Inhibition of mitogen-activated protein kinase (MAPK) and cyclin-dependent kinase 2 (Cdk2) by platinum(II) phenanthroline complexes

Emma S. Child · Savvas N. Georgiades ·
Kirsten N. Rose · Verity S. Stafford · Chirag B. K. Patel ·
Joachim H. G. Steinke · David J. Mann · Ramon Vilar

Received: 30 July 2010 / Accepted: 9 February 2011 / Published online: 26 February 2011
© Springer-Verlag 2011

Abstract Inhibition of protein kinases in the fight against disease remains a constant challenge for medicinal chemists, who have screened multitudes of predominantly planar organic scaffolds, natural and synthetic, to identify potent—albeit not always selective—kinase inhibitors. Herein, in an effort to investigate the potential biological utility of metal-based compounds as inhibitors against the cancer-relevant targets mitogen-activated protein kinase and cyclin-dependent kinase 2, we explore various parameters in planar platinum(II) complexes with substituted phenanthroline ligands and aliphatic diamine chelate co-ligands, to identify combinations that yield promising inhibitory activity. The individual ligands' steric requirements as well as their pattern of hydrogen bond donors/acceptors appear to alter inhibitory potency when modulated.

Keywords Platinum · Kinase · Cdk · Bioinorganic · MAPK · ERK · Inhibitor

Electronic supplementary material The online version of this article (doi:10.1007/s12154-011-0059-5) contains supplementary material, which is available to authorized users.

E. S. Child · D. J. Mann (✉)
Division of Cell and Molecular Biology,
Imperial College London,
South Kensington,
London SW7 2AZ, UK
e-mail: d.mann@imperial.ac.uk

S. N. Georgiades · K. N. Rose · V. S. Stafford · C. B. K. Patel ·
J. H. G. Steinke · R. Vilar (✉)
Department of Chemistry, Imperial College London,
South Kensington,
London SW7 2AZ, UK
e-mail: r.vilar@imperial.ac.uk

Introduction

Many fundamental biological events are regulated by protein phosphorylation. Such phosphorylation is catalysed by members of the protein kinase family and these enzymes are often deregulated in disease. Consequently, there is much interest in developing inhibitors for individual protein kinases for therapeutic purposes. Most protein kinase inhibitors bind to the enzyme at the ATP binding site. Since many protein kinases share similar structurally conserved binding sites, this can lead to undesired off-target effects [1, 2]. This approach has been successfully employed to develop a large number of kinase inhibitors (see Fig. 1 for selected examples). Many kinase inhibitors feature a planar aromatic core that occupies the same space in the protein kinase ATP binding site normally filled by adenine. Specificity is determined largely by addition of hydrogen bond donor/acceptor groups and flexible substituents to these cores (see Fig. 1).

While organic compounds have been extensively studied in this context, far less is known about the therapeutic properties of metal complexes. The structural versatility of metal centres provides a unique opportunity to explore chemical diversity for the design of drugs. Indeed, there are several recent reports of metal complexes that are able to inhibit the activity of specific enzymes [3–5]. For example, vanadium complexes have been shown to act as phosphatase inhibitors [6, 7], platinum complexes to inhibit topoisomerases [8] and kinases [9], and gold complexes to be active against the proteasome [10] and selected phosphatases [11]. Particularly relevant here is the work by Meggers who has developed a series of metal complexes that are very potent inhibitors of different kinases (see Fig. 1b) [12–16]. In these complexes (inspired by the

Fig. 1 **a** Selected examples of organic compounds known to be good kinase inhibitors. **b** Ruthenium complex previously shown to be an ATP-competitive kinase inhibitor

structure of the naturally-occurring protein kinase inhibitor staurosporine—see Fig. 1a), the metal centre plays a structural role organising different organic fragments in the right orientation for optimal interaction with the enzyme. Inspired by this design approach, we engaged in exploring whether platinum(II) complexes (see Scheme 1) containing a planar aromatic ligand (i.e. phenanthroline) substituted

with simple hydrogen bond donor/acceptors (i.e. amines, ureas) plus a chelating hydrophobic amine co-ligand (i.e. ethylenediamine and propylenediamine) could act as kinase inhibitors. As model kinases we have employed mitogen-activated protein kinase (MAPK, also known as extracellular signal-regulated kinase) and cyclin-dependent kinase 2 (Cdk2). p42 and p44 MAPKs are key members of the

