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Communication

Iridium complexes inhibit tumor necrosis factor-α by utilizing light and mixed ligands

Elodie Sauvageot [a, b], Pierre Lafite [c, d], Eric Duverger [c, d, e], Ronan Marion [a, b], Matthieu Hamel [f], Sylvain Gaillard [a, b, **], Jean-Luc Renaud [a, b, ***], Richard Daniellou [c, d, *]

[a] Normandie University, University of Caen Normandie, Laboratoire de Chimie Moléculaire et Thioorganique, 14050 Caen, France
[b] CNRS, UMR 6507, 14050 Caen, France
[c] Univ. Orléans, CNRS, Institut de Chimie Organique et Analytique, 45067 Orléans, France
[d] CNRS, UMR 7311, 45067 Orléans, France
[e] Glycodiag, rue de Chartres, 45067 Orléans, France
[f] CEA, LIST, Laboratoire Capteurs et Architectures Electroniques, 91191 Gif-sur-Yvette Cedex, France

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ABSTRACT

We report herein a large study of the inhibition of tumor necrosis factor-α with 51 iridium(III) complexes, thus highlighting the influence of the nature of the ligands around the metal, their synergic effect and the role of the light.

1. Introduction

Since the discovery of the well-known cis-platin and its analogues, organometallic complexes have been widely used as anticancer drugs [1] and their biological studies are growing exponentially. This intense interest has been summarized in many reviews which nicely depict the state of the art of these researches in many diseases [2]. The principle advantage of transition metal complexes is their highly versatility for drug design. Besides variations in the metal and its oxidation state, metal ions have a range of geometries and coordination numbers that allow the fine-tuning for biological activities.

The tumor necrosis factor (TNF) superfamily plays highly diversified roles in the body but all members, without exception, exhibit pro-inflammatory activity, in part through activation of the transcription factor NF-κB [3]. Amongst them, the fascinating and heavily studied TNF-α demonstrated to play roles in inflammation, cellular proliferation, apoptosis and morphogenesis. In addition, its expression also proved to be linked with a wide variety of diseases, including cancer, diabetes, obesity, cardiovascular, neurologic, pulmonary, and autoimmune disorders. Thanks to that, TNF-α has become a tremendous active target for drug development in the last years. Novel therapies including anti-TNF antibodies such as Enbrel®, Humira®, Remicade® or Cimzia®, have significantly improved disease outcomes in patients suffering from a range of important inflammatory conditions including rheumatoid arthritis, psoriasis and Crohn's disease for examples. However and despite their success in the clinic, such antibodies suffer from important drawbacks, including poor tissue penetration, high manufacturing costs and frequent secondary effects like increase of cancer. As a consequence, there is an urgent need for alternatives. Following the pioneering work of He et al. with the compound SPD304 (Fig. 1) [4], small molecule modulation of this specific protein-protein interaction, *i.e.* able to transform an active TNF-α trimer into an inactive

* Corresponding author. Univ. Orléans, CNRS, Institut de Chimie Organique et Analytique, 45067 Orléans, France.
** Corresponding author. Normandie University, University of Caen Normandie, Laboratoire de Chimie Moléculaire et Thioorganique, 14050 Caen, France.
*** Corresponding author. Normandie University, University of Caen Normandie, Laboratoire de Chimie Moléculaire et Thioorganique, 14050 Caen, France.
E-mail addresses: sylvain.gaillard@ensicaen.fr (S. Gaillard), jean-luc.renaud@ensicaen.fr (J.-L. Renaud), richard.daniellou@univ-orleans.fr (R. Daniellou).

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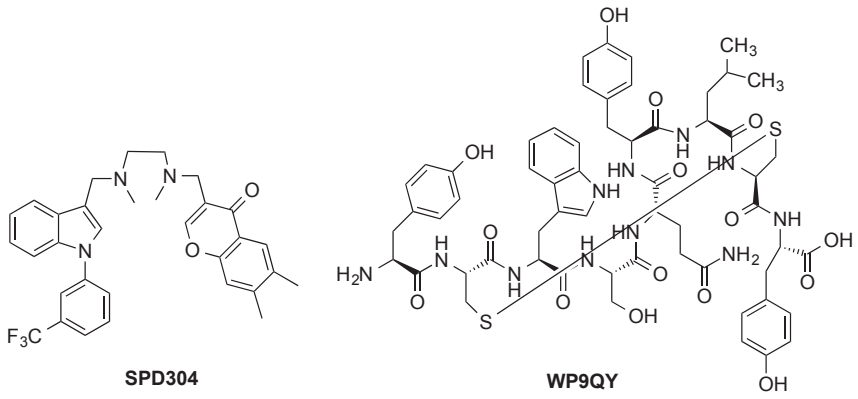

Fig. 1. Chemical structures of SPD304 and WP9QY.

