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Synthesis and antiproliferative evaluation of novel hydroxypropyl-ferrociphenol derivatives, resulting from the modification of hydroxyl groups

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ABSTRACT

As previously reported, the ferrocenyl derivative $HO(CH_2)_3C(Fc) = C(C_6H_4OH)_2$ (**2**) shows an excellent cytotoxic effect against MDA-MB-231 (TNBC) cancer cell lines. Building on an analysis of this molecular framework, a series of novel hydroxypropyl-ferrociphenol derivatives with modified terminal hydroxyl groups were synthesized, and their antiproliferative activities against MDA-MB-231 cell lines were evaluated. Biological results showed that compound **8**, whose terminal hydroxyl was protected by acetylation, exhibited the greatest cytotoxic effect among this series of hydroxypropyl derivatives. Furthermore, the impact of acetyl as a protecting group on the cytotoxicity of hydroxypropyl-ferrociphenol compounds by incorporating it at alkyl or phenyl hydroxyl positions of the core structure has been studied. Several of the compounds presented in this study revealed lipophilicity more suitable for formulation in lipid nanocapsules (LNCs) for subsequent in vivo studies. They also inhibit the cancer cell growth of MDA-MB-231 at a submicromolar IC_{50} value, providing an interesting potential for further development as innovative anticancer agents.

1. Introduction

Since it was first proposed as a topic in 1985 [1], bio-organometallic chemistry has become increasingly popular owing to the unveiling of several therapeutically active organometallic compounds currently undergoing clinical trials [2–4]. In this context, compounds possessing metal-carbon covalent bonds have been widely studied as drug candidates or imaging agents for the treatment of various diseases [5–11]. It provides a broad space for the Medicinal Chemist to develop novel molecules that are different from conventional drugs, in terms of chemical structure and mechanisms of biological action [12–17]. We have previously developed a series of compounds, entitled ferrociphenols, resulting from the incorporation of a ferrocenyl group into the tamoxifen backbone, and have shown the [ferrocenyl-ene-phenol] motif to be sensitive to an oxidative environment [18]. Many of these organometallic compounds exhibit a marked antiproliferative effect against MDA-MB-231 triple negative breast cancer cells (TNBC); an archetypical example is compound **1** with an IC_{50} value around 0.6 μM (Fig. 1). Moreover, compounds in this series inhibit the proliferation of breast cancer cells but have no cytotoxic effect on normal cells ($IC_{50} > 100–200$ μM) [19,20], which makes them more attractive in terms of selective toxicity as compared to platinum complexes. We have also demonstrated that the ferrociphenols exert their cytotoxic activity by formation of quinone methides (such as **1-QM**) [21–23] that are easily attacked by nucleophiles present in the cancer cell [24], at least partly affecting the cellular redox balance [25]. It should be emphasized that the ferrocenyl group plays a significant role in the oxidation of ferrociphenols to give the corresponding quinone methides [21,24].

More recently, based on an investigation of the metabolites of ferrocenyl derivatives, we have prepared the hydroxypropyl derivative of ferrociphenol, $HO(CH_2)_3C(Fc) = C(C_6H_4OH)_2$ (**2**), which displays excellent antiproliferative activity against a range of primary and metastatic neoplasms including MDA-MB-231 TNBC cancer cells (IC_{50} value approximately 0.11 μM) [26]. Formation of

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the new tetrahydrofuran-substituted quinone methide (**2-QM**) via internal cyclization of the hydroxy-alkyl chain may explain its greater cytotoxic activity. The stronger and broad-spectrum anti-tumor effect of hydroxypropyl-ferrociphenol **2** prompted us towards further chemical exploration of this framework to elucidate the active motif. An increase in the lipophilicity of compound **2** by modification of its hydroxyl group may increase the quantity of this molecule encapsulated in lipid nanocapsules (LNCs), thus increasing the quantity of drug available for administration [27]. As part of this examination, we first chose to examine the influence of its alkyl chain, for example, by replacing the terminal hydroxyl substituent with its classical bioisosteres including chloro- and thiol- to test their influence on cytotoxic activity (Fig. 2). Hydroxamate-based analogues [28] or oxime ether fragments [29–31] are widely applied in medicinal chemistry as ubiquitous pharmacophores that display a broad range of biological activity. By combining our hydroxypropyl-ferrociphenol skeleton with some N-substituted hydroxylamine derivatives, we were also able to probe how such substituents influence the lipophilicity and cytotoxicity of these types of molecules. Protecting groups are often used in drug development to improve the stability and bioavailability of molecules; these so-called prodrugs take action only after *in situ* hydrolysis by appropriate enzymes in vivo. Inspired by this concept, two different acyl protecting groups were incorporated at the hydroxyl position with the aim of monitoring the formation of their quinone methides, and also their cytotoxic behavior.

We here present the syntheses and antiproliferative evaluation of a series of novel hydroxypropyl-ferrociphenol derivatives with modified hydroxyl substituents. The effect of several pharmacophores, such as chloro, thiol and hydroxamate, on the lipophilicity and cytotoxicity of ferrocenyl-containing molecules is explored. The selective introduction of one or more acyl groups at different positions within the hydroxypropyl-ferrociphenol skeleton, and the comprehensive structure-activity-relationship of the resulting compounds is also reported.

