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Porphyrin conjugates of Ibuprofen and their antiproliferative activity against human prostate and breast cancer cells

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ABSTRACT

Four porphyrin-dendrimer-conjugates of ibuprofen were synthesized in this work: conjugates with four, eight and thirty-two ibuprofen moieties in the structure, the yields of synthesis to obtain the conjugates of ibuprofen with porphyrin-dendrimers with polyamidoamine dendritic arms were good. Biological activity tests showed that the synthesized compounds have high potential activity against PC-3 and MCF-7 cancer cells. Inhibition of cancer cell proliferation was enhanced in the presence of 4, 8 and 32 ibuprofen-moiety-substituents in the dendritic branches in comparison to the results obtained with free ibuprofen. Based on the morphological changes of the cells and the blue fluorescence exhibited by the cells treated with the dendrimer-conjugate, the cellular internalization of the ibuprofen-conjugates ocurred. When the PC-3 cells were irradiated at 365 nm, the cells exhibited significant morphological changes, an increase in cell volume and showed strong internal movements. These movements were not observed for the PC-3 cells without conjugates after irradiation.

Keywords: *Porphyrin; Ibuprofen; Dendrimers; Conjugates; Anticancer activity.*

1. INTRODUCTION

In the last years, interest in the synthesis of new nanomolecules for biomedical applications has been growing rapidly. New smart macromolecules for pharmacological use as carriers of drugs are being studied to offer the possibility to control the drug-release process [1,2]. In this field, the research is directed towards the development of molecular carriers which are also able to recognize the diseased tissues in which the drug must act [3]. The most widely adopted drug-carriers, namely: dendrimers, polymers, liposomes, micelles, etc., are provided with suitable molecular cavities in which the drug species can remain to be transported [4-8]. These new nano-systems are easy to characterize analytically. Most importantly, it is desirable for the new smart molecules to be able to recognize specifically the sick tissues and release the drugs only where necessary [9].

For this aim, porphyrin derivatives are fascinating systems. *Meso*-substituted porphyrins are macrocyclic tetrapyrrole compounds with different substituents and high potential for medical applications. Several meso-substituted porphyrin derivatives display high selectivity for tumor tissues [10]. For several years, interest has been growing in the use of porphyrins and their derivatives as therapeutic drugs. They are applied in medicine for cancer detection and as photosensitizers in the photodynamic therapy of cancer [11]. Porphyrins are stable molecules that accumulate in cancer tissues. Porphyrins are used as chemical sensors of pH [12-15] and/or as chirogenetic probes for the analysis of amino acids and proteins[16-18] due to the spectroscopic properties of porphyrins, such as high molar absorption, strong fluorescence and good quantum yields.

Ibuprofen is an anti-inflammatory with anticancer activity [2,19,20]. In this work, the anti-proliferative activity of porphyrin-ibuprofen conjugates is demonstrated against cancer cells in vitro, which is significantly higher in comparison to the free ibuprofen. We suggest that the higher cytotoxic effect results from the highly effective uptake of the conjugate by the cells.

2. EXPERIMENTAL SECTION

Materials. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-300 MHz with tetramethylsilane (TMS) as an internal reference. Infrared (IR) spectra were measured on a Nicolet FT-SSX spectrophotometer. Elemental analysis was determined by Thermo Scientific, model Flash 2000. FAB+ mass spectra were taken on a JEOL JMS AX505 HA instrument. Electrospray mass spectra were taken on a Bruker Daltonic, Esquire 6000. MALDI-TOF mass spectra were taken on a Bruker Omni FLEX using 9-nitroanthracene (9NA) as a matrix. The UV-vis absorption spectra were obtained at room temperature with a Shimadzu 2401 PC spectrophotometer.

2-(4-Isobutylphenyl) propionic acid (ibuprofen), (98%, Sigma-Aldrich, St. Louis, MO, USA), 5,10,15,20-tetrakis(4-hydroxyphenyl)-21H,23H-porphine (99%, Sigma-Aldrich, St. Louis, MO, USA), hydrochloric acid (36.5–38% Aldrich), diethylenetriamine (Sigma-Aldrich, St. Louis, MO, USA), ethylenediamine (Sigma-Aldrich, St. Louis, MO, USA), methyl bromoacetate (Sigma-Aldrich, St. Louis, MO, USA) and methyl acrylate (Sigma-Aldrich, St. Louis, MO, USA) were used without additional purification. Triton™ X-100 solution (Sigma-Aldrich, St. Louis, MO, USA), and ethanol (Sigma-Aldrich, St. Louis, MO, USA) were used without additional drying. Tetrahydrofuran THF

was dried with Na. N,N-Dimethylformamide (DMF, Sigma-Aldrich, St. Louis, MO, USA) and dimethylsulfoxide (DMSO) (99.8%, Sigma-Aldrich, St. Louis, MO, USA) were used.

