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The role of cobalt, copper, nickel, and zinc in the DNA replication inhibitory activity of *p*-aminophenyl triphenylporphyrin

Efraín Barragán[1], Barbara Gordillo[1]*, Genaro Vargas[1] and Luis Velazco[2]

[1]Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado Postal 14-740, 07000 Mexico DF, Mexico
[2]Instituto de Química, Circuito Exterior, Ciudad Universitaria, Universidad Nacional Autónoma de México, Mexico DF 04510, Mexico

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Cationic porphyrins have been widely used as tumor localizers in cancer therapies. When cationic porphyrins are flat they intercalate with double-stranded DNA, duplexes of RNA or RNA–DNA. The antitumor activity of some cationic porphyrins depends on their interaction with human telomeric quadruplexes. Here, we report that noncationic *meso*-(4-aminophenyl)triphenylporphyrin (H_2TPPNH_2) (3) and its cobalt, copper, nickel, and zinc metallo derivatives (4–7) have DNA replication inhibitory activity in B16 mouse melanoma line cells. By means of quantification of ^{3}HdTT radio-labeled DNA, we observed that the nonplanar porphyrin [$CoTPPNH_2$] has the highest activity against carcinogenic DNA replication. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: porphyrins; metalloporphyrins; DNA replication inhibition; DNA binding; mouse melanoma B16 cell lines; radio-labeled DNA

INTRODUCTION

For more than 50 years, a subject of interest in the chemistry of metal (M) ions, is the way that they interact with DNA.[1] Knowledge of the way in which an M–DNA complex can be formed is essential to the rational design of antitumor or antiviral drugs.[2]

Nucleotides, the monomeric units of DNA, have specific sites by means of which they interact with metals. The basic difference between the four nucleotides in DNA (thymidine, cytosine, adenosine and guanosine 3′ or 5′ monophosphates) is in the nucleobase. Scheme 1 attempts to summarize the specific primary sites of the nucleobases where the metal species directly coordinates. Whereas alkaline earth metal cations tend to be linked to the oxygen of the deoxyribose sugar or the phosphate bridging groups, the transition metals, at high concentrations, form complexes with DNA through the nucleobases.

*Correspondence to: Barbara Gordillo, Departmento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado Postal 14-740, 07000 México DF, México.
E-mail: ggordill@mail.cinvestav.mx
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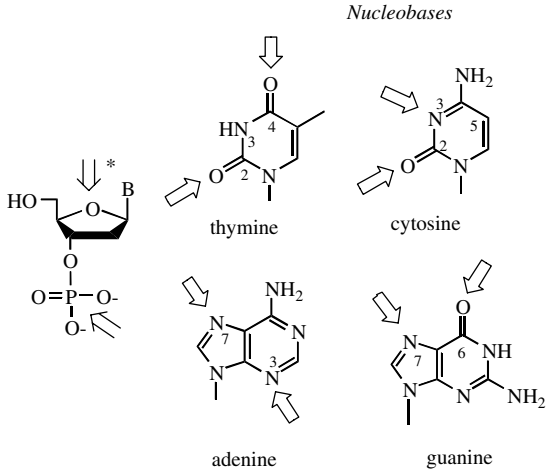
From these data it can be deduced that, the hard M–phosphate interaction gives stability to the double helix; however, the soft M–DNA coordinative linking can break hydrogen bonds between the complementary strands of DNA, thus leading to DNA cross-links or even to unwound single DNA strands. This property of transition metals can be exploited in the design of drugs that inhibit the growth of DNA in carcinogenic cells, such as for cisplatin and their derivatives.[3] Binding characteristics of several transition metals, such as platinum(II), palladium(II), copper(II), nickel(II), cobalt(II), iron(II), and zinc(II), to nucleobases are known through the X-ray analysis of model 9-substituted purines or 1-substituted pyrimidines.[4]

