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Synthesis, structure and in vitro cytostatic activity of ferrocene—Cinchona hybrids

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ABSTRACT

Exploring copper(I)- and ruthenium(II)-catalyzed azide–alkyne cycloadditions and a Sonogashira protocol, novel cytostatic ferrocene-cinchona hybrids were synthetized displaying significant in vitro activity on HepG-2 and HT-29 cells. Preliminary SAR studies disclosed that compounds incorporating linkers with 1,2,3-triazole and chalchone residues can be considered as promising lead structures. According to the best of our knowledge this is the first letter on the incorporation of ferrocene nucleus in the reputed cinchona family via triazole and chalcone linkers with established pharmaceutical profile.

It is a generally accepted view that chemotherapy is one of the essential tools for treatment of malignancies. However, cisplatin, one of the most commonly used chemotherapeutics in the treatment of cancer[1] capable of covalently binding to DNA,[2] causes severe side-effects.[3] In order to overcome the toxic limitations and to broaden the set of treatable malignancies, an intense search was initiated for alternative agents with remarkable structural diversity and led to the development of carboplatin and oxaliplatin, the further emblematic representatives of clinically approved classical metal-complexes,[2b] and a wide variety of organometallic and organic potential cytostatic agents. Among organometallics, due to their nontoxic character and chemical stability, ferrocene derivatives with diverse molecular architectures are of pronounced importance. A concept taken into account for the design of compounds was that replacement of an aromatic nucleus of certain organic compounds for a three-dimensional ferrocene unit with tuneable redox character can lead to products possessing unexpected properties which are absent or less manifested in the parent molecule. This view was supported by a number of reviewed examples[4] including ferrocene-containing analogues of the non-steroidal selective estrogen receptor modulator hydroxytamoxifen[5] which display strong cytotoxic and cytostatic effects on hormone-independent MDA-MB-231 breast tumour cells.

Chalcones represent a privileged molecular fragment having a number of interesting biological properties such as antioxidant, cytotoxic, anticancer, antimicrobial, antiprotozoal, antiulcer, antihistaminic and anti-inflammatory activities.[6] There are also remarkable examples of active metallocene derivatives with chalcone analogue structures. For instance, ferrocenylprop-2-en-1-ones were also demonstrated to strongly inhibit the HepG2 cells while having no toxicity towards healthy human fibroblasts.[7]

The potential of simple ferrocene-based heterocycles in fighting cancer, ferrocenylalkyl-substituted azoles were also found to exhibit in vivo antitumor activity presenting up to 100% of tumour growth inhibition associated with lower toxicity when compared to clinically used drugs.[8] In this regard, even metal-free organic 1,4-disubstituted 1,2,3-triazoles exhibit significant antiproliferative effect against a wide range of human malignant cell lines.[9] On the other hand, 1,5-disubstituted 1,2,3-triazole analogues of combretastatin A-4 were also found to display marked cytotoxicity on several cancer cell lines.[10]

The structural versatility of deserving therapeutic agents is further demonstrated by several members of natural alkaloids including vinblastine, camptothecine, staurosporine and ellipticin.[11] It is also well-documented that the application of quinine derivatives in the field of cancer diagnosis[12] and in chemotherapy[13] goes far back to the past. Accordingly, we have also prepared cinchona-ferrocene conjugates with amide and urea spacers displaying

