Tetrahedron 67 (2011) 2149–2154

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

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

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ARTICLE INFO

Article history:
Received 22 November 2010
Received in revised form 30 December 2010
Accepted 5 January 2011
Available online 12 January 2011

Keywords:
Iron chelator
Cancer
Iron chelation therapy
Triethylene-tetramine
BHPTC
Thioamide

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).

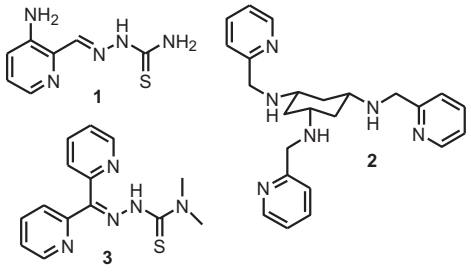

Fig. 1. Structures of triapine **1**, tachpyridine **2**, and Dp44mT **3**.

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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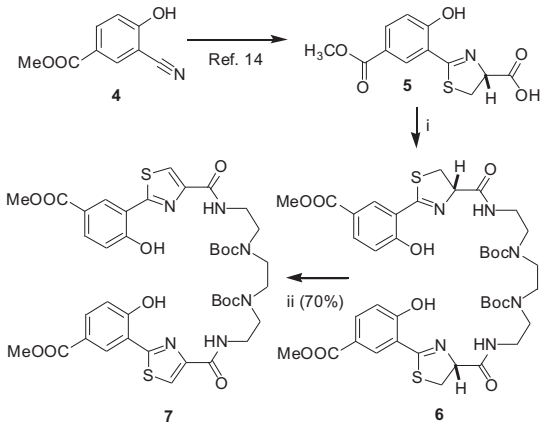
0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.01.008

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.

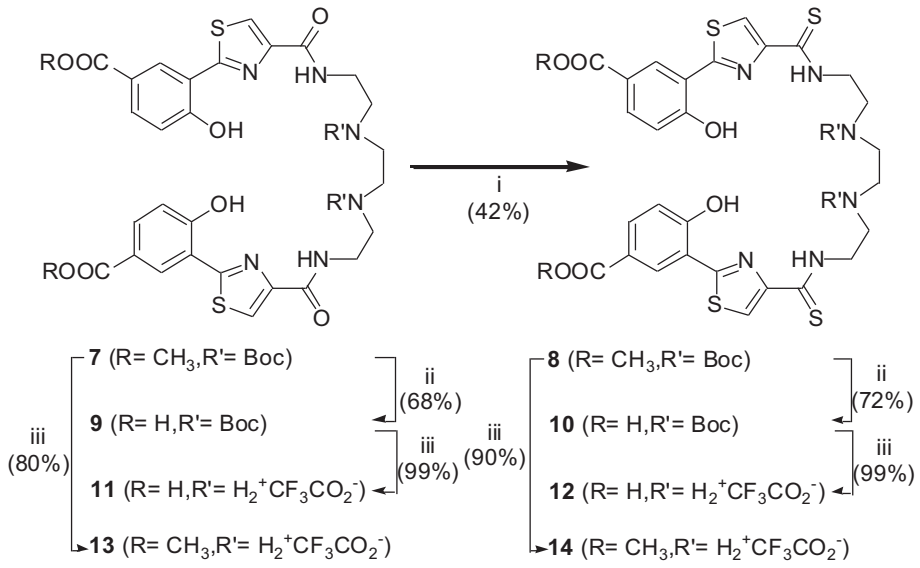
In a first approach, the aromatic ring was substituted with a methylester function in *para* from phenol. This function may be converted easily into many other organic functions. This strategy makes possible, at one and at the same time, to tune the acido-basic/chelating properties of the phenol function 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



Scheme 2. Synthesis of compounds **7**–**14**. (i) Lawesson reagent, toluene, reflux. (ii) KOH_{aq} 1 N, dioxane, 60 °C. (iii) CH_2Cl_2, TFA 5%, 20 °C.

