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Porphyrin–aminoquinoline conjugates as telomerase inhibitors

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Received 22nd November 2002, Accepted 16th January 2003
First published as an Advance Article on the web 17th February 2003

A series of metalloporphyrins was prepared in order to target the G-quadruplex structure of telomeric DNA for the design of antitelomerase compounds. The initial cationic tetramethylpyridiniumyl porphyrin was modified by the replacement of one or two methylpyridiniumyl groups by one or two 4-aminoquinoline moieties, at the meso position, in order to increase the cell penetration and the quadruplex affinity. The porphyrins were either metallated by manganese or by nickel. The degradation of quadruplex DNA was assayed *in vitro* with the manganese redox-active derivatives. All porphyrins complexes were capable of inhibiting the telomerase enzyme with IC_{50} values in the micromolar range (TRAP assay).

Introduction

Telomeres consist of highly conserved DNA repeat G-rich-sequences, associated with proteins, at the end of chromosomes. The extreme terminal 3′-region of the G-rich strand is single-stranded and is elongated by the telomerase enzyme.[1,2] The single-stranded regions of the telomeres can fold to generate four-stranded guanine quadruplex secondary structures *in vitro* at physiological salt concentrations (Na^+ or K^+).[3,4] The identification of proteins in eukaryotic cells that recognize, generate or alter G-quadruplexes *in vitro* with remarkable specificity suggests that these structures are formed *in vivo*.[5–9] Following the discovery of aberrant telomere length regulation in numerous neoplasias, G-quadruplexes are being considered as a new molecular target for cancer therapeutics. Telomerase activity has been found in 85–90% of all human tumors but not in adjacent normal cells.[10] It has been hypothesized that for a tumor cell to undergo sustained proliferation beyond the limits of cellular senescence, telomerase should be reactivated in order to maintain telomere length. This fact makes telomerase a target for the development of novel therapeutic agents. The different approaches to telomerase inhibition currently consist of targeting the RNA component of telomerase with antisense oligonucleotides,[11] peptide nucleic acids,[12] ribozymes[13] or disturbing the telomeric structure and blocking the telomere/telomerase interaction with G-quartet affine agents. Compounds able to target the G-quadruplex structures *in vitro*[14–22] and compounds able to inhibit telomerase[23–25] have recently been reported. For a review concerning these various strategies see ref 26.

The tetracationic porphyrin, *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin, H_2–TMPyP, possesses the appropriate physico-chemical properties to interact with the G-quadruplexes.[15,27–31] It was shown to be an inhibitor of human telomerase *in vitro*.[15] CD and UV thermal melting studies together with photocleavage experiments showed that this porphyrin binds to and stabilizes the human telomeric DNA quadruplex $(GGGTTA)_4$ by external π-stacking with the last guanine tetrad. The corresponding manganese derivative, Mn–TMPyP (Fig. 1), is a powerful chemical nuclease.[32–34] It can be activated by an oxygen atom donor, potassium monopersulfate, $KHSO_5$, into a very reactive high-valent oxo-manganese porphyrin,

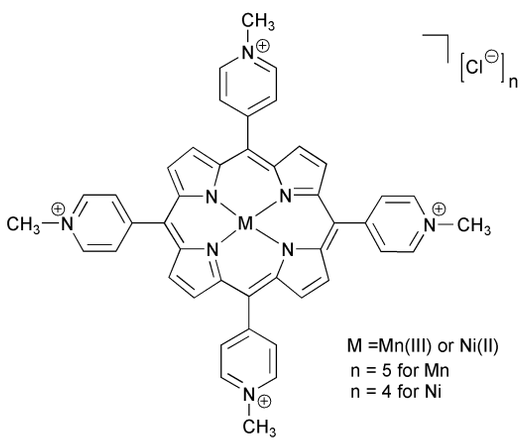

Fig. 1 Manganese (III)– or nickel (II)–bis(aqua)-*meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin (Mn– or Ni–TMPyP). The manganese complex exhibits two water molecules as axial ligands and is surrounded by five negative counter-ions.

Mn(V)=O species,[35] that is able to oxidize deoxyriboses[32,33] or guanine residues[34] within double-stranded DNA. We also reported recently the efficient oxidative degradation mediated to the human telomeric sequence by this oxo-manganese porphyrin.[36] The activation of Mn–TMPyP into a Mn(V)=O species should be possible *in vivo* with molecular oxygen and a source of electrons.[35]

In the present work, new porphyrin derivatives with aminoquinoline residues covalently linked to the cationic porphyrin core were synthesized. The $pK_a = 6.8$ for the 4-aminoquinoline moiety makes this compound particularly suitable for crossing cell membrane. Furthermore, since the aminoquinoline is known to interact with nucleic acids, the association of the metalloporphyrin and the 4-aminoquinoline moieties by a covalent link may increase the overall affinity of the modified porphyrin for the telomeric structures. Manganese and nickel porphyrin series were prepared. Depending on the nature of the metal inserted within the porphyrin cycle the mechanism of action of these molecules toward telomeres of cancer cells is expected to be different. The manganese porphyrin is redox-active, it is a potential DNA damaging agent. The nickel porphyrin is inert with respect to redox processes and has to be considered only as a G-quadruplex interacting agent. We describe the preparation of manganese and nickel porphyrins

