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Synthesis and characterization of new ferrocenyl compounds with different alkyl chain lengths and functional groups to target breast cancer cells

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ABSTRACT

A new family of organometallic compounds bearing chains of different lengths and with different functional groups was synthesized and evaluated against triple negative MDA-MB-231 and hormone-dependent MCF-7 breast cancer cells. Biological results comparing suberamides (six-methylene chain length) and succinamides (two methylene chain length) showed that chain shortening does not dramatically impact on their antiproliferative effects. Cytotoxicity of primary amides is not dependent on chain length, as suberamide and succinamide showed almost identical activity against both types of breast cancer cells. However, the possibility that some of the cytotoxic activity of hydroxamides could be related to enzyme inhibition, e.g. histone deacetylase (HDAC) inhibition, is not excluded. This is based on the fact that compounds bearing a longer alkyl chain showed IC_{50} values lower than those with shorter alkyl chains. Succinic and adipic carboxylic acids and a succinimide were also tested and they also showed cytotoxic activity. Interestingly, succinimide was the most active compound against hormone-dependent MCF-7 breast cancer cells, presumably owing to an antagonist effect with the alpha form of the estrogen receptor (ERα). Some new and interesting side chain influences related to anti-proliferative effects can therefore be observed.

1. Introduction

Organometallic compounds show particular physicochemical properties depending on the nature of the metal moieties. Their use in catalysis is very well known and their application in drug synthesis constitutes one of the most common practices of organic chemists. However, at the end of the last century, it was demonstrated that these compounds could themselves be used to treat diseases [1]. Redox properties and 3D structures of organometallic compounds are some of the countless features that have attracted the attention of researchers to design new drug candidates and to explore new mechanisms of action [2]. Recently, we have incorporated the pharmacophore of the known drug (N^1-hydroxy-N^8-phenylsuberamide) **SAHA** into the ferrocifen (**FcTAM3**) molecule as a strategy to explore the mechanism of action of ferrocifen derivatives (Fig. 1) [3]. **SAHA** is a histone deacetylase inhibitor (HDACi) drug used to treat primary cutaneous T-cell lymphoma [4,5] while **FcTAM3** and derived compounds are organometallic tamoxifen-type species which proved to be more active than the corresponding organic analogs against hormone-dependent MCF-7 breast cancer cells. Moreover, they were also found to be strongly cytotoxic against triple negative MDA-MB-231 breast cancer cells, where tamoxifen (**TAM**) and related organic compounds are completely inactive under the same conditions [6].

Biological studies of the resulting organometallic hybrid **FcTAM-SAHA** on triple negative MDA-MB-231 breast cancer cells showed that the combination of both features gave them beneficial effects in terms of antiproliferative activity. Analyzing their cytotoxicities separately, one can see that, in the case of **SAHA**, the presence of the organometallic moiety 2-ferrocenyl-1-phenylbut-1-en-1-yl, as a substituent on the 4 position of its cap, enhanced its activity more than five times (from $IC_{50} = 3.64$ μM to 0.70 μM) while in the case of **FcTAM3**, the presence of the 8-hydroxyamino-8-oxooctanamido group instead of its dimethylaminoalkoxy chain made it over three times more effective (from $IC_{50} = 2.62$ μM to 0.70 μM).

Regarding hormone-dependent MCF-7 breast cancer cells, the positive effect of the structural combination of **SAHA** and **FcTAM3**

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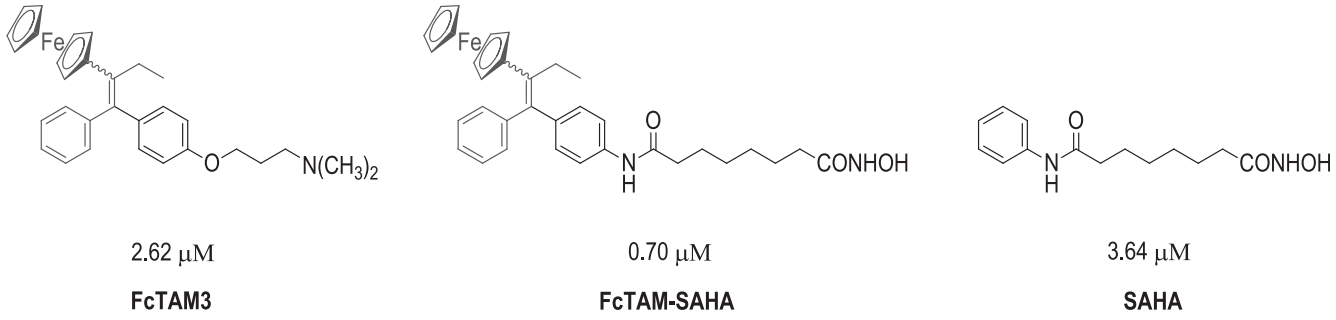

Fig. 1. Structures of **FcTAM3**, **SAHA** and **FcTAM-SAHA** with their corresponding IC_{50} values against triple negative MDA-MB-231 breast cancer cells.

was only observed in the case of the latter. Such modification more than doubled the activity of **FcTAM3** (from IC_{50} = 4.41 μM to 2.01 μM). However, **SAHA** modification did not increase its antiproliferative activity against MCF-7, since on these hormone-dependent breast cancer cells the organometallic derivative **FcTAM-SAHA** remained little more than half as active as **SAHA** (IC_{50} = 2.01 μM for **FcTAM-SAHA** *vs* 1.04 μM for **SAHA** in this study). Interestingly, this behavior on MCF-7 was also reported for two other organometallic **SAHA** analogs, the N^1-ferrocenyl-N^8-hydroxysuberamide (**JAHA**) prepared by Spencer et al. [7] and the tricarbonyl[(8-hydroxyamino-8-oxooctanamido)-η^5-cyclopentadienyl]rhenium(I) prepared by Can et al. [8] (Fig. 2).

