A Metal-Based Inhibitor of NEDD8-Activating Enzyme

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Abstract

A cyclometallated rhodium(III) complex [Rh(ppy)2(dppz)]+ (1) (where ppy = 2-phenylpyridine and dppz = dipyrido[3,2-α:2′,3′-c]phenazine dipyridophenazine) has been prepared and identified as an inhibitor of NEDD8-activating enzyme (NAE). The complex inhibited NAE activity in cell-free and cell-based assays, and suppressed the CRL-regulated substrate degradation and NF-κB activation in human cancer cells with potency comparable to known NAE inhibitor MLN4924. Molecular modeling analysis suggested that the overall binding mode of 1 within the binding pocket of the APPBP1/UBA3 heterodimer resembled that for MLN4924. Complex 1 is the first metal complex reported to suppress the NEDDylation pathway via inhibition of the NEDD8-activating enzyme.

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Introduction

The serendipitous discovery of the chemotherapeutic properties of the now well-known anticancer drug cisplatin has aroused considerable interest in the area of medicinal inorganic chemistry [1–8]. Cisplatin or its analogues bind DNA and disrupt its double helical conformation, thereby impairing DNA transcription or replication processes and ultimately promoting cell death [9]. However, the adverse side effects and drug resistance associated with the prolonged use of cisplatin has prompted the development of novel bioactive metal complexes displaying distinct mechanisms of action to complement the existing arsenal of platinum-derived cytotoxics.

The application of rhodium complexes as chemotherapeutics has attracted much less attention in contrast to their ruthenium activities on substrates regulated by CRLs, such as IκBα and p27. These proteins have important roles in DNA replication and repair, NF-κB signal transduction, cell cycle regulation and inflammation. Targeting a specific E3 such as the CRLs compared to a more upstream enzyme would have the potential to only stabilize a particular subset of proteins, possibly resulting in an improved selectivity profile [43]. The NAE inhibitor MLN4924 [43] (Figure 1) was recently reported to be effective against both solid (colon, lung) and hematological (myeloma, lymphoma) human cancer cells. We have previously employed high-throughput virtual screening to identify 6,6′-biapigenin as only the second inhibitor of NEDD8-activating enzyme from a natural product and natural product-like database [44]. While transition metal complexes have been widely utilized for the treatment of cancer ubiquitin E3 ligases (CRLs) by NEDD8, a ubiquitin-like protein, is known to be essential for the CRL-mediated ubiquitination of downstream targets in the ubiquitin-proteasome system [34,35], which is critically involved in protein homeostasis. The NEDD8-activating enzyme (NAE) plays an analogous role to the ubiquitin E1 enzyme [36]. NAE is involved in the first step of CRL activation, through activation of NEDD8 and its subsequent transfer to Ubc12, the E2 conjugating enzyme of the NEDD8 pathway. NEDD8 then becomes conjugated to a conserved lysine residue near the C-terminus of the cullin proteins of the CRLs. This covalent modification is required for the cullin complex to recruit an ubiquitin-charged E2 enzyme in order to facilitate the polyubiquitination of proteins, yielding substrates for proteasomal degradation [37–42]. Thus, the targeted inhibition of NAE could mediate the rate of ubiquitination and the subsequent degradation of substrates regulated by CRLs, such as IκBα and p27. These proteins have important roles in DNA replication and repair, NF-κB signal transduction, cell cycle regulation and inflammation.
their activity against NEDD8-activating enzyme has not been explored. Inspired by the above findings as well as pioneering works from the Meggers’s group on the design of structurally rigid octahedral ruthenium(II) [40–53] and iridium(III) [53–55] complexes as shape-complementary inhibitors of protein kinases, we sought to investigate the biological effects of a series of cyclometallated rhodium(III) complexes on the NEDD8 pathway. Cyclometallated rhodium(III) complexes containing the dipyr- ido[3,2-a,3"-c]
planar or tetrahedral symmetry, thus allowing much larger complex adopts an octahedral geometry rather than a square planar scaffold with limited conformational freedoms of the co-
supported. In contrast, the octahedral geometry of this rhodium complex provides a globular and rigid scaffold with limited conformational freedoms of the co-
trials, and complete inhibition at 12.5 μM (Figure 2b). However, no significant inhibition of Ubc-9-SUMO formation was observed at the same concentrations tested. This result shows that complex 1 was able to suppress the activity of NAE in a cellular system but not the closely related SAE, which is consistent with the result from cell-free western blotting.

