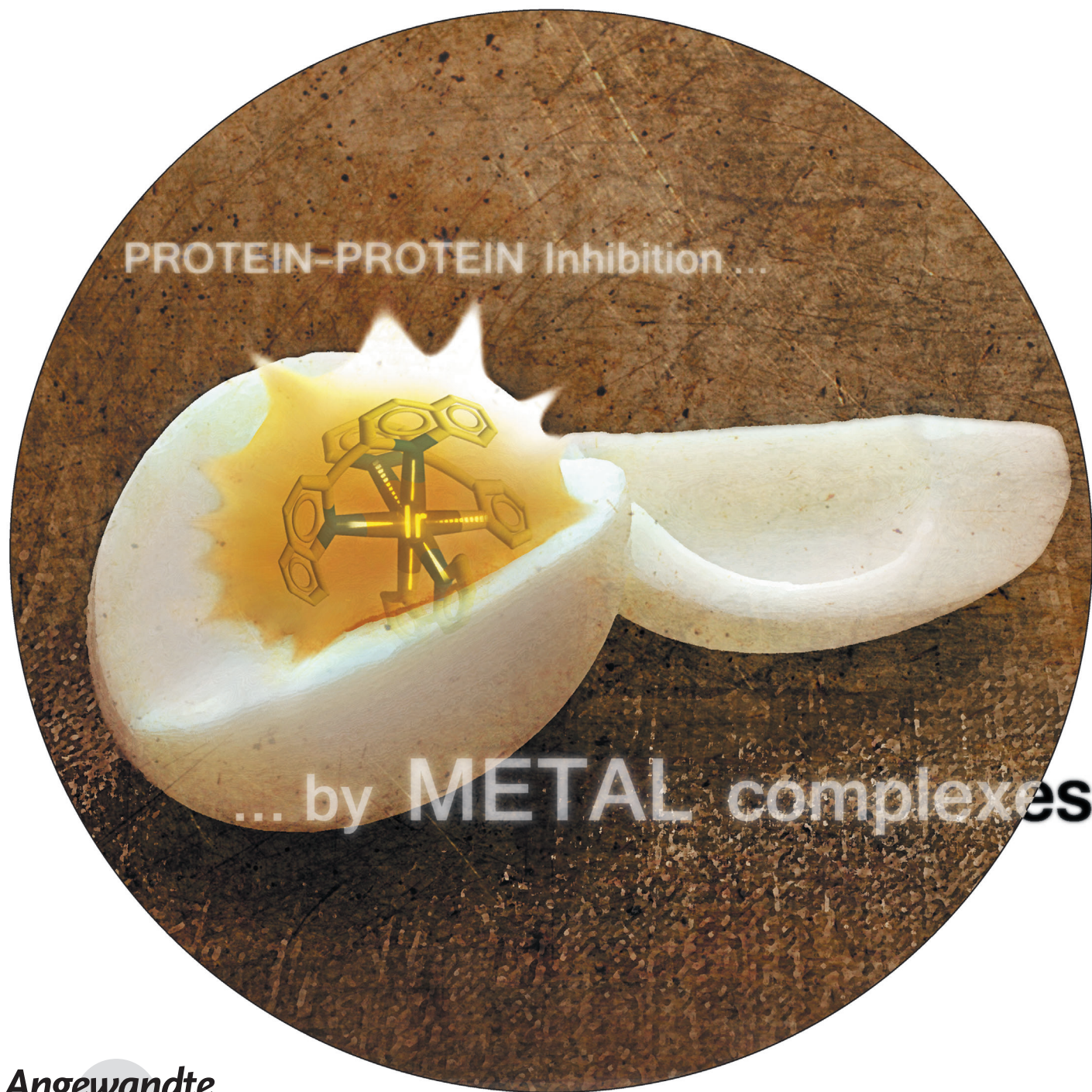


A Metal-Based Inhibitor of Tumor Necrosis Factor- α **

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Tumor necrosis factor- α (TNF- α) is a pro-inflammatory cytokine that plays a critical role in key biological processes, including haematopoiesis, immunity, and inflammation.^[1] Aberrant TNF- α activity has been associated with septic shock, diabetes, tumorigenesis, transplant rejection, viral replication, and autoimmune diseases such as rheumatoid arthritis, psoriatic arthritis, and Crohn's disease.^[2] The synthetic therapeutic antibodies etanercept, infliximab, and adalimumab bind to TNF- α directly, inhibiting the interaction between TNF- α and the tumor necrosis factor receptor (TNFR).^[3] Clinical trials of infliximab and etanercept have shown the success of TNF- α inhibitors in the stabilization of ovarian cancer.^[4] However, disadvantages of synthetic antibodies include their ability to elicit an autoimmune, anti-antibody response and the potential weakening of the immune defenses to opportunistic infections. This has stimulated the development of alternative small-molecule-based therapies as inhibitors of TNF- α .^[5] Most such small-molecule inhibitors target TNF- α indirectly, by down-regulating the expression of TNF- α .^[6] Only a few small molecules have been reported to directly disrupt the TNF- α /TNFR interaction, such as the polysulfonated naphthylurea, suramin and its analogues,^[7] the indole-linked chromone SPD304,^[8] and the isoindolo[2,1-a]quinazoline derivatives.^[9] High-throughput virtual screening (HTVS) represents an alternative approach in discovering potential therapeutic agents that targeting various forms of DNA, proteins, and protein–protein interfaces.^[10] In this endeavor, we have recently reported the discovery of direct TNF- α small-molecule inhibitors using

HTVS of natural product and FDA-approved drug databases.^[11]

