DNA replication inhibition and fluorescence microscopy of non-cationic porphyrins in malignant cells

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Abstract

The synthesis of non-cationic *meso*-(4*a*-bromoacetamidophenyl)triphenyl-porphyrin $H_2TPPNHCOCH_2Br$ (**6**) and its zinc, copper, nickel and cobalt metallo derivatives (**7-10**) is reported. By means of ^3H assays we observed that non-cationic porphyrins have activity against DNA replication inhibition in mouse melanoma B16 cells. The intercalation of copper (II) porphyrin (**3**) with plasmid DNA was supported by the hypochromicity observed in the Soret maximum in UV-vis spectroscopy. Fluorescence microscopy corroborates that porphyrins accumulate in the nucleus of the cell.

Keywords: Non-cationic porphyrins, confocal fluorescence microscopy, DNA replication inhibition, DNA intercalation

Introduction

Porphyrins are useful bio-ligands that control oxygen transport and storage processes in the cells of mammals.[1] For the last fifty years, studies related to the use of porphyrins as photosensitizers in medical cancer therapy[2] have brought a wealth of knowledge about the pathways these substances take, once they are delivered into the organism. As a very active field of research, photodynamic therapy (PDT)[3] has emerged since then as a method for the diagnosis and clinical treatment of tumors. The technique is based in the selective delivery of the photosensitizer to the

damaged tissue or organ, and the subsequent irradiation by visible or near-visible light to provoke photodamage of the tumor.

In the design of an active porphyrin-based photosensitizer, it is worth while to take into account the intracellular transport properties of the porphyrin, and its efficiency in the energy transfer process that produces singlet oxygen, the therapeutic agent that causes tissue photonecrosis.[4] Cationic porphyrins (CP) are well-known tumor localizers. The efficient transport of CP through the plasma and nuclear membranes is ascribed to their amphiphilic nature.[5] When CP accumulate in the nucleus of the cell, they bind to double helix DNA or to telomeres of mutagenic tissues or organs by means of stabilizing non-covalent interactions.[6] For example, CuTMPyP4 [copper(II) *meso*-tetra(N-methyl-4-pyridyl)porphyrin], a cationic metalloporphyrin, binds to duplex DNA by intercalation between the C and G nucleobases (π-π stacking) and by cationic-anionic electrostatic interaction between the *meso* groups of the porphyrin and the phosphates located in the groove of DNA.[7] On the other hand, it has been reported that TMPyP4 and its analogs bind to telomeres by stacking on the G tetrads at the core of the DNA quadruplex.[6,8,9] It is worth of mention that hydrogen bonding and van der Waals interactions can also promote intercalation of non-planar molecules with DNA.[10,11]

Even though spectroscopic methods do not support the intercalation of non-aggregating porphyrins[12] and the fact that non-cationic porphyrins (NCP) lack the potential for cationic-anionic electrostatic interaction with the DNA phosphates, *meso*-tetra(*m*-hydroxyphenyl) *m*-THPP and *meso*-tetra(*p*-hydroxyphenyl)porphyrin *p*-THPP are, among others, good examples of neutral NCP's which are good light absorbers and selective towards tumor cells, and therefore conveniently used as photosensitizers in PDT.[5,13] On the other hand, we have recently reported that non-cationic *meso*-(4-aminophenyl)triphenylporphyrin (H_2TPPNH$_2$) (**1**); and its Co, Cu, Ni, and Zn metallo derivatives (**2-5**) have activity against carcinogenic DNA replication. We observed that the octahedrally complexed porphyrin [CoTPPNH$_2$] is the most active compound.[14] From these results and those cited above it is clear that NCP's and their metallo derivatives also interact with DNA of tumor cells although less likely do so by intercalation. In this work we report the DNA replication inhibitory activity of a series of porphyrins (**6-10**) (Scheme 1), by using ^{3}H radio-labeled DNA in B16 mouse melanoma line cells. A comparison of the DNA replication inhibitory activity of **2-5** and **6-10**, led us to confirm the importance of the non-covalent interactions between DNA and the porphyrins in the replication of these malignant cells. In support to our results, we report the intracellular localization of porphyrins by using confocal fluorescence microscopy.