We then screened three analogues of complex 1 in order to establish a brief structure-activity relationship for the observed effects against NAE activity in the cell-free assay (Figure S2). Interestingly, the results showed that functionalizing the dppz ligand with methyl groups (complex 2) or the ancillary ppy ligands with aldehyde functionalities at the ppy ligands (complex 4) resulted in the weakest NAE inhibitory activity (IC50 = ca. 13 μM). Furthermore, the uncoordinated dppz ligand was found to be inactive against Ubc12-NEDD8 conjugation at a relative high concentration tested.
NF-κB, presumably via its ability to inhibit NAE activity. p27, a cell cycle inhibitor, has been found to block the degradation of CRL substrates (i.e. IκB). In summary, complex 1 resulted in accumulation of CRL substrates [43]. To examine this, we first investigated the ability of complex 1 to inhibit NAE-regulated IκB degradation. Caco-2 cells were stimulated with TNF-α to induce IκB degradation, which was monitored using Western blot analysis (Figure 3a). Encouragingly, we observed that inhibition of IκB degradation by TNF-α was blocked by complex 1 in a dose-dependent manner, with potency comparable to MLN4924.

Figure 2. Complex 1 inhibits NAE activity in a dose-dependent manner. Western blots show dose-dependent inhibition of a) Ubc-12-NEDD8 formation in a cell-free system and b) cellular Ubc-12-NEDD8 levels by 1. MLN4924 was included for comparison.

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Western blot analysis (Figure 3a). Encouragingly, we observed that the induction of IκB degradation by TNF-α was blocked by 1 in a dose-dependent manner, with potency comparable to the control compound MLN4924 at 2.5 μM. Similarly, treatment of Caco-2 cells with 1 resulted in accumulation of p27 protein, with comparable effects to MLN4924. In summary, complex 1 has been found to block the degradation of CRL substrates (i.e. IκB and p27), presumably via its ability to inhibit NAE activity.

Since the accumulation of IκB would be expected to repress NF-κB activity, we next investigated the ability of complex 1 to interfere with NF-κB signaling in human cells. Caco-2 cells transfected with the luciferase reporter plasmid harboring NF-κB-luciferase gene were pre-incubated with 1 prior to TNF-α activation. The inhibition of TNF-α-induced NF-κB signaling is manifested as a reduction in luciferase activity. We observed a dose-dependent reduction of NF-κB activity by 1, with an estimated IC50 value of ca. 0.77 μM (Figure 4). The potencies of 1 and MLN4924 were found to be similar in a parallel experiment, with 90% inhibition of NF-κB activity at a concentration of 2.5 μM. These results suggest that complex 1 inhibits NF-κB signaling in human cells, and are consistent with the observed accumulation of IκB induced by 1 as described previously.

The cytotoxicity of complex 1 towards human cancer cells was investigated using an MTT assay. Complex 1 inhibited the growth of the Caco-2 cells with an IC50 value of ca. 0.3 μM, which was approximately ten-fold more potent than MLN4924 in a parallel experiment (Figure S3). We believe that the cytotoxicity of 1 against cancer cells is due, at least in part, to its ability to up-regulate p27 protein level and attenuate NF-κB signaling as described previously. p27 is a cell cycle regulator which controls cell cycle progression during the G1 phase [67]. The loss of function in p27 can lead to uncontrolled cell proliferation and the development of cancer.
highest-scoring binding pose of complex 1 overlapped considerably with that of MLN4924 (Figure 5b) [68]. The dppz ligand of 1 was predicted to occupy the hydrophobic pocket near Met101 and Ile148 as well as the ribose binding region located between Asp100 and Asp 167, contacting similar residues as the indan and dihydropyrrolopyrimidine systems of MLN4924. We presumed that the structural and electronic similarity between the dppz moiety and the aromatic ring systems of MLN4924 possibly contributes to the favourable binding interaction between the rhodium(III) complex and UBA3 subunit. The rhodium(III) metal centre is situated in the central canyon-like groove of NAE in a similar region to that occupied by the γ-phosphate group of