Table 1
In vitro antiproliferative activity on 13 cancer cell lines after 72 h exposition to compounds **7**–**14** at 10 μM and 1 μM

Cell lines	**7**[a,b]	**8**[a,b]	**9**[a,b]	**10**[a,b]	**11**[a,b]	**12**[a,b]	**13**[a,b]	**14**[a,b]	DFO[a,b]
KB	79/0	92/26	0/0	37/0	0/0	0/0	0/0	96/4	16/0
HCT116	44/0	92/8	1/0	24/0	0/0	0/0	1/0	78/11	20/0
HT29	18/2	22/0	1/1	4/0	9/2	0/0	3/0	57/0	42/0
HCT15	30/0	85/0	23/19	35/9	0/5	0/2	13/0	75/0	46/0
MCF7	0/0	63/0	0/0	13/0	0/0	0/0	0/0	69/0	0/0
MCF7R	28/0	63/0	0/0	9/0	0/0	0/0	5/0	41/0	9/0
A549	64/0	90/72	5/6	27/7	5/0	0/0	4/1	71/16	50/2
PC-3	53/7	60/9	7/0	20/7	6/10	0/0	0/6	32/10	44/1
SF268	22/0	42/0	1/0	18/0	0/0	1/0	0/0	75/18	33/0
SK-OV-3	56/19	58/3	9/0	0/0	12/4	0/0	4/3	60/0	52/5
HL60	44/0	82/14	0/0	7/0	0/0	0/0	0/0	39/3	0/0
K562	53/7	74/0	10/9	0/0	4/1	0/0	7/4	72/0	0/0
HepG2	23/0	65/20	0/0	9/10	0/0	10/3	0/0	28/16	0/0

[a] Percentage of cell proliferation inhibition at 10^{-5} M/10^{-6} M.
[b] Values are means of triplicate experiments.

cannot be extended to thiocarboxamide **12**, which appears as ineffective in inhibiting proliferation as the carboxamide **11**. Both molecules are probably not lipophilic enough to cross the cell membrane and compete for intracellular iron pool.[20a] This observation is in good accordance with the fact that the lipophilic uncharged dithiocarboxamide chelator **8** is the most active compound among the eight BHPTC synthesized. In this context, the activity of compound **14** is intriguing: this chelator is as efficient as **8** even if **14** is highly hydrosoluble. Further biological investigations to explain this result are currently underway. All these informations may lead to the design of antiproliferative BHPTC iron chelators bearing preferably thiocarboxamide function along with increased lipophilic properties. A further synthetic diversification of the three chelators **7**, **8**, and **14** may improve their proliferation inhibition properties, reaching activities below the micromolar concentration reported for the chelators of the PIH family.[20] Iron chelators have a number of biological targets (pleiotropism) in human beings. This accounts for both their high efficacy for treating diseases and their toxicity when used over long periods or during particularly heavy therapies. Thus, even DFO promoted ICT has provided effective symptom relief in patients with chronic iron overload, this compound generates severe side-effects for long-term treatment.[21] The same problems were reported with the two main other ICT drugs, deferiprone and *Exjade* (deferasirox or ICL670).[22] Our experiments proved that chelators **9**, **11**, **12**, and **13** are less toxic than DFO whatever the organ concerned. Thus, apart the development of new antiproliferative chelators, our results may pave the way to the synthesis of new chelators designed for ICT.

3. Conclusion

We have described in this article an efficient synthetic route to eight gemini bis-tridentate iron chelators of the BHPTC (bis-hydroxyphenyl-thiazole-carboxamides or -thiocarboxamides) family. Compounds **7**–**14** were efficiently synthesized and proved to form one to one complexes with iron(III). These compounds were tested for their antiproliferative activity on 13 cancerous cell lines. Among these eight molecules, three of them (e.g., compounds **7**, **8**, and **14**) proved to have a higher antiproliferative activity than desferrioxamine (DFO). These results suggest that the BHPTC scaffold is a promising synthetic platform for the design of new iron chelators for anticancer chemotherapy. Surprisingly, the four compounds **9**, **11**, **12**, and **13** showed to be less toxic than DFO and may be therefore interesting leads for the design of new ICT devoted iron chelators. In the light of these results, the complexation of iron by bis-tridentate molecule appears to be a promising avenue of research in the development of a new generation of chelators for therapeutic purposes. Thus, based on the BHPTC molecular scaffold,

the synthesis of new chelating molecules suitable either for cancer treatment or ICT is currently underway in our group.