Results and Discussion

Synthesis
The synthesis of porphyrin H_2TPPNH$_2$ (**1**) and its metallo derivatives **2-5** is described somewhere else.[14] The reaction of **1-5** with bromoacetyl bromide using pyridine as a base led to

porphyrins MTPPNHCOCH$_2$Br **6-10**. The progress of reaction was followed by thin layer chromatography. The compounds were obtained with yields better than 86%, in reaction times between 2.5 h to 4 h, as shown in Scheme 1. Alternatively, compound **6** was obtained from **1** and its metallation with Zn (II), Cu (II), Ni (II) and Co (III) was accomplished with the corresponding metal (II) acetate, (Scheme 1); however yields were lower (55 % - 70%) and reaction times longer (12 h), in every case.[15] Purity of compounds **6-10** was ascertained by high resolution FAB mass spectrometry.

1

2, M= Zn (II)
3, M= Cu (II)
4, M= Ni (II)
5, M= Co (III)

6

7, M= Zn (II)
8, M= Cu (II)
9, M= Ni (II)
10, M= Co (III)
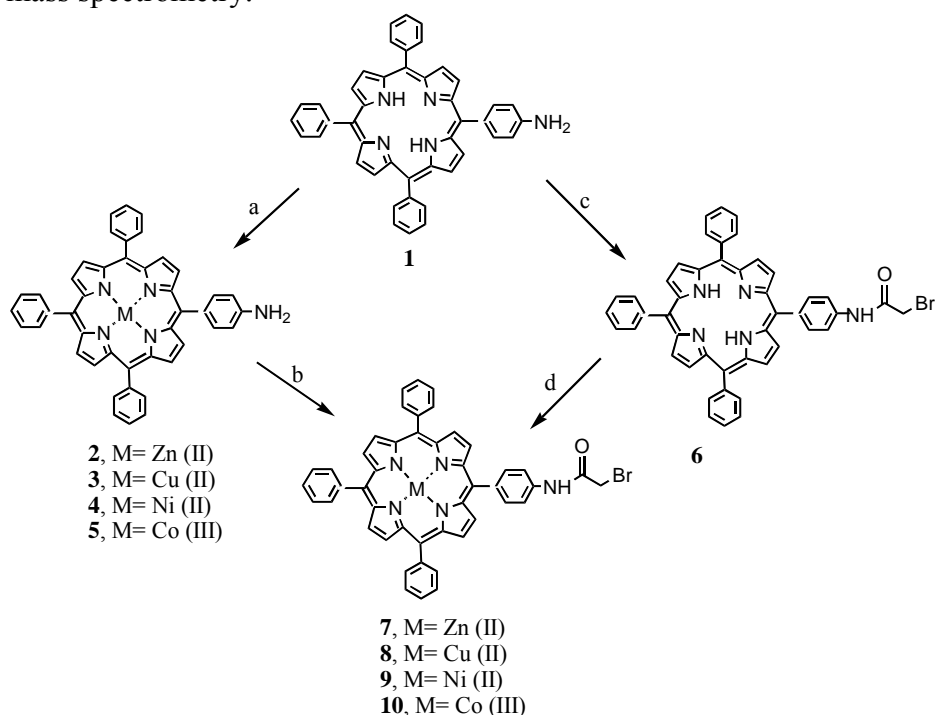
(a) M(AcO)$_2$/MeOH-CH$_2$Cl$_2$; (b) BrCH$_2$COBr, THF-Pyridine, 0-25 ^{o}C, ~ 3 h, (86%-90%);
(c) BrCH$_2$COBr, THF-Pyridine, 0-25 ^{o}C, 2.5 h, (92%); (d) M(OAc)$_2$/MeOH-CH$_2$Cl$_2$,12 h, (55%-70%).

Scheme 1

The characterization of compounds **6-10** was performed by ^{1}H and ^{13}C NMR, IR and UV spectroscopy, as well as by high resolution FAB mass spectrometry. In the porphyrinyl complexes of Cu (II) (**8**) and Co (III) (**10**), the ^{1}H NMR signals were broad and therefore they are not reported. The ^{1}H NMR signal of the pyrrol NH group of porphyrin **6** was displayed at – 2.73 ppm. In most of the cases, the aa´bb´ proton system for the substituted phenyl ring appeared as a double doublets at frequencies between 7.9-8.3 ppm.