COX (ovine/human) Inhibitor. COX (ovine/human) Inhibitor, the major metabolite of arachidonic acid metabolism, was measured by PGF2α produced in the COX reaction (Cayman Chemical, Ann Arbor, MI) using conditional cell culture medium according to the protocol provided by manufacturer. Measurements were made in triplicate in separate experiments.

Anticancer Screening. The experimental methodology started with the growth of the tumor cell lines (U251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), K-562 (human chronic myelogenous leukemia cells), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), SKLU-1 (human lung adenocarcinoma) were acquired from the National Cancer Institute (NCI, USA). HGF human gingival fibroblasts were purchased from Sigma Aldrich (St. Louis, MO, USA)) and grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 μM L-glutamine. For a typical screening experiment, cells are inoculated into 96-well microtiter plates in 1000 μL of cells suspension aliquots ranging from 5000–10,000 cells per well. After cell inoculation, the microtiter plates are incubated at 37 ◦C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to the addition of compounds. After 24 h, two plates of each cell line were fixed in situ with trichloroacetic acid (TCA), to measure the cell population for each cell line at the time of drug addition (time zero (Tz)). Stock solutions of test compounds initially dissolved in DMSO (20 μM) were prepared and further diluted in growth medium to produce the desired concentrations. The single dose screen is carried out at a concentration of 25 μM. Following drug addition, the plates were incubated for 48 h under the conditions mentioned above. For adherent cells, the assay was terminated by the addition of cold trichloroacetic-acid. Cells are fixed in situ by addition of 50 μL of cold 50% (w/v) trichloroacetic-acid and incubated for 1 h at 4 ◦C. Sulforhodamine (SRB) solution (100 μL) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were placed on a shaked for 5 min at room temperature. Absorbance was read on an automated Ultra Microplate Reader (Elx 808: Bio-Tek Instruments, Inc., Winooski, VT, USA) at a wavelength of 515 nm. Using the time zero (Tz), control growth (C) and test growth in the presence of the drug at a concentration of 10 μM, the percentage of growth is calculated [25, 26].

Chemical Data

Synthesis of porphyrins with polyaminoamide dendritic arm dendrimers. Porphyrin-polyamidoamine-dendritic arms **1** and **2** were synthesized and characterized as reported by Gómez-Vidales et al. [21], Dendrimers **3** and **4** were obtained as reported by Garfias-Gonzalez et al. and Martínez-Klimov et al. [22-24] (Chart 1).

Synthesis of ibuprofen conjugates of first and second generation

Porphyrin-polyamidoamine-dendrimers (0.0525 mmol) in methanol (40 mL) were heated at 80°C. After 20 minutes, ibuprofen (0.63 mmol) was added to the mixture, after that the temperature was increased to 120 °C the mixture was stirred for 24 h. The solvent was evaporated and the resulting solid was dissolved in methanol and precipitated by EtOAc (Chart 2).

***Conjugate* 5.** Purple solid in 85 % yield. m.p: > 300 °C. FTIR (pellet, KBr, cm^{-1}): 3297, 2938, 1651, 1545, 1470, 1348, 1290, 1235, 1176, 1120, 797, 731, 587. UV-vis (CH_2Cl_2): λ_{max} 243, 278, 346, 422, 519, 577, 591, 654. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.74 (s 24H, CH_3), 1.37 (s, 12H, CH_3), 1.68 (m, 4H, CH), 2.26 (s, 8H, CH_2), 2.98 (br, 8H, CH_2-N), 3.31 (m, 8H, CH_2-N), 3.52 (br, 4H, CH), 4.09 (s, 8H, CH_2-O), 6.92 (d, 8H, *J*= 6.6 Hz, Ar_{ibu}), 7.16 (d, 8H, *J*= 6.6 Hz, Ar_{ibu}), 7.27 (br, 8H, Ar_{por}), 7.97 (d, 8H, *J*= 7.2 Hz, Ar_{por}), 8.73 (br, 8H, pyrrole). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 20.27 (CH_3), 23.1 (CH_3) 31.3 (CH_2-NH_2), 40.4 (CH), 46.4 (CH_2-N), 47.6 (CH), 69.0 (CH_2-O), 114.8 (Ar), 121.3 (Ar_{ipso}), 128.7 (Ar), 134.4 (Ar), 137.2 (pyrrole), 141.1 (Ar), 142.2 (Ar), 158.9 (Ar_{ipso}), 159.6 (Ar_{ipso}), 172.0 (C=O), 182.5 (C=O). Electro spray m/z: 1831 (M^+). Anal. Calcd for $C_{112}H_{126}N_{12}O_{12}$. C 73.42 %, H 6.93 %, N 9.17 %. Found: C, 73.40; H, 6.91; N 9.95 %.