From the biological point of view, transition metals are important because they are the key linkers of complex enzymes or proteins. These conglomerates perform their substrate–receptor molecular recognition activities through metals.[5] For example, myoglobin and hemoglobin cannot act as the oxygen storage and carrier respectively in the organism without the presence of iron(II) domed in the heme unit of these essential-to-life proteins.[6] The redox properties, the coordination number and the way the metal is held by the bio-ligand, determine the binding activity and properties used in the life processes.[7]



⇒ alkaline earth metals and/or main group metal cations

⇨ transition metals [Cu (II), Pt (II), Pd (II), Ni (II), Co (II), Ru(III), Rh (II) among others]

* Cu (II), Sn (IV), Os (IV) also coordinate

Scheme 1.

Porphyrins are the natural ligands of heme groups in hemoglobin or electron carriers as cytochromes.[8] Chlorophylls, which are responsible for the photosynthesis process in plants, are also magnesium(II) metalloporphyrins. Porphyrins are particularly important ligands because of their ubiquitous electronic properties:[9] they can coordinate almost every metal; therefore, they have found application in diverse fields of science, e.g. in physics for the design of optoelectronic molecular devices,[10–12] in chemistry for the development of new catalysts[13,14] that work as efficiently as the coenzyme B_{12},[15] and in medicine as carriers of energy- or photo-sensitizers delivered specifically to tumors in human organs for cancer treatments, as boron neutron capture therapy (BNCT)[16] and photodynamic therapy (PDT)[17,18] respectively. In these techniques, porphyrins have been chosen as carriers not only for their amazingly good capacity to associate selectively to membranes of malignant cells *in vitro* or *in vivo* studies, but also because of their proved low toxicity, since they can be easily cleared from tissues and blood fluids. Thus, we consider it of interest to analyze the biological activity of *meso*-(4-aminophenyl)triphenylporphyrin (H_2TPPNH_2) (**3**), a polar, partially water-soluble porphyrin that can promote a hydrogen bonding interaction with DNA, and their cobalt, copper, nickel, and zinc metallo derivatives (**4**–**7**, see Scheme 2), towards the inhibition of DNA replication in B16 mouse melanoma cells. The results are discussed in terms of the geometry and coordinative properties of the metal complexes and their likely interaction with the nucleobases of DNA.

EXPERIMENTAL

General

Proton nuclear magnetic resonance (NMR) spectra were recorded on Jeol Eclipse 400 and Bruker Avance 300 spectrometers, at 399.8 MHz and 300.1 MHz respectively. The abbreviations used for the signals are: s = singlet, d = doublet, m = complex pattern, b = broad. UV–Vis spectra were determined on a Perkin Elmer Lambda 12 spectrophotometer. IR spectra were registered on Perkin Elmer lambda 12 spectrophotometer. The abbreviations used for IR absorption frequencies are: w = weak, m = moderately strong, s = strong, b = broad. High-resolution fast atom bombardment (FAB) mass spectra were measured on a Jeol JMS-SX 102A spectrometer using electron impact (EI) at 70 eV. All chemicals were reagent grade and were purchased from Aldrich Chemical Co. Solvents were dried and purified according to literature procedures.[19] Flash column chromatography was performed on silica gel 200–400 mesh. ^{3}HdTT incorporation in DNA was measured on a Stap Fax 303 plus spectrophotometer.