2. Results and discussion

2.1. Synthesis

An efficient synthesis for the desired alkenes was established based on McMurry cross-coupling by the method previously developed in our group. As depicted in Scheme 1, 4,4'-dihydroxybenzophenone was coupled with 4-chloro-1-ferrocenylbutan-1-one or methyl 4-oxo-4-ferrocenylbutanoate using $TiCl_4$/Zn in dry THF, to give compounds **3** [32] or **14**, respectively, as brown solids. Compound **4** bearing a terminal thiol unit was prepared *via* a two-step procedure involving an isothiuronium intermediate. After initial nucleophilic substitution of chloride in **3** by thiourea in the presence of KI, followed by removal of solvent, the crude isothiuronium obtained was then hydrolyzed in NaOH solution (EtOH/H_2O) to give compound **4** in an overall yield of 89% for the two steps. Similarly, the isoindoline-1,3-dione analog **5** was synthesized by nucleophilic displacement of Cl in **3**. Compounds **6** and **7**, respectively, were obtained by treatment of **5** with a solution of hydrazine hydrate in acetone/methanol, or by the alcoholysis in methanol.

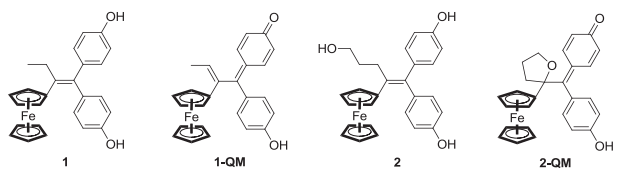

Fig. 1. Structures of ferrocenyl compounds **1**, **2** and their corresponding quinone methides.

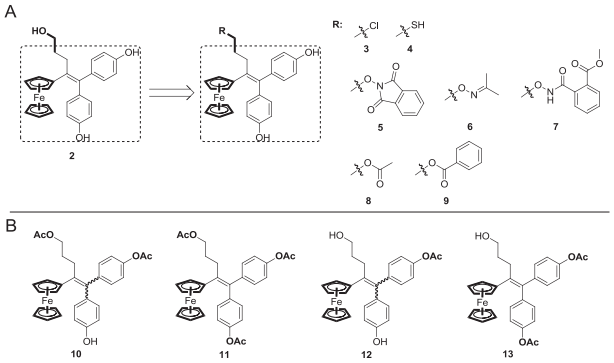

Fig. 2. New hydroxypropyl-ferrociphenol compounds. **A**. Structural modification of the terminal hydroxyl on the alkyl chain of compound **2**; **B**. A range of hydroxypropyl-ferrociphenol derivatives possessing multiple acetyl groups.

Hydroxypropyl-ferrociphenol, **2**, was obtained from the ester **14** by reduction using $LiAlH_4$ in THF solution. However, since compound **2** possesses one aliphatic and two phenolic hydroxyl groups, it was necessary to find suitable reagents to control acetylation at only the desired positions. Attempted acetylation at a single phenolic site, as in **12**, by treatment of compound **2** with AcCl or Ac_2O in the presence of TEA gave instead the triacetylated molecule **11** as the major product. There was no selectivity of the esterification between aliphatic and phenolic hydroxyl groups when using the Ac_2O/TEA system. Gratifyingly, when the weaker base pyridine was allowed to react with one equivalent of **2** and two equivalents of Ac_2O, the mono- and di-acetylated compounds **12** (32%) and **13** (42%) were selectively obtained, and could be separated by chromatography. In general, the phenols are predominantly acylated in the presence of aliphatic alcohols due to the greater nucleophilicity of phenols under basic conditions. Fortunately, reversal of chemoselectivity has been reported in a number of Lewis acid catalyzed esterifications [33,34]. Thus, for compounds **8** and **10** whose phenolic –OH groups were apparently exposed, the Lewis acid trimethylsilyl hydroxyl group using two equivalents of Ac_2O [34] to give compounds **8**, **10** and **11** that were easily purified by chromatography. When the same method was used for benzoylation, the single product was compound **9** (82% yield), whereby reaction occurred only at the aliphatic site.

2.2. Biological evaluation of new hydroxypropyl-ferrociphenol derivatives

The antiproliferative effect of the new hydroxypropyl ferrociphenol derivatives were evaluated on the triple negative breast cancer cell line MDA-MB-231, and the IC_{50} and $\log P_{o/w}$ values of these compounds are summarized in Table 1. All of the hydroxypropyl ferrociphenol derivatives tested inhibit the growth of MDA-MB-231 cancer cells from the micromolar to the submicromolar level, except for compound **4** which possesses a terminal thiol group. This compound does not show a significant cytotoxic effect on MDA-MB-231 cells even at the concentration of 20 μM; moreover, its $\log P_{o/w}$ value was 9.5 indicating the high hydrophobic property of this molecule. Mass spectrometric experiments have shown that the molecule present in the incubation medium for cancer cells during IC_{50} test and the stock solution for lipophilicity measurement was the dimer of compound **4**, formed by the oxidative coupling of thiols. This observation not only provides a reasonable explanation of its high $\log P_{o/w}$ value, but also the

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