4. Experimental

4.1. General procedures

All the reactions were carried out under an inert argon atmosphere. Analytical grade solvents were used. Reactions were monitored by thin-layer chromatography (TLC), using Merck precoated silica gel 60 F_{254} (0.25 mm). Column chromatographies were performed with demetalated Merck kieselgel 60 (63–200 μm).[23] NMR spectra were recorded either on a Bruker Avance 200 (200 MHz for ^{1}H and 50 MHz for ^{13}C) or Avance 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C). Mass spectra were recorded in the *Service Commun d'Analyse* (*SCA*) *de la Faculté de Pharmacie de l'Université de Strasbourg* and were measured after calibration in ES-TOF experiments on a *Bruker Daltonic* MicroTOF mass spectrometer.

4.2. Protocols, analytical, and spectral data

*4.2.1. Benzoic acid, 3,3′-[1,2-ethanediylbis[[[(1,1-dimethylethoxy)carbonyl]imino]-2,1-ethanediyliminocarbonyl-4,2-thiazolediyl]]-bis[4-hydroxy],1,1′-dimethyl ester (**7**).* To a solution of 2,2′-{ethylene-bis[(*tert*-butoxycarbonyl)imino]}diethan-1-amine[16] (271 mg, 0.78 mmol) and 2-(2-hydroxy-5-methoxycarbonyl-phenyl)-4,5-dihydro-thiazole-4-carboxylic acid **5** (440 mg, 1.56 mmol)[14] in CH_2Cl_2 (15 mL), solid EDCI (359 mg, 1.87 mmol) was added and then stirred for 16 h at 20 °C under argon. The solvent was evaporated under reduced pressure before adding water (30 mL) and then adjusting pH to 2.0 with 1 N aqueous HCl. The aqueous phase was extracted with CH_2Cl_2 (3×30 mL) and the collected organic phases were dried over Na_2SO_4, filtered, and the solvents were eliminated under reduced pressure. The foamy residue (composed of a mixture of the diastereoisomers **6**) was dissolved in CH_2Cl_2 (40 mL), then DBU (0.41 mL, 2.74 mmol) and $CBrCl_3$ (0.30 mL, 3.04 mmol) were successively added at 0 °C. After addition, the mixture was stirred for 16 h at 25 °C under argon. The mixture was adsorbed on demetalated silica gel and purified by chromatography on a demetalated silica gel column (97:3→95:5 CH_2Cl_2/EtOH) to afford the compound **7** (475 mg, 70% yield in two steps from thiazoline **5**) isolated as a yellow amorphous solid. R_f=0.38 (95:5 CH_2Cl_2/EtOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.02 (br s, 2H), 8.90 (d, 2H, *J*=2.2 Hz), 8.61–8.47 (m, 2H), 8.28 (s, 2H), 7.91 (dd, 2H, *J*=8.6 Hz), 7.12 (d, 2H), 3.83 (s, 6H), 3.50–3.20 (m, 12H), 1.31 (s, 18H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 165.8, 161.5, 160.9, 159.0, 155.0, 154.8, 149.1, 132.2, 129.6, 124.6, 121.0, 119.3, 116.6, 78.9, 78.6, 51.9, 39.9, 39.1, 37.6, 27.9; ESI-MS *m*/*z* 769.2 ([M−Boc+2H]$^+$), 867.1 ([M−H]$^-$). High resolution ESI-MS: *m*/*z* found 869.2822 ([M+H]$^+$); *m*/*z* calcd for $C_{40}H_{49}N_6O_{12}S_2$: 869.2844.