^{3}HdTT assays

B16 mouse melanoma cells were obtained from American Type Culture Collection (ATCC, cat. no. CRL-6323). B16 sparse (1×10^5 cells/cm^2) monolayers, between the 60th and 90th passage, were grown at 36.5 °C in P-15 culture dishes (95% air, 5% CO_2 atmosphere) and 2 ml of Dulbecco's modified Eagle's basal medium (DMEM; D1152 Sigma Chemical Co., St Louis, MO) and 10% iron-supplemented certified calf serum (Gibco BRL, 10371-029, Grand Island, NY). Cells were washed twice with phosphate-buffered saline (PBS) and exposed to 20 μM solution (dimethylsulfoxide (DMSO)) of each porphyrin (**3**–**7**) for 12 h in darkness. Cells were washed twice with PBS, after that 1.5 ml of culture media with ^{3}HdTT (1.5 μCi ml^{-1}) was added to each culture dish. Cells were incubated for 4 h and washed twice with 2 ml of PBS, each. Cells were detached with 500 μl of trypsin, and centrifuged at 5000 rpm for 5 min (Eppendorf centrifuge). The pellets were washed with 1 ml of PBS. They were incubated with 200 μl of 0.4 M perchloric acid, 15 μl of PBS and 0.1% of fetal bovine serum, at −20 °C for 1 h. Cells were then centrifuged at 5000 rpm for 2 min and their hydrolysis was performed with 250 μl of (1 M) perchloric acid. Each sample was sonicated (three beats of 30 s each) and then incubated at 70 °C for 30 min. Samples were centrifuged at 3000 rpm for 2 min and aliquots of 200 μl were taken and incubated with a solution of 200 μl of the chromogen (see below) for 18 h. Aliquots of 300 μl were taken and their absorbance was measured at 600 nm. Aliquots of 200 μl were placed in vials that contained a sparkling liquid and incorporation of ^{3}HdTT was measured.

The chromogen was obtained by mixing 20 ml of solution A (prepared from 1.5 g of diphenylamine in 100 ml glacial acetic acid and 1.5 ml of concentrated sulfuric acid) with

0.1 ml of solution B (prepared from 0.5 ml of acetaldehyde in 25 ml of water).

Synthesis

*5,10,15,20-Tetraphenyl-21*H,*23*H*-porphyrin (**1**)*

To a 1 l round-bottomed flask equipped with a magnetic stirring bar and covered with aluminum foil, were added 1.52 ml of benzaldehyde (15 mmol), 1.04 ml of pyrrole (15 mmol), and dry methylene chloride (1 l). The solution was stirred and 0.4 ml of a solution of boron trifluoride diethyl etherate (2.5 M in methylene chloride) was added. The red colored mixture was stirred at room temperature for 1 h and 2.77 g of *p*-chloranil (11 mmol) added. The flask was immersed in an oil bath heated to 45 °C for an additional 1 h. The solvent was removed with a rotary evaporator and the resulting dark residue was washed with methanol (500 ml). The purple solid was purified by flash chromatography on silica gel using chloroform as eluent to afford 1.4 g of tetraphenylporphyrin (55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ −2.73 (s, 2H, NH-pyrrole), 7.75–7.80 (m, 12H), 8.24–8.26 (m, 8H), 8.88 (s, 8H, β-pyrrole).

*5(*p*-Nitrophenyl),10,15,20-triphenyl-21*H,*23*H*-porphyrin (2)*

To a 250 ml, three-necked flask equipped with magnetic stirrer and addition funnel, was added a solution of 1 g of **1** (1.63 mmol) in 100 ml of chloroform. After the solution was cooled to 0 °C, 1.06 mL of fuming nitric acid (27 mmol) was added dropwise. The solution was stirred at room temperature for 2 h, during which it became dark green. The reaction mixture was quenched with water (3 × 100 ml) and the organic layer dried over sodium sulfate, filtered and concentrated in the presence of a small amount of silica gel. The nitrophenyltriphenylporphyrin was purified by flash column chromatography on silica gel using chloroform as eluent to give 0.54 g (46%) of product **2**. ^{1}H NMR (300 MHz, $CDCl_3$): δ −2.78 (s, 2H, NH-pyrrole), 7.75–7.84 (m, 9H, *meta*/*para*-phenyls), 8.21–8.23 (m, 6H, *ortho*-phenyls), 8.41 (d, 2H, J = 8.63 Hz, nitrophenyl), 8.63 (d, 2H, J = 8.6 Hz, nitrophenyl), 8.75 (d, 2H, J = 4.7 Hz, β-pyrrole), 8.87 (s, 4H, β-pyrrole), 8.90 (d, 2H, J = 4 Hz, β-pyrrole).

