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Synthesis and anticancer activity of ruthenium half-sandwich complexes comprising combined metal centrochirality and planar chirality

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ABSTRACT

Reported herein is the synthesis and evaluation of anticancer properties of a well-established organometallic protein kinase inhibitor scaffold in which the stereochemical complexity is increased. The investigated ruthenium η^5-cyclopentadienyl half-sandwich complexes contain a bidentate pyridocarbazole and a monodentate CO ligand, thereby leading to four different stereoisomers due to the combined presence of ruthenium-centered chirality and planar chirality of the π -coordinated trisubstituted cyclopentadienyl moiety (two diastereomers as mixtures of enantiomers). While one of the two racemic diastereomers turns out to be nontoxic towards cancer cells, the second racemic diastereomer displays high cytotoxicities towards different cancer cell lines *in vitro*, thus demonstrating the intertwining of organometallic stereochemistry and biological activity.

1. Introduction

Over the last one to two decades, we and others have exploited the high versatility of inert metal complexes as powerful structural templates for targeting proteins, with a large variety of reactive and inert protein-binding metal-containing compounds being available today as tools for chemical biology or as drug candidates [1–9]. For example, recent studies confirm the aptitude of replacing organic moieties by ferrocene in different types of bioactive compounds such as ferrocene derivatives of the anti-cancer drugs erlotinib and geftinib [10], and the histone deacetylase inhibitor SAHA [11,12], or oxindole containing ferrocenes, inhibiting DYRK3-4 and VEGFR2 [13]. Beyond ferrocene, ruthenocene and ruthenium(II) pentamethylcyclopentadienyl benzene sandwich complexes as potent carbonic anhydrase inhibitors have been reported recently [14–16], revealing that not only the structure, but also the metal itself has an impact on the inhibition potency [17]. However, not every incorporation of a metal-containing moiety leads to an improvement in affinity or activity [18,19]. Negative effects on the recognition of non-steroidal antiandrogen drugs by the androgen receptor were reported for ferrocene derivatives of the drugs flutamide and bicalutamide, and are probably due to their increased or modulated space demand [19]. The modulated cellular uptake of these compounds may also play a crucial role as shown with a ferrocene-containing bioorganometallic inspired by the antibiotic platensimycin, which, according to modeling, fits nicely into the target FabF, but shows no antibacterial activity up to 200 μg/mL [20]. Besides sandwich-compounds, half-sandwich complexes are popular metal-containing scaffolds for the design of protein binders, including recent reports of arene-$Cr(CO)_3$-containing analogues of the antibiotic platensimycin [21], $Cr(CO)_3$ coordination to anti-inflammatory diterpenes [22], $Re(CO)_3$- and $^{99m}Tc(CO)_3$-cyclopentadienyl-complexes for targeting of human carbonic anhydrase IX [23] or amino acid transporters [24]. In recent reports, η^6-arene ruthenium half-sandwich complexes were used as templates for a broad range of metal-based bioactive compounds, such as piano-stool complexes inhibiting carbonic anhydrase II [25], selective organoruthenium inhibitors of protein tyrosine phosphates 1B [26], as well as for the development of organoruthenium antagonists of human A_3 adenosine receptor [27]. A η^5-cyclopentadienyl ruthenium half-sandwich complex was recently reported as a single digit nanomolar inhibitor for the human repair enzyme 8-oxo-dGTPase [28].

Recently, our laboratory introduced inert metal complexes as structural scaffolds for the design of highly potent and selective inhibitors of protein kinases [29–46]. Whereas octahedral coordination geometries provide a large structural complexity [45], half-sandwich complexes offer the advantage that they are typically much easier to synthesize [32,34–36]. Since we experienced that, as expected, the stereochemistry strongly affects the kinase

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inhibition properties [32,34,35,40,41,45], we became interested in adding another stereochemical aspect to the metal-centered chirality of our established metallo-pyridocarbazole half-sandwich scaffold. The impact of the stereochemistry of ruthenium half-sandwich complexes towards its biological effect has been previously shown by other groups. Examples include induced-fit DNA recognition with dynamic stereogenic centers or the anticancer activity of complexes with chiral arene ligands [47]. In our approach, using trisubstituted cyclopentadienyl moieties, η^5-coordination to ruthenium provides planar chirality, so that the combined presence of metal-centered and planar chirality leads to two diastereomers as pairs of enantiomers. Interestingly, as presented in a proof-of-principle study herein, we identified a derivative in which the two diastereomers differ significantly in their anticancer properties in several cancer cell lines, thus demonstrating the merit of exploiting the stereochemical options of half-sandwich scaffolds for the design of bioactive organometallics (Fig. 1).

2. Results and discussion

Starting with sandwich compound **1** [48] the racemic metal precursor **4** was obtained over four steps in a procedure that is analogous to a recent report from us [35]. Accordingly, the ethyl ester **1** was hydrolysed with K_2CO_3 to the analogous carboxylic acid **2** (90%) and subsequently protected with 2-(trimethylsilyl)ethanol by EDCl coupling (69%) to yield the ester **3**. Following photochemical replacement of the benzene by three acetonitrile ligands and CO conversion (quantitative over 2 steps) led to the desired complex **4** (see Scheme 1).