While transition metal complexes have been widely utilized for the treatment of various diseases such as cancer, inflammation, and Alzheimer's disease,^[12] their activity for direct TNF- α inhibition has not yet been explored. As demonstrated recently by the group of Meggers and in our previous work, kinetically inert octahedral metal complexes can be used as structurally diverse molecular scaffolds for the design of potent and selective enzyme inhibitors.^[13] Herein, we have investigated the cyclometalated iridium(III) complex [Ir(ppy)₂(biq)]PF₆ containing the bidentate 2-phenylpyridinato (ppy) ligand and 2,2'-biquinoline (biq) ligand for the following reasons: 1) the iridium(III) complex adopts an octahedral geometry rather than square-planar or tetrahedral symmetry, thus allowing a larger structural complexity; 2) the octahedral geometry of the iridium complex provides a large conformational flexibility, which may allow previously inaccessible regions of the chemical space in the TNF- α binding site to be sampled;^[13c,14] 3) the complex [Ir(ppy)₂(biq)]PF₆ can be synthesized conveniently and rapidly; and 4) the extended aromatic biq ligand can potentially form favorable interactions with the hydrophobic binding interface of TNF- α . We describe herein the first application of the octahedral iridium complex **1** (Figure 1) as a direct inhibitor of TNF- α . To our knowledge, this is the first example of a metal-based inhibitor of TNF- α .

The biologically active TNF- α complex consists of a trimer of identical subunits. Previously reported small-molecule inhibitors of TNF- α have been postulated to bind to the TNF- α dimer, blocking the association of the third subunit and the formation of the active trimer complex.^[6] Similar to most protein–protein interfaces, the binding site on the TNF- α dimer is mostly hydrophobic and featureless, primarily consisting of glycine, leucine, and tyrosine subunits. Consequently, the interaction between small molecules and the TNF- α dimer has been presumed to be mainly hydrophobic and shape-driven. We anticipated that the aromatic ligands of the iridium(III) complex **1**, particularly biq, could facilitate favorable hydrophobic-binding interactions with the TNF- α dimer binding interface.

