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Synthesis of gemini triethylene-tetramine bridged bis-tridentate iron(III) chelators

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ABSTRACT

Eight gemini bis-2-(2-hydroxyphenyl)-thiazole-4-carboxamide and -thiocarboxamide (BHPTC) chelators were efficiently synthesized. Mass spectrometry showed these compounds all form 1:1 complexes with iron(III). Three of these chelators exhibit promising antiproliferative activities when tested on human cancerous cell lines.

1. Introduction

The predominance of iron in the active sites of enzymes catalyzing crucial metabolic processes accounts for the promising bioactive properties of iron chelators in medicine. Two types of pathologies are historically more concerned with the applications of iron chelators: iron chelation therapy (ICT) for patients suffering of iron overload and the approaches in the treatment of cancer.[1] Two key characteristics of cancer cells are their rapid multiplication and loss of contact inhibition, leading to tumor development or the proliferation of leukemic cells. Cell division is a complex process involving a large number of iron-dependent enzymes and regulatory proteins.[2,3] Iron depletion by chelators can compromise the entire process of cell division. Thus, iron chelators can be used to decrease DNA synthesis, stop cell division (antiproliferative and cytostatic activity), promote apoptosis (cytotoxic activity) simultaneously, thereby containing the proliferation of cancer cells, and lowering the risk of metastatic dissemination.[4] The first molecules tested in this context were originally developed for ICT like desferrioxamine (also called Desferal or DFO) and ICL670.[5,6] However a new generation of iron chelators, compatible with the specific needs of cancer treatment, gradually emerged.[4] In this context, triapine **1** (or 3-AP),[7] tachpyridine **2**,[8] di-2-pyridyl thiosemicarbazones like Dp44mT[9] **3**, and the pyridoxal isonicotinoyl hydrazone (PIH) family[10] appear to be promising molecules for this application (Fig. 1).

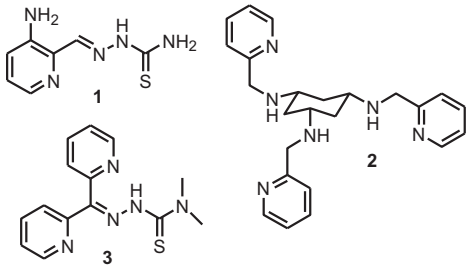

Fig. 1. Structures of triapine **1**, tachpyridine **2**, and Dp44mT **3**.

All these compounds are efficient tridendate iron(III) chelators.[11] Astonishingly, among the huge diversity of chelators developed to date,[12] only rare examples of bis-tridentate chelators for therapeutic purposes were reported.[13] In this context, we described recently the synthesis of the first generation of chelators of the bis-hydroxyphenyl-thiazole-carboxamide (BHPTC) family. These chelators were characterized by two 2-(2-hydroxyphenyl)-thiazole-carboxylic acid chelating moieties connected through amide functions with a spacer arm derived from 2,2′-(ethylenedioxy)-bis-ethylamine.[14] These compounds proved to be hexacoordinate bis-tridentate chelators forming 1:1 complexes with iron(III) and able to bind efficiently this metal ion in physiological media.[14] The in vitro biological

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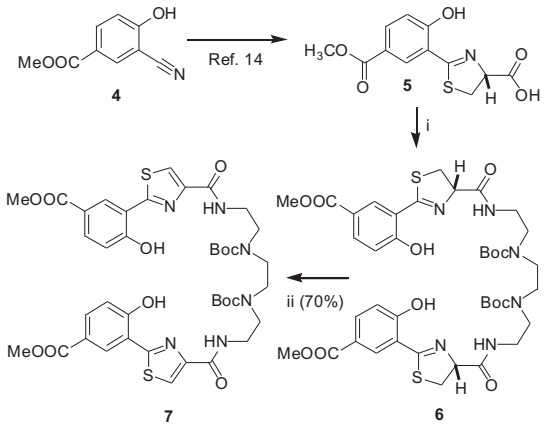
tests suggested that this first generation of BHPTC chelators are promising lead compounds to develop either new antiproliferative chelator or, for some of them, suitable for iron chelation therapy (ICT). These results prompted us to develop new generations of BHPTC with increased therapeutic potential for each of both these two applications. The antiproliferative chelators target the intracellular iron pool and should be able to cross efficiently the membranes to compete with metallo- and iron storage-proteins in tumors. On the opposite, it was observed that hydrophilic chelators are more suitable for ICT since they are able to harvest preferably the extracellular chelatable iron pool with as a consequence a lesser intracellular toxicity. The present article reports the synthesis and the preliminary antiproliferative properties of eight iron chelators of the bis-hydroxyphenyl-thiazole-carboxamide and -thiocarboxamide (BHPTC) family with various lipophilicity/hydrophilicity profiles. In a preliminary biological evaluation, three of these new compounds present promising antiproliferative properties.