*4.2.2. Benzoic acid, 3,3′-[1,2-ethanediylbis[[[(1,1-dimethylethoxy)carbonyl]imino]-2,1-ethanediyliminocarbonothioyl-4,2-thiazolediyl]]-bis[4-hydroxy], 1,1′-dimethyl ester (**8**).* The compound **7** (114 mg, 0.13 mmol) and Lawesson's reagent (131 mg, 0.32 mmol) were suspended in anhydrous toluene (10 mL). The suspension was refluxed for 3 h before being cooled down to room temperature. The solvent was evaporated under reduced pressure. The oily residue was dissolved in CH_2Cl_2 (15 mL), washed successively with water, a saturated aqueous solution of $NaHCO_3$, and finally brine. The organic phase was dried over Na_2SO_4, filtered, and the solvents were eliminated under reduced pressure. The residue was chromatographed on a demetalated silica gel column (99:1 CH_2Cl_2/EtOH) leading to the dithioamide **8** (49 mg, 42% yield) isolated as a yellow amorphous solid. R_f=0.74 (95:5 CH_2Cl_2/EtOH); ^{1}H NMR (300 MHz, DMSO-d_6, 333 K) δ 11.97 (br s, 2H), 10.54–10.32 (m, 2H), 8.99–8.86 (m, 2H), 8.46 (s, 2H), 7.92 (dd, 2H, *J*=2.1, 8.7 Hz), 7.13 (d, 2H), 4.04–3.86 (m, 4H), 3.83 (s, 6H), 3.62–3.51 (m, 4H), 3.41 (s, 4H), 1.32 (s, 18H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 186.4, 165.8, 161.1, 159.1, 155.3, 154.6, 152.8, 132.3, 129.8, 127.1, 121.0, 119.3, 116.5, 79.1, 78.6, 51.9, 44.6, 44.1, 43.4, 27.9; ESI-MS *m*/*z* 923.8 ([M+Na]$^+$), 899.0 ([M−H]$^-$), 802.0 ([M−Boc+2H]$^+$), 801.0 ([M−Boc]$^-$). High resolution ESI-MS: *m*/*z* found 901.2362 ([M+H]$^+$); *m*/*z* calcd for $C_{40}H_{49}N_6O_{10}S_4$: 901.2388.

*4.2.3. Benzoic acid, 3,3′-[1,2-ethanediylbis[[[(1,1-dimethylethoxy)carbonyl]imino]-2,1-ethanediyliminocarbonyl-4,2-thiazolediyl]]-bis[4-hydroxy] (**9**).* The compound **7** (100 mg, 0.12 mmol) was suspended in a mixture of dioxane (6.4 mL) and aqueous KOH 1 M (6.4 mL). The resulting suspension was stirred for 5 h at 60 °C. The mixture was then cooled to room temperature, diluted with water (25 mL), washed with CH_2Cl_2 (25 mL) before adding aqueous HCl (1 N) until pH 1.0. The resulting precipitate was filtered off and dried under vacuum, affording the compound **9** (66 mg, 68% yield) isolated as a pale yellow powder. R_f=0.62 (50:48:2 acetone/AcOEt/AcOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.63 (br s, 2H), 12.05 (br s, 2H), 8.89 (d, 2H, *J*=2.0 Hz), 8.64–8.46 (m, 2H), 8.28 (s, 2H), 7.90 (dd, 2H, *J*=8.6 Hz), 7.11 (d, 2H), 3.56 (s, 4H), 3.52–3.18 (m, 8H), 1.30 (s, 18H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 166.9, 161.8, 160.9, 158.7, 154.9, 149.1, 132.4, 129.8, 124.5, 122.1, 119.1, 116.4, 78.9, 78.5, 40.3, 39.5, 37.6, 27.9; ESI-MS *m*/*z* 863.0 ([M+Na]$^+$), 839.0 ([M−H]$^-$), 796.0 ([M−COOH]$^-$), 741.1 ([M−Boc+2H]$^+$), 739.0 ([M−Boc]$^-$), 641.0 ([M−2Boc+3H]$^+$). High resolution ESI-MS: *m*/*z* found 841.2514 ([M+H]$^+$); *m*/*z* calcd for $C_{38}H_{45}N_6O_{12}S_2$: 841.2531.