*5(*p*-Aminophenyl),10,15,20-triphenyl-21*H,*23*H*-porphyrin (3)*

To a 250 ml round-bottomed flask equipped with magnetic stirrer was added a solution of 250 mg of **2** (0.38 mmol) in concentrated hydrochloric acid (10 ml). The mixture was stirred at room temperature, then 260 mg of tin(II) chloride dihydrate (1.15 mmol) was added and the suspension warmed to 65–70 °C for 2 h. After cooling to room temperature, 30 ml of water was added to the mixture followed by dropwise addition of concentrated ammonium hydroxide until pH 8 was reached. The aqueous phase was extracted with chloroform (3 × 50 ml) and the combined organic layers were concentrated in a rotary evaporator. The product was purified by flash column chromatography on silica gel and a mixture of hexane/CH_2Cl_2 (7 : 3) was used as eluent to afford 248 mg (94%) of aminophenyltriphenylporphyrin. ^{1}H NMR (300 MHz, $CDCl_3$): δ −2.78 (s, 2H, NH-pyrrole), 4.02 (s, 2H, NH_2) 7.06 (d, 2H, J = 8.3 Hz, aminophenyl), 7.75–7.84 (m, 9H, *meta*/*para*-phenyls), 8.08 (d, 2H, J = 8.3 Hz, aminophenyl), 8.22 (m, 6H, *ortho*-phenyl), 8.75 (d, 2H, J = 4.7 Hz, β-pyrrole), 8.87 (s, 4H, β-pyrrole), 8.90 (d, 2H, J = 4.7 Hz, β-pyrrole). IR (KBr, cm^{-1}): ν 3698 (w), 3464 (s, b), 3053 (m), 2851 (w), 1617 (s, b), 1470 (m), 1352 (m), 996 (m), 800 (m), 733 (m), 702 (m).

*5(*p*-Aminophenyl),10,15,20-triphenyl-21*H,*23*H*-porphyrin M(II) (4–7) general procedure*

A 250 ml, three-necked flask equipped with magnetic stirrer and addition funnel was charged with 200 mg of **3** and 20 ml of methylene chloride. The mixture was stirred at room temperature, then a solution of 400 mg of the corresponding M(II) acetate in 20 ml of methanol was added dropwise. The reaction was monitored by thin-layer chromatography using a mixture of CH_2Cl_2/hexane (7 : 3) as eluent. The solution was stirred for 12 h for zinc, copper and cobalt acetates and for 5 days for nickel acetate. During evaporation of the CH_2Cl_2 in a rotary evaporator, methanol was added gradually and a red solids were formed for the copper, nickel or cobalt porphyrins, and a blue solid for the zinc porphyrin. Products were purified by flash column chromatography on silica gel, using a mixture of CH_2Cl_2/hexane (7 : 3) as eluent. Metalloporphyrins were obtained in 90–98% yields.

*5(*p*-Aminophenyl),10,15,20-triphenyl-21*H,*23*H*-porphyrin zinc(II) (4)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.79 (b, 2H, NH_2), 6.90 (d, 2H, J = 8.2 Hz, aminophenyl), 7.72 (m, 9H, *meta*/*para*-phenyls), 7.87 (d, 2H, J = 8.2 Hz, aminophenyl), 8.20 (m, 6H, *ortho*-phenyls), 8.82 (m, 6H, β-pyrrole), 9.0 (d, 2H, J = 4.8 Hz, β-pyrrole). IR (KBr, cm^{-1}): ν 3698 (w), 3448 (s, b), 2924 (w), 1619 (m), 1352 (m), 1007 (b), 795 (w), 702 (m). FABMS (m/z) calc. 693.1266; found 693.1152.