Scheme 1 Reaction scheme for the synthesis of ligands and complexes used in this study

signalling cascade functioning downstream of established oncogenes such as Ras and Raf [17]. Cdks are enzymes that play crucial regulatory roles in cell cycle progression and are often hyperactive in cancer [18]. Thus, both of these protein kinases are compelling targets for the development of anticancer drugs [19–22].

Materials and methods

Materials and general procedures All chemicals used in this study were purchased from Sigma-Aldrich (except for K_2PtCl_4 which was kindly donated by Johnson Matthey). The purity of the organic chemicals was verified by ^1H NMR spectroscopy. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 MHz Ultrashield NMR spectrometer. Infrared spectra were recorded on a Perkin-Elmer FTIR spectrometer. Electrospray ionisation mass spectra were recorded on Bruker Daltronics Esquire 3000 spectrometer. Compounds **1**, **2**, **4** and **5** were prepared following literature procedures [23–25].

Synthesis of L^3 A suspension of 1,10-phenanthroline-5-amine (0.20 g, 1.0 mmol) in dry CH_2Cl_2 (20 mL) was heated at 40 °C for 30 mins under a nitrogen atmosphere. Phenylisocyanate (0.11 mL, 1.0 mmol) was then added at the same temperature and the mixture was refluxed for 12 h. After that time, a yellow precipitate formed which was filtered and washed with CH_2Cl_2 (10 mL) to yield L^3 as a pale yellow solid (0.32 g, 98% yield). ^1H NMR (400 MHz, d_6 -DMSO)— δ (ppm): 9.18 (s, 1 H), 9.16 (dd, 1 H, $^3J_{\text{HH}}=4.1$ Hz, $^4J_{\text{HH}}=1.2$ Hz), 9.03 (s, 1 H), 8.99 (dd, 1 H, $^3J_{\text{HH}}=4.3$ Hz, $^4J_{\text{HH}}=2.0$ Hz), 8.68 (dd, 1 H, $^3J_{\text{HH}}=8.4$ Hz, $^4J_{\text{HH}}=1.7$ Hz), 8.43 (s, 1 H), 8.4 (dd, 1 H, $^3J_{\text{HH}}=8.0$ Hz, $^4J_{\text{HH}}=1.7$ Hz), 7.91 (dd, 1 H, $^3J_{\text{HH}}=8.1$ Hz, $^3J_{\text{HH}}=3.9$ Hz), 7.72 (dd, 1 H, $^3J_{\text{HH}}=8.0$ Hz, $^3J_{\text{HH}}=3.9$ Hz), 7.55 (d, 2 H, $^3J_{\text{HH}}=7.2$ Hz), 7.34 (dd, 2 H, $^3J_{\text{HH}}=7.9$ Hz), 7.03 (t, 1 H, $^3J_{\text{HH}}=7.6$ Hz). ^{13}C NMR (100 MHz, d_6 -DMSO)— δ (ppm): 153.6, 150.4, 148.9, 146.2, 143.6, 140.1, 135.8, 132.8, 130.7, 129.6, 124.3, 123.3, 122.6, 119.0, 115.7. IR (KBr, cm^{-1}): 3,202, 3,034 (ν_{NH}), 1,710 (ν_{CO}), 1,623 ($\nu_{\text{C=N}}$), 1,546, 1,498 ($\nu_{\text{C=C}_{\text{ring}}}$), 1,073, 738, 693 ($\nu_{\text{C-C}_{\text{ring}}}$). ES(+)-MS: 315 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}\cdot\frac{1}{2}\text{CH}_2\text{Cl}_2$: C, 65.64; H, 4.21; N, 15.71. Found: C, 65.21; H, 3.78; N, 15.66.