*4.2.4. Benzoic acid, 3,3′-[1,2-ethanediylbis[[[(1,1-dimethylethoxy)carbonyl]imino]-2,1-ethanediyliminocarbonothioyl-4,2-thiazolediyl]]-bis[4-hydroxy] (**10**).* The compound **8** (124 mg, 0.14 mmol) was suspended in a mixture of dioxane (7.5 mL) and an aqueous solution of KOH 1 M (7.5 mL). The suspension was stirred for 6 h at 60 °C and cooled down to room temperature. The mixture was diluted with water (25 mL) and washed with CH_2Cl_2 (25 mL) before adding HCl (1 N) to reach pH 1.0. The resulting precipitate was filtered off and dried under vacuum, affording the compound **10** (88 mg, 72%) isolated as a yellow powder. R_f=0.84 (50:48:2 acetone/AcOEt/AcOH); ^{1}H NMR (300 MHz, DMSO-d_6, 333 K) δ 12.51 (br s, 2H), 11.82 (br s, 2H), 10.50–10.32 (m, 2H), 8.95–8.85 (m, 2H), 8.45 (s, 2H), 7.91 (dd, 2H, *J*=8.7, 1.8 Hz), 7.12 (d, 2H), 4.02–3.86 (m, 4H), 3.59–3.50 (m, 4H), 3.39 (s, 4H), 1.32 (s, 18H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 186.5, 166.9, 161.4, 158.8, 153.0, 152.8, 132.5, 130.0, 126.9, 122.2, 119.1, 116.3, 79.0, 44.6, 44.3, 43.9, 27.9; ESI-MS *m*/*z* 871.4 ([M−H]$^-$), 855.3 ([M−OH]$^-$), 772.4 ([M−Boc]$^-$). High resolution ESI-MS: *m*/*z* found 871.1928 ([M−H]$^-$); *m*/*z* calcd for $C_{38}H_{43}N_6O_{10}S_4$: 871.1929.

*4.2.5. Benzoic acid, 3,3′-[1,2-ethanediylbis(imino-2,1-ethanediyliminocarbonyl-4,2-thiazolediyl)]-bis[4-hydroxy],2,2,2-trifluoroacetate (1:2) (**11**).* The compound **9** (64 mg, 22.3 μmol) was dissolved in a 5% solution of TFA in CH_2Cl_2 (2 mL). The resulting solution was stirred for 3 h at 20 °C before being evaporated under reduced pressure. The oily residue was dissolved in water (5 mL) and freeze-dried to afford compound **11** (65 mg, 99% yield) isolated as a white bis-TFA salt. R_f=0.59 (4:1:1 *n*-BuOH/H_2O/AcOH); ^{1}H NMR (200 MHz, DMSO-d_6) δ 12.77 (br s, 2H), 12.15 (br s, 2H), 8.93 (br s, 2H), 8.85–8.70 (m, 2H), 8.70–8.46 (m, 2H), 8.36 (s, 2H), 7.92 (br d, 2H, *J*=8.6 Hz), 7.15 (d, 2H), 3.74–3.56 (m, 4H), 3.32 (s, 4H), 3.32–3.16 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.0, 162.3, 162.2, 159.3, 148.5, 132.6, 129.8, 125.3, 122.1, 119.1, 116.5, 47.2, 42.9, 35.6; ESI-MS *m*/*z* 641.1 ([M+H]$^+$), 321.1 ([M+2H]$^{2+}$), 312.1 ([M−OH+H]$^{2+}$), 303.1 ([M+2OH]$^{2+}$). High resolution ESI-MS: *m*/*z* found 641.1497 ([M+H]$^+$); *m*/*z* calcd for $C_{28}H_{29}N_6O_8S_2$: 641.1483.