*5(*p*-Aminophenyl),10,15,20-triphenyl-21*H,*23*H*-porphyrin copper(II) (5)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.76 (b, 2H, NH_2), 6.70 (broad, 2H, aminophenyl), 7.50 (broad, 11H, aminophenyl, *meta*/*para*-phenyls), 8.22 (b, 14H, β-pyrrole, *ortho*-phenyls). IR (KBr, cm^{-1}): ν 3721 (w), 3447 (s, b), 3053 (w), 2920 (w), 1619 (m), 1346 (m), 1001 (s), 800 (m), 769 (m), 705 (m). FABMS (m/z) calc. 691.2926; found 691.2187.

*5(*p*-Aminophenyl),10,15,20-triphenyl-21*H,*23*H*-porphyrin nickel(II) (6)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 2H, NH_2) 6.97 (d, 2H, J = 8.1 Hz, aminophenyl), 7.69 (m, 9H, *meta*/*para*-phenyls), 7.78 (d, 2H, J = 8.1 Hz, aminophenyl), 8.01 (m, 6H, *ortho*-phenyls), 8.75 (m, 6H, β-pyrrole), 8.84 (d, 2H, J = 4.8 Hz, β-pyrrole). IR (KBr, cm^{-1}): ν 3782 (w), 3719 (w), 3446 (s, b), 3051 (m), 2861 (m), 1616 (m), 1352 (m), 1005 (m), 794 (m), 749 (m), 702 (m). FABMS (m/z) calc. 686.4366; found 686.4352.

*5(*p*-aminophenyl),10,15,20-triphenyl-21*H*,23*H*-porphyrin cobalt(III) (7)*

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21 (s, 2H, NH_2) 7.33–8.56 (b, 13H, aminophenyl, *meta/para*-phenyls), 8.98–9.12 (b, 6H, *ortho*-phenyls), 9.56–9.87 (b, 8H, β-pyrrole). IR (KBr, cm^{-1}): ν 3421 (s, b), 2930 (m), 1625 (m), 1346 (m), 1001 (m). FABMS m/z calc. 686.6798; found 687.6763 $[M + 1]^+$.

RESULTS

Synthesis

The condensation of pyrrole and benzaldehyde using BF_3 as catalyst and *p*-chloranil as oxidant led to H_2TPP (**1**) in 55% yield.[20,21] This compound was treated with an excess of fuming nitric acid to afford the *p*-nitrophenyl monosubstituted porphyrin (**2**) in 46% yield, in a regioselective manner.[22] Reduction of the nitro group was carried out with a mixture of hydrochloric acid/tin chloride to give the H_2TPPNH$_2$ (**3**) in 94% yield.[22] Metallation of **3** with Co^{III}, Ni^{II}, Cu^{II} and Zn^{II} was accomplished with the corresponding metal(II) acetate,[23] as shown in Scheme 2.

The characterization of compounds **3**–**7** was performed by ^{1}H NMR and IR spectroscopy as well as by high-resolution FAB mass spectrometry. The ^{1}H NMR signal of the pyrrol NH group of the free porphyrin was displayed at −2.73 ppm. In most cases, the aa′bb′ proton system for the substituted phenyl ring appeared as double doublets at frequencies other than the rest of the phenyl rings. Table 1 compiles the chemical shift and coupling constants of these protons. In the porphyrinyl complexes of copper(II) (**5**) and cobalt(III) (**7**) the signals were broad; therefore, coupling constants could not be determined.