To investigate the mechanism of action of the metal complexes towards the TNF- α dimer, we first used molecular modeling to analyze the interaction of the two enantiomers of complex **1** (Δ -**1** and Λ -**1**) with TNF- α using the X-ray co-crystal structure of the TNF- α dimer with SPD304

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[**] This work is supported by Hong Kong Baptist University (FRG2/10-11/008 and FRG2/11-12/009), Environment and Conservation Fund (ECF Project 3/2010), Centre for Cancer and Inflammation Research, School of Chinese Medicine (CCIR-SCM, HKBU), the Research Fund for the Control of Infectious Diseases (RFCID/11101212), the Research Grants Council (HKBU/201811), and the University of Macau (Start-up Research Grant to C.-H. Leung), MYRG091(Y1-L2)-ICMS12-LCH and MYRG121(Y1-L2)-ICMS12-LCH). We especially thank Prof. Eric Meggers and his colleagues for providing Δ -**1** and Λ -**1** for us to perform the biological assays.



Supporting information for this article (experimental details) is available on the WWW under <http://dx.doi.org/10.1002/anie.201202937>.

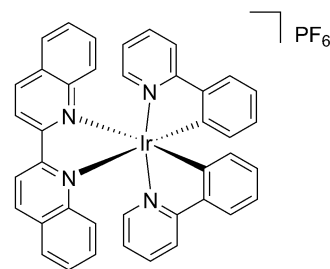


Figure 1. Chemical formula of [Ir(ppy)₂(biq)]PF₆ (**1**). Only one enantiomer (Δ -**1**) is shown for clarity.

(PDB code: 2AZ5).^[8] The binding interaction was evaluated using the Molsoft ICM method (ICM-Pro 3.6-1d molecular docking software).^[15] The geometry of both isomers was optimized using density functional theory (DFT) calculations. The ligands were docked to a grid representation of the receptor and assigned a score reflecting the quality of the complex. In the lowest-energy binding pose of Δ -**1** and Λ -**1** to the TNF- α dimer, both isomers are observed to occupy the same binding pocket of SPD304 (Figure 2). The big ligand of Δ -**1** contacts the β -strand of the A subunit of TNF- α , while the two ppy moieties interact with the β -strand from the B subunit inside the binding pocket (Figure 2a). In comparison, the Λ -**1** enantiomer is predicted to occupy the same binding pocket with a different binding position. The big ligand of Λ -**1** spans the β -strands of both A and B subunits, while one of the ppy ligands contacts the β -strands from both subunits of the TNF- α dimer complex (Figure 2b). Because of the lack of hydrogen bonding or salt-bridge interactions in the molecular model, we presume that the interaction between the β -strands of the TNF- α dimer with Δ -**1** or Λ -**1** is predominantly hydrophobic in nature, consistent with previous models. The calculated binding score for Δ -**1** ($-37.21 \text{ kJ mol}^{-1}$) reflects a strong interaction between the iridium complex and the TNF- α dimer complex, while the binding score of Λ -**1** was calculated to be $-35.44 \text{ kJ mol}^{-1}$. As a reference, we calculated the binding score of SPD304 to be $-32.9 \text{ kJ mol}^{-1}$. The molecular docking results suggest that both enantiomers of **1** are able to interact with the binding pocket of TNF- α (Figure 2c).

We also analyzed the interaction of the Δ - and Λ -forms of the rhodium(III) analogue **1b** with the TNF- α dimer by molecular modeling (Supporting Information, Figure S1). Intriguingly, although both iridium(III) and rhodium(III) complexes were predicted to occupy the same binding pocket as SPD304, the binding scores for Δ -**1b** ($-23.61 \text{ kJ mol}^{-1}$) and Λ -**1b** ($-21.52 \text{ kJ mol}^{-1}$) were less negative than those for the corresponding iridium(III) complexes (Figure S2). This suggests that the rhodium(III) congeners bind to TNF- α less avidly, presumably because of their inability to form effective interactions with both subunits of the TNF- α dimer (Figure S2a,b). The predicted binding coordinates of SPD304 in the binding pocket (Figure 2d) are within 1.0 Å root-mean-square deviation (RMSD) of those reported from the protein X-ray crystal structure.