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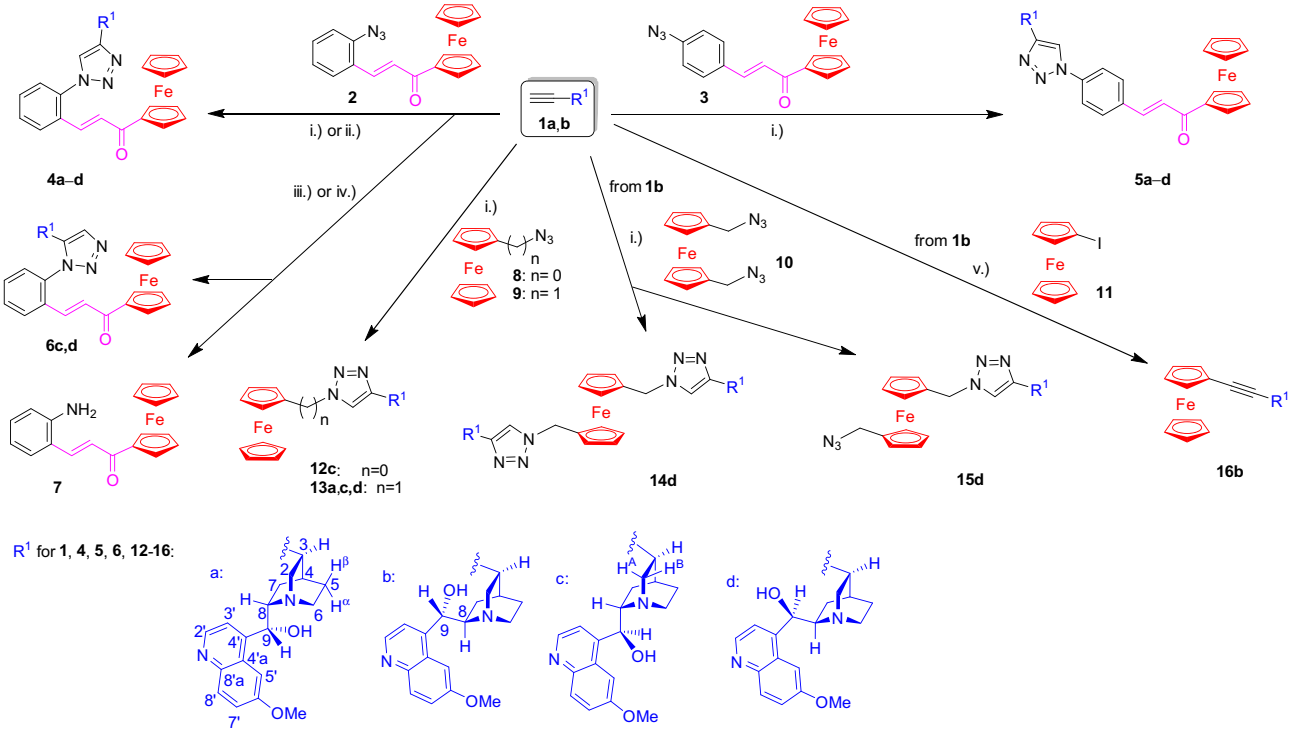
significant in vitro activity against MCF-7 breast adenocarcinoma, HepG2 hepatoma, HL-60 leukemia and SH-SY5Y neuroblastoma human cell cultures characterized by IC_{50} values in the range of 0.1–4.2 μM concentrations.[14] It must be pointed out here that alkaloid chemistry utilizing click strategy seems to have a real potential in searching novel lead structures as demonstrated by the significant in vitro antiproliferative effects of cinchona hybrids with 1,4-disubstituted 1,2,3-triazole linker containing nucleoside[9a] or polyether residue.[9b]

Taking all of the aforementioned precedence into account, on the basis of the concept of fragment-based drug design[15] we envisaged the synthesis of novel hybrids of enhanced activity by reasonable chemical combination of modules having themselves well-documented anticancer effects providing a possibility for a synergic mode of action on appropriate biological targets. Thus, here we report on the synthesis, brief structural characterization and in vitro evaluation of the first representatives of ferrocene-cinchona hybrids tethered via chalcone- and 1,2,3-triazole moieties along with seven chalcone-free triazole derivatives and a chalcone- and triazole-free alkyne serving as reference molecules to establish preliminary structure–activity relationships.