On the other hand, both **SAHA** and **FcTAM-SAHA** were shown to chelate metal ions such as Fe^{3+} and they are believed to bind Zn^{2+} as well [9]. This feature plays an important role in biochemistry since these chelating effects could be exploited to inactivate enzymes [10]. For example **FcTAM-SAHA** was shown to be active as HDACi [3]. In this context, it is important to state that some members of the **JAHA** family, which are also ferrocenic, were reported to be effective HDACi against some enzyme isoforms [11].

In an attempt to determine whether the *N*-hydroxamide function on **FcTAM-SAHA** was structurally necessary to produce cytotoxic effects, and thus to estimate the effect of the **SAHA** pharmacophore in the hybrid molecule, the primary amide **FcTAM-PSA** (Fig. 3) was also synthesized and evaluated. Surprisingly, this molecule also showed strong antiproliferative activity against both types of breast cancer cells, with IC_{50} values even slightly lower than those of **FcTAM-SAHA**. Further, its organic analog, the *N*-phenylsuberamide (**PSA**) was very far from achieving the same cytotoxic effects even at higher concentrations (>10 μM).

Interestingly, both **PSA** and **FcTAM-PSA** exhibited neither chelating capacities nor any HDACi activity, seeming to indicate that cytotoxic effects are not strongly dependent on the functionalization on the lateral alkyl chain of both suberamides to produce almost the same response. This also means that the two compounds may share at least one particular mechanistic feature which is responsible for their strong cytotoxic effects. This would correlate with the fact that both **FcTAM-SAHA** and **FcTAM-PSA** were able to generate the expression of the tumor suppressor gene p21 [3].

On the other hand, it is possible to find in the literature that one of the most common strategies in structural–activity relationship studies of biologically active molecules is chain length modification. For example, an idoxifene analog bearing an alkyl chain modified by the presence of eight methylene groups instead of two did not show any improvements in terms of *in vitro* antiestrogenicity or antitumor activity [12]. However, it has been suggested that the chemical nature of the residue located at the end of the long side chain of other related antiestrogens may be an important factor. Another example is the evaluation of 4-phenylbutyric acid analogs, which were believed to act as HDACi. Data suggested that there was no correspondence between their HDACi activity and their cytotoxic effects against a mouse erythroleukemia cell line [13]. The chain length increase, from four carbons to ten, resulted in greater antiproliferative activity but a decline in their HDACi effects, suggesting that they may differ in their mechanism of action. Other studies on derivatives of **SAHA** have explored the chain-length dependence of enzymatic activity [14]. Biological evaluations of compounds combining the bioavailability of short-chain fatty acids such as butyric acid with the binding ability of the hydroxamic function in **SAHA** showed, with modest results, that the most effective species consisted of compounds bearing longer chains with hydroxamic acid groups [15]. Finally, some studies seeking potent multi-acting molecules targeting HDAC, the epidermal growth factor receptor (EGFR) and the human epidermal growth factor receptor 2 (HER2) at the same time for the treatment of cancer, showed that chain length is an important motif for HDAC inhibition, the eight-carbon long-chain analogs being the most active agents [16,17]. Our group has also reported the effects of side-chain modification in structure–activity relationship studies of the ferrocifen family [6,18].

The aim of the present investigation is to explore the impact of this effect in ferrocenyl hydroxy and primary amides derived from adipic and succinic acids on their antiproliferative activity against breast cancer cells. Carboxylic acids and a succinimide are also included in this study in order to evaluate other chemical functionalities.

2. Results and discussion

2.1. Synthesis of adipic compounds

The synthesis of the three adipic derivatives that were tested in biological assays was performed as described in the literature (Scheme 1) [3]. Aniline **1** was previously obtained as a mixture of *Z* and *E* isomers (*Z*/*E* ratio = 85/15) from a McMurry cross coupling between 4-aminobenzophenone and propionylferrocene [3,19]. To obtain carboxylic acid **2**, aniline **1** reacted with adipoyl chloride ($ClCOCH_2CH_2)_2$) in 20 min at room temperature to afford 51% of product. As reported, lower temperatures did not significantly increase the yield, and bisamide **3** is always obtained as byproduct. After aniline **1** was consumed, the mixture was allowed to react with sodium hydroxide (NaOH) to ensure that chlorides transformed into carboxylates and consequently acidification was needed to favor the soluble form in organic solvents for extraction.

To obtain hydroxamide **4**, a solution of hydroxylamine was first prepared at 0 °C from 4 equivalents of hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) and 8 equivalents of potassium hydroxide (KOH). Simultaneously, carboxylic acid **2** reacted with 2 equivalents of ethyl chloroformate ($ClCO_2Et$) and 2.5 equivalents of triethylamine (TEA) to result in a carbonate intermediate. This latter was attacked