To validate the results of our molecular modeling, we used an ELISA to determine the half-maximal inhibitory concentration (IC_{50}) value of complex **1** against the TNF- α /TNFR-1 interaction (Figure 3). Under our test conditions, the potencies of complexes Δ -**1** and Λ -**1** were found to be comparable with SPD304 ($IC_{50} = \text{ca. } 22 \mu\text{M}$), one of the strongest small-molecule inhibitors of TNF- α reported to date.^[8] Addition of a racemic mixture of complex **1** (*rac*-**1**) was found to produce similar inhibition of TNF- α compared to the enantiopure complexes.

We next investigated the ability of complex **1** to inhibit TNF- α signaling in the human hepatocellular carcinoma cell line HepG2. TNF- α was pre-incubated with *rac*-**1**, Δ -**1**, Λ -**1**, or SPD304 prior to its addition to HepG2 cells stably transfected with the NF- κ B-luciferase gene (Figure 4). The Δ -**1** and Λ -

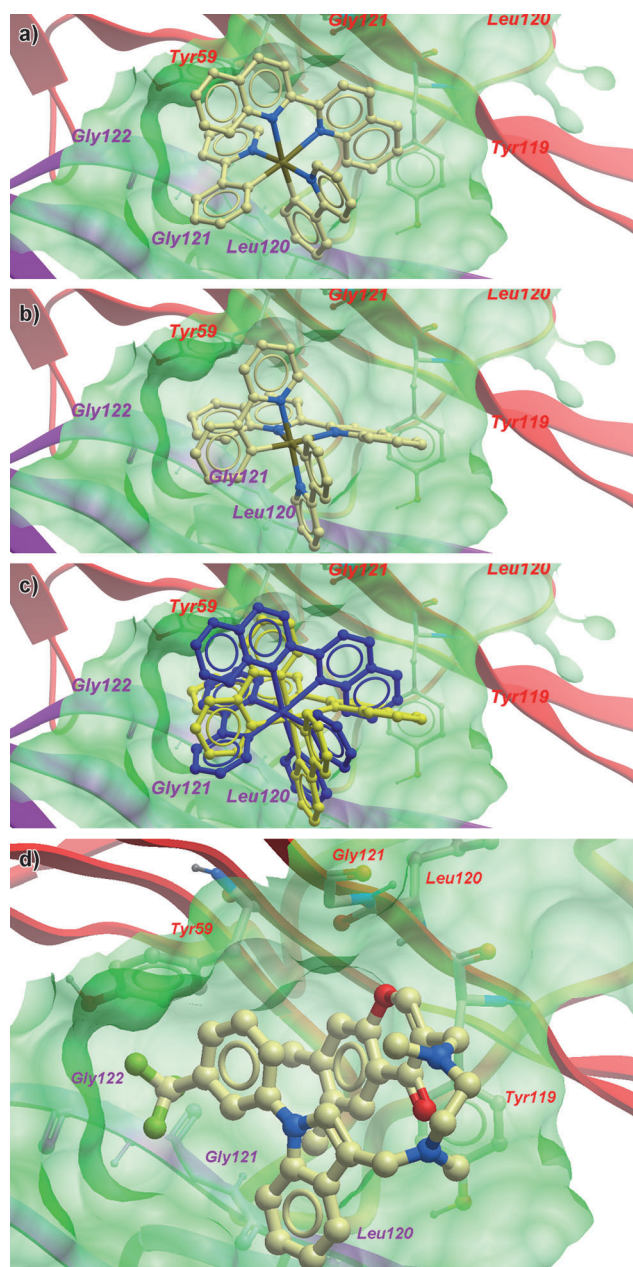


Figure 2. Low-energy binding conformations of a) Δ -**1**, b) Λ -**1**, c) superimposed Δ -**1** (blue) and Λ -**1** (yellow), and d) SPD304 bound to TNF- α dimer generated by virtual ligand docking. The two subunits of the TNF- α dimer are depicted in ribbon form and are colored purple (A subunit) and red (B subunit). The binding pocket of the TNF- α dimer is a translucent green surface. The small molecules are depicted as ball-and-stick models, shown as: C yellow, Ir dark green, O red, N blue, F green.