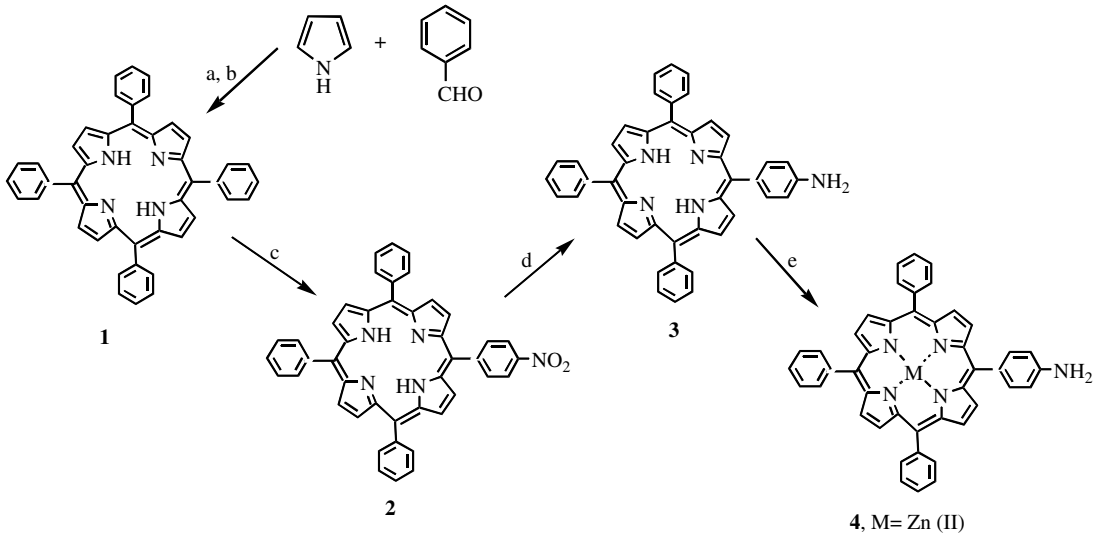
Table 1. Chemical shift (ppm) for the aa′bb′ system for compounds **3**–**7**

Porphyrin	aa′	bb′
3	8.08 (d, $^3J = 8.3$ Hz)	7.06 (d, $^3J = 8.3$ Hz)
4	7.87 (d, $^3J = 8.2$ Hz)	6.90 (d, $^3J = 8.2$ Hz)
5	7.50 (broad)	6.70 (broad)
6	7.78 (d, $^3J = 8.1$ Hz)	6.97 (d, $^3J = 8.1$ Hz)
7	7.33–8.56[a]	7.33–8.56[a]

[a] Broad and overlapped signals.

UV–Vis spectroscopy

As observed for cationic porphyrins and their metallo derivatives,[24,25] porphyrins **3**–**7** were characterized by an intense Soret band around 420 nm. Table 2 summarizes the molar extinction coefficient of each band found in the visible region of porphyrins **3**–**7**. The nonmetallo H_2TPPNH$_2$ porphyrin (**3**) has an extra four small visible bands in the region 515–650 nm; these bands became two upon coordination of the metals. As observed by D'Souza and co-workers for zinc porphyrins,[26,27] we found a red-shifted Soret and visible β and α bands for ZnTPPNH$_2$ porphyrin (**4**) in DMSO, as compared with in methylene chloride [λ_{DMSO} (nm): 428, 561, 603 ($\varepsilon_\alpha/\varepsilon_\beta = 0.90$) versus $\lambda_{CH_2Cl_2}$ (nm): 420, 553, 593 ($\varepsilon_\alpha/\varepsilon_\beta = 0.50$)], suggesting that DMSO is bound to the zinc, leading to a five-coordinate metalloporphyrin. On



(a) BF_3/CH_2Cl_2, 1 h; (b) *p*-chloranil, 45 °C, 1 h, (55%); (c) fuming $HNO_3/CHCl_3$, 2h, 0 °C (46%); (d) $SnCl_2$/HCl (conc.), 2 h, 65-70 °C (94%); (e) $M(OAc)_2/CHCl_3$, MeOH, 2 h.

Scheme 2.