dimer, has also been recognized as a promising approach in drug discovery [5]. For example, the antagonist cyclic peptide WP9QY (Fig. 1) demonstrated its efficiency in preventing both inflammatory bone destruction and systemic bone loss in rheumatoid arthritis [6]. Structured-based screening of chemical library of natural products has also led to the discovery of two potent small inhibitors, namely quinuclidine and indoloquinolizidine [7]. Moreover the screening of the SPECS database allowed the identification of original scaffolds with promising biological properties [8]. Besides, group 9 organometallic compounds have recently received an increased attention for their therapeutic and bioanalytical applications, mainly due to their ease of preparation, their stability in water and air, and their high solubility [9]. Amongst them, the team of Leung recently reported that the racemic (or even each enantiomer) iridium(III) complex, [Ir(ppy)$_2$(biq)][PF$_6$], was able to prevent the active trimer association through its interaction with β-strands of the TNF-α dimer, and exhibited an IC$_{50}$ of 22 μM in ELISA experiments against the TNF-α/TNFR1 interaction, comparable with SPD304 [10].

Since several years, we have developed a research program devoted to the synthesis [11], the coordination [12], and the reactivity of metal complexes coordinated to dipyridylamine derivatives. We have recently reported the synthesis and the characterization of a series of neutral and cationic cyclometalated iridium(III) complexes bearing both C^N ligands and a dipyridylamine motif as N^N ligand [13,14]. They proved to be efficient catalysts under photoredox conditions in a model aza-Henry reaction [13]. Herein, we wish to report the inhibition properties of TNF-α with 51 iridium(III) heteroleptic organometallic complexes.

2. Material and methods

2.1. General procedure GP1 for the synthesis of [Ir(C^N)$_2$(N^N)][PF$_6$] complexes

In a dry flamed Schlenk tube under argon atmosphere, iridium dimers (1 eq.) and N^N ligands (2.2 eq.) were introduced in degassed 2:1 mixture of dichloromethane/methanol (8 mL). The reaction mixture was stirred at 50 °C for 6 h. After cooling down the solution to room temperature, excess of KPF$_6$ (10 eq.) was added affording a precipitate. The inorganic solid was filtered off and the filtrate was evaporated. The solid was washed on a frit with diethyl ether (3 × 5 mL) and dried under vacuum to afford pure cationic iridium [Ir(C^N)$_2$(N^N)][PF$_6$] complexes.

2.2. General procedure GP2 for the synthesis of neutral [Ir(C^N)$_2$(acac)] complexes

In a flamed-dried Schlenk tube under argon atmosphere, iridium dimers (1 eq.) and acac ligands (5 eq.) were introduced in a degassed solution of ethoxyethanol with K$_2$CO$_3$ (10 eq.). The reaction mixture was stirred at 100 °C for 24 h. After cooling down the solution to room temperature, water was added affording a precipitate. The resulting precipitate was collected on a frit and washed with water (5 mL) and diethyl ether (5 mL) and finally dried under vacuum to afford pure complexes.

2.3. TNF-α–TNFR-1 binding ELISA procedure

This assay was carried following a known protocol [10]. Briefly, microtiter plates were coated overnight with TNF-α (0.625 μg/mL) in 100 μL PBS at 4 °C. The wells were washed three times with 200 μL PBS/0.05% Tween 20 (PBST), blocked with 200 μL PBST containing 1% BSA for 60 min and washed as before. Dilutions of the test compounds (100 μM) in 50 μL PBS containing 2% DMSO were added to the wells and the microtiter plates were incubated with shaking for 20 min. TNFR-1 (0.2 μg/mL) in 50 μL PBS was added to the wells and the plates were incubated for a further 120 min. The plates were washed as before and incubated for 120 min with TNFR-1 antibody (1:1000) in 100 μL PBST containing 1% BSA. The plates were washed three times with PBST and incubated for 90 min with anti-rabbit cross adsorbed-peroxidase secondary antibody (8 μg/mL) in 100 μL PBS. The plates were washed as before and incubated with 100 μL TMB solution, quenched with 100 μL 2 N chlorhydric acid and the absorbance was measured at λ = 450 nm.

2.4. Influence of light on ELISA procedure

Microtiter plates were coated overnight with TNF-α (0.625 μg/mL) in 100 μL PBS at 4 °C. The wells were washed three times with 200 μL PBS/0.05% Tween 20 (PBST), blocked with 200 μL PBST containing 1% BSA for 60 min and washed as before. Test compounds (100 μM) in 50 μL PBS containing 2% DMSO were added to the wells and the microtitre plates were incubated with shaking for 20 min, under blue LED strip (FlexLed inspire®, 2.4 W/m) or obscurity. TNFR-1 (0.2 μg/mL) in 50 μL PBS was added to the wells and the plates were incubated for a further 120 min under blue LED strip or obscurity. The plates were washed as before and incubated for 120 min under blue LED strip or obscurity with TNFR-1 antibody (1:1000) in 100 μL PBST containing 1% BSA. The plates were washed three times with PBST and incubated for 90 min under blue LED strip or obscurity with anti-rabbit cross adsorbed-peroxidase