1 enantiomers showed comparable activities against TNF- α -driven NF- κ B signaling, with approximate IC_{50} values of 2.5 and 4 μM , respectively, and were both slightly more potent than SPD304 ($IC_{50} = 10 \mu\text{M}$) in a side-by-side assay. The *rac*-**1** had comparable potency to the enantiopure complexes under our test conditions. Nearly complete inhibition of NF- κ B activity was measured at 25 μM of the complexes (Figure 4).

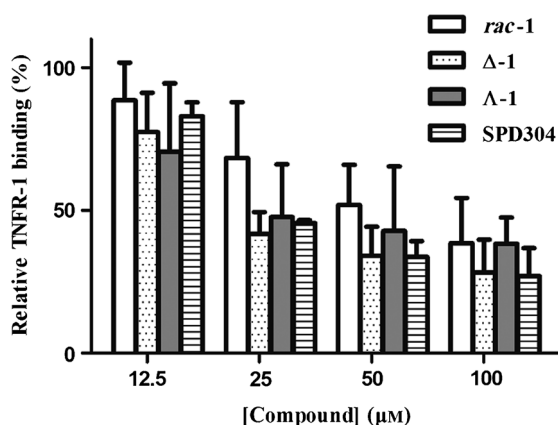


Figure 3. Compound inhibition of TNFR-1 binding to immobilized TNF- α (ELISA). Microtiter plates coated with TNF- α were incubated with TNFR-1 together with *rac*-1, Δ -1, Λ -1, or SPD304 at the indicated concentrations. TNFR-1 binding was detected using anti-TNFR antibody and horseradish peroxidase-conjugated secondary antibody. Approximate IC_{50} values: Δ -1: 20 μ M, Λ -1: 25 μ M, SPD304: 23 μ M. Error bars represent the standard deviations of the results from three independent experiments.

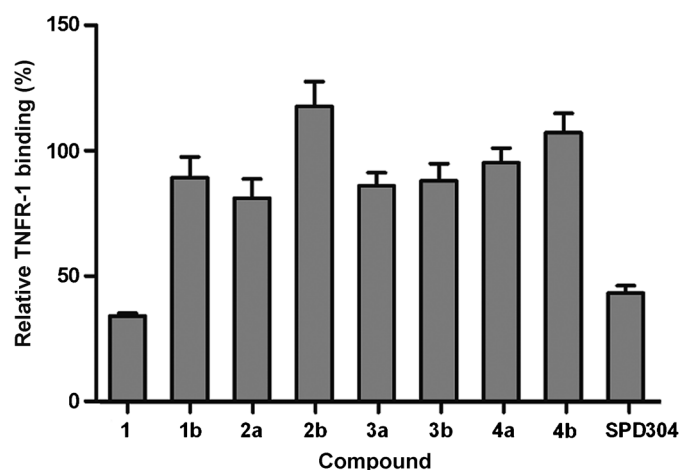


Figure 5. Inhibition of TNFR-1 binding to immobilized TNF- α (ELISA) by the cyclometalated Ir^{III} and Rh^{III} complexes at a concentration of 50 μ M (see Supporting Information for chemical formulas of inhibitor complexes). SPD304 was used as a reference compound and all complexes used were a racemic mixture of enantiomers. Error bars represent the standard deviations of the results from three independent experiments.