Absorption and fluorescence spectra

As in the case of porphyrins 1-5,14 the absorption spectra of porphyrins 6-10 are characterized by an intense Soret band around 420 nm. The molar extinction coefficients of the visible absorption bands are shown in Table 1. Mean while the free porphyrin 6 has an extra four small

visible bands in the region 516-646 nm, the metalloporphyrins 7-10 have only two. For the ZnTPPNHCOCH2Br porphyrin (7) a red-shift of Soret and visible β and α bands was observed on going from DMSO to CH2Cl2 solvent [λDMSO (nm): 429, 561, 601= (εα/εβ = 0.60) versus λCH2Cl2 (nm): = 419, 548, 586 (εα/εβ = 0.33)] suggesting that DMSO in porphyrin 7 is coordinated to Zn leading to a five-coordinate porphyrin.[16] Metal ion in porphyrin 10 is most likely Co (III) than Co (II) since we bubbled O2 through the DMSO solution and observed that the Soret maximum was practically equal (435 nm).[17] Soret absorption bands for Ni and Cu porphyrins were close to 420 nm, therefore we conclude that they are four-coordinate porphyrins.[18]

The emission spectra of porphyrins **1**, **2**, **4**, **6**, **7** and **9** shown in Figure 1 were taken in CH$_2$Cl$_2$, exciting the sample at the Soret absorption frequency for each porphyrin (Table 1). The fluorescence emission maxima are summarized in Table 2. The paramagnetic metal ions Cu (II) and Co (III) extinguished the fluorescence.[19] A Stokes shift of 11 nm was observed for the porphyrin **7**, this value is in agreement with the Stokes shift observed for similar Zn (II) acetoamidophenyl porphyrins.[15a]

Table 1. Molar extinction coefficients [ε $^{\lambda(nm)}$ M^{-1} cm^{-1}] in dry DMSO for porphyrins **6-10**

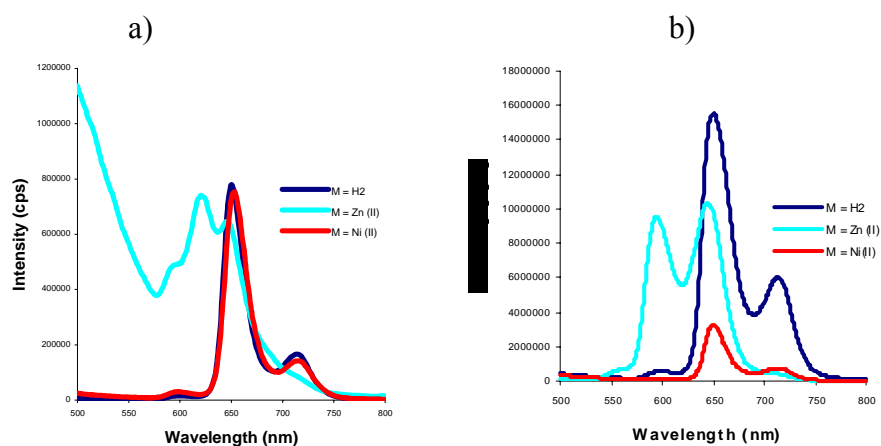| 6 (M= NH$_2$)	7 (M= Zn)	8 (M= Cu)	9 (M= Ni)	10 (M= Co)
ε^{420}= 4.12x10^5	ε^{429}= 1.42x10^5	ε^{419}= 2.87x10^5	ε^{417}= 3.42x10^5	ε^{436}= 2.96x10^5
ε^{516}= 1.67x10^4	ε^{561}= 4.17x10^3	ε^{542}= 1.25x10^4	ε^{531}= 2.54x10^4	ε^{547}= 2.08x10^4
ε^{551}= 8.33x10^3	ε^{601}= 2.50x10^3	ε^{589}= 2.08x10^3	ε^{600}= 4.17x10^3	ε^{586}= 1.67x10^4
ε^{592}= 4.17x10^3	-	-	-	-
ε^{646}= 4.17x10^3	-	-	-	-

Figure 1. Fluorescence emission spectra of (a) amino porphyrin **1** (dark blue line); Zn (II) porphyrin **2** (light blue line); Ni (II) porphyrin **4** (red line) and (b) bromoamido porphyrin **6** (dark blue line); Zn (II) porphyrin **7** (light blue line) and Ni (II) porphyrin **9** (red line). See Table 2 for experimental conditions.