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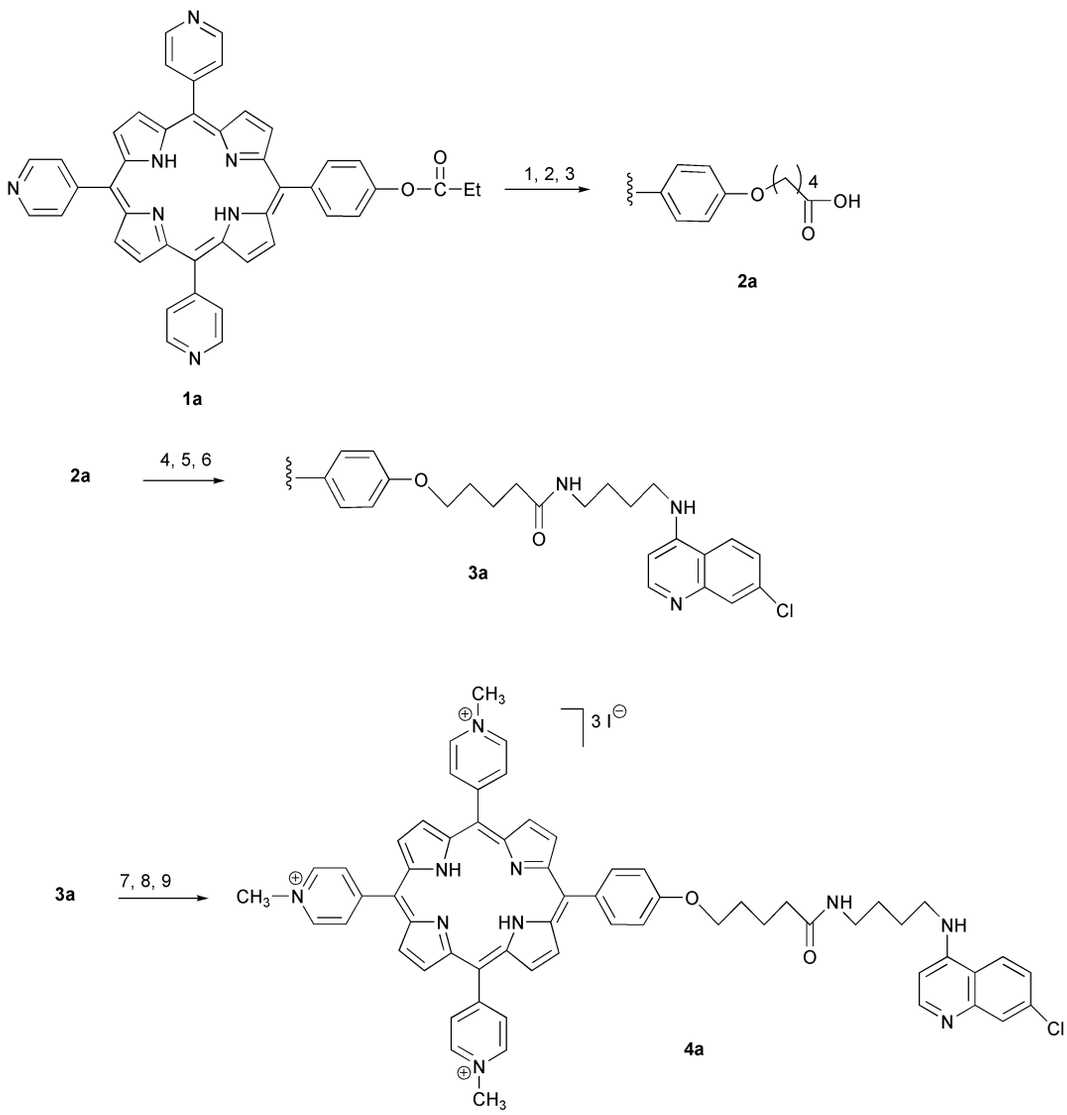
DOI: 10.1039/b211634k



Scheme 1 Synthesis of **4a** (1) NaOH (excess), (2) ethyl 5-bromovalerate (1.5 eq.), (3) H_2O, (4) NMM (2 eq.), (5) BOP (2 eq.), (6) 7-chloro-4-[*N*-(4-aminobutyl)amino]quinoline (2 eq.), (7) $HCl.OEt_2$ (1 eq.), (8) MeI (excess), (9) Et_3N.

carrying one or two aminoquinoline residues, the results of the G-quadruplex cleavage of the human telomeric sequence by the manganese porphyrins *in vitro* as well as the inhibition of telomerase activity for both series, the manganese and the nickel porphyrins.

Results and discussion

Synthesis of porphyrin–aminoquinoline hybrid molecules

The tetrapyridiniumyl-porphyrins, Mn–TMPyP and Ni–TMPyP, were prepared as previously described.[37] Synthesis of the porphyrins modified with one or two 4-aminoquinoline moieties was accomplished as described in Scheme 1. All the porphyrins were prepared by total synthesis starting from pyrrole and 4-hydroxybenzaldehyde.[38] The synthesis of the porphyrins having either three pyridyl groups and a propionyl-protected *p*-hydroxyphenyl substituent (**1a**) or two *cis* pyridyl groups and two *cis* propionyl-protected *p*-hydroxyphenyl substituents (**1b**) at the meso positions was previously described.[38,39] Series **a** or **b** refer to porphyrins with one or two aminoquinoline moieties, respectively. The synthesis of 7-chloro-4-[*N*-(4-aminobutyl)amino]quinoline was performed according to a previously published procedure.[40]

The phenolate functions of porphyrins **1a** or **1b** were generated by alkali in dry DMF. Addition of 1.5 equivalent of ethyl 5-bromovalerate per phenolate function led to the formation of porphyrins carrying one or two tether(s) with a terminal carboxylic ester function. The ester function was hydrolyzed to the corresponding acid, giving porphyrins **2a** and **2b**, that were obtained after precipitation and purification by silica gel chromatography. The yield for the preparation of **2a** and **2b** from **1a** and **1b** was 65 and 73%, respectively. The formation of an amide bond between these carboxylic acid functions and the primary amine of the aminobutyl arm of 7-chloro-4-[*N*-(4-aminobutyl)amino]quinoline was performed after the activation of the carboxylic acid functions by BOP in the presence of *N*-methylmorpholine leading to compounds **3a** and **3b** (yield = 95% after purification by silica gel chromatography). The pyridine substituents of these aminoquinoline-coupled porphyrins **3a** and **3b** were then quantitatively *N*-methylated with an excess of methyl iodide in the presence of one equivalent of HCl per aminoquinoline moiety to afford the cationic porphyrins **4a** and **4b** after precipitation. The products of these different steps were analysed by NMR and elemental analyses as detailed in the Experimental section. Metallation of **4a** and **4b** with nickel or manganese diacetate led to compounds Ni–**4a**, Ni–**4b** or Mn–**4a**, Mn–**4b**, respectively. The metallated porphyrin derivatives were analyzed by electrospray mass spectrometry. After anion exchange with a resin (chloride form) the counter ions of all tested porphyrins were chlorides.

