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Metallocene-uracil conjugates: Synthesis and biological evaluation of novel mono-, di- and tri-nuclear systems

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ABSTRACT

The chemistry and biology of metallocene-nucleobase conjugates has been recognized as an increasingly important area of bioorganometallic chemistry. In this study, we have developed a two-step synthetic route to prepare mono-, di-, and tri-nuclear metallocene-uracil derivatives. Our synthetic procedure uses the Sonogashira cross-coupling and the PEMB-mediated reduction reactions as key steps. Successful application of PEMB as a mild and selective reducing agent allowed us to obtain with high yields a series of metallocenethymines bearing saturated linker groups. The compounds have been characterized by spectroscopic methods and the molecular structures of [3-(*N*1-(5iodouracilyl)propionyl]ferrocene (**18**) and [3-(*N*1-(5-iodouracilyl)propionyl]ruthenocene (**19**) were determined by X-ray diffraction. The anti-cancer activity of the metallocene-nucleobase products has been tested against estrogen receptor negative MDA-MB-231 mammary carcinoma, human HT-29 colon carcinoma and non-tumor mouse L929 fibroblast cells. This study has emphasized the cytotoxic activity of [3-(*N*1-(5-ferrocenylethynyl) propionyl]ferrocene (**7**) and [3-(*N*1-(5-methyluracilyl)propyl]-1′-diferocenylmethane (**12**). Notably, compound **12** was more cytotoxic against HT-29 cells than cisplatin. Moreover the undesired cytotoxicity of **12** against healthy L929 fibroblast cells was at the same level as cisplatin. Our results confirm the importance of dinuclear organometallic systems as anticancer agents.

Introduction

The chemistry of nucleosides, nucleotides and nucleobases has received a great deal of attention from academia and pharmaceutical industries. Marketed drugs like 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV) [1], 3′-azido-3′-deoxythymidine (zidovudine, AZT) [2a,2b], 5-fluorouracil (5-FU) [3] and others [4] reflect this interest.

Organometallic derivatization of drugs [5], and of primary and secondary metabolites [6] is a common strategy utilized by bio-organometallic chemists [7]. In most cases, this strategy is aimed at obtaining compounds with anticancer [8], antibacterial [9], or antiparasitic [10] activity, but other biological applications have been also explored [11]. Due to the well-developed synthetic chemistry, stability and its reversible electrochemistry, ferrocene [12] has became an attractive derivatizing agent, widely utilized in bioorganometallic chemistry [7a,7b,8a,8b,13].

Nucleobases constitute an attractive target for ferrocenyl derivatization. A literature search shows only a modest number of reports dealing with ferrocenyl-nucleobase conjugates [14]. In addition there is a big gap in number of papers reporting on the synthesis of ferrocenyl-nucleobase compounds and those reporting on their biological properties. Representative examples of the latter are provided by the groups of Schmalz [14i], Štěpnička [14h], Laguna [14q] and Tucker [14r].

In view of continuing our program in the biological function of organometallic compounds [5a,5b,9b,15], we became interested in ferrocenyl derivatives of nucleobases [16]. Initially, we discovered that a Michael addition reaction of acryloylferrocene to uracils gave easy access to novel ferrocenyl-nucleobase adducts of the general structure **1** (Fig. 1). In the following studies, we reported on the synthesis of alcohol (**2**) and olefin (**3**) derivatives (Fig. 1) [16b].

Bioconjugates of classes **1**–**3** were tested for anticancer and antibacterial activity against human estrogen receptor-responsive breast adenocarcinoma MCF-7, and HT-29 colon carcinoma cells and against Gram-positive methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), vancomycin-

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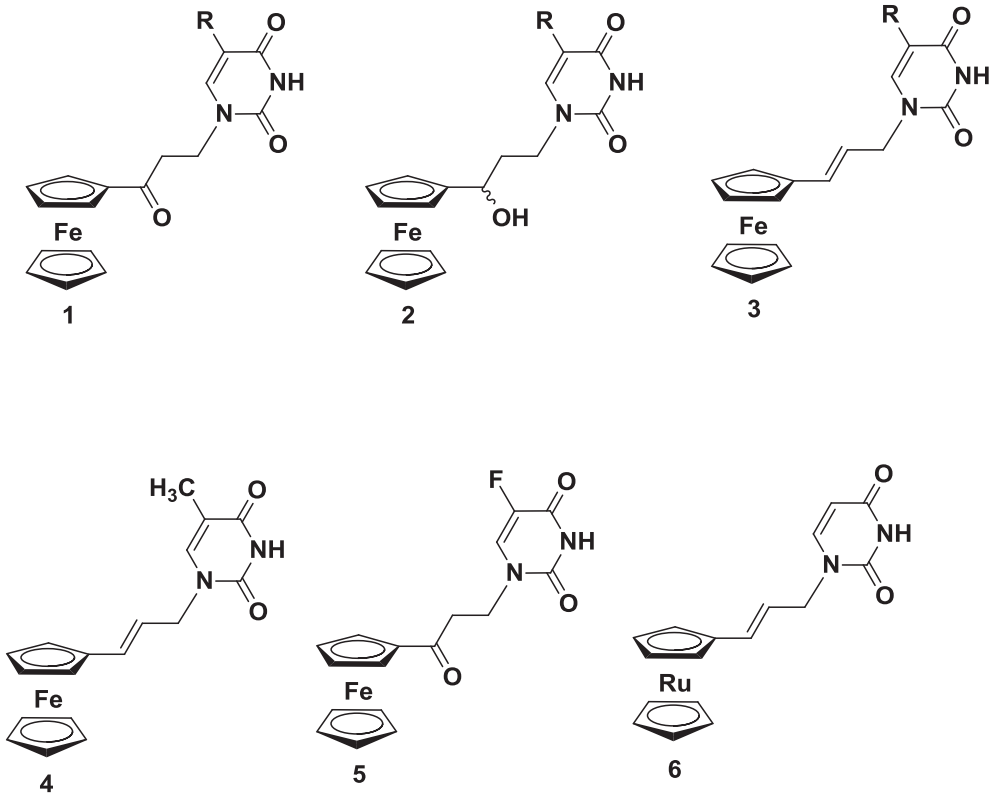