***Conjugate* 6.** Purple solid in 85 % yield. m.p: > 300 °C. FTIR (pellet, KBr, cm^{-1}): 3297, 2938, 1651, 1545, 1470, 1348, 1290, 1235, 1176, 1120, 797, 731, 587. UV-vis (CH_2Cl_2): λ_{max} 243, 278, 346, 422, 519, 577, 591, 654. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.73 (s 48H, CH_3), 1.36 (s, 24H, CH_3), 1.67 (m, 8H, CH), 2.26 (s, 16H, CH_2), 2.88 (br, 32H, CH_2-N), 3.31 (m, 28H, CH_2-N), 3.52 (br, 20H, CH_2-N), 3.60 (br, 8H, CH), 4.38 (s, 8H, CH_2-O), 6.93 (d, 16H, *J*= 7.8 Hz, Ar_{ibu}), 7.16 (d, 16H, *J*= 7.8 Hz, Ar_{ibu}), 7.27 (br, 8H, Ar_{por}), 7.97 (d, 8H, *J*= 7.2 Hz, Ar_{por}), 8.75 (br, 8H, pyrrole). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 20.83 (CH_3), 23.7 (CH_3) 32.3 (CH_2-NH_2), 40.1 (CH), 47.0 (CH_2-N), 49.3 (CH), 69.9 (CH_2-O), 115.6 (Ar), 122.7 (Ar_{ipso}), 129.3 (Ar), 131.0 (Ar), 137.6 (pyrrole), 141.7 (Ar), 142.8 (Ar), 160.2 (Ar_{ipso}), 172.8 (C=O), 183.3 (C=O). Electro spray m/z: 3325 (M^+). Anal. Calcd for $C_{196}H_{250}N_{24}O_{24}$. C 70.77 %, H 7.58 %, N 10.11 %. Found: C, 70.79; H, 7.56; N 10.12 %.

Conjugate 7. Purple solid in 85 % yield. m.p: > 300 °C. FTIR (pellet, KBr, cm^{-1}): 3297, 2938, 1651, 1545, 1470, 1348, 1290, 1235, 1176, 1120, 797, 731, 587. UV-vis (CH_2Cl_2): λ_{max} 243, 278, 346, 422, 519, 577, 591, 654. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.78 (s 48H, CH_3), 1.40 (s, 24H, CH_3), 1.71 (m, 8H, CH), 2.30 (s, 16H, CH_2), 2.93 (br, 28H, CH_2-N), 3.57 (m, 12H, CH, CH_2-N), 4.15 (s, 8H, CH_2-O), 6.97 (d, 16H, *J*= 7.8 Hz, Ar_{ibu}), 7.21 (d, 16H, *J*= 7.8 Hz, Ar_{ibu}), 7.33 (br, 8H, *J*= 7.8 Hz, Ar_{por}), 8.04 (d, 8H, *J*= 7.2 Hz, Ar_{por}), 8.80 (br, 8H, pyrrole). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 20.26 (CH_3), 23.1 (CH_3) 31.8 (CH_2-NH_2), 40.5 (CH), 46.4 (CH_2-N), 47.7 (CH), 69.3 (CH_2-O), 71.6 (CH_2-O), 115.1 (Ar), 122.1 (Ar_{ipso}), 128.7 (Ar), 130.4 (Ar), 137.0 (pyrrole), 141.1 (Ar), 142.2 (Ar), 159.7 (Ar_{ipso}), 172.2

(C=O), 182.8 (C=O). Electro spray m/z: 2756 (M^+). Anal. Calcd for $C_{172}H_{210}N_{16}O_{16}$. C 74.91 %, H 7.68 %, N 8.13 %. Found: C, 74.93; H, 7.68; N 8.11 %.