Synthesis of **3** A hot solution (50 °C) of L^3 (0.10 g, 0.32 mmol) in DMSO (10 mL) was added to a solution (50 °C) of K_2PtCl_4 (0.13 g, 0.32 mmol) in water (1 mL) and DMSO (3 mL). The solution was heated (75 °C) for 2.5 h and then was left to cool to room temperature. Upon cooling, a bright, fine yellow solid precipitated. The solid (which proved to be insoluble in common solvents such as DMSO, DMF, water, and acetone) was collected, washed

with DMSO and acetone, and dried under reduced pressure at 90 °C for 2 h (0.12 g, 66% yield). IR (KBr, cm^{-1}): 3,310, 3,086 (ν_{NH}), 1,731 (ν_{CO}), 1,640 ($\nu_{\text{C=N}}$), 1,551, 1,499 ($\nu_{\text{C=C}_{\text{ring}}}$), 1,004, 716, 692 ($\nu_{\text{C-C}_{\text{ring}}}$). 326 ν (Pt-Cl). FAB(+)-MS: 580 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{OCl}_2\text{Pt}$: C, 39.32; H, 2.43; N, 9.65. Found: C, 39.22; H, 2.35; N, 9.53.

Synthesis of **6** This compound was prepared from **2** by an analogous method to the one reported for the synthesis of complex **5** (using propylenediamine instead of ethylene diamine). ^1H NMR (400 MHz, d_6 -DMSO)— δ (ppm): 9.29 (d, 1 H, $^3J_{\text{HH}}=8.3$ Hz), 9.22 (d, 1 H, $^3J_{\text{HH}}=5.5$ Hz), 8.75 (d, 1 H, $^3J_{\text{HH}}=5.5$ Hz), 8.63 (d, 1 H, $^3J_{\text{HH}}=8.3$ Hz), 8.22 (dd, 1 H, $^3J_{\text{HH}}=8.3$ Hz, $^3J_{\text{HH}}=5.5$ Hz), 7.92 (dd, 1 H, $^3J_{\text{HH}}=8.3$ Hz, $^3J_{\text{HH}}=5.5$ Hz), 7.15 (s, 2 H, NH), 7.05 (s, 1 H), 6.71 (broad signal, 4 H, NH), 2.85 (broad triplet, 4 H), 1.51 (multiplet, 2 H). ES⁺-MS: 532 $[\text{M}+\text{H}]^+$. Anal. calcd. for $\text{C}_{15}\text{H}_{19}\text{Cl}_2\text{N}_5\text{Pt}\cdot\frac{3}{4}\text{H}_2\text{O}$: C, 32.83; H, 3.76; N, 12.76. Found: C, 32.56; H, 3.99; N, 13.19.

Synthesis of **7** Ethylenediamine (1.2 mL, 17 mmol) was added to a suspension of compound **3** (0.10 g, 0.17 mmol) in water (10 mL). The solution was heated to 75 °C and stirred for 3 h. During this time, the yellow solid initially in suspension went into solution; this was followed by precipitation of an orange/red solid. The solid was filtered, washed with water and dried in air (0.030 g, 35.7% yield). ^1H NMR (400 MHz, D_2O with trace d_6 -DMSO)— δ (ppm): 9.14 (distorted t, 2 H), 8.97 (distorted t, 2 H), 8.53 (s, 1 H), 8.23 (m, 1 H), 8.13 (m, 1 H), 7.94 (s, 1 H), 7.51 (bs, 5 H), 7.31 (bs, 1 H), 3.41 (bs, 2 H), 3.03 (bs, 2 H). IR (KBr, cm^{-1}): 3,417, 3,010 (ν_{NH}), 1,708 (ν_{CO}), 1,630 ($\nu_{\text{C=N}}$), 1,552, 1,469 ($\nu_{\text{C=C}_{\text{ring}}}$), 1,017, 717, 659 ($\nu_{\text{C-C}_{\text{ring}}}$). ES⁺-MS: 567 $[\text{M-H-2Cl}]^+$. Anal. calcd. for $\text{C}_{21}\text{H}_{22}\text{N}_6\text{OCl}_2\text{Pt}$: C, 39.38; H, 3.46; N, 13.12. Found: C, 39.30; H, 3.55; N, 13.11.