Cleavage of human telomeric sequence by the manganese porphyrin series (Mn–TMPyP, Mn–4a, and Mn–4b)

The used telomeric DNA target (39-mer) was composed of four human telomeric repeats (5′-GGGTTA). It is able to fold into an intramolecular quadruplex in buffers containing K^+ ions and is stabilized by a double-stranded stem as shown previously (Fig. 2).[30] An intramolecular G-quadruplex was clearly evidenced by dimethylsulfate (DMS) footprinting analysis under the reaction conditions used in this work (Tris/KCl buffer: 50 mM Tris/HCl buffer pH 7.2, KCl 200 mM). Single-stranded or stem-loop structure was proposed in the Tris buffer medium (50 mM Tris/HCl buffer pH 7.2).[30,36]

5'-CATGGTGGTTT**GGGTTAGGGTTAGGGTTAGGGTTA** CCAC-3'

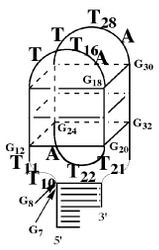


Fig. 2 The 39-mer oligonucleotide of the human telomeric DNA sequence. Possible folding of this oligonucleotide into an intramolecular quadruplex structure.

The oxidative reactivity of the manganese porphyrins toward the human telomeric sequence was assayed with the 39-mer telomeric target at 1 μM concentration in the presence of 1 μM of the porphyrin derivatives in Tris/KCl and in Tris buffers. Initiation of the reaction was achieved by addition of $KHSO_5$ (final concentration 100 μM). The reaction time was 1 h at 0 °C. Resulting products were analyzed by electrophoresis on a 20% denaturing polyacrylamide gel (Fig. 3). The lesions were revealed as discrete cleavage bands by a piperidine treatment (Fig. 3, lanes 1–6) that co-migrated with Maxam–Gilbert sequencing bands (Fig. 3, lane G) and thus corresponded to fragments bearing a 3′-phosphate terminus. This stands also for the cleavage fragments at T-residues (corresponding Maxam–Gilbert sequencing lane not shown).

In the Tris buffer, in the absence of K^+ cation, (Fig. 3, lane 1–3), the cleavage sites of Mn–TMPyP (lane 1) or the porphyrin–aminoquinoline, Mn–**4a** (lane 2) were located at almost all the guanines of the four telomeric repeats, G_{24} and G_{12} being the most reactive ones. In the case of the Mn–TMPyP derivative (lane 1), some cleavage at thymine residues, T_{10}, T_{11}, T_{21}, T_{22} were also observed. The double-stranded region was not cleaved (not shown). Under this unsalted Tris buffer condition, the probable stem-loop structure of DNA made all the guanines of the single-stranded loop accessible to the oxo-manganese porphyrin. The degradation of the telomeric sequence reached 40% and 10% for Mn–TMPyP and Mn–**4a**, respectively. The manganese porphyrin covalently modified with two aminoquinoline moieties, Mn-**4b**, was not efficient for DNA degradation (lane 3).

When the oxidation reaction was performed in Tris buffer with 200 mM of K^+ ions (Fig. 3, lane 4–6), Mn–TMPyP (lane 4) was able to mediate oxidative damage at the T_{10}, T_{11}, G_{12}, T_{21}, T_{22} and G_{24} residues. This result confirmed that Mn–TMPyP could act as a probe for quadruplex DNA.[36] The cleavage sites were compatible with a quadruplex folding proposed in Fig. 2 for the tested 39-mer sequence. The cleavage sites of Mn–TMPyP were located in the vicinity of the last tetrad formed by G_{12}, G_{20}, G_{24} and G_{32} bases (Fig. 2). This suggested that Mn–TMPyP interacted with the intramolecular quadruplex by a one-side external π-stacking with the G_{12}–G_{20}–G_{24}–

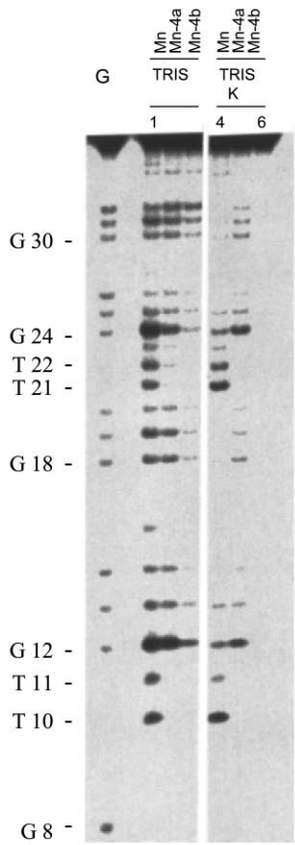


Fig. 3 Polyacrylamide gel electrophoresis analysis of the cleavage of the 5′-[^{32}P]-labeled 39-mer telomeric DNA sequence by the manganese porphyrin series. Mn stands for Mn–TMPyP, Mn–**4a**, and Mn–**4b** refer to the porphyrin derivatives carrying one or two 4-aminoquinoline moieties, respectively. G is the G-lane of Maxam and Gilbert sequencing reaction. TRIS: lanes 1–3 correspond to the reaction of the porphyrins in Tris buffer (50 mM Tris/HCl buffer pH 7.2). TRIS–K: lanes 4–6 correspond to the reaction in Tris/KCl buffer (50 mM Tris/HCl buffer pH 7.2 and 200 mM KCl). Telomeric sequence cleavage reactions were performed at a DNA final concentration of 1 μM. The DNA samples were heated at 90 °C for 10 min and slowly cooled to room temperature before the addition of the metalloporphyrin in order to let the oligonucleotide fold into its secondary structure. The DNA was preincubated with the metalloporphyrin (1 μM) during 1 h at 0 °C. DNA cleavage reactions were initiated by adding a freshly prepared solution of $KHSO_5$ (final concentration 500 μM). After 1 h at 0 °C, the reactions were stopped by addition of Hepes buffer pH 8 (48 mM, final concentration). The DNA samples were then precipitated and subjected to piperidine treatment before analysis.