**Figure 3.** Dose-dependent inhibition of IkBa degradation by 1. Caco-2 cells were pre-incubated with indicated concentrations of 1 for 16 hours and then stimulated with 5 ng/ml of TNF-α at indicated time intervals. Whole cell lysates were analyzed by Western blot using anti-IkBα antibody. Densitometry estimates of IkBa levels normalized with GAPDH are shown under each lane. b) Caco-2 cells were treated with 1 or MLN4924 for 16 h. The cell lysates were immunoblotted to analyse the level of CRL substrate p27. doi:10.1371/journal.pone.0049574.g003

**Figure 4.** Complex 1 suppresses NAE-regulated NF-κB-dependent luciferase reporter gene expression in a dose-dependent manner. Caco-2 cells were transfected with the NF-κB-dependent luciferase reporter p3EnhConA-Luc gene, treated with 1 for 16 hours, and then stimulated with 5 ng/ml of TNF-α for 3 hours. Luciferase expression was measured and normalized with β-galactosidase activity. Results are expressed as fold change compared to TNF-α stimulation alone and the errors bar show the standard derivation of triplicate results. MLN4924 was included for comparison. doi:10.1371/journal.pone.0049574.g004
ATP. Interestingly, the three-dimensional structural arrangement of the ligands means that one of the phenylpyridine moieties of 1 is closer to the APPBP1 subunit of NAE compared to MLN4924, allowing potential hydrophobic interactions to form with the residues near Lys124 and Asp273. On the other hand, the other phenylpyridine ligand is located closer to NEDD8, in a similar area to that of the ribose ring of MLN4924. The lowest-energy binding mode of 1 was also significantly similar to that for ATP (Figure 5c). For reference, the binding score for 1 with NAE was calculated to be –32.89, compared to –30.3 and –30.8 for ATP and MLN4924, respectively. Based on the strong calculated binding score of 1 to the active site of NAE, as well as the multiple Van der Waals interactions predicted between 1 with the UBA3, APPBP1 and NEDD8 subunits, we propose that 1 may act as a reversible ATP-competitive inhibitor of NAE by occupying the ATP-binding domain. Other possible binding poses of 1 and their corresponding docking scores are also included in the Supporting Information (Table S1).

Conclusion

In summary, we have identified the rhodium(III) complex 1 as a new inhibitor of NAE. The identification of the metal-based inhibitor 1 represents, to our knowledge, the first reported example of NAE inhibition by a transition metal complex and only the third example of a small-molecule inhibitor of NAE. Complex 1 was found to inhibit NAE activity in a cell-free assay and also reduced Ubc12-NEDD8 conjugate levels in human cancer cells. Significantly, complex 1 blocked CRL substrate degradation and repressed NF-κB activation in human cancer cells with comparable potency to MLN4924, the strongest NAE inhibitor reported to date. Our brief structure-activity relationship analysis and molecular modeling results suggest that the unique structural features of the octahedral coordination geometry of the Rh(III) complex 1 allows it to form optimal interactions with NAE, which is envisaged to contribute significantly to its binding potency and selectivity for NAE over the closely-related enzyme SAE. Based on our findings, we believe that this bioactive complex can potentially be developed as a useful lead to generate more potent analogues for chemotherapeutic or autoimmune/inflammatory applications.

Materials and Methods

Material and Cell Lines

All the chemicals, unless specified, were purchased from Sigma-Aldrich and were used as received. NEDD8 Conjugation Initiation Kit and anti-Ubc12 rabbit polyclonal antibody was obtained from Boston BioChem (Cambridge, MA, USA). Caco-2 cells were purchased from American Type Culture Collection (Manassas, VA, USA), catalog number: HTB-37. Cells were cultured in Minimum Essential Media containing 10% fetal bovine serum and Minimum Essential Media containing 10% fetal bovine serum and were incubated at 37°C/5% CO₂. Deuterated solvents for NMR purposes were obtained from Armar and used as received.

General Experimental

All ¹H and ¹³C NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz. The ¹H and ¹³C chemical shifts were referenced internally to solvent shift (CDCl₃: ¹H δ 7.26, ¹³C δ 77.2; MeOD: ¹H δ 3.31, ¹³C δ 49.15; d₆-DMSO: ¹H δ 2.50, ¹³C δ 39.5; CD₃CN: ¹H, δ 1.94, ¹³C δ 118.7). Chemical shifts are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ¹H and ±0.05 for ¹³C. Coupling constants are typically ±0.1 Hz for ¹H-¹H and ±0.5 Hz for ¹H-¹³C couplings.