Table 2. Fluorescence emission data in methylene chloride solution (λ_{exc} around 420 nm)

Compound (*meso* substituent, M)	Concentration [moles/L]	Fluorescence λ_{max} (nm)
1	2.6×10^{-6}	650
(NH$_2$, H$_2$)		718
2	1.0×10^{-6}	620
(NH$_2$, Zn)		647
4	3.2×10^{-6}	653
(NH$_2$, Ni)		715
6	3.0×10^{-6}	594
(NHCOCH$_2$Br, H$_2$)		653
		717
7	3.0×10^{-6}	597
(NHCOCH$_2$Br, Zn)		646
9	3.0×10^{-6}	653
(NHCOCH$_2$Br, Ni)		717

Biological assays

The ability of porphyrins **6-10** to inhibit the replication of the DNA of tumoral cells was tested in B16 mouse melanoma line cells by counting the tritium incorporated into DNA as thymidine-^{3}H obtained by inoculation in the presence or absence of porphyrin compounds.[20,21]

The amino substituent, in the *p*-position of a *meso* phenyl group, of porphyrins **1-5** renders the porphyrin with a partial hydrophilic character, as well as the ability to act as a donor group in a hydrogen bonding interaction. On the other hand, porhyrins **6-10** have a bromomethyl amido group capable of acting as an acceptor in a hydrogen bonding interaction. We examined the DNA replication inhibitory activity of these series of porphyrins in B16 mouse melanoma line cells (Table 3). Cells were incubated with thymidine-^{3}H, and the ability of porphyrins **6-10** to inhibit the replication of DNA was analyzed by counting the β radiations, in DPM [decay per minute] for the radioactive decay of tritium. The incorporation of ^{3}H is inversely related to the inhibitory ability of the porphyrin.

Table 3. DNA replication inhibition, by porhyrins **1-10**, in mouse melanoma B16 cells

M	% inhibitory activity Porphyrins **1-5**[14]	% inhibitory activity Porphyrins **6-10**
Control[a]	0.0	0.0
H$_2$	7.06 (**1**)	15.19 (**6**)
Zn (II)	6.53 (**2**)	53.07 (**7**)
Cu (II)	11.90 (**3**)	10.80 (**8**)
Ni (II)	23.31 (**4**)	11.16 (**9**)
Co (III)	43.36 (**5**)	34.09 (**10**)

[a] Cells incubated without porphyrin (see experimental part for details)

Studies of the interaction of porphyrins **1-5** with DNA, isolated from plasmid sample, by UV-Vis spectroscopy were also carried out. We found hypochromicity (% H) in the Soret maximum of most of the studied porphyrins; observing somewhat more hypochromicity in the flat Cu (II) porphyrin (**3**) (30 % H) than in the non-axially substituted Co (III) porphyrin (**5**) (16 % H) or Zn (II) porphyrin (**2**) (7 % H). The hypochromicity observed in the Soret band of the Cu (II) porphyrin (**3**) is shown in Figure 2. Soret band hypochromicity has been taken as a proof of the interaction of cationic porphyrins with double helix DNA by intercalation.[8a,17a] Since we also observed hypochromicity for porphyrins **3-7**, we can conclude that there is indeed interaction of these non-cationic porphyrins with DNA and very likely by intercalation. In most of the cases, the percentages of hypochromicity calculated for porphyrins **1-5** were smaller than those obtained for cationic porphyrins [i.e Ni (II)TMpyP-4= 38 % H *vs.* Ni(II)TPPNH$_2$= 4 % H (this work)].[17a] These results might be expected because of the lacking of the attractive electrostatic interaction between the porphyrins and phosphate DNA groups for non-cationic porphyrins that may lead to a decreased interaction with DNA.
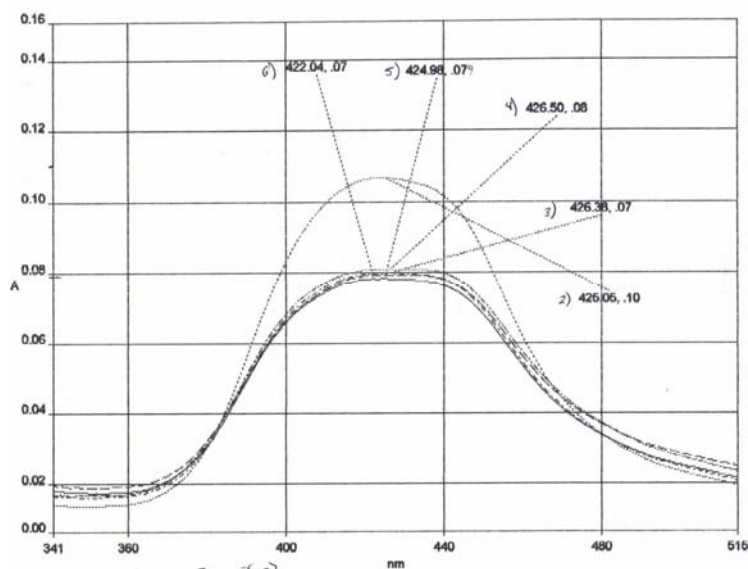
2) porphyrin
3) Porphyrin/DNA (2/1)
4) Porphyrin/DNA (1/1)
5) Porphyrin/DNA (1/2)
6) Porphyrin/DNA (1/3)