The synthetic pathway leading to fundamental target structures incorporating the entire set of the envisaged building blocks is terminated by the widely used copper(I)-mediated regioselective Sharpless [2+3] cycloaddition,[16] involving the readily available diastereomeric cinchona-derived alkynes **1a**,**b** and ferrocene-containing azido components **2** and **3** (Scheme 1) prepared by base-mediated condensation of acetylferrocene with the corresponding azidobenzaldehyde (for experimental details: cf. Supporting information).

The reactions of alkynes **1a**,**b**[17] with azide components **2** and **3**, respectively, conducted in H_2O/*n*-BuOH (1:1, v/v) for 10 h at room temperature in the presence of low catalyst loading (1 mol % of $CuSO_4$, 5 mol % of Na-ascorbate) (Method *i.*) afforded mixtures of diastereomers, which were separated (isolated yields: 23/51% for **4a**/**4c**; 21/39% for **4b**/**4d**; 23/48% for **5a**/**5c**; and 29/44% for **5b**/**5d**) with significant dominance of the epimerized products stabilized by intramolecular hydrogen bridge between the hydroxyl group and the quinuclidine N1-atom.[18] The partial epimerization of C9 sterogenic center in the cinchona residue takes probably place via reversible deprotonation enabled by the coordination of Cu(I)-species to quinoline N1-atom. Supporting this view, a higher catalyst loading (4 mol % of $CuSO_4$) even with a shorter reaction time (2 h) (Method *ii*) gave rise to increase in both conversion and epimerization (isolated yields: 12/82% for **4a**/**4c**; 23/54% for **4b**/**4d**; 17/80% for **5a**/**5c**; and 24/69% for **5b**/**5d**).

In order to obtain further hybrids with complete set of the envisaged building blocks including 1,5-disubstituted 1,2,3-triazole fragment, we performed click reactions of **1a**,**b** and **2** using complex Cp*RuCl(COD) as catalyst (Method *iii*: Scheme 1).[19] Both reactions proceeded with concomitant Ru(II)-mediated C9-epimerization resulting in the corresponding cinchona product in low-to-reasonable yields (26% for **6c** and 48% for **6d**). The facile epimerization accompanying cycloadditions might again be interpreted in terms of coordination between metal-containing species and the quinoline N1-atom. Besides hybrids **6c**,**d** aminochalchone **7**, resulted from the reduction of the azide component **2**, was also formed under the applied conditions and could be isolated in low yield (16–28%). We also attempted to couple **1a**,**b** and **2** in dioxane at 60 °C in the presence of complex Cp*RuCl(PPh_3)$_2$ (Method *iv*). While the reaction of **1b** afforded **6d** in acceptable yield (38%) along with a substantial amount of **7** (35%), under the same conditions **1a** proved to be almost completely resistant to ruthenium-catalysis and the reaction led to the isolation of **7** in low yield (22%). Cinchona hybrid **6c** could only be detected by ^{1}H NMR in traces in the crude product. These findings suggest that—strongly depending on the nature of the spectator ligands—9-OH group might coordinate to the Ru(II)-centre to form the catalytically



Reaction conditions: i.) $CuSO_4{\cdot}5H_2O$ (1 mol%), Na-ascorbate (5 mol%), H_2O/*n*-BuOH (1:1), rt, 10 h; ii.) $CuSO_4{\cdot}5H_2O$ (4 mol%), Na-ascorbate (20 mol%), H_2O/*n*-BuOH (1:1), rt, 2 h; iii.) Cp*RuCl(COD) (5 mol%), DCE, 1 h, 45 °C, Ar; iv.) Cp*RuCl(PPh_3)$_2$ (5 mol%), dioxane, 12 h; 60 °C, Ar; v.) CuI (2 mol%), $PdCl_2(PPh_3)_2$ (5 mol%), DIEA, 24 h, 25 °C, Ar.

Scheme 1. Synthesis of the targeted ferrocene-cinchona hybrids.