Table 2. Molar extinction coefficients [$\varepsilon^{\lambda(nm)}$ M^{-1} cm^{-1}] in dry DMSO for porphyrins **3**–**7**

3 (M = NH_2)	**4** (M = Zn)	**5** (M = Cu)	**6** (M = Ni)	**7** (M = Co)
$\varepsilon^{419} = 2.87 \times 10^5$	$\varepsilon^{428} = 1.38 \times 10^4$	$\varepsilon^{419} = 1.80 \times 10^5$	$\varepsilon^{418} = 1.21 \times 10^4$	$\varepsilon^{437} = 1.70 \times 10^5$
$\varepsilon^{516} = 1.25 \times 10^4$	$\varepsilon^{561} = 4.17 \times 10^2$	$\varepsilon^{543} = 1.25 \times 10^4$	$\varepsilon^{531} = 1.67 \times 10^3$	$\varepsilon^{550} = 1.75 \times 10^4$
$\varepsilon^{552} = 4.16 \times 10^3$	$\varepsilon^{603} = 3.75 \times 10^2$	$\varepsilon^{585} = 5.00 \times 10^3$	$\varepsilon^{637} = 8.33 \times 10^2$	$\varepsilon^{590} = 1.25 \times 10^4$
$\varepsilon^{590} = 2.79 \times 10^3$	—	—	—	—
$\varepsilon^{647} = 2.79 \times 10^3$	—	—	—	—

the other hand, based on the Soret maximum for $CuTPPNH_2$ and $NiTPPNH_2$ (around 418 nm) and by comparison with the Soret maximum of analogous metalloporphyrins,[23] we assume that copper(II) and nickel(II) derivatives (**5** and **6** respectively) are four-coordinate planar porphyrins. In the case of $CoTPPNH_2$ porphyrin, to differentiate whether the metal ion was a cobalt(II) or cobalt(III) species we bubbled O_2 through the DMSO solution and observed that the Soret maximum did not change; therefore, we conclude that porphyrin **7** is a cobalt(III) species and very likely a six-coordinate axial ligand complex.[24,28]

Biological assays

The ability of porphyrins **3**–**7** to inhibit the replication of the DNA of carcinogenic cells was tested in mouse melanoma B16 line cells by counting the tritium associated to DNA as thymidine-^{3}H obtained by inoculation. Incorporation of ^{3}HdTT is inversely related to the inhibitory ability of the drug analyzed.[29,30] On the other hand, nucleotides quantitation in DNA samples was performed by a standard colorimetric method using diphenylamine as the chromogen.[31,32] Calf thymus DNA was used to standardize the technique (the calculated error in the measurement of the absorbance is 0.0069).

Incorporation of thymidine-^{3}H

Cells were incubated with 20 μM solution (DMSO) of porphyrins for 12 h in darkness, according to the procedure mentioned in the Experimental section. A culture medium with ^{3}HdTT (1.5 μCi ml^{-1}) was added to cultures and incubation continued for an additional 4 h. Incorporated ^{3}HdTT was measured by counting the β radiation, in decays per minute (DPM), for the radioactive decay of tritium. Measurement of nucleotide absorbance for each sample, achieved by colorimetry, permitted the counting of radioactive decay (DPM) per microgram of DNA. The percentage efficiency in the incorporation of ^{3}HdTT to DNA was adjusted to 100% efficiency, assigned to the decay radiations found for a control sample (a sample without porphyrin). The DNA replication inhibitory activity of porphyrins **3**–**7** is reported in Table 3.

Table 3. DNA replication inhibition, by porphyrins **3**–**7**, in mouse melanoma B16 cells

M in porphyrin	Efficiency (%)	Inhibitory activity[a] (%)
Control	100.0	0.0
H_2	92.94	7.06
Zn(II)	93.47	6.53
Cu(II)	88.10	11.90
Ni(II)	76.69	23.31
Co(III)	56.64	43.36

[a] Calculated as (100 − Efficiency).