G_{32} tetrad. Both G_7 and G_8 guanines of the double-stranded region were not touched. The interaction of the metalloporphyrin with this 39-mer telomeric sequence was thus stronger with the G-tetrad plane than with the double-stranded stem. As discussed previously,[36] the absence of binding on the "top" side of this quadruplex structure (at the G_{14}–G_{18}–G_{26}–G_{30} tetrad, Fig. 2) was also observed for the non-metallated porphyrin, H_2–TMPyP. It was found by molecular modeling studies that the external stacking of H_2–TMPyP with the G_{14}–G_{18}–G_{26}–G_{30} tetrad was extremely unfavorable compared to the interaction on the other side.[30]

When the porphyrin modified with one aminoquinoline moiety, Mn–**4a**, was reacted with telomeric DNA in the presence of 200 mM K^+ ions (Fig. 3, lane 5), oxidative damage was seen mainly at the G_{12} and G_{24} residues. These two G-residues belong to the "bottom" tetrad of the quadruplex and were oxidized also in the case of Mn–TMPyP. However, on the contrary to Mn–TMPyP, no cleavage occurred with Mn–**4a** at the previously observed T-residues of the loops (T_{10}, T_{11}, T_{21}, T_{22}). The extent of cleavage reached 10% of degradation of the full-length DNA for both porphyrins, Mn–TMPyP and Mn–**4a**,

Table 1 TRAP assay, inhibition of telomerase by manganese and nickel porphyrin derivatives. IC_{50} values (μM)

Porphyrin derivative	IC_{50}/μM
Mn–TMPyP	25.9
Ni–TMPyP	5
Mn–**4a**	11.5
Ni–**4a**	7.3
Mn–**4b**	8.6
Ni–**4b**	39

under the used experimental conditions, but the patterns of the quadruplex cleavage sites were different. The common binding site for both porphyrins for the quadruplex structure seems to be at the "bottom" tetrad (G_{12}–G_{20}–G_{24}–G_{32}).

The Mn–**4b** derivative, corresponding to a manganese porphyrin carrying two aminoquinoline moieties, was completely inactive on quadruplex DNA (Fig. 3, lane 6) and poorly reactive on single-stranded DNA (Fig. 3, lane 3). This was probably due to an intramolecular stacking of the aminoquinolines with the metalloporphyrin core that precluded any activation and/or reaction of the metalloporphyrin.

The telomeric DNA cleavage by manganese porphyrins was assayed to test whether the new porphyrin derivatives, with one or two aminoquinoline(s), showed encouraging quadruplex DNA cleaving efficiencies. The yield of DNA degradation was not increased by the presence of aminoquinoline substituents. The Mn–**4b** derivative even proved to be unable to degrade quadruplex DNA. However, these experiments revealed the location of the porphyrin moiety within the quadruplex structure for Mn–TMPyP and Mn–**4a**. Mn–**4a** probably interacted, as the previously studied Mn–TMPyp, with the last tetrad of the quadruplex (G_{12}–G_{20}–G_{24}–G_{32}) and was able to oxidize guanine bases of the tetrad but not the residues located on the loops. A general observation was that the aminoquinoline moieties lowered the oxidizing reactivity of the metalloporphyrins, probably by shielding one or two faces of the metalloporphyrin. This test did not allow us to evidence an increase of binding affinity for quadruplex DNA for the new porphyrins. However, a strong interaction of a porphyrin derivative may not be associated with a high level of DNA damage. Furthermore, the axial ligands of nickel porphyrins are labile, compared to their manganese counterparts, so their interaction with the G-tetrads may be different. This is why the Mn- and Ni-compounds were directly assayed for their capacity to inhibit telomerase.

Inhibition of telomerase

The cell-free enzyme-based telomeric repeat amplification protocol (TRAP) assay was performed with the nickel and the manganese porphyrins to determine whether these new molecules could be telomerase inhibitors. The compounds caused inhibition of telomerase-mediated telomere extension at concentrations in the micromolar range. The IC_{50} values are given in Table 1. The influence of the covalent attachment of a 4-aminoquinoline group on the porphyrin core or the nature of the metal did not seem to influence, in a significant way, the efficiency of these metalloporphyrins as telomerase inhibitors.

Conclusion

A new series of metalloporphyrins was prepared in order to target the quadruplex structure of telomeric DNA. The cationic tetramethylpyridiniumyl porphyrins were modified by replacement of one or two methylpyridiniumyl groups by one or two 4-aminoquinoline moietie(s), at the meso position(s), in order to increase the cell penetration and the quadruplex affinity. The porphyrins were either metallated by manganese or by nickel. All porphyrin derivatives of the series showed inhibition of telomerase with IC_{50} values in the micromolar range. Structural and modelling studies are under current investigation to understand the different parameters controlling the interaction of these molecules with telomere sequence. These data are providing knowledge for further investigation on porphyrin–quinoline interaction with DNA in order to modify the design of this type of telomerase inhibitors and to improve their activity.

Experimental

Proton attributions for ^{1}H NMR analyses are shown in Scheme 2. Coupling constants values (J) are given in Hz.