Synthesis of **8** This compound was prepared from **3** using an analogous synthetic procedure to that followed for the preparation of **7** (using propylenediamine instead of ethylenediamine). ^1H NMR (400 MHz, d_6 -DMSO)— δ (ppm): 8.90 (m, 2 H), 8.62 (d, 1 H, $J=6.6$ Hz), 8.06 (t, 1 H, $J=7.3$ Hz), 7.69 (t, 2 H, $J=7.4$ Hz), 7.62 (t, 1 H, $J=7.4$ Hz), 7.53 (d, 2 H, $J=7.4$ Hz), 7.27 (bs, 1 H), 6.61 (bs, 3 H), 6.38 (d, 1 H, $J=6.6$ Hz), 2.82 (triplet, 4 H, $J=7.5$ Hz), 1.86 (multiplet, 2 H). IR (KBr, cm^{-1}): 1,051, 1,176, 1,312, 1,430, 1,463, 1,558, 1,595, 1,613, 3,000 (br), 3,375 (br). ES⁺-MS 584.18 $[\text{M} + \text{H}]^+$ for $[\text{C}_{22}\text{H}_{24}\text{N}_6\text{OPT}]^{2+}$. Anal. Calcd for: $\text{C}_{22}\text{H}_{24}\text{N}_6\text{OCl}_2\text{Pt}\cdot\text{H}_2\text{O}\cdot\frac{1}{2}[\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}_2]$: C, 37.78; H, 4.15; N, 13.13. Found: C 38.15; H 4.55; N 13.24 (Note: the presence of non-coordinated $\text{H}_2\text{N}(\text{CH}_2)_3\text{NH}_2$ in this sample was confirmed by ^1H NMR spectroscopy).

Fig. 2 Comparison of percent-
age activity of MAPK with the
different ligands and platinum
complexes. Purified MAPK was
pre-incubated with 1 mM of the
indicated compounds and then
kinase assays were performed
against substrate MBP. After
separation by PhosTag-
containing SDS-PAGE, gels
were stained and the proportion
of phosphoMBP determined by
its retarded mobility. Data are
the average of three independent
experiments and are depicted as
a percentage activity of the
solvent-only control

Kinase expression and purification Activated p42 MAPK was isolated from bacteria as described by Khokhlatchev et al. [26]. Sf9 cells were grown in Graces insect media (PAA) supplemented with 10% foetal bovine serum (PAA) and incubated at 27 °C. Sf9 cells were infected with baculoviruses directing the expression of cyclin E1 and GST-Cdk2 [27]. Three days post-infection, the cells were harvested by centrifugation at 1,000 rpm at 4 °C for 5 min. Cells were lysed in 10 mL of NETN (20 mM Tris–HCl, pH 8, 150 mM NaCl, 1 mM EDTA, 0.5% NP40) by repeated passage through a 21-gauge needle. Debris was removed by centrifugation at 5,000 rpm at 4 °C for 5 min and the lysate was then incubated with glutathione–sepharose beads for 2 h at 4 °C. Non-bound protein was removed by repeated washing with NETN.

Kinase assays Kinase assays were performed in kinase buffer (25 mM HEPES, pH 7.9, 5 mM MgCl₂, 0.1% 2-mercaptoethanol, 0.1 mM EDTA). One unit of cyclin E1/Cdk2 [27] in 5 µL was incubated for 10 mins at 30 °C with either a compound or the appropriate solvent control

(2 µL of 1:1 DMSO/H₂O or kinase buffer). The enzyme activity was then assayed following addition of a substrate mix (1 µg histone H1 from Roche and 1 µCi γ-32P-ATP in kinase buffer; total reaction volume 17 µL) for 15 min at 30 °C. Reactions were terminated by adding sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer. The protein samples were separated by SDS-PAGE, visualised by autoradiography and quantified by Phosphorimaging. MAPK assays were performed as described above except 2.5 µg myelin basic protein (MBP) was used as substrate together with 1 µM ATP. Reactions were terminated as above and resolved using 12% SDS-PAGE gels doped with 100 µM of Mn(II)-Phos-Tag™ acrylamide. The gels were cast using the components shown in Table S.1 (see Supplementary Information).