Figure 2. Absorption spectral change of Cu TPPNH$_2$ (**3**) on the addition of DNA from plasmid sample. The concentration of porphyrin was 3 x10^{-6} M. The spectra were recorded in buffer solution [10 mM sodium phosphate, 1 mM EDTA, 0.1 M NaCl (pH = 7.0)].

Fluorescence microscopy

Even though it is well-known that many tumors fluoresced spontaneously, porphyrin fluorescence and photosensitization has been widely used for the accurate localization and irradiation of malignant tumors in clinical PDT treatments.[22] Therefore, in accordance with the results of inhibitory activity measurements collected in Table 3, the intracellular accumulation of porphyrins **1** and **7** in the nucleus of the cell is clearly seen from their fluorescent pattern obtained by confocal fluorescence microscopy. B16 cells were treated with sytox dye that exclusively dyes the nucleus of the cell with a green color. Free and metal porphyrins (**1** and **7** respectively) are highly fluorescent, with intense red color emission; therefore it is possible to trace and to localize them into the cell. When porphyrins have reached the nucleus, the combination of green and red colors gives a yellow color. Figure 3 shows images of confocal fluorescence microscopy for compound **1**, a barely active compound against DNA replication, and **7** [Zn(II) in Table 3] the more active porphyrin, respectively.

Even though the yellow color appeared in both micrographies, the fluorescence for compound **1** is perinuclear with a local area of accumulation adjacent to the nuclear membrane; on the contrary, fluorescence accumulation for **7** takes place across the nucleus.
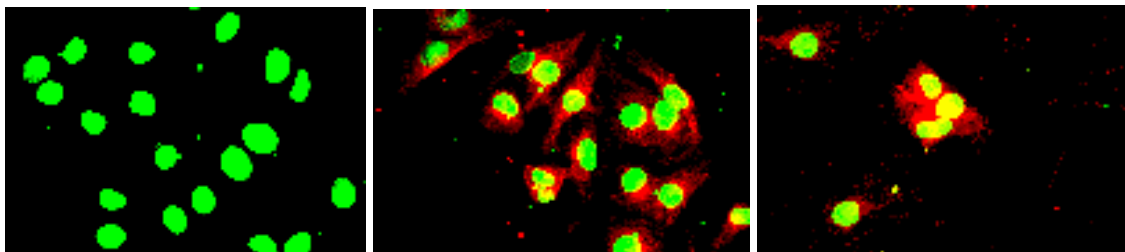

Figure 3. Intracellular localization of porphyrins (red) and sytox (green) in B16 cell lines of mouse melanoma determined by confocal fluorescence microscopy. Left: B16 cells dyed with sytox (control); center: B16 cells incubated with 20 μM of compound **1** for 12 h; right: B16 cells incubated with 20 μM of compound **7** for 12 h.