Synthesis of porphyrins with one tether with an ester function (1a) or two tethers (1b)[38,39]

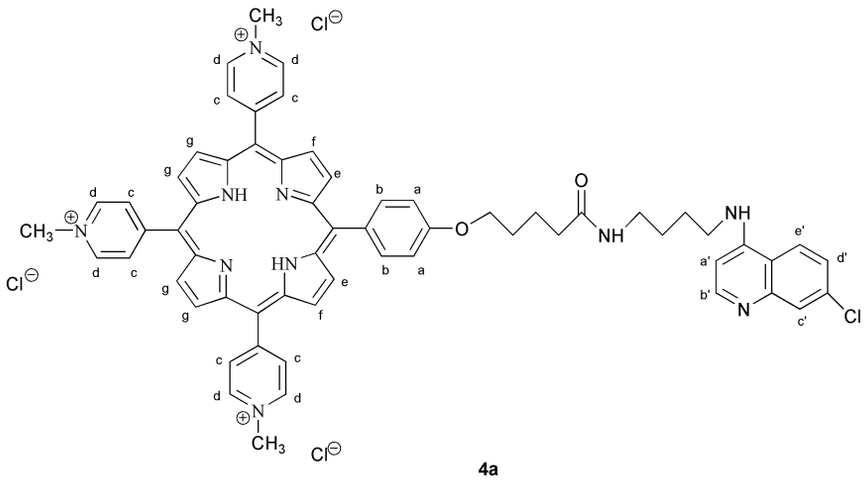
The two porphyrins **1a** and **1b** were separated by silica gel chromatography from the same reaction mixture. Porphyrin **1b**: (Found: C, 74.16; H, 4.45; N, 10.79. $C_{48}H_{32}N_6O_4{\cdot}H_2O$ requires C, 74.41; H, 4.42; N, 10.85%); δ_H (250 MHz, $CDCl_3$, Me_4Si) −2.89 (s, 2H, $NH_{pyrrole}$), 1.41 (t, $^3J_{HH}$ 7.5, 6H, CH_3), 2.79 (q, $^3J_{HH}$ 7.5, 4H, CH_2), 7.51 (d, $^3J_{HH}$ 8.5, 4H, H_b), 8.16 (d, $^3J_{HH}$ 5.9, 4H, H_c), 8.20 (d, $^3J_{HH}$ 8.5, 4H, H_a), 8.81 (d, $^3J_{HH}$ 4.9, 2H, $H_{e\ or\ f}$), 8.84 (s, 2H, $H_{g\ or\ h}$), 8.90 (s, 2H, $H_{g\ or\ h}$), 8.93 (d, $^3J_{HH}$ 4.9, 2H, $H_{e\ or\ f}$), 9.04 (d, $^3J_{HH}$ 5.9, 4H, H_d).

Synthesis of porphyrins with one tether with an acid function (2a) or two tethers (2b)

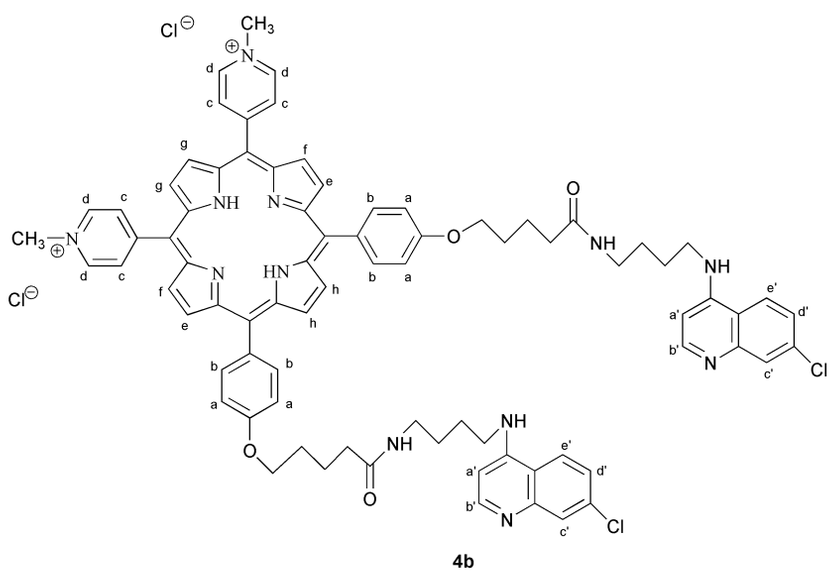
The synthesis of **2a** was performed as previously described.[38,39] For the preparation of **2b**, the same conditions as for preparation of **2a** were used with 50 equivalents of NaOH for 3 h. Then 3 equivalents of ethyl 5-bromovalerate were added and the mixture was stirred for 24 h at room temperature. Compound **2b** was obtained in 73% yield (Found: C, 68.19; H, 5.15; N, 8.94. $C_{52}H_{44}N_6O_6{\cdot}3.5\ H_2O$ requires C, 68.48; H, 5.64; N, 9.21%); δ_H (250 MHz, $CDCl_3$, Me_4Si) −2.96 (s, 2H, $NH_{pyrrole}$), 1.83 (m, 8H, O–CH_2–CH_2–CH_2), 2.41 (t, $^3J_{HH}$ 6.9, 4H, CH_2–COOH), 4.25 (t, $^3J_{HH}$ 6.0, 4H, O–CH_2), 7.35 (d, $^3J_{HH}$ 8.5, 4H, H_b), 8.09 (d, $^3J_{HH}$ 8.5, 4H, H_a), 8.24 (d, $^3J_{HH}$ 5.7, 4H, H_c), 8.83 (d, $^3J_{HH}$ 5.0, 2H, $H_{e\ or\ f}$), 8.86 (s, 2H, $H_{g\ or\ h}$), 8.89 (s, 2H, $H_{g\ or\ h}$), 8.91 (d, $^3J_{HH}$ 5.0, 2H, $H_{e\ or\ f}$), 9.03 (d, $^3J_{HH}$ 5.7, 4H; H_d), 12.1 (s, 2H, COOH).