Phos-Tag™ acrylamide was synthesised by small modifications of a previously reported method (see [Supplementary Information](#) for synthetic details) [28, 29]. Gels were subsequently stained with Coomassie Brilliant Blue and digitised and quantified using a Fuji LAS 5000 and Aida software.

Fig. 3 Comparison of percent-
age activity of MAPK with
platinum complexes 4–8. Puri-
fied MAPK was pre-incubated
with 63 µM of the indicated
compounds and then kinase
assays were performed against
substrate MBP and analysed as
described in the legend to
Fig. 2

Fig. 4 Dose response assays of MAPK to the compounds **5–8** (no plots are included for **4** and the three free ligands L^1 – L^3 since they showed very little activity). Assays were performed as described in the legend to Fig. 2 except 1 mM, 250 μ M and 63 μ M compound were used. The upper panels show representative stained gels for a single dose response data set with L^x denoting ligands at 1 mM. *Line* denotes assays performed in the absence of ATP, *positive sign* denotes assays performed with addition of the appropriate volume of solvent (1:1 DMSO:H₂O)

Results and discussion

Synthesis of platinum(II) complexes The square planar platinum(II) complexes **4–8** (see Scheme 1) were synthesised with the aim of exploring the influence that hydrogen bonding groups attached to the backbone of the planar phenanthroline

ligand would have on the inhibition of kinases MAPK and Cdk2. In these complexes, the remaining two coordination sites around the platinum(II) centre are occupied by a chelating aliphatic amine (either ethylenediamine or propylenediamine).

Complexes **4** and **5** were prepared from the corresponding $[Pt(L^x)Cl_2]$ precursor (**1** and **2** respectively) following a

previously reported procedure (see Scheme 1). Compound **6** was prepared in an analogous fashion to **5**, by reacting [Pt(L²)Cl₂] (**2**) with propylenediamine. Complexes **7** and **8** were prepared from precursor [Pt(L³)Cl₂] (**3**), which was derived from the new phenanthroline–urea ligand L³ as shown. The prepared complexes were characterised by ¹H NMR and IR spectroscopies (see the “Materials and methods” section for details). In addition, the new compounds were analysed by mass spectrometry (using either FAB(+) or ES(+) ionisation), which showed the expected mass for the complexes. Elemental analysis further confirmed their formulation and purity.

Kinase inhibition assays The inhibitory activity of these complexes was investigated using MAPK with MBP as the substrate. The complexes were added to MAPK and incubated for 10 min at 30 °C before adding the substrate-ATP mix with further incubation for 15 min at 30 °C. The MAPK activity remaining after treatment with different concentrations (vide infra) of the different compounds was measured. Figures 2 and 3 summarise the results of the kinase inhibition assays. From Fig. 2, it is clear that the platinum(II) complexes are much better inhibitors than their respective phenanthroline ligands (L¹, L² or L³). Figure 3 compares the inhibitory activity of the five different platinum complexes at 63 μM concentration. From this data, the most potent inhibitor appears to be compound **6**, an average size complex that reconciles a small H-bonding substituent (NH₂) on the planar phenanthroline system with the most sterically demanding of the two diamine co-ligands used (propylenediamine). The activity of complex **5** (which is also a derivative of the amino-substituted phenanthroline ligand) also shows good inhibitory activity. These two complexes are followed by **7** and **8** (complexes with the urea-substituted phenanthroline) which show only a modest degree of activity. Finally, complex **4** (with the unsubstituted phenanthroline) showed a very poor inhibitory activity against MAPK (see Fig. 3).

To study further the inhibitory ability of these complexes, dose response assays were carried out. As expected, increasing the compound concentration led to higher MAPK inhibition. This was particularly clear for complexes **5–8** (see Fig. 4).