Figure 5. Low-energy binding conformations of a) 1, b) MLN4924 and c) both 1 and ATP bound to NAE heterodimer generated by virtual ligand docking. Proteins APPBP1 (blue), UBA3 (purple) and NEDD8 (yellow) are displayed in ribbon form. Small molecules are depicted as a ball-and-stick model showing showing carbon (yellow), hydrogen (grey), oxygen (red), nitrogen (blue), phosphorus (orange) and sulfur (green). Non-polar hydrogens were not shown.

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The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. All NMR data were acquired and processed using standard Bruker software (Topspin). MALDI-MS analysis was performed using a Bruker Autoflex II mass spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser (337 nm, wavelength; 3 ns pulse width) operated in reflectron mode with accelerating voltage, grid voltage and delayed extraction time set to 19 kV, 90%, and 120 ns, respectively. Unless otherwise stated, each mass spectrum was acquired as an average of 200 laser shots at 10.0 Hz frequency.

Synthesis of Rhodium(III) Complexes

Preparation of dipyrido[3,2-a:2',3'-c]phenazine (dppz) derivatives. A mixture of 1,10-phenanthroline-5,6-dione (2 mmol) and appropriate o-phenylenediamine
derivate (2.4 mmol) in ethanol (200 mL) was stirred at 50°C for 2 h and then at room temperature overnight. The precipitate was collected by filtration and washed with cold ethanol (3 × 15 mL). The crude product was then recrystallized from methanol.

11-Nitrotripyrdo[3,2-a',3'-c]phenazine. Yellow solid, 557.1 mg, 85.1%. 1H NMR (400 MHz, CDCl3): 9.57 (t, J = 8.0 Hz, 2H), 9.30 (t, J = 8.0 Hz, 2H), 9.23 (s, 1H), 8.65 (d, J = 8.0 Hz, 2H), 8.46 (d, J = 8.0 Hz, 2H), 7.43-7.05 (m, 2H). 13C NMR (400 MHz, CDCl3): 152.3, 148.3, 147.7, 143.9, 140.0, 139.7, 133.8, 133.6, 128.3, 127.9, 104.4. Maldi-TOF-HRMS: Calcd for C42H25N7O4Rh [M-OTf]+: 721.1582 Found: 721.1552.

Complex 2. Yellow solid, 29.8 mg, 35.3%. 1H NMR (400 MHz, CD3CN): 9.65 (s, 2H), 9.48 (d, J = 8.0 Hz, 2H), 8.39 (s, 2H), 8.29 (d, J = 8.0 Hz, 2H), 8.16-8.10 (m, 6H), 7.69-7.70 (m, 2H), 8.25 (s, 2H), 7.8 (d, J = 8.0 Hz, 2H). 13C NMR (400 MHz, CD3CN): 150.6, 146.3, 140.7, 132.7, 131.0, 126.2, 125.9, 123.9, 123.1, 121.3, 77.1480 Found: 777.1462.

Complex 3. Yellow solid, 27.6 mg, 48.8%. 1H NMR (400 MHz, CD3CN): 9.23 (s, 2H), 9.68 (d, J = 8.0 Hz, 2H), 8.53 (s, 2H), 8.39 (s, 2H), 8.14-8.10 (m, 6H), 7.75-7.70 (m, 2H), 7.90-7.86 (m, 4H), 7.57 (s, 2H), 8.00 (s, 2H), 7.99 (s, 2H). 13C NMR (400 MHz, CD3CN): 191.3, 166.8, 162.5, 151.3, 150.1, 149.6, 147.5, 143.9, 141.1, 139.6, 138.9, 136.2, 135.3, 133.9, 132.9, 127.2, 126.5, 125.4, 121.9, 20.2. Maldi-TOF-HRMS: Calcd for C42H25N7O4Rh [M-OH]−: 794.1017 Found: 794.1048.

Preparation of the complexes 2–4. The procedure for preparation of complex 1 was adopted using the corresponding precursor complex and the dpbz derivative.