Synthesis of 3a and 3b

Coupling reaction of porphyrin 2a with one quinoline fragment. To a solution of 100 mg of porphyrin **2a** (0.136 mmol) in 15 cm^3 of freshly distilled CH_2Cl_2 were added 20 equivalents of *N*-methylmorpholine (300 mm^3, 2.725 mmol) and 2 equivalents of BOP (124 mg, 0.272 mmol). When the activation reaction was achieved (0.5 h, controlled by analytical TLC plates, eluent : CH_2Cl_2/EtOH, 94 : 6, v : v), 2 equivalents of 7-chloro-4-[*N*-(4-aminobutyl)amino]quinoline were added (70 mg, 0.272 mmol). The reaction mixture was stirred for 0.5 h at room temperature (controlled by analytical TLC plates, eluent CH_2Cl_2/EtOH, 94 : 6, v : v with NH_4OH atmosphere) then washed with 3 × 30 cm^3 of water, dried with Na_2SO_4 purified by silica gel chromatography (eluent CH_2Cl_2/EtOH, 93 : 7, with NH_4OH 0.1%). Analytically pure product was obtained after CH_2Cl_2/Et_2O (1 : 20) precipitation at 4 °C. The product **3a** was dissolved in a mixture of CH_2Cl_2/EtOH (90 : 10, v : v) and dried (124.1 mg, 0.129 mmol, 95%). δ_H (250 MHz, $CDCl_3$, Me_4Si) −2.88 (s, 2H, $NH_{pyrrole}$), 1.79 (m, 4H, C(O)–NH–CH_2–CH_2–CH_2), 2.05 (m, 4H, O–CH_2–CH_2–CH_2), 2.42 (t, $^3J_{HH}$ 6.3, 2H, CH_2–C(O)–NH), 3.41 (m, 4H, C(O)–NH–CH_2 and CH_2–NHquinoline), 4.27 (t, $^3J_{HH}$ 6.1, 2H, O–CH_2), 5.85 (t, $^3J_{HH}$ 6.0 Hz, 1H, NH_{tether}), 5.92 (t, $^3J_{HH}$ 6.1, 1H, NH_{tether}), 6.39 (d, $^3J_{HH}$ 5.5, 1H, $H_{a'}$), 7.27 (d, $^3J_{HH}$ 8.6, 2H, H_b), 7.35 (dd, $^3J_{HH}$ 9.0, $^4J_{HH}$ 2.2, 1H, $H_{d'}$), 7.88 (d, $^3J_{HH}$ 9.0, 1H, $H_{e'}$), 7.91 (d, $^4J_{HH}$ 2.2, 1H,



4a



4b

Scheme 2 Proton attributions for ^{1}H NMR analyses.

$H_{c'}$), 8.09 (d, $^3J_{HH}$ 8.6, 2H, H_a), 8.15 (m, 6H, H_c), 8.47 (d, $^3J_{HH}$ 5.5, 1H, $H_{b'}$), 8.80 (d, $^3J_{HH}$ 4.8, 2H, $H_{e\ or\ f}$), 8.85 (s, 4H, H_g), 8.95 (d, $^3J_{HH}$ 4.8, 2H, $H_{e\ or\ f}$), 9.04 (m, 6H, H_d).

Coupling reaction of porphyrin 2b with two quinoline fragments. The coupling reaction with **2b** was carried out under the same conditions as those described for **2a** except that the reaction was performed in dry DMF under argon with twice more NMM, BOP and quinoline derivative. The crude product **3b** was purified by silica gel chromatography (eluent: CH_2Cl_2/EtOH, 90 : 10, v : v with 0.1% NH_4OH). Analytically pure product was obtained after CH_2Cl_2/MeOH/Et_2O (1 : 1 : 20) precipitation at 4 °C. The product **3b** was dissolved in a mixture of MeOH/H_2O (50 : 50, v : v) and dried (145.7 mg, 0.112 mmol, 95%). δ_H (250 MHz, $CDCl_3$, Me_4Si) −2.96 (s, 2H, $NH_{pyrrole}$), 1.62 (m, 8H, C(O)–NH–CH_2–CH_2–CH_2), 1.83 (m, 8H, O–CH_2–CH_2–CH_2), 2.24 (t, $^3J_{HH}$ 6.3, 4H, CH_2–C(O)–NH), 3.16 (td, $^3J_{HH}$ = $^3J_{HH}$ = 5.4, 4H, C(O)–NH–CH_2 or CH_2–NHquinoline), 3.28 (td, $^3J_{HH}$ =$^3J_{HH}$ = 5.7, 4H, C(O)–NH–CH_2 or CH_2–NHquinoline), 4.18 (m, 4H, O–CH_2), 6.45 (d, $^3J_{HH}$ 5.5, 2H, $H_{a'}$), 7.28 (d, $^3J_{HH}$ 7.9, 4H, H_b), 7.37 (t, $^3J_{HH}$ 5.4, 2H, NH_{tether}), 7.41 (dd, $^3J_{HH}$ 9.0, $^4J_{HH}$ 2.2, 2H, $H_{d'}$), 7.74 (d, $^4J_{HH}$ 2.2, 2H, $H_{c'}$), 7.93 (t, $^3J_{HH}$ 5.7, 2H, NH_{tether}), 8.04 (d, $^3J_{HH}$ 7.9, 4H, H_a), 8.22 (d, $^3J_{HH}$ 5.0, 4H, H_c), 8.27 (d, $^3J_{HH}$ 9.0, 2H, $H_{e'}$), 8.34 (d, $^3J_{HH}$ 5.5, 2H, $H_{b'}$), 8.81 (d, $^3J_{HH}$ 4.9, 2H, $H_{e\ or\ f}$), 8.85 (s, 4H, $H_{g\ and\ h}$), 8.88 (d, $^3J_{HH}$ 4.9, 2H, $H_{e\ or\ f}$), 9.00 (d, $^3J_{HH}$ 5.0, 4H, H_d).