In order to extend our observations, we performed kinase assays with cyclin E1/Cdk2. Again, the free ligands were ineffective inhibitors of kinase activity, but their platinum (II) complexes **4–8** exhibited similar activity against cyclin E1/Cdk2 as that observed against MAPK. Interestingly, complex **6** (featuring the amine-substituted phenanthroline) was also found to be the most effective cyclin E1/Cdk2 inhibitor (see Supplementary Information, Figures S1 and S2).

Conclusions

In this study, we have shown that square planar platinum(II) complexes containing substituted phenanthrolines (a planar aromatic ligand) and a chelating aliphatic diamine are able to inhibit two distinct protein serine/threonine kinases, MAPK and Cdk2 at micromolar concentrations, suggesting such compounds may describe a general scaffold for kinase inhibitor design. The presence of hydrogen bond donor/acceptor groups (namely amine or urea) on the backbone of the phenanthroline ligand is significant for enhancing the inhibitory activity of the complexes relative to an unsubstituted ligand, presumably by engaging in additional interactions with the ATP binding site residues. The results also suggest that in complex **8** (the bulkiest of the compounds studied) we are reaching the spatial limits of the kinases' ATP binding site. Future studies will concentrate on introducing less sterically demanding hydrogen-bonding substituents (as compared to that in L³) on the phenanthroline backbone as well as exploring substituted diamine co-ligands in this type of complex.

Acknowledgements The UK's Engineering and Physical Sciences Research Council (EPSRC) is thanked for financial support. Johnson Matthey PLC is thanked for a generous loan of platinum and we thank Dr Melanie Cobb, University of Texas Southwestern Medical Center for the MAPK expression system.

References

1. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) The protein kinase complement of the human genome. *Science* 298:1912
2. Elphick LM, Lee SE, Gouverneur V, Mann DJ (2007) Using chemical genetics and ATP analogues to dissect protein kinase function. *ACS Chem Biol* 2:299
3. Davies CL, Dux EL, Duhme-Klair A-K (2009) Supramolecular interactions between functional metal complexes and proteins. *Dalton Trans* 46:10141
4. Meggers E (2009) Targeting proteins with metal complexes. *Chem Comm* 9:1001
5. Che C-M, Siu F-M (2010) Metal complexes in medicine with a focus on enzyme inhibition. *Curr Opin Chem Biol* 14:255
6. Rosivatz E, Matthews JG, McDonald NQ, Mulet X, Ho KK, Lossi N, Schmid AC, Mirabelli M, Pomeranz KM, Erneux C, Lam EWF, Vilar R, Woscholski R (2006) A small-molecule inhibitor for phosphatase and tensin homologue deleted on chromosome 10 (PTEN). *ACS Chem Biol* 1:780
7. Schmid AC, Byrne RD, Vilar R, Woscholski R (2004) Bisphosphonate compounds are potent PTEN inhibitors. *FEBS Lett* 566:35
8. Lo Y-C, Ko T-P, Su W-C, Su T-L, Wang AHJ (2009) Terpyridine-platinum(II) complexes are effective inhibitors of mammalian