Considering that octahedral metalloporphyrins **5**, or **10** (Co) or five-coordinated porphyrin **7** (Zn) inhibit the replication of the DNA of tumoral cells (Table 3) more actively than non-axially substituted porphyrins (M= H_2, Cu, Ni) which would be expected to intercalate better with DNA, It seems likely that the dominant effect in the interaction of these porphyrins with B16 cells is coordination of transition metals with extra-helical structural nucleobases of duplex DNA. Additionally, taking into consideration that in cancerous damaged cells, telomeres (chromosomes with four stranded DNA arrangements in which guanines are predominant) are also expressed,[23] one can hypothesize that Zn or Co porphyrins bind to quadruplex DNA, through the interaction with G tetrads. It is worth of mention that the antitumor activity of cationic porphyrins has been ascribed to the inhibition of the enzyme telomerase that replicates telomeres.[6,8,9]

The combination of hydrophobic and hydrophilic substituents in *meso* substituted cationic porphyrins facilitates membrane penetration producing better accumulation in subcellular compartments.[24] In a recent study of fluorescence microscopy of platinum anticancer drugs, it was pointed out the importance of the intracellular transport in the mechanism of their action.[25] Thus, the expectation that the series of porphyrins **1-5** are more hydrophilic than porhyrins **6-10** can be used to explain the relative transport through plasma or nucleus membrane and therefore their relative activities.[26] Micrographies in Figure 3 show a higher accumulation of porphyrin **7** relative to porphyrin **1** in the nucleus of the cell, therefore we can conclude that the *meso* group in the porphyrin and/or the metal contributes to mobility of the Zn (II) porphyrin through the membrane. Lipids and proteins compose membranes, so it seems likely that the porphyrin **7**, penetrates the membrane to the nucleus much more than porphyrin **1**, resulting in greater inhibition of DNA replication by porphyrin **7**. Alternatively, since **7** can form an amido group with an α-amino-acid residue of the lipoprotein, they might react through a covalent interaction. Independently of non-covalent or covalent nature of the interaction, the binding of porphyrin **7** with the lipoprotein can give rise to the complex required to catalyze the transport process.[27] It is also worth mentioning that aminoacyl-analogues of anthracenedione derivatives target DNA in chemotherapy.[28]

In summary, we have found by confocal fluorescence microscopy that non cationic porphyrins **1** and **7** are accumulated in the nucleus of the cell. Porphyrins **1-10** show DNA replication inhibitory activity against malignant cells. Hypochromicity of the Soret maximum was observed for Cu (II) porphyrin (**3**) in the presence of plasmid DNA indicating the likely interaction by intercalation between them.

Experimental section

General procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded on Jeol Eclipse 400 and Bruker Avance 300 spectrometers, at 399.8 and 300.1 MHz respectively for ^{1}H NMR and at 100.5 and 74.5 MHz respectively for ^{13}C NMR. The abbreviations used for the signals are: s=singlet, d=doublet, m=multiplet, b=broad. The NMR signals of compounds **8** and **10** are very broad because they are paramagnetic metalloporphyrins. UV/Vis spectra were registered on Perkin Elmer lambda 12 spectrophotometer and IR on a FT-IR Perkin Elmer Spectrum GX. The abbreviations used for IR absorption frequencies are: w=weak, m=moderately strong, s=strong, b=broad. Fluorescence spectra were registered on a Spex Fluoro-Max-3 fluorimeter (Jobin-Yvon Horiba) and processed with Data Max 2.2 software. High resolution FAB mass spectra and electron impact (EI) at 70 eV were measured on a Jeol JMS-SX 102A spectrometer. All chemicals were of reagent grade and were purchased from Aldrich Chemical Co. Solvents were dried and purified according to literature procedures.[29] Flash column chromatography was performed on silica gel 200-400 mesh. [3]H incorporation in DNA was measured on a Stap Fax 303 plus spectrophotometer. Cells were incubated for 12 hr with 20 μm solution of porphyrins **1** and **7** and the intracellular fluorescence of the monolayers was examined using a confocal

microscope (MCR-600, Bio-Rad) with a krypton-argon laser [519 nm (green filter) and 572 nm (red filter) emission line]. Excitation source [495 nm (green filter) and 547 nm (red filter)].

Biological assays. Incorporation of ^{3}H into DNA as thymidine-^{3}H is inversely related to the inhibitory ability of the analyzed drug.[20,21] On the other hand, quantitation of nucleotides DNA samples, was performed by a standard colorimetric method using diphenylamine as chromogen.[30,31] Calf thymus DNA was used to standardize the technique (the calculated error in the measurement of the absorbance is 0.0069). Fluorescence microscopy of samples **1** and **7** was taken in sytox treated B16 mouse melanoma cells cultures as shown below.