Preparation of methylated porphyrin derivatives 4a and 4b

Methylation of pyridine moieties of 3a. Porphyrin **3a** (124 mg, 0.129 mmol) was dissolved in 20 cm^3 of dry DMF, then 1 equivalent of HCl 1M in diethyl oxide was added (130 mm^3, 0.129 mmol). The reaction mixture was stirred for 0.5 h at room temperature then 50 equivalents of methyl iodide (400 mm^3, 6.473 mmol) were added. After 6 h stirring, solvent and excess MeI were evaporated under reduced pressure. The dark brown solid residue was dissolved in 5 cm^3 of DMF and stirred for 30 min with 1 equivalent of Et_3N (18 mm^3, 0.129 mmol). The product was precipitated by addition of 15 cm^3 of 2-propanol and 30 cm^3 of diethyl oxide. After 10 min of centrifugation, the supernatant was eliminated and the solid pellet was dissolved in H_2O/MeOH (80 : 20, v : v). Pure **4a** was dried under vacuum (170.5 mg, 0.118 mmol, 91%). δ_H (250 MHz, $CDCl_3$, Me_4Si) −3.01 (s, 2H, $NH_{pyrrole}$), 1.64 (m, 4H, C(O)–NH–CH_2–CH_2–CH_2), 1.87 (m, 4H, O–CH_2–CH_2–CH_2), 2.27 (t, $^3J_{HH}$ 6.3, 2H, CH_2–C(O)–NH), 3.17 (m, 2H, C(O)–NH–CH_2 or CH_2–NHquinoline), 3.45 (m, 2H, C(O)–NH–CH_2 or CH_2–NHquinoline), 4.28 (m, 2H, O–CH_2), 4.73 (s, 9H, N–CH_3), 6.73 (d, $^3J_{HH}$ 6.5, 1H, $H_{a'}$), 7.42 (d, $^3J_{HH}$ 8.5, 2H, H_b), 7.63 (dd, $^3J_{HH}$ 9.0, $^4J_{HH}$ 1.9, 1H, $H_{d'}$), 7.80 (d, $^4J_{HH}$ 1.9, 1H, $H_{c'}$), 7.95 (s, 4H, H_g), 8.14 (d, $^3J_{HH}$ 8.5, 2H, H_a), 8.43 (d, $^3J_{HH}$ 9.0, 1H, $H_{e'}$), 8.47 (d, $^3J_{HH}$ 6.5, 1H, $H_{b'}$), 9.00 (d, $^3J_{HH}$ 6.2, 6H, H_c), 9.12 (s, 2H, $H_{e\ or\ f}$), 9.25 (s, 2H, $H_{e\ or\ f}$), 9.51 (d, $^3J_{HH}$ 6.2, 6H, H_d). The NH protons of the tether were not observed.

Methylation of pyridine moiety of 3b. The same procedure as previously described for the methylation of **3a** was used. After evaporation of solvent and excess MeI, the product was dissolved in a minimum volume of MeOH/H_2O (50 : 50, v : v), then 3 volumes of 2-propanol and 6 volumes of diethyl oxide were added and the product **4b** precipitated (95%). δ_H (250 MHz, $CDCl_3$, Me_4Si) −2.93 (s, 2H, $NH_{pyrrole}$), 1.62 (m, 8H, C(O)–NH–CH_2–CH_2–CH_2), 1.85 (m, 8H, O–CH_2–CH_2–CH_2), 2.26 (t, $^3J_{HH}$ 6.3, 4H, CH_2–C(O)–NH), 3.15 (m, 4H, C(O)–NH–CH_2 or CH_2–NHquinoline), 3.54 (m, 4H, C(O)–NH–CH_2 or CH_2–NHquinoline), 4.26 (t, $^3J_{HH}$ 4.6, 4H, O–CH_2), 4.71 (s, 6H, N–CH_3), 6.89 (d, $^3J_{HH}$ 7.2, 2H, $H_{a'}$), 7.38 (d, $^3J_{HH}$ 8.6, 4H, H_b), 7.74 (dd, $^3J_{HH}$ 9.0, $^4J_{HH}$ 2.0, 2H, $H_{d'}$), 7.81 (d, $^4J_{HH}$ 2.0, 2H, $H_{c'}$), 7.96 (t, $^3J_{HH}$ 5.5, 2H, NH_{tether}), 8.12 (d, $^3J_{HH}$ 8.6, 4H, H_a), 8.49 (d, $^3J_{HH}$ 9.0, 2H, $H_{e'}$), 8.51 (d, $^3J_{HH}$ 7.2, 2H, $H_{b'}$), 8.92 (s, 2H, $H_{g\ or\ h}$), 9.02 (m, 8H, H_{c+e+f}), 9.14 (s, 2H, $H_{g\ or\ h}$), 9.31 (t, $^3J_{HH}$ 5.4, 2H, NH_{tether}), 9.47 (d, $^3J_{HH}$ 6.6 Hz, 4H, H_d).

Preparation of metallated porphyrins Mn-4a, Ni-4a, Mn-4b, Ni-4b

Metallation reaction of 4a. A solution of **4a** (50 mg, 0.036 mmol) in 3 cm^3 of DMF/H_2O (50 : 50, v : v) was mixed with a solution of manganese or nickel diacetate tetrahydrate (6 equivalents, 0.216 mmol) and 2,4,6-collidine (7 equivalents, 0.251 mmol) in 1.2 cm^3 of DMF and heated at 100 °C. The completion of the reaction was monitored spectrophotometrically (λ_{max} (**4a**) = 428 nm, shifted to 466 nm in the presence of HCl; λ_{max} Mn–**4a** = 466 nm; λ_{max} Ni–**4a** = 428 nm, no shift observed in the presence of HCl). The reaction was complete within 3 h. Then 15 cm^3 of 2-propanol and 30 cm^3 of diethyl oxide were added to the reaction mixture to precipitate the metallated product. After 10 min of centrifugation the supernatant was eliminated and the solid pellet was dissolved in H_2O/MeOH (80 : 20, v : v). Solvents were evaporated and the product was dried under vacuum. The powder (green in the case of Mn and red in the case of Ni) was suspended in 40 cm^3 of THF and stirred overnight at room temperature to eliminate the excess of salts. The mixture was centrifuged and the supernatant was discarded. The solid residue was dissolved in 30 cm^3 of MeOH/H_2O (50 : 50, v : v) and DOWEX 1 × 8–200 resin beads were added to exchange the counter-ions by chlorides. The mixture was mechanically stirred for 24 h, then the beads were filtered off and washed with 10 cm^3 of MeOH/H_2O (50 : 50, v : v). The filtrate was recovered and solvents were evaporated. The solid residue was dissolved in a minimum of DMF (one volume) and the product was precipitated by addition of 1/10 volume of H_2O, 3 volumes of 2-propanol and 6 volumes of diethyl oxide. The precipitation was achieved during 1 h at 4 °C. The mixture was then centrifuged and the supernatant was discarded. The solid residue was dissolved in MeOH/H_2O (50 : 50, v : v) and dried under vacuum. The Ni derivative (Ni–**4a**) was obtained in good yield (34.5 mg, 0.029 mmol, 82%). *m*/*z* (ES-MS, positive mode) 355.3 ($[M\text{-}3Cl]^{3+}$/3), 525.3 ($[M\text{-}3Cl\text{–}CH_3]^{2+}$/2), 532.3 ($[M\text{-}2Cl]^{2+}$/2). The Mn derivative (Mn–**4a**) was also obtained in good yield (32.8 mg, 0.028 mmol, 78%). *m*/*z* (ES-MS, positive mode) 354.5 ($[M\text{-}4Cl]^{3+}$/3), 349.5 ($[M\text{-}4Cl\text{–}CH_3]^{3+}$/3), 548.2 ($[M\text{-}3Cl]^{2+}$/2).