The reaction of **4** with TBS-protected isoquinolinocarbazole **5** [30] in the presence of K_2CO_3 and 2-(trimethylsilyl)ethanol provided the two diastereomeric half-sandwich complexes **6a** and **6b** in 69% yield, which were easily separated *via* HPLC on a silica gel stationary phase using hexane/ethyl acetate (93:7) as eluting solvent. Deprotection of the maleimide and the carboxylic acid of the complexes **6a** and **6b** was subsequently achieved with TBAF treatment, providing **7a** and **7b** in quantitative yields. In this step, we were unable to remove some aliphatic impurities from the deprotection, however those did not affect the following steps and could be removed after the conversion to the final product. The modification of the carboxylic acid attached to the cyclopentadienyl moiety was achieved most rapidly by conversion to activated *N*-hydroxysuccinimidyl (NHS) esters **8a** (96%) and **8b** (83%), followed by the conversion to amides or esters simply by addition of the corresponding amine or alcohol. In addition, this activated ester offered the opportunity to derivatize the cyclopentadienyl very quickly and to synthesize amide or ester libraries which are then applicable to different types of biological screenings [35,49]. In this way, for each diastereomer, an amide library of 31 members (denoted as libraries **9a** and **9b** in Scheme 2) was built by the addition of primary amines to a DMF solution of the NHS-ester followed by a reaction at 20 °C for 16 h (see Supporting information for more details).

We have recently reported anticancer activities of inert ruthenium half-sandwich complexes [37,50]. Cytotoxicity and structure–activity relationships of metal-containing compounds have been studied and reviewed extensively over the last couple of years [8,51–59], revealing that many anticancer compounds under evaluation were either discovered through cytotoxicity screening or showed cytotoxicity against cancer cells in later stages of their investigation. In order to evaluate the differences in biological activity of the half-sandwich complexes resulting from opposite planar chirality, the diastereomeric amide libraries **9a** and **9b** were screened in cell viability assays *via* the MTT method in the human colorectal adenocarcinoma cell line HT-29 at a concentration of 1 μM (Supporting information). The most promising compound concerning cytotoxicity against the HT-29 cancer cell line in the above mentioned libraries, was identified as complex **1b** (Scheme 2b), while its diastereomer **1a** displayed no cytotoxicity under the same conditions. Both diastereomers were re-synthesized for full characterisation and more biological testing. For this, the NHS-esters **8a** and **8b** were reacted with ethanolamine to yield the diastereomeric complexes **1a** (78%) and

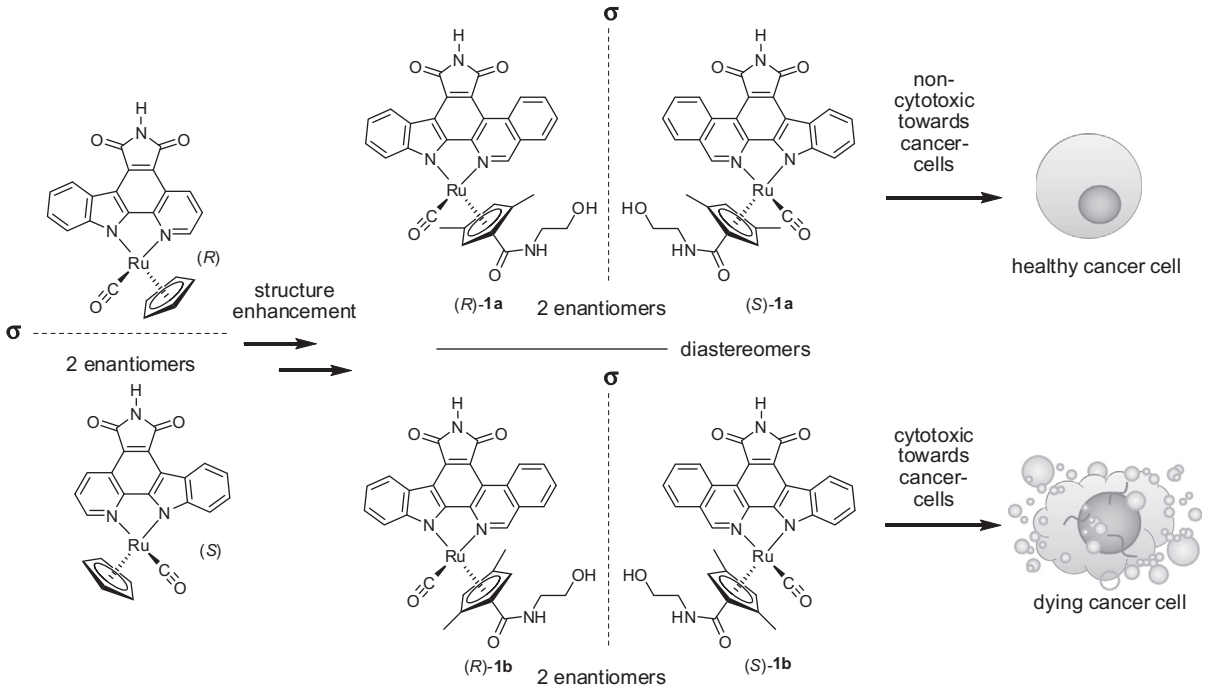

Fig. 1. Diastereomers of metallo-pyridocarbazole complexes with combined metal-centered and planar chirality differ in their anticancer activities.