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


Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that plays a critical role in the 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 with 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 body’s 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 reported in the literature 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 naphthyliurea suramin and its analogues,7) the indole-linked chromone SPD304,8) and the isoidenol[2,1-a]quinazoline derivatives.9) We have recently reported the discovery of direct TNF-α small-molecule inhibitors using high-throughput virtual screening of natural product and FDA-approved drug databases.10)

While transition metal complexes have been widely utilized for the treatment of cancer,11) their activity for direct TNF-α inhibition has not yet been explored. As demonstrated recently by the group of Meggers, kinetically inert octahedral metal complexes can be utilized as structurally diverse molecular scaffolds for the design of potent and selective enzyme inhibitors.12) Herein, we have pursued the investigation of the cyclometalated iridium(III) complex [Ir(ppy)₂(biq)]PF₆ containing the 2,2′-biqunoline (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;13-15) (3) the complex [Ir(ppy)₂(biq)]PF₆ can be synthesized conveniently and rapidly; and (4) the extended aromatic biq ligand can potentially form favourable interactions with the hydrophobic binding interface of TNF-α. We describe the first application of the octahedral iridium complex 1 (Figure 1) as a direct inhibitor of TNF-α. To the best of 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.16) Similar to most protein-protein interfaces, the binding site on the TNF-α dimer is mostly hydrophobic and

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

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

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To investigate the mechanism of actions of the metal complexes towards the TNF-α dimer, we first used molecular modeling to analyze the interaction of Δ-1 and Λ-1 with TNF-α using the X-ray co-crystal structure of the TNF-α dimer with SPD304 (PDB code: 2AZ5).13 The binding interaction was evaluated using the Molsoft ICM method [ICM-Pro 3.6-1d molecular docking software].14 The geometry of both isoforms 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 isoforms are observed to occupy the same binding pocket of SPD304 (Figure 2). The biq ligand of Δ-1 contacts the β-strand of A subunit, while the two phenylpyridine moieties interact with the β-strand from 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 pose. The biq ligand of Λ-1 spans the β-strands of both A and B subunits, while one of the phenylpyridine ligands contacts the β-strands from both subunits of TNF-α dimer complex (Figure 2b). Due to the lack of hydrogen bonding or salt bridge interactions in the molecular model, we presume that the interaction between β-strand 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 kJ/mol) 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. As a reference, we calculated the binding score of SPD304 to be ~32.9. 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 (Figure S1). Intriguingly, although both rhodium(III) and rhodium(III) complexes were predicted to occupy the same binding pocket as SPD304, the binding scores for Δ-1b (~23.61) and Λ-1b (~21.52) 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 due to their inability to form effective interactions with both subunits of the TNF-α dimer (Figure S2a and 2b). The predicted binding

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 (subunit A) and red (subunit B). The small molecules are depicted as a ball-and-stick model showing carbon (yellow), iridium (dark green), oxygen (red), nitrogen (blue), and fluorine (green) atoms. Hydrogen bonds are depicted as dotted lines. The binding pocket of the TNF-α dimer is represented as a translucent green surface.

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.

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 IC₅₀ values: Δ-1: 20 μM, Λ-1: 25 μM, SPD304: 23 μM. rac-1 was included for comparison. Error bars represent the standard deviations of the results from three independent experiments.
coordinates of SPD304 in the binding pocket (Figure 2d) are within 1.0 Å RMSD of those reported as observed from the protein X-ray crystal structure.

In order to validate the results of our molecular modeling, we used an ELISA to determine the half-maximal inhibitory concentration (IC₅₀) 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 (IC₅₀ = ca. 22 μM), one of the strongest small-molecule inhibitors of TNF-α to date. Addition of 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-xB–luciferase gene (Figure 4). The Δ-1 and Λ-1 enantiomers showed comparable activities against TNF-α-driven NF-xB signaling, with approximate IC₅₀ values of 2.5 and 4 μM, respectively, and were both slightly more potent than SPD304 (10 μM) in a side-by-side assay. The racemic mixture of 1 had comparable potency to the enantiopure complexes under our test conditions. Nearly complete inhibition of NF-xB activity was observed at 25 μM of the complexes (Figure 4).

To establish a preliminary structure-activity relationship (SAR) analysis for complex 1, a series of related iridium(III) and rhodium(III) cyclometallated 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 aqua ligands (4a) also significantly reduced the potency of the complex. This is consistent with the results of the molecular modeling analysis, where the aqua ligands of both Δ-1 and Λ-1 were predicted to play important roles in contacting the β-strands of the TNF-α subunits via hydrophobic interactions. Furthermore, the isoelectronic rhodium(III) complexes displayed comparable (3b and 4b) or weaker potencies (1b and 2b) against the TNF-α–TNFR1 binding compared to their corresponding iridium(III) analogues. Significantly, the rhodium(III) congener 1b exhibited only ca. 15% inhibition of TNF-α binding at 50 μM, compared to ca. 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 decreased previously. Taken together, these results suggest that the size of the complex, the character of the metal centre 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.

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A cyclometallated iridium biquinoline complex was found to target the protein-protein interface of TNF-α trimer. Both enantiomers display comparable \textit{in vitro} potency to the strongest small molecule inhibitor of TNF-α reported to date.