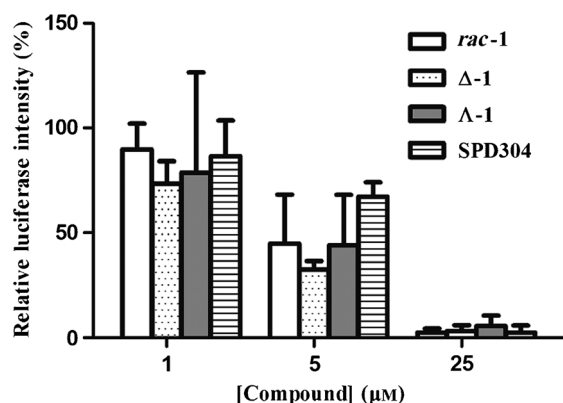


Figure 4. Compound inhibition of cellular TNF- α -induced NF- κ B activity. HepG2 cells stably transfected with the NF- κ B-luciferase gene were stimulated with TNF- α that was pre-incubated with the indicated concentrations of *rac*-1, Δ -1, Λ -1, or SPD304. Cell lysates were analyzed for luciferase activity to determine the extent of NF- κ B inhibition. Approximate IC_{50} values: Δ -1: 2.5 μ M, Λ -1: 4 μ M, SPD304: 10 μ M. Error bars represent the standard deviations of the results from three independent experiments.

To establish a preliminary structure–activity relationship (SAR) analysis for complex **1**, a series of related iridium(III) and rhodium(III) cyclometalated analogues (Figure S1) were screened by ELISA to evaluate their ability to interfere with TNF- α /TNFR-1 binding (Figure 5). The results showed that the dimeric iridium(III) complexes **2a** and **3a** exhibited significantly decreased activities against TNF- α binding compared to complex **1**. Presumably, the dimeric complexes were too bulky to fit into the binding pocket of the TNF- α dimer and were thus, unable to prevent formation of the biologically active trimer complex. This suggests that the inhibition of TNF- α activity by **1** involves shape complementarity between the metal complex and the protein-binding pocket and is not

due to an unspecific hydrophobic effect. Substitution of biq with aqua ligands (**4a**) also significantly reduced the potency of the complex. This is consistent with the results of the molecular modeling analysis, where the biq ligands of both Δ -1 and Λ -1 were predicted to play important roles in contacting the β -strands of the TNF- α subunits through hydrophobic interactions. Furthermore, the isoelectronic rhodium(III) complexes displayed comparable (**3b** and **4b**) or weaker potencies (**1b** and **2b**) against the TNF- α /TNFR-1 binding compared to their corresponding iridium(III) analogues. Significantly, the rhodium(III) congener **1b** exhibited only about 15% inhibition of TNF- α binding at 50 μ M, compared to about 70% inhibition by the iridium(III) complex **1** at the same concentration. The lower in vitro potency of **1b** is consistent with the molecular modeling results discussed previously. Taken together, these results suggest that the size of the complex, the character of the metal center, and the nature of auxiliary ligands are important determinants for TNF- α inhibitory activity.

In summary, we have developed the first metal-based direct inhibitor of TNF- α based on the iridium(III) biquinoline complex **1**. Molecular modeling analysis revealed a predominantly hydrophobic binding mode of Δ -1 and Λ -1 to the TNF- α dimer similar to that of SPD304, one of the strongest small-molecule inhibitors of TNF- α reported to date. Significantly, the potency of both Δ -1 and Λ -1 were comparable to SPD304 in side-by-side cell-free and cellular models of TNF- α inhibition, with activities in the low micromolar range in the cell-based luciferase assay. The discovery of **1** could serve as a promising scaffold for the development of more potent organometallic complexes as protein–protein interaction (PPI) inhibitors.