*4.2.6. Benzoic acid, 3,3′-[(1,12-dithioxo-2,5,8,11-tetraazadodecane-1,12-diyl)bis(4,2-thiazolediyl)]-bis[4-hydroxy], 2,2,2-trifluoroacetate (1:2) (**12**).* The compound **10** (60 mg, 68 μmol) was dissolved in

a 5% solution of TFA in CH_2Cl_2 (2 mL). The solution was stirred for 3 h at 20 °C then solvents were evaporated under reduced pressure. The oily residue was dissolved in water (5 mL) and freeze-dried, leading to compound **12** (61 mg, 99%) isolated as a white bis-TFA salt. R_f=0.55 (4:1:1 *n*-BuOH/H_2O/AcOH); ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.24 (br s, 2H), 12.21 (br s, 2H), 8.79 (br t, 2H, *J*=5.7 Hz), 8.95 (d, 2H, *J*=2.4 Hz), 9.06–8.82 (m, 2H), 8.55 (s, 2H), 7.93 (dd, 2H, *J*=8.7 Hz), 7.14 (d, 2H), 4.21–4.08 (m, 4H), 3.47–3.38 (m, 4H), 3.33 (s, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 187.7, 167.0, 161.6, 159.1, 152.6, 132.7, 129.9, 128.0, 122.1, 119.1, 116.5, 45.2, 42.9, 41.2; ESI-MS *m*/*z* 673.1 ([M+H]$^+$). High resolution ESI-MS: *m*/*z* found 673.1025 ([M+H]$^+$); *m*/*z* calcd for $C_{28}H_{29}N_6O_6S_4$: 673.1026.

*4.2.7. Benzoic acid, 3,3′-[1,2-ethanediylbis(imino-2,1-ethanediyliminocarbonyl-4,2-thiazolediyl)]-bis[4-hydroxy],1,1′-dimethyl ester,2,2,2-trifluoroacetate (1:2) (**13**).* The compound **7** (113 mg, 0.13 mmol) was dissolved in a solution of 5% TFA in CH_2Cl_2 (2 mL). The solution was stirred for 5 h at 20 °C before being evaporated under reduced pressure. The oily residue was dissolved in water (5 mL) and freeze-dried to afford the compound **13** (93 mg, 80% yield) isolated as a white bis-TFA salt. R_f=0.49 (10:1:1 $CH_3CN/H_2O/NH_4OH$); ^{1}H NMR (300 MHz, CD_3OD) δ 8.65 (d, 2H, *J*=2.4 Hz), 8.23 (s, 2H), 7.89 (dd, 2H, *J*=8.7 Hz), 6.97 (d, 2H), 3.89 (s, 6H), 3.84–3.80 (m, 4H), 3.65 (s, 4H), 3.48–3.44 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD) δ 168.1, 165.5, 165.3, 160.7, 149.0, 133.8, 131.2, 126.4, 123.0, 120.0, 117.6, 52.7, 49.9, 44.9, 37.4; ^{19}F NMR (188 MHz, CD_3OD) δ −73.4 (TFA salt); ESI-MS *m*/*z* 669.1 ([M+H]$^+$), 667.0 ([M−H]$^-$). High resolution ESI-MS: *m*/*z* found 669.1805 ([M+H]$^+$); *m*/*z* calcd for $C_{30}H_{33}N_6O_8S_2$: 669.1796.

*4.2.8. Benzoic acid, 3,3′-[1,2-ethanediylbis(imino-2,1-ethanediyliminocarbonothioyl-4,2-thiazolediyl)]-bis[4-hydroxy],1,1′-dimethyl ester,2,2,2-trifluoroacetate (1:2) (**14**).* The compound **8** (68 mg, 75 μmol) was dissolved in a 5% solution of TFA in CH_2Cl_2 (2 mL). The solution was stirred for 6 h at 20 °C and solvents were subsequently evaporated under reduced pressure. The oily residue was dissolved in water (5 mL) before being freeze-dried to afford the compound **14** (63 mg, 90%) isolated as a white bis-TFA salt. R_f=0.48 (10:1:1 $CH_3CN/H_2O/NH_4OH$); ^{1}H NMR (300 MHz, CD_3OD) δ 8.89 (d, 2H, *J*=2.4 Hz), 8.50 (s, 2H), 7.94 (dd, 2H, *J*=8.7 Hz), 7.04 (d, 2H), 4.29 (br t, 4H, *J*=5.7 Hz), 3.90 (s, 6H), 3.56 (br t, 4H), 3.54 (s, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 187.6, 165.9, 161.4, 159.4, 152.7, 132.5, 129.8, 128.0, 121.0, 119.3, 116.7, 52.1, 45.3, 43.2, 41.5; ESI-MS *m*/*z* 701.2 ([M+H]$^+$). High resolution ESI-MS: *m*/*z* found 701.1339 ([M+H]$^+$); *m*/*z* calcd for $C_{30}H_{33}N_6O_6S_4$: 701.1339.