- topoisomerases and human thioredoxin reductase 1. *J Inorg Biochem* 103:1082
9. Williams DS, Carroll PJ, Meggers E (2007) Platinum complex as a nanomolar protein kinase inhibitor. *Inorg Chem* 46:2944
 10. Milacic V, Chen D, Ronconi L, Landis-Piowar KR, Fregona D, Dou QP (2006) A novel anticancer gold(III) dithiocarbamate compound inhibits the activity of a purified 20S proteasome and 26S proteasome in human breast cancer cell cultures and xenografts. *Cancer Res* 66:10478
 11. Karver MR, Krishnamurthy D, Kulkarni RA, Bottini N, Barrios AM (2009) Identifying potent, selective protein tyrosine phosphatase inhibitors from a library of Au(I) complexes. *J Med Chem* 52:6912
 12. Anand R, Maksimoska J, Pagano N, Wong EY, Gimotty PA, Diamond SL, Meggers E, Marmorstein R (2009) Toward the development of a potent and selective organoruthenium mammalian sterile 20 kinase inhibitor. *J Med Chem* 52:1602
 13. Atilla-Gokcumen GE, Williams DS, Bregman H, Pagano N, Meggers E (2006) Organometallic compounds with biological activity: a very selective and highly potent cellular inhibitor for glycogen synthase kinase 3. *ChemBiochem* 7:1443
 14. Bregman H, Carroll PJ, Meggers E (2006) Rapid access to unexplored chemical space by ligand scanning around a ruthenium center: discovery of potent and selective protein kinase inhibitors. *J Am Chem Soc* 128:877
 15. Debreczeni JE, Bullock AN, Atilla GE, Williams DS, Bregman H, Knapp S, Meggers E (2006) Ruthenium half-sandwich complexes bound to protein kinase pim-1. *Angew Chem Int Ed* 45:1580
 16. Xie P, Williams DS, Atilla-Gokcumen GE, Milk L, Xiao M, Smalley KSM, Herlyn M, Meggers E, Marmorstein R (2008) Structure-based design of an organoruthenium phosphatidylinositol-3-kinase inhibitor reveals a switch governing lipid kinase potency and selectivity. *ACS Chem Biol* 3:305
 17. Kolch W (2005) Coordinating ERK/MAPK signaling through scaffolds and inhibitors. *Nat Rev Mol Cell Biol* 6:827
 18. Harper JW, Adams PD (2001) Cyclin-dependent kinases. *Chem Rev* 101:2511
 19. Coley HM (2008) Targeting the cell cycle as a strategy for treating cancer. In: Yoshida K (ed) *Research Signpost Trends Cell Cycle Res*, p. 237
 20. Diaz-Padilla I, Siu LL, Duran I (2009) Cyclin-dependent kinase inhibitors as potential targeted anticancer agents. *Investig New Drugs* 27:586
 21. Krystof V, Uldrijan S (2010) Cyclin-dependent kinase inhibitors as anticancer drugs. *Curr Drug Targ* 11:291
 22. Morphy R (2010) Selectively nonselective kinase inhibition: striking the right balance. *J Med Chem* 53:1413
 23. Krause-Heuer AM, Gruenert R, Kuehne S, Buczkowska M, Wheate NJ, Le Pevelen DD, Boag LR, Fisher DM, Kasparkova J, Malina J, Bednarski PJ, Brabec V, Aldrich-Wright JR (2009) Studies of the mechanism of action of platinum(II) complexes with potent cytotoxicity in human cancer cells. *J Med Chem* 52:5474
 24. McFadyen WD, Wakelin LPG, Roos IAG, Leopold VA (1985) Activity of platinum(II) intercalating agents against murine leukemia L1210. *J Med Chem* 28:1113
 25. Kemp S, Wheate NJ, Buck DP, Nikac M, Collins JG, Aldrich-Wright JR (2007) The effect of ancillary ligand chirality and phenanthroline functional group substitution on the cytotoxicity of platinum(II)-based metallointercalators. *J Inorg Biochem* 101:1049
 26. Khokhlatchev A, Xu S, English J, Wu P, Schaefer E, Cobb MH (1997) Reconstitution of mitogen-activated protein kinase phosphorylation cascades in bacteria. Efficient synthesis of active protein kinases. *J Biol Chem* 272:11057
 27. Elphick LM, Lee SE, Child ES, Prasad A, Pignocchi C, Thibaudeau S, Anderson AA, Bonnac L, Gouverneur V, Mann DJ (2009) A quantitative comparison of wild-type and gatekeeper mutant Cdk2 for chemical genetic studies with ATP analogues. *ChemBiochem* 10:1519
 28. Kinoshita E, Kinoshita-Kikuta E, Takiyama K, Koike T (2006) Phosphate-binding tag, a new tool to visualize phosphorylated proteins. *Mol Cell Proteomics* 5:749
 29. Kinoshita E, Yamada A, Takeda H, Kinoshita-Kikuta E, Koike T (2005) Novel immobilized zinc(II) affinity chromatography for phosphopeptides and phosphorylated proteins. *J Sep Sci* 28:155