Preparation of the complexes 2–4. The procedure for preparation of complex 1 was adopted using the corresponding precursor complex and the dpbz derivative.
global-energy optimization method consists of: 1) a change in conformation, as a result of the random changes in the free variables according to a predefined continuous probability distribution; 2) the local-energy minimization of analytical differentiable terms; 3) calculation of the complete energy, where the nondifferentiable terms, such as entropy and solvation energy, are included; 4) the acceptance or the rejection of the total energy based on the Metropolis criterion and return to step (1). The binding between the complex I and NAE-NEDD8 was evaluated with the use of binding energy, which includes grid energy, continuum electrostatic, and entropy terms. The initial model of NAE was built from the X-ray crystal structure of the quaternary APPBP1-UBA3- NEDD8-ATP complex (PDB: 1R4N) [70], according to a previously reported procedure. [44] Hydrogen and missing heavy atoms were added to the receptor structure followed by local minimization by using the conjugate gradient algorithm and analytical derivatives in the internal coordinates. In the docking analysis, the binding site was assigned across the entire structure of the protein complex. The complex I was assigned the MMFF force field atom types and charges, and the generated structure was then subjected to Cartesian minimization. The ICM docking was performed to find the most favorable orientation. The resulting trajectories of the complex between the complex I and the quaternary protein complex were energy minimized, and the interaction energies were computed.

Supporting Information

Figure S1 Synthetic scheme for the preparation of complexes 1-4. (TIF)

Figure S2 Inhibition of Ubc12-NEDD8 conjugation in vitro by the cyclometallated Rh(III) complexes 2-4. (PNG)

Figure S3 MTT cytotoxicity assay showing the cell viability as a function of the concentration of complex 1 and MLN4924. (PNG)

Table S1 Lower-ranking binding conformations of 1 to NAE and their corresponding docking scores generated by virtual ligand docking. (DOCX)

Author Contributions

Conceived and designed the experiments: DLM CHL HMW. Performed the experiments: HJZ HY DSHC. Analyzed the data: HJZ HY DSHC. Contributed reagents/materials/analysis tools: DLM CHL. Wrote the paper: DSHC DLM CHL. Directed the research: DLM CHL. HMW. Contributed reagents/materials/analysis tools: DLM CHL. Wrote the paper: HJZ HY DSHC. Directed the research: DLM CHL.

References

1. Wang D, Lippard SJ (2005) Cellular processing of platinum anticancer drugs. Nat Rev Drug Discov 4: 307–320.
2. Hartinger CG, Dyson PJ (2009) Bioorganometallic chemistry-from teaching paradigms to medicinal applications. Chem Soc Rev 38: 391–401.
3. Meggers E (2009) Targeting proteins with metal complexes. Chem Commun: 1001–1010.
4. Klein AV, Hambly TW (2009) Platinum Drug Distribution in Cancer Cells and Tumors. Chem Rev 109: 4911–4920.
5. Nobili S, Mini E, Landini I, Gabbiani C, Casini A, et al. (2010) Gold compounds as anticancer agents: chemistry, cellular pharmacology, and preclinical studies. Med Res Rev 30: 530–580.
6. Che C-M, Sia F-M (2010) Metal complexes in medicine with a focus on enzyme inhibition. Curr Opin Chem Biol 14: 255–261.
7. Liu H-K, Sadler PJ (2011) Metal Complexes as DNA Intercalators. Acc Chem Res 44: 349–359.
8. Noffke AL, Habtemariam A, Pizarro AM, Sadler PJ (2012) Designing organometallic compounds for catalysis and therapy. Chem Commun 48: 5219–5246.
9. Jung Y, Lippard SJ (2007) Direct Cellular Responses to Platinum-Induced DNA Damage. Chem Rev 107: 1387–1407.
10. Dorcier A, Ang WH, Bolan˜o S, Gonsalvi L, Juillerat-Jeannerat L, et al. (2006) In Vitro Evaluation of Rhodium and Osmium RAPTA Analogues: The Case for Organometallic Anticancer Drugs Not Based on Ruthenium. Organometallics 25: 4960–4966.
11. Angulo-Boza AM, Cháfofides HT, Aguirre JD, Choiou A, Fu PKL, et al. (2006) Dihiodo[II,II] Complexes: Molecular Characteristics that Affect in Vitro Activity. J Med Chem 49: 6841–6847.
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12. Aguirre JD, Angeles-Boza AM, Chassai A, Turco G, Pellois JP, et al. (2009) Anticancer activity of heterodimeric dinuclear complexes of rhodium: A study of intercalating properties, hydrophobicity and in cellular activity. Dalton Trans: 10806 –10812.