Conjugate 8. Purple solid in 85 % yield. m.p: > 300 °C. FTIR (pellet, KBr, cm^{-1}): 3297, 2938, 1651, 1545, 1470, 1348, 1290, 1235, 1176, 1120, 797, 731, 587. UV-vis (CH_2Cl_2): λ_{max} 243, 278, 346, 422, 519, 577, 591, 654. ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.76 (s 192H, CH_3), 1.35 (s, 96H, CH_3), 1.69 (m, 32H, CH), 2.29 (s, 64H, CH_2), 2.89 (br, 32H, CH_2-N), 3.22 (m, 128H, CH_2-N), 3.52 (m, 96H, CH, CH_2-N), 4.10 (m, 8H, CH_2-O), 6.94 (d, 64H, *J*= 7.2 Hz, Ar_{ibu}), 7.18 (d, 64H, *J*= 6.6 Hz, Ar_{ibu}), 7.40 (br, 8H, Ar_{por}), 8.049 (d, 8H, *J*= 6.6 Hz, Ar_{por}), 8.81 (br, 8H, pyrrole). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 22.2 (CH_3), 22.6 (CH_3) 29.3 (CH_2-NH_2), 29.8 (CH_2-NH_2), 31.9 (CH_2-NH_2), 37.1 (CH), 45.0 (CH-N), 47.1 (CH), 47.7 (CH), 68.3 (CH_2-O), 69.8 (CH_2-O), 127.5 (Ar), 129.0 (Ar), 135.2 (pyrrole), 139.6 (Ar), 140.2 (Ar), 154.4 (Ar_{ipso}), 169.2 (C=O), 173.6 (C=O), 181.3 (C=O). Electro spray m/z: 9789 (M^+). Anal. Calcd for $C_{596}H_{834}N_{64}O_{56}$. C 73.11 %, H 8.59 %, N 9.16 %. Found: C, 73.13; H, 8.62; N 9.14 %.

3. RESULTS SECTION

The porphyrin-dendrimers **1**-**4** (Chart 1) were synthetized in agreement with the literature reports [22-24].

Porphyrin-polyamidoamine-dendrimers (0.0525 mmol) in methanol (40 mL) were heated at 80°C. After 20 minutes, ibuprofen (0.63 mmol) was added to the mixture, after that the temperature was increased to 120 °C the mixture was stirred for 24 h. The solvent was evaporated and the resulting solid was dissolved in methanol and precipitated by EtOAc (Chart 2).