Fig. 1. Ferrocenyl-uracils studied to date by our group.

resistant *S. aureus* (VRSA), and *S. epidermidis* bacterial strains, respectively [16b]. Screening studies show complex **4** (Fig. 1) to be the most active compound against estrogen receptor-responsive breast adenocarcinoma MCF-7 cells with IC_{50} of 23.8 μM, while complex **5** (Fig. 1) is the most active against all bacterial strains tested with the lowest MIC value of 16 μg mL^{-1} against *S. epidermidis* bacterial strain [16b]. In order to get more insight into the mechanism of action, we used atomic absorption spectroscopy (AAS) for measurements of the cellular up-take of the model ruthenocenyl derivative **6** (Fig. 1). The AAS experiments confirmed that complex **6** enter the cancerous cells in a time-dependent manner. However the cellular concentrations of **6** were low, which may account for the moderate cytotoxic activity of the investigated compounds. These preliminary results prompted us to pursue our program in ferrocenyl-uracil chemistry.

The primary goal of this work was to explore the synthetic chemistry of metallocene-uracil systems. In that respect we report here the synthesis of the novel di- and tri-metallic ferrocenyl-uracil derivatives **7**, **8**, **9** and complexes **10**, **11**, **12** bearing a $-CH_2CH_2CH_2-$ linker to bridge the metallocenyl and the thymine end-groups. We also report the metallocenyl furanopyrimidones **13** and **14** obtained as by-products during the synthesis of **7** and **8**. Fig. 2 shows the structures of the entire series of compounds **7**–**14**. The secondary goal of our work encompassed anticancer and antibacterial activity studies of these newly obtained metallocene-nucleobase derivatives. In this paper we also disclose the X-ray crystal structures of complexes **18** and **19**.

Our motivation to synthesise derivatives **7**–**9** and **12** was spurred by the recent reports on the anticancer and antibacterial activity of di- and tri-nuclear organometallic compounds [15b,17]. We thought that introduction of an additional metallocene nucleus might enhance the anticancer activity of ferrocenyl-nucleobase systems. In addition, derivatives **10**-**12** complete the series of complexes **1**-**3** with the systems bearing aliphatic linker groups.

Results and discussion

*Synthesis of **7**–**9** and **10**–**12***

The synthetic strategy for the preparation of ferrocenyl-uracils **7**–**9** consists of two steps (Scheme 1). In the first step, Michael adducts **18**–**20** were obtained, following a published method [16b], by reaction of (3-chloropropionyl)-metallocenes **15**–**17** with 5-iodouracil in yields of 77, 51 and 84%, respectively. The second step involved a Sonogashira cross-coupling reaction between derivatives **18**–**20** and ethynylferrocene. A combination of the tetrakis(triphenylphosphine)palladium(0) catalyst, copper(I) iodide as co-catalyst, and triphenylphosphine was used in a solvent mixture of triethylamine-dimethylformamide (TEA-DMF). The addition of DMF was not necessary for the synthesis of complex **9** due to the good solubility of the iodo-derivative **20** in TEA.

Apart from the expected products **7**–**9** (obtained with 73, 54 and 54% isolated yield, respectively), the Sonogashira reaction yielded furanopyrimidones **13** and **14** as by-products. Noticeably, we did not isolate a by-product in the reaction of the dinuclear complex **20** and ethynylferrocene. The formation of similar furanopyrimidones during the course of the Sonogashira coupling of ethynylferrocene with 5-iodouracil derivatives has been reported by Coutouli-Argyropoulou and co-workers [18]. According to their mechanistic proposal, the formation of furanopyrimidones originates from the negatively charged cyclic transition products which subsequently couple to the ethynylferrocene molecule [18]. The synthetic approach towards compounds **10**, **11** and **12** is outlined in Scheme 2. In a first step, the Michael adducts **21**, **22** and **23** were synthesized from the (3-chloropropionyl)-metallocenes **15**–**17** and thymine in 64, 69 and 63% yields respectively. In the second step, complexes **21**–**23** were treated with 5-ethyl-2-methylpyridine borane (PEMB) in acetic acid for 2 h at room temperature. The PEMB is an established reagent for the reductive amination of