Metallation reaction of 4b. Metallation reactions were perfomed under the same conditions as previously described for **4a**. The Ni derivative (Ni–**4b**) was obtained in 75% yield. *m*/*z* (ES-MS, positive mode) 698.4 ($[M\text{-}2Cl]^{2+}$/2), 1381.4 ($[M\text{-}2Cl\text{–}CH_3]^{+}$). The Mn derivative (Mn–**4b**) was obtained with 66% yield. (ES-MS, positive mode) 464.3 ($[M\text{-}3Cl]^{3+}$/3), 475.6 ($[M\text{-}2Cl]^{3+}$/3).

Synthesis, purification and labeling of the human telomeric target

The human telomeric sequence was synthesized by standard solid-phase β-cyanoethylphosphoramidite chemistry. Purification was performed by electrophoresis on a 20% polyacrylamide denaturing gel. DNA concentration was determined by UV measurements at 260 nm. The 5′-end of the telomeric target was labeled by [^{32}P] using standard procedures with T4 polynucleotide kinase and [γ-^{32}P]ATP purchased from New England Biolabs and ICN Biomedical, respectively.

Oxidation of the human telomeric sequence by metalloporphyrins/$KHSO_5$

Telomeric sequence cleavage reactions were performed with 5′-labeled target (final concentration 1 μM, 100000–150000 cpm) in Tris/HCl buffer pH 7.2 (50 mM) and KCl (200 mM) (Tris/KCl buffer) or in 50 mM Tris/HCl buffer pH 7.2 (Tris buffer). DNA was heated at 90 °C for 10 min and slowly cooled to room temperature before the addition of the metalloporphyrin. DNA was preincubated with the metalloporphyrin (1 μM) for 1 h at 0 °C. DNA cleavage reactions were initiated by adding a freshly prepared solution of $KHSO_5$ (final concentration 500 μM, Curox® from Interox). Final volume was 20 mm^3. After 1 h at 0 °C, the reactions were stopped by addition of Hepes buffer pH 8 (48 mM, final concentration). The DNA samples were then diluted with 10 mm^3 of 3.5 M sodium acetate buffer (pH 5.2) and 1 mm^3 of yeast tRNA at 10 mg cm^{-3} and precipitated with 300 mm^3 of cold ethanol overnight at −20 °C. After centrifugation (15 min at 4 °C, 12 × 10^3 rpm), the DNA pellet was washed twice with 70% cold ethanol, dried under vacuum (speedvac), and subjected to piperidine treatment (50 mm^3, 1 M, 30 min at 90 °C). Piperidine treatment was followed by lyophilization of the piperidine solution.

Polyacrylamide gel electrophoresis analysis

After ethanol precipitation of the reaction samples or after piperidine treatment, the dry DNA pellet was diluted in formamide with marker dyes. The samples were incubated 2 min at 90 °C, chilled in ice and run on a 20% denaturing polyacrylamide gel (7 M urea) for 3 h at 2000 V. The DNA fragments were visualized either by autoradiography using Kodak BioMax MR-1 film or by phosphorimagery (Molecular Dynamics) using Image Quant Software. Each gel included a Maxam–Gilbert sequencing (G-lane).

Telomeric repeat amplification protocol, TRAP assay

Exponentially growing HeLa cell cultures were trypsinized, washed in PBS and S-100 extracts obtained as described.[41] Stock protein concentration was adjusted to 5 μg mm^{-3}, flash-frozen and stored at −80 °C. To assess telomerase activity, compounds were serially diluted in lysis buffer (range, 100 μM to 30 nM) and mixed 1:1 with 1.25 μg of HeLa cell extract (final volume, 5 mm^3). Extension and amplification reactions and electrophoresis were carried out as described.[41] For quantification, autoradiographs were scanned in a Storm 860 scanner and signal intensity of telomerase ladder and PCR internal control (ITAS) measured using ImageQuant v1.2 software. Dose-dependent PCR inhibition was observed with compounds Ni–**4a** and Ni–TMPyP (diminished or absent internal control (ITAS) signal at concentrations >20 μM); these datapoints were therefore excluded from analysis for these two compounds. Compounds Mn–**4a**, Ni–**4b**, Mn–**4b**, Mn–TMPyP showed no PCR inhibition up to the highest tested concentration (100 μM). Regression curves and IC_{50} values were calculated using Excel software, and values expressed as % of activity of an equal amount of nontreated HeLa cell extract. Negative controls were included in all assays by preincubating HeLa extracts with RNAse for 10 min at 30 °C prior to the extension reaction. All compounds were assayed in at least two separate TRAP assays; regression curves for each compound were highly reproducible.

Acknowledgements

C.V. is deeply indebted to the "Ligue Nationale contre le Cancer, Section du Gers" for a PhD fellowship. S.F. is a pre-doctoral student of the Ministry of Health, Spain. Research at the laboratory of M.A.B. is funded by grants PM97–0133 from the MCYT, 08.1/0030/98 from CAM, and by grants EURATOM/991/0201, FIGH-CT-1999–00002 and FIS5-1999–00055, from the European Union, and by the Department of Immunology and Oncology (DIO).

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