DISCUSSION

The intercalation of porphyrins with DNA has been investigated thoroughly by spectroscopic methods.[33] From that study, and some reported X-ray structures,[34] it is well known that porphyrins intercalate with the nucleobases of DNA. In the case of cationic porphyrins, the intercalation is favored by two factors: the electrostatic interaction between the cationic fragment of the porphyrin and the anionic phosphate groups located in the grooves of DNA, and the stabilizing $\pi-\pi$ stacking contacts between the aromatic parts of the porphyrins and purines. A third factor has to be taken into consideration in the interaction of DNA with cationic metalloporphyrins i.e. the coordinative ability of the metal, so that intercalation is only observed with planar nonaxially substituted porphyrins (M = Cu, Ni, Au, and Pd).[35] Flat cationic porphyrins also intercalate with duplexes of RNA or RNA–DNA.[36] In viral or cancerous damaged cells, where single DNA strands are more likely,[37] coordination of transition metals with nucleobases might be enhanced because they can coordinate to primary sites of purines and pyrimidines, as shown in Scheme 1. The proliferation and immortality of cancerous cells is associated with the replication of telomeres. Recently, it was found that cationic porphyrins interact with four-stranded DNA telomeres through guanines.[38] In addition, Debnath *et al*.[39] found that several neutral porphyrins have anti-HIV activity.

In the case of porphyrins **3**–**7**, it is interesting to note that even though they are not cationic porphyrins they display DNA inhibitory activity on tumor cells (Table 2), indicating that they also form complexes with DNA; therefore, they interfere in the incorporation of ^{3}HdTT into the DNA growing

chain. On the other hand, in results to be published elsewhere, we have also examined the interaction of porphyrins **3**–**7** with DNA isolated from a plasmid and observed hypochromicity of the Soret band, which is evidence of the interaction of DNA with these porphyrins. The fact that porphyrin **7** ($CoTPPNH_2$) is the most active inhibitor, and that the planar porphyrins **3, 5**, and **6** (M = H_2, Cu, and Ni respectively) are less active, is suggestive of intercalation not being the prevalent noncovalent interaction between them and DNA.[36] The amino group in these porphyrins may interact by hydrogen bonding to phosphoryl groups of DNA phosphate bridging groups[40] and the metals will form complexes with extra-helical structural purines or pyrimidines, as many metal-active sites of enzymes usually do,[7,41] or will help in the binding of porphyrins to DNA quadruplexes in telomeres. These complexes will destabilize the DNA duplex, quadruplex, or tetraplex, altering the pathways for the DNA enzymatic functions such as replication, transcription, etc. Of course, this is particularly important in malignant cells, because the porphyrin–DNA complexes can inhibit the enzyme telomerase responsible of the replication of DNA.[37] The enzyme telomerase is also expressed in tumor cell lines.[42] Therefore, taking into consideration that $CoTPPNH_2$ (**7**) is more active than $ZnTPPNH_2$ (**4**) (inhibition: for **7** is 43.4% and for **4** is 6.34%), we can conclude that cobalt(III) will tend to form stronger complexes with extra-helical DNA guanines, of damaged double-helix DNA, than zinc(II). Perhaps this is related to the ability of cobalt(III) to coordinate six ligands, compared with zinc(II) which only coordinates five (see above). This finding is in line with the inhibition of the growing of malignant human melanoma cells in the presence of cobalt-complexes reported by Czuchajowski and Niedbala.[43]

CONCLUSIONS

This study reports on the DNA replication inhibitory activity of *p*-aminophenyl triphenylporphyrin (**3**) and its metallo derivatives (M = Zn, Cu, Co, Ni) in mouse melanoma B16 cells. Planar porphyrins (M = H_2, Ni, Cu) were less active than cobalt(II) porphyrin (**7**); therefore, we conclude that these noncationic porphyrins interact with DNA of carcinogenic cells, presumably by coordination with extra-helical purines of double-helix DNA, or by stacking externally on the G-tetrads of telomeres, rather than by intercalation. The decrease in ^{3}HdTPP incorporation into the growing tumorous DNA observed for the DNA–$CoTPPNH_2$ complex compared with DNA–$ZnTPPNH_2$ complex might indicate that the octahedral cobalt(III) complex creates more abnormal topological defects in DNA of mutagenic cells than pyramidal zinc(II) complex.

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