Received: April 17, 2012

Revised: June 8, 2012

Published online: July 13, 2012

Keywords: inhibitors · iridium(III) complexes · metal-based inhibitors · protein–protein interactions · tumor necrosis factor

- [1] H. Wajant, K. Pfizenmaier, P. Scheurich, *Cell Death Differ.* **2003**, *10*, 45–65.
- [2] B. B. Aggarwal, *Nat. Rev. Immunol.* **2003**, *3*, 745–756.
- [3] K. Chatzantoni, A. Mouzaki, *Curr. Top. Med. Chem.* **2006**, *6*, 1707–1714.
- [4] a) S. Madhusudan, S. R. Muthuramalingam, J. P. Braybrooke, S. Wilner, K. Kaur, C. Han, S. Hoare, F. Balkwill, T. S. Ganesan, *J. Clin. Oncol.* **2005**, *23*, 5950–5959; b) M. L. Harrison, E. Obermueller, N. R. Maisey, S. Hoare, K. Edmonds, N. F. Li, D. Chao, K. Hall, C. Lee, E. Timotheadou, K. Charles, R. Ahern, D. M. King, T. Eisen, R. Corringham, M. DeWitte, F. Balkwill, M. Gore, *J. Clin. Oncol.* **2007**, *25*, 4542–4549.
- [5] M. A. Palladino, F. R. Bahjat, E. A. Theodorakis, L. L. Moldawer, *Nat. Rev. Drug Discov.* **2003**, *2*, 736–746.
- [6] a) R. Alzani, A. Corti, L. Grazioli, E. Cozzi, P. Ghezzi, F. Marcucci, *J. Biol. Chem.* **1993**, *268*, 12526–12529; b) H. S. Rasmussen, P. P. McCann, *Pharmacol. Ther.* **1997**, *75*, 69–75; c) M. R. Lee, C. Dominguez, *Curr. Med. Chem.* **2005**, *12*, 2979–2994; d) S. Haraguchi, N. Day, W. Kamchaisatian, M. Beigier-Pompadre, S. Stenger, N. Tangsinmankong, J. Sleasman, S. Pizzo, G. Cianciolo, *AIDS Res. Ther.* **2006**, *3*, 8; e) C.-H. Leung, S. P. Grill, W. Lam, W. Gao, H.-D. Sun, Y.-C. Cheng, *Mol. Pharmacol.* **2006**, *70*, 1946–1955.
- [7] a) F. Mancini, C. M. Toro, M. Mabilia, M. Giannangeli, M. Pinza, C. Milanese, *Biochem. Pharmacol.* **1999**, *58*, 851–859; b) J. R. Burke, M. A. Pattoli, K. R. Gregor, P. J. Brassil, J. F. MacMaster, K. W. McIntyre, X. Yang, V. S. Iotzova, W. Clarke, J. Strnad, Y. Qiu, F. C. Zusi, *J. Biol. Chem.* **2003**, *278*, 1450–1456.
- [8] M. M. He, A. S. Smith, J. D. Oslob, W. M. Flanagan, A. C. Braisted, A. Whitty, M. T. Cancilla, J. Wang, A. A. Lugovskoy, J. C. Yoburn, A. D. Fung, G. Farrington, J. K. Eldredge, E. S. Day, L. A. Cruz, T. G. Cachero, S. K. Miller, J. E. Friedman, I. C. Choong, B. C. Cunningham, *Science* **2005**, *310*, 1022–1025.
- [9] K. S. Kumar, P. M. Kumar, K. A. Kumar, M. Sreenivasulu, A. A. Jafar, D. Rambabu, G. R. Krishna, C. M. Reddy, R. Kapavarapu, K. Shivakumar, K. K. Priya, K. V. L. Parsa, M. Pal, *Chem. Commun.* **2011**, *47*, 5010–5012.