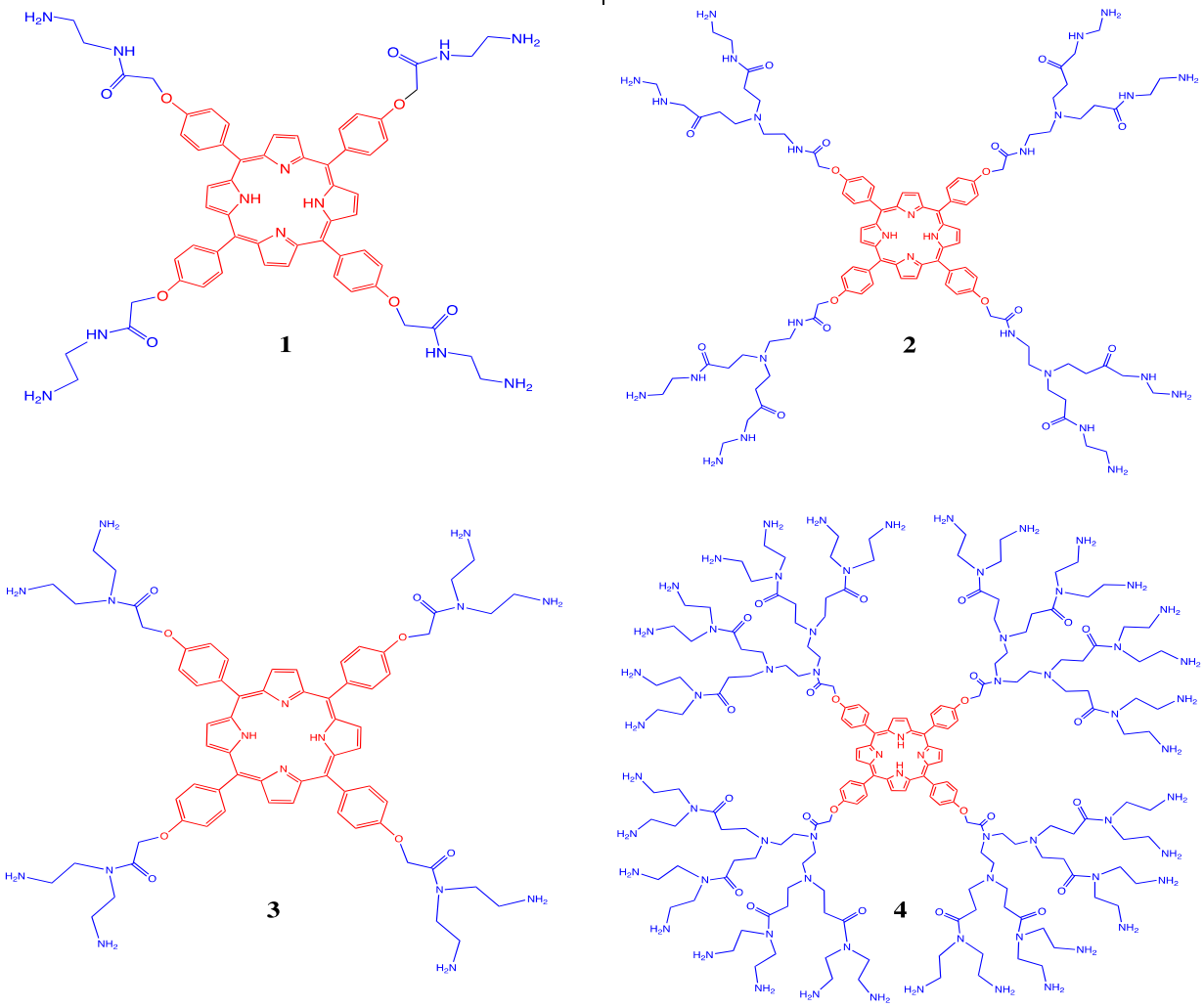

Chart 1. Porphyrin-polyamidoamine dendrimers **1**-**4**.

The structures of the latter products were confirmed based on their analytical and spectral data. Thus, the ^1H NMR spectra of the conjugate **5** and **6** is shown in Figure 1. In the spectra, the most important signals are: two signals at δ_H 0.74 corresponding to the isopropyl group of the ibuprofen, two signals at δ_H 1.37 corresponding to the methyl group, one multiplet at δ_H 1.68 due to the CH group, one doublet at δ_H 2.27 due the CH_2 group of ibuprofen, two broad signals at δ_H 2.93 and 3.31 for the CH_2-N groups, two doublets at δ_H 6.92 and 7.16 due to the phenyl at the ibuprofen with coupling constants of *J*= 7.8 and *J*= 7.5 Hz,

respectively, and three signals due the porphyrin and the pyrrolic protons of the macrocycle.

The full functionalization of the amine groups with ibuprofen also was confirmed for electrospray mass spectrometry and the compound **6** showed one ion molecular peak at 3325 m/z and for compound **8**, one peak at 9789 m/z (Figure 2).

