t, 2H, $J$ = 9.6 Hz, –NC $H_2$), 4.54 (t, 2H, $J$ = 9.6 Hz, –OC $H_2$). Anal. Calc. (Found): C 25.87 (25.65); H 3.91 (3.51); N 6.03 (6.01)%.



 

15 Full details on the X-ray structural characterisation of complex **3** will be published separately: Gossage, R. A.; Jenkins, H. A. *unpublished results*.
16 L. J. Farrugia, *J. Appl. Crystallogr.*, 1997, **30**, 565.
17 (*a*) For example: P. N. Yadav, R. A. Gossage and A. Decken, *Anal. Sci.*, 2008, **24**, x301; (*b*) A. Albinati, H. Moriyama, H. Rüegger, P. S. Pregosin and A. Togni, *Inorg. Chem.*, 1985, **24**, 4430; (*c*) R. A. Michelin, R. Bertani, M. Mozzon, G. Bombieri, F. Benetollo and R. J. Angelici, *Organometallics*, 1991, **10**, 1751; (*d*) U. Belluco, R. Bertani, F. Meneghetti, R. A. Michelin, M. Mozzon, G. Bandoli and A. Dolmella, *Inorg. Chim. Acta*, 2000, **300**, 912; (*e*) Y. Chen, Z. Guo, S. Parsons and P. J. Sadler, *Chem.–Eur. J.*, 1998, **4**, 672.
18 Human ovarian carcinoma cell lines A2780 were maintained in exponential growth as monolayers in Advanced DMEM supplemented with 2.5 mM glutamine and 2% fetal calf serum at 37 °C in 5% $CO_2$. Complexes tested were prepared as 250 μM aq. solutions containing 1% DMF immediately prior to the assay. Cytotoxicity was determined using the MTT assay as previously described.[19] Single cell suspensions were obtained by trypsinisation of monolayer cultures, with cell counts performed using a heamocytometer counter (*Weber*). Approximately $2 \times 10^4$ cells in 100 μL culture medium were seeded onto each well of flat bottomed 96-well plates (*Becton Dickinson*) and allowed to attach overnight. Platinum complex solutions were diluted in culture medium such that 5 to 40 μL of each drug solution added to quadruplicate wells produced the final desired concentrations spanning a 4-log range (final DMF concentrations were limited to 0.3%). Following incubation of the cells for 72 h, MTT (1.0 mM) was added to each well and they were incubated for a further 4 h. The culture medium was then removed from each well and DMSO (150μL) (*Sigma*) was added, the plate shaken for 5 s and the absorbance measured immediately at 600 nm in a Victor$^3$V microplate reader (*Perkin Elmer*). $IC_{50}$ values were determined as the drug concentration that reduced the absorbance to 50% of that in untreated control wells.
19 J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna and J. B. Mitchell, *Cancer Res.*, 1987, **47**, 936.
20 M. V. Keck and S. J. Lippard, *J. Am. Chem. Soc.*, 1992, **114**, 3386.
21 S. F. Bellon, J. H. Coleman and S. J. Lippard, *Biochemistry*, 1991, **30**, 8026.
22 R. A. Gossage in *Experiments in Green and Sustainable Chemistry* (ed.: H. W. Roesky and D. K. Kennepohl), Wiley-VCH, Weinheim, 2009, Ch. 4.