13. Burtis R, Doboschek M, Triller A, Ott I, Spehr M, et al. (2010) Cell-Selective Apoptosis-inducing Rhodium(III) Crown Triether Complexes. ChemMedChem: 5: 1123 –1133.

14. Ernst RJ, Komor AC, Barton JK (2011) Selective Cytotoxicity of Rhodium Metallosensors in Mismatch Repair-Deficient Cells. Biochemistry: 50: 10919 –10928.

15. Geldmacher Y, Rubbiani R, Wefelmeier P, Prokop A, Ott I, et al. (2011) Synthesis and DNA-binding properties of apoptosis-inducing cytoxic half-sandwich rhodium(II) complexes with methyl-substituted polypyrrolidy ligands. J Organomet. Chem: 706: 1021 –1031.

16. Sui F-M, Liu W-S, Yan K, Lok C-N, Low K-H, et al. (2012) Anticancer dirhodium(II) carboxylates as potent inhibitors of ubiquitin-proteasome system. Chem Sci 3: 1785 –1793.

17. Dieckmann S, Riedel R, Harns K, Meggers E. (2012) Pyridocarbaole-Rhodium(III) Complexes as Protein Kinase Inhibitors. Eur J Inorg Chem 2012: 813 –821.

18. Erck A, Raimen L, Whiteyman J, Chang I-M, Kimball AP, et al. (1974) Studies on the Interaction of Rhodium(II) Acetate and Rhodium(III) Acetate in Tumor-bearing Mice. Cancer Res 36: 2204 –2209.

19. Chifotides HT, Dunbar KR (2005) Interactions of Metal–Metal-Bonded Anticancer Active Complexes with DNA Fragments and DNA. Acc Chem Res: 38: 146 –156.

20. Dunham SU, Chifotides HT, Mikulis K, Burr AE, Dunbar KR (2004) Covalent Binding and Interstrand Cross-Linking of Duplex DNA by Dirhodium(II) Carboxylate Complexes. Biochemistry 44: 996 –1003.

21. Chifotides HT, Koonen JM, Kang M, Tichy SE, Dunbar KR, et al. (2004) Binding of DNA Purine Sites to Dirhodium Comounds Probed by Mass Spectrometry. Inorg Chem 43: 6177 –6180.

22. Clarke MJ, Zhu F, Frasca DR (1999) Non-Platinum Chemotherapeutic Metallotherapeutics. Chem Rev 99: 2511 –2543.

23. Espo’sito BP, Faljoni-Ala’rio A, de Menezes JFS, de Brito HF, Najjar R (1999) A Metalloinsertor in Mismatch Repair-Deficient Cells. Biochemistry 44: 996 –1003.

24. Yang D, Tan M, Wang G, Sun Y (2012) The p21-Dependent Radiosensitization Mechanism of Human Breast Cancer Cells by MLN4924, an Investigational Inhibitor of the NEDD8-Activating enzyme. Mol Cancer Ther 11: 942 –950.

25. Howard RA, Spring TG, Bear JL (1976) The Interaction of Rhodium(II) complexes and human serum albumin. J Inorg Biochem 75: 55 –61.

26. Howard RA, Spring TG, Bear JL (1976) The Interaction of Rhodium(II) Carboxylates. Enzymes of the NEDD8 Conjugation Pathway. J Biol Chem 274: 8841 –8848.

27. Swords RT, Kelly KR, Smith PG, Garnsey JJ, Mahalingam D, et al. (2010) Inhibition of NEDD8-activating enzyme: a novel approach for the treatment of acute myeloid leukemia. Blood 115: 3796 –3800.

28. Watson IR, Irwin MS, Ohl M (2011) NEDD8 Pathways in Cancer, Sine Quibus Non. Cancer Cell 19: 168 –176.

29. Wang M, Mederos RC, Erba HP, DeAngelo DJ, Giles FJ, et al. (2011) Targeting protein neddylation: a novel therapeutic strategy for the treatment of cancer. Expert Opin Ther Targets 15: 253 –264.