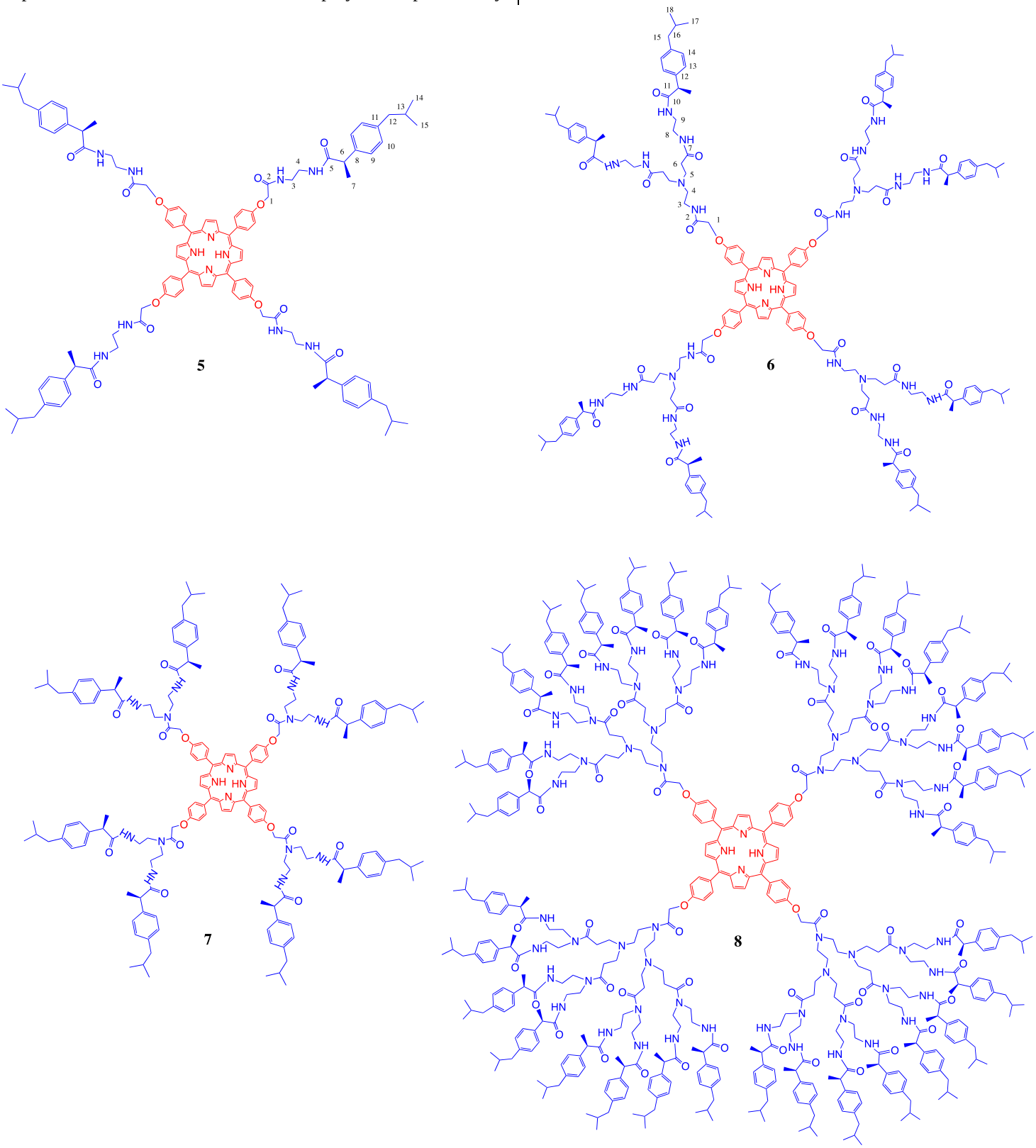

Chart 2. Porphyrin-ibuprofen conjugates **5-8**.

COX (ovine/human) Inhibitor. The inhibition of ovine COX-1 and human COX-2 were evaluated. Ibuprofen is a nonselective inhibitor of COX as was observed in our results (Table 1) (COX-1 IC50; 1.0 ± 0.07 μg/ml, COX-2; 13±5.3 μg/ml). Ibuprofen was **13** times more active against COX-1 than COX-2. Conjugate **8** was a potent inhibitor tested against COX-2 (IC50; 0.03 ± 0.01 μg/ml), COX-1 (0.12 ± 0.01 μg/ml) and also showed the greatest selectivity for COX-2 (ratio 0.25). Interestingly, in this study, we observed that the porphyrin-conjugate ibuprofen induced cancer cell death, irrespectively of its ability to block COX-2.