Cell culture. B16 mouse melanoma cells were obtained from American Type Culture Collection (ATCC, cat. no. CRL-6323). Cells between the 60th and 90th passage, were grown at 36.5 ^{o}C in disposable plastic bottles (Costar 3151, Cambridge, MA) with a 95% air, 5% CO_2 atmosphere (Stericult 200, Forma Scientific, Marrieta, OH) and 2 mL of Dulbecco's modified Eagle's basal medium (DMEM; D1152 Sigma Chemical Co., St. Louis MO) with penicillin (100 IU mL; Eli Lilly, México) and 10% iron supplemented certified calf serum (Gibco BRL, 10371-029, Grand Island, NY). Cells were harvested with trypsin-EDTA (In Vitro, México) and plated at sparse (1 x 10^5 cells/cm^2).

Thymidine-^{3}H incorporation. B16 cells were grown in P-15 culture dishes, they were washed 2 times with PBS (phosphate-buffered saline) and exposed to 20 μM solution (DMSO) of each porphyrin (**6-10**) for 12 hr in the darkness. Cells were washed 2 times with PBS, after that 1.5 mL of culture media with ^{3}H (1.5 μCi/mL) was added to each culture dish. Cells were incubated for 4 hr and washed 2 times 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 tablets were washed with 1 mL of PBS. They were incubated with 200 μL of perchloric acid (0.4 M), 15 μL of PBS and 0.1% of fetal bovine serum, at –20 ^{o}C for 1 hr. Cells were then centrifuged at 5000 rpm for 2 min and their hydrolysis was performed with 250 μL of perchloric acid (1 M). Each sample was sonicated (1x10^{-4} kHz) and then incubated at 70 ^{o}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 hr. Aliquots of 300 μL were taken and their absorbance was measured at 600 nm. Aliquots of 200 μL were placed in vials containing a spark liquid and incorporation of ^{3}H was measured.

Te chromogen was obtained by mixing 20 mL of solution A (prepared from 1.5 g of diphenylamine in 100 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).

Confocal fluorescence microscopy. B16 sparse monolayers cultured on glass cover slips were incubated with 20 μM solution (DMSO) of each porphyrin for 12 hr. They were extensively washed in PBS and fixed in 2% aqueous solution of *p*-formaldehyde for 30 min at 4 ^{o}C. The monolayers were incubated with a 1μM solution of Sytox green nucleic acid stain (S-720, Molecular Probes, Inc., Eugene, Oregon, USA) for 20 min at 4 ^{o}C. They were washed 5 times

with PBS and one time with filtered distilled water. The monolayers on the glass cover slips were mounted with the antifade reagent Vectashield (Vector Laboratories Inc., Burlingame, CA).

Synthesis. General procedures

1. To a 100 mL round bottomed flask equipped with a magnetic stirring bar and covered with aluminum foil, were added 0.16 mmol of the corresponding porphyrin (**1-5**), followed by 0.015 mL of pyridine (0.19 mmol), and 3 mL of dry THF. The solution was stirred and 0.017 mL of a solution of bromoacetyl bromide (0.19 mmol) in dry THF (2 mL) was added. The mixture was stirred at room temperature for 3 hr and quenched with water (5 mL).[32] The mixture was concentrated in a rotary evaporator and CH_2Cl_2 (50 mL) was added to the solid residue. The organic layer was washed with water (5 x 25 mL), dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography on silica gel using CH_2Cl_2:MeOH (9:1) as eluent.

A 250-mL, three-necked, flask equipped with magnetic stirrer and addition funnel was charged with 50 mg of **6** and 5 mL of methylene chloride. The mixture was stirred at room temperature, then a solution of 100 mg of the corresponding M(II) acetate in 10 mL of methanol was added drop-wise. The solution was stirred for 12 hr. The product was concentrated in a rotary evaporator. The residue was washed twice with water and the organic layer extracted with chloroform (15 mL each). Products were purified by flash column chromatography on silica gel, using a mixture of CH_2Cl_2:MeOH (9:1). Metalloporhyrins were obtained in 55% - 70% yields.