2. Results and discussion

These newly synthesized chelators conserve the gemini structure made of two tridentate 2-(2-hydroxyphenyl)-thiazole-carboxylic acid bridged with a flexible spacer arm. The 2,2′-(ethylenedioxy)-bis-ethylamine linker of the first BHPTC generation was, in this approach, replaced by a spacer arm derived from the triethylenetetramine. Molecular modeling proved that the length and flexibility of both linkers are identical, inducing an optimal organization of the two tridentate moieties around iron(III). However the protonation of two secondary amine functions embedded in the triethylene-tetramine linker should lead to chelators with increased water solubility. On the opposite, these secondary amines could be acylated to generate chelators with increased lipophilic properties. The starting material for the synthesis of our chelators is 3-cyano-4-hydroxymethylbenzoate **4**.[14,15] The conversion of nitrile **4** into 2-(2-hydroxy-5-methoxycarbonyl-phenyl)-4,5-dihydro-thiazole-4-carboxylic acid **5** was previously described by our group.[14] The crude thiazoline **5** was used as it is to be coupled with 2,2′-{ethylenebis[(*tert*-butoxycarbonyl)imino]}diethan-1-amine[16] in the presence of EDCI. The resulting crude mixture of diastereoisomers **6** was then treated with $CBrCl_3$ in the presence of DBU,[17] leading to the corresponding dithiazole compounds **7** (Scheme 1).



Scheme 1. Synthesis of compound **7**. (i) EDCI, CH_2Cl_2, 20 °C ii. $CBrCl_3$, DBU, CH_2Cl_2, 20 °C.

In a first approach, the aromatic ring was substituted with a methylester function in *para* from phenol. This function may be converted easily into many other organic functions. This strategy makes possible, at one and at the same time, to tune the acido-basic/chelating properties of the phenol function and the solubility of our chelators in physiological medium. The two tridentate moieties are connected to each other by a spacer arm through amide/thioamide groups. Thus, chelator **7** was prepared from nitrile **4** in 60% overall yield on three combined steps with only one final chromatographic purification. Carboxamide **7** was then converted into the corresponding thiocarboxamide **8** using Lawesson's reagent.[18] A saponification of compounds **7** and **8**, converted these two diesters into the corresponding diacids **9** and **10**. The treatment of compounds **7**–**10** with 5% TFA in dichloromethane leads to the free diamines **11**–**14** isolated as the bis-ammonium trifluoroacetate salts (Scheme 2).

The more remarkable point of these syntheses is the perfect regioselectivity of the thionation reaction promoted by the Lawesson reagent. Thus for the molecules **7** and **8** bearing three different carbonyl functions (amide, ester, and carbamate) no side products resulting from the thionation of the ester and carbamate functions, were detected.

In order to control the ability of our chelators to complex iron (III), compounds **7**–**14** were treated with a hydromethanolic solution of iron trichloride. The corresponding dark complexes were isolated and a mass spectrometry analysis showed that the ferric chelates were formed in a 1:1 stoichiometry, usually as major products. However, in the case of chelators **7**–**10** some additional chelates were observed, resulting from the partial cleavage of Boc groups induced probably by the treatment with iron trichloride. Finally, as described previously for the first generation of BHPTC chelators,[14] the desulfurization products, which may be expected by the reaction of dithiocarboxamide compounds **8**, **10**, **12**, and **14** with a Lewis acid such iron trichloride were neither observed nor isolated in our experiments.

We therefore assessed the cytotoxicity of these molecules and their antiproliferative activity in several human cancer cell lines. Compounds **7**–**14** were tested at the two concentrations – 10 μM and 1 μM—for their antiproliferative activities on the following 13 human cancerous cell lines: KB (epidermoid carcinoma), HCT116, HT29, and HCT15 (colon adenocarcinoma cells), MCF7 (breast adenocarcinoma), MCF7R (doxorubicin chimio-resistant MCF7), SK-OV-3 (ovary adenocarcinoma), HepG2 (hepatocarcinoma), PC-3 (prostate adenocarcinoma), A549 (lung carcinoma), HL60 (promyeocytic leukemia), K562 (chronic myelogenous leukemia), and finally SF268 (glioblastoma). The antiproliferative activity of these eight compounds was compared to the desferrioxamine (DFO) (Table 1).

In our experiments, DFO was used as a control since, this chelator, used for decades and commercially available, still the gold standard in the field of iron chelation for therapeutic purpose. DFO has only an average to poor antiproliferative activity at 10 μM and only a low impact on proliferation at 1 μM. Besides, compounds **7**, **8**, and **14** appear to be the most interesting molecules since they show a high activity at 10 μM especially on cancer cells from the respiratory system (KB, A549) and colon cancer (HCT116). Although this effect is drastically lowered at 1 μM (except compound **8** for the A549 cell line), the antiproliferative activities of compounds **7**, **8**, and **14** are higher than those described for DFO or the other commercially available chelators deferiprone[19] or deferasirox.[6] Among all the chelators synthesized, **9**, **10**, **11**, **12**, and **13** are generally as or less active as DFO whatever the concentrations or the cell lines considered. The compounds **7**–**14** are characterized by a common molecular architecture but they differ drastically by the heteroatoms involved in the metal coordination shell (carboxamide vs thiocarboxamide) and the solubility (lipophilic vs charged/hydrophilic) of the chelator. The comparison of the structures with the antiproliferative activities measured could give us the structural guidelines leading to the next generation of antiproliferative BHPTC. In this context thiocarboxamide compounds (**8**, **10**, and **14**) appear to have a higher antiproliferative activity than the corresponding carboxamide chelators (**7**, **9**, and **13**). This observation