- [10] a) H.-M. Lee, D. S.-H. Chan, F. Yang, S.-C. Yan, C.-M. Che, D.-L. Ma, *Chem. Commun.* **2010**, *46*, 4680–4682; b) D.-L. Ma, D. S.-H. Chan, C.-H. Leung, *Chem. Sci.* **2011**, *2*, 1656–1665; c) C.-H. Leung, D. S.-H. Chan, H. Yang, R. Abagyan, D.-L. Ma, *Chem. Commun.* **2011**, *47*, 2511–2513; d) T. Geppert, S. Bauer, J. A. Hiss, E. Conrad, M. Reutlinger, P. Schneider, M. Weisel, B. Pfeiffer, K.-H. Altmann, Z. Waibler, G. Schneider, *Angew. Chem.* **2012**, *124*, 264–268; *Angew. Chem. Int. Ed.* **2012**, *51*, 258–261; e) D.-L. Ma, V. P.-Y. Ma, D. S.-H. Chan, K.-H. Leung, H.-J. Zhong, C.-H. Leung, *Methods* **2012**, DOI: 10.1016/j.ymeth.2012.1002.1001.
- [11] a) D. S.-H. Chan, H.-M. Lee, F. Yang, C.-M. Che, C. C. L. Wong, R. Abagyan, C.-H. Leung, D.-L. Ma, *Angew. Chem.* **2010**, *122*, 2922–2926; *Angew. Chem. Int. Ed.* **2010**, *49*, 2860–2864; b) C.-H. Leung, D. S.-H. Chan, M. H.-T. Kwan, Z. Cheng, C.-Y. Wong, G.-Y. Zhu, W.-F. Fong, D.-L. Ma, *ChemMedChem* **2011**, *6*, 765–768.
- [12] a) T. W. Hambley, *Dalton Trans.* **2007**, 4929–4937; b) S. P. Fricker, *Dalton Trans.* **2007**, 4903–4917; c) P. C. A. Bruijninx, P. J. Sadler, *Curr. Opin. Chem. Biol.* **2008**, *12*, 197–206; d) A. Levina, A. Mitra, P. A. Lay, *Metallomics* **2009**, *1*, 458–470; e) C. G. Hartinger, P. J. Dyson, *Chem. Soc. Rev.* **2009**, *38*, 391–401; f) A. V. Klein, T. W. Hambley, *Chem. Rev.* **2009**, *109*, 4911–4920; g) A. Casini, C. Hartinger, A. Nazarov, P. Dyson, *Vol. 32* (Eds.: G. Jaouen, N. Metzler-Nolte), Springer Berlin/Heidelberg, **2010**, pp. 57–80; h) H.-K. Liu, P. J. Sadler, *Acc. Chem. Res.* **2011**, *44*, 349–359; i) N. J. Farrer, P. J. Sadler, *Bioinorganic Medicinal Chemistry* (Ed.: E. Alessio), Wiley-VCH, Weinheim, **2011**, pp. 1–47; j) B. Y.-W. Man, H.-M. Chan, C.-H. Leung, D. S.-H. Chan, L.-P. Bai, Z. Cheng, Z.-H. Jiang, H.-W. Li, D.-L. Ma, *Chem. Sci.* **2011**, *2*, 717–721.
- [13] a) A. Wilbuer, D. H. Vlecken, D. J. Schmitz, K. Kräling, K. Harms, C. P. Bagowski, E. Meggers, *Angew. Chem.* **2010**, *122*, 3928–3932; *Angew. Chem. Int. Ed.* **2010**, *49*, 3839–3842; b) L. Feng, Y. Geisselbrecht, S. Blanck, A. Wilbuer, G. E. Atilla-Gokcumen, P. Filippakopoulos, K. Kräling, M. A. Celik, K. Harms, J. Maksimoska, R. Marmorstein, G. Frenking, S. Knapp, L.-O. Essen, E. Meggers, *J. Am. Chem. Soc.* **2011**, *133*, 5976–5986; c) E. Meggers, *Chem. Commun.* **2009**, 1001–1010; d) C.-H. Leung, H. Yang, V. P.-Y. Ma, D. S.-H. Chan, H.-J. Zhong, Y.-W. Li, W.-F. Fong, D.-L. Ma, *Med. Chem. Commun.* **2012**, *3*, 696–698.
- [14] a) E. Meggers, *Curr. Opin. Chem. Biol.* **2007**, *11*, 287–292; b) S. P. Mulcahy, E. Meggers, *Top. Organomet. Chem.* **2010**, *32*, 141–153.
- [15] M. Totrov, R. Abagyan, *Proteins Struct. Funct. Genet.* **1997**, *29*, 215–220.