5-(4α-Bromoacetamidophenyl)-10,15,20-triphenyl-21H,23H-porphyrin (6). Purple amorphous powder, yield 92% (from general procedure 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ -2.73 (s, 2H, NH-pyrrole), 4.18 (s, 2H, CH_2) 7.76 (m, 9H, *meta*/*para*-phenyls), 7.93 (d, 2H, *J*=8.4 Hz), 8.24 (m, 8H), 8.46 (b, 1H, NH), 8.87 (s, 8H, *β*-pyrrole). ^{13}C NMR (400 MHz, $CDCl_3$) δ 29.7, 118.3, 119.2, 120.3, 126.8, 127.8, 131.3, 134.7, 135.2, 136.8, 139.2, 142.2, 163.8. IR (KBr) ν cm^{-1} 3471 (s, b), 1662 (s), 1596 (m), 1524 (m), 965 (m), 797 (m), 721 (m), 700 (m). UV-Vis λ (DMSO) (nm): 420 (Soret band). HRMS-FAB FABMS *(m/z)* Calc. for $C_{46}H_{32}N_5OBr$, 750.6957; Found 751.6953 [M+1][+].

5-(4α-Bromoacetamidophenyl)-10,15,20-triphenyl-21H,23H-porphyrin zinc (II) (7). Pink-reddish amorphous powder, yield 86% (from general procedure 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17 (s, 2H, CH_2) 7.76 (m, 9H, *meta*/*para*-phenyls), 7.90 (d, 2H, *J*=8.4 Hz), 8.30 (m, 8H), 8.43 (b, 1H, NH), 8.85 (s, 8H, *β*-pyrrole). ^{13}C NMR (400 MHz, $CDCl_3$) δ 29.8, 118.4, 119.3, 120.4, 126.8, 127.8, 131.4, 134.7, 135.3, 136.8, 139.2, 142.3, 163.7. IR (KBr) ν cm^{-1} 3475 (s, b), 1669 (s), 1590 (w), 1530 (m), 970 (m), 795 (w), 720 (w), 705 (w). UV-Vis λ (DMSO) (nm): 429 (Soret band). HRMS-FAB FABMS *(m/z)* Calc. for $C_{46}H_{30}N_5OBrZn$, 814.0599; Found 815.0596 [M+1][+].

5-(4a-Bromoacetamidophenyl)-10,15,20-triphenyl-21H,23H-porphyrin copper(II) (8). Reddish amorphous powder, yield 90% (from general procedure 1). IR (KBr) ν cm^{-1} 3465 (s, b), 1659 (s), 1578 (m), 1530 (m), 970 (m), 796 (m), 732 (m), 720 (m). UV-Vis λ (DMSO) (nm): 419

(Soret band). HRMS-FAB FABMS *(m/z)* Calc. for $C_{46}H_{30}N_5OBrCu$, 812.2259; Found 813.2252 $[M+1]^+$.

5-(4*a*-Bromoacetamidophenyl)-10,15,20-triphenyl-21*H*,23*H*-porphyrin nickel (II) (9). Orange-reddish amorphous powder, yield 88% (from general procedure 1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (s, 2H, CH_2) 7.69 (m, 9H, *meta*/*para*-phenyls), 7.84 (d, 2H, *J*=8.4 Hz), 8.01 (m, 8H), 8.40 (b, 1H, NH), 8.76 (s, 8H, *β*-pyrrole). ^{13}C NMR (400 MHz, $CDCl_3$) δ 29.7, 118.5, 119.2, 127.0, 127.9, 132.1, 132.3, 132.4, 133.8, 134.4, 134.7, 137.9, 140.9, 163.7. IR (KBr) ν cm^{-1} 3472 (s, b), 1660 (s), 1585 (m), 1529 (m), 975 (m), 792 (m), 725 (w), 715 (w). UV-Vis λ (DMSO) (nm): 417 (Soret band). HRMS-FAB FABMS *(m/z)* Calc. for $C_{46}H_{30}N_5OBrNi$, 807.3699; Found 808.3694 $[M+1]^+$.

5-(4*a*-Bromoacetamidophenyl)-10,15,20-triphenyl-21*H*,23*H*-porphyrin cobalt (III) (10). Dark reddish amorphous powder, yield 90% (from general procedure 1). IR (KBr) ν cm^{-1} 3459 (s, b), 1660 (s), 1570 (m), 1529 (m), 972 (m), 795 (d), 730 (w), 720 (w). UV-Vis λ (DMSO) (nm): 435 (Soret band). HRMS-FAB FABMS *(m/z)* Calc. for $C_{46}H_{30}N_5OBrCo$, 807.6131; Found 807.6128 $[M]^+$.

Acknowledgements

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