30. Luo Z, Yu G, Lee HW, Li L, Wang I, et al. (2012) The Nedd8-activating enzyme inhibitor MLN4924 induces apoptosis and suppresses liver cancer cell growth. Cancer Res: DOI: 10.1158/0008-5472.can-1112-0388.

31. McMillin DW, Jacobs HM, Delmore JE, Buon L, Hunter ZR, et al. (2012) Potent and Selective Protein Kinase Inhibitors. J Am Chem Soc 134: 5976 –5986.

32. Wiens A, Vlecken DH, Schmitz DJ, Kra¨ling K, Harms K, et al. (2010) Iridium Complex with Antiangiogenic Properties. Proc Natl Acad Sci USA 107: 14129 –14134.

33. Podust VN, Brownell JE, Gladysheva TB, Luo R-S, Wang C, et al. (2000) A Metalloinsertor of Glycogen Synthase Kinase 3. J Am Chem Soc 122: 8771 –8779.

34. Wilbuer A, Vlecken DH, Schmitz DJ, Kra¨ling K, Harms K, et al. (2004) An Organometallic Inhibitor for Glycogen Synthase Kinase 3. J Am Chem Soc 126: 11395 –11398.

35. Podust VN, Brownell JE, Meggers E. (2009) 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 130: 877 –804.

36. Tripathi P, Aggarwal A (2006) NF-kB transcription factor: a key player in the promotion of immune responses. Curr Sci 90: 518 –523.

37. McDaniel ND, Coughlin FJ, Tinker LL, Bernhard S (2007) Cyclometalated Iridium(III) Aquo Complexes: Efficient and Tunable Catalysts for the enumerative Oxidation of Ammonium. J Am Chem Soc 130: 201 –207.

38. Lobry MS, Hudson WR, Pascal RA, Bernhard S (2004) Accelerated Luminescence Discovery through Combinatorial Synthesis. J Am Chem Soc 126: 14129 –14133.

39. Bieda R, Dobroschke M, Triller A, Ott I, Spehr M, et al. (2010) Cell-Selective, Sandwich Rhodium(III) Carboxylates with Methyl-substituted Polypyridyl ligands. J Am Chem Soc 132: 10812 –10815.

40. Ghosh S, Baltimore D (1990) Activation in vitro of NF-κB by phosphorylation of its inhibitory subunit IκBα. Nature 365: 182 –185.

41. Henkel T, Machleidt T, Alkalay I, Kronke M, Ben-Neriah Y, et al. (1993) Rapid IκBα degradation is necessary for activation of transcription factor NF-κB. Proc Natl Acad Sci USA 90: 5658 –5662.

42. Wilbuer A, Vecken DH, Schmitz DJ, Kra¨ling K, Harms K, et al. (2010) Iridium Complex with Antiangiogenic Properties. Proc Natl Acad Sci USA 107: 14129 –14134.

43. Podust VN, Brownell JE, Gladysheva TB, Luo R, Wang C, et al. (2000) A Metalloinsertor of Glycogen Synthase Kinase 3. J Am Chem Soc 122: 8771 –8779.

44. Wilbuer A, Vecken DH, Schmitz DJ, Kra¨ling K, Harms K, et al. (2004) Accelerated Luminescence Discovery through Combinatorial Synthesis. J Am Chem Soc 126: 14129 –14133.

45. Podust VN, Brownell JE, Gladysheva TB, Luo R-S, Wang C, et al. (2000) A Metalloinsertor of Glycogen Synthase Kinase 3. J Am Chem Soc 122: 8771 –8779.

46. Podust VN, Brownell JE, Gladysheva TB, Luo R-S, Wang C, et al. (2000) A Metalloinsertor of Glycogen Synthase Kinase 3. J Am Chem Soc 122: 8771 –8779.
69. Totrov M, Abagyan R (1997) Flexible protein-ligand docking by global energy optimization in internal coordinates. Proteins Suppl. 1: 215–220.

70. Walden H, Podgorski MS, Huang DT, Miller DW, Howard RJ, et al. (2003) The Structure of the APPBP1-UBA3-NEDD8-ATP Complex Reveals the Basis for Selective Ubiquitin-like Protein Activation by an E1. Mol Cell 12: 1427–1437.