*4.2.9. Ferric complexes of chelators (**7**)–(**14**).* To solutions of compounds **7**–**14** (7 μmol) in MeOH (2.5 mL), was added 0.22 M $FeCl_3$ (prepared by dilution of a commercial aqueous 2.2 M solution, 32 μL, 7.1 μmol). A dark blue suspension was obtained, which was stirred for 16 h at 20 °C. The solvent was evaporated and the residue suspended in water and sonicated for 2 min. Water was eliminated by freeze-drying, to obtain the corresponding ferric chelates. Ferric complex of chelator **7**: ESI-MS *m*/*z* found 922.2 ([M(**7**)−2H+Fe]$^+$). Ferric complex of chelator **8**: ESI-MS *m*/*z* found 954.2 ([M(**8**)−2H+Fe]$^+$). Ferric complex of chelator **9**: ESI-MS *m*/*z* found 894.1 ([M(**9**)−2H+Fe]$^+$). Ferric complex of chelator **10**: ESI-MS *m*/*z* found 926.1 ([M(**10**)−2H+Fe]$^+$). Ferric complex of chelator **11**: ESI-MS *m*/*z* found 232.1 ([M(**11**)+2H+Fe]$^{3+}$). Ferric complex of chelator **12**: MS *m*/*z* found 242.7 ([M(**12**)+2H+Fe]$^{3+}$). Ferric complex of chelator **13**: ESI-MS *m*/*z* found 241.4 ([M(**13**)+2H+Fe]$^{3+}$). Ferric complex of chelator **14**: MS *m*/*z* found 252.1 ([M(**14**)+2H+Fe]$^{3+}$).

4.3. Screening of antiproliferative activities

The antiproliferative activity of 13 cancer cell lines was evaluated at the *Ciblothèque Cellulaire de l'Institut de Chimie des Substances Naturelles* at Gif-sur-Yvette (France). The human cell lines KB (mouth epidermoid carcinoma) and HepG2 (hepatocarcinoma) were obtained from ECACC (Salisbury, UK) and grown in DMEM medium supplemented with 10% fetal calf serum (FCS), in the presence of penicillin, streptomycin, and fungizone, in a 75 cm^2 flask, under an atmosphere containing 5% CO_2. By contrast, HCT116, HT29, and HCT15 (colon adenocarcinoma), MCF7 (breast adenocarcinoma), MCF7R (MCF7 cell lines resistant to doxorubicine), SK-OV-3 (ovary adenocarcinoma from NCI), PC-3 (prostate adenocarcinoma), A549 (lung carcinoma), HL60 (promyelocytic leukemia), K562 (chronic myelogenous leukemia), and SF268 (glioblastoma from NCI) cells were grown in RPMI medium. Cells were plated in 200 μL of medium, in 96-well tissue culture microplates and were treated 24 h later with compounds dissolved in DMSO at concentrations of 1 and 10 μM, using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h of incubation, MTS reagent (Promega) was added and the plates incubated for 3 h at 37 °C. Absorbance was monitored at 490 nm and the results are expressed as the inhibition of cell proliferation, calculated as the ratio [(1−(OD_{490} treated/OD_{490} control))×100] in triplicate experiments.

Acknowledgements

G.L.A.M., D.R.L., and I.J.S. would like to thank the Centre National de la Recherche Scientifique (CNRS) and the Agence Nationale pour la Recherche (ANR-05-JCJC-0181-01) for global financial support. Authors would like to thank Région Alsace for financial support and for providing a postdoctoral fellowship to D.R.L.

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