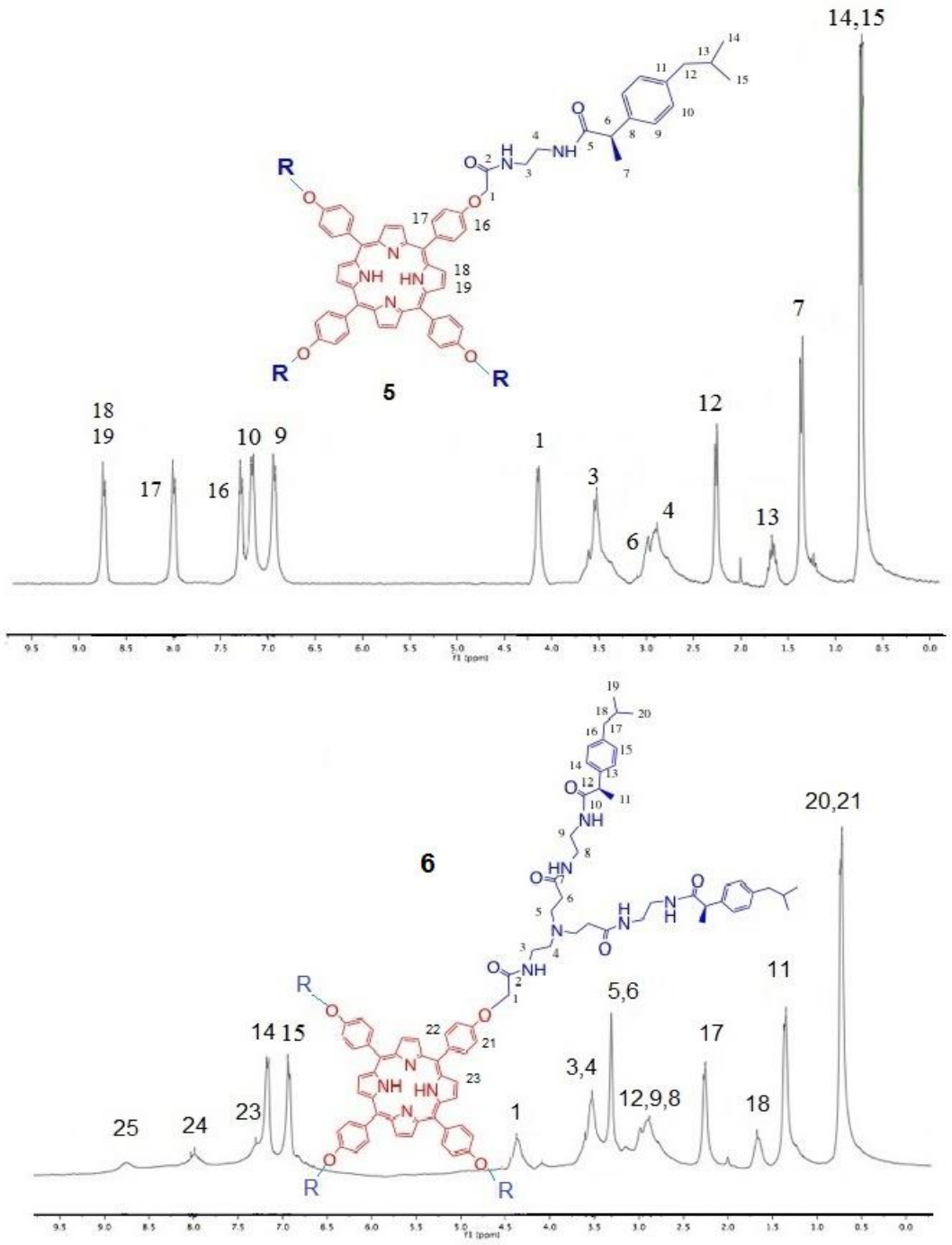

Figure 1. ^{1}H NMR spectrum of the conjugates 5 and 6.

Table 1. IC50 values for ibuprofen and conjugate **8** on COX-1 and COX-2 activity.

IC50 µg/ml			
Compound	COX-1	COX-2	COX-2/COX-1
Ibuprofen	1.0 ± 0.07	13 ± 5.3	13±5.5
Conjugate **8**	0.12 ± 0.01	0.03 ± 0.01	0.25±0.10

The data show the mean ± the SD calculated from triplicate determinations.

Cytotoxicity of ibuprofen conjugates. All compounds prepared in this work were tested for anticancer activity. The first obtained cytotoxic screening data at 25 µM of the porphyrin and all the intermediates including the dendrimers of first and second generation dendrimers **1-4** were tested against six different cancer cell lines: U251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), K-562 (human chronic myelogenous leukemia cells), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), SKLU-1 (human lung adenocarcinoma) and HGF human gingival fibroblasts. The cytotoxic screening data showed that at 25 µM, the porphyrin-dendrimers **1-4** produced inhibition (%) of the growth in the HGF cell line, indicating their low cytotoxicity compared to the reference drug cisplatin. Free ibuprofen showed no detectable cytotoxicity towards the HGF cell line. The intermediates did not show anti-cancer activity against U251, K-562, HCT-15 and SKLU-1 (see Table 2). The conjugates **5-8** at 25 µM were more active than the compounds **1-4** for the two lines PC-3 and MFC-7 and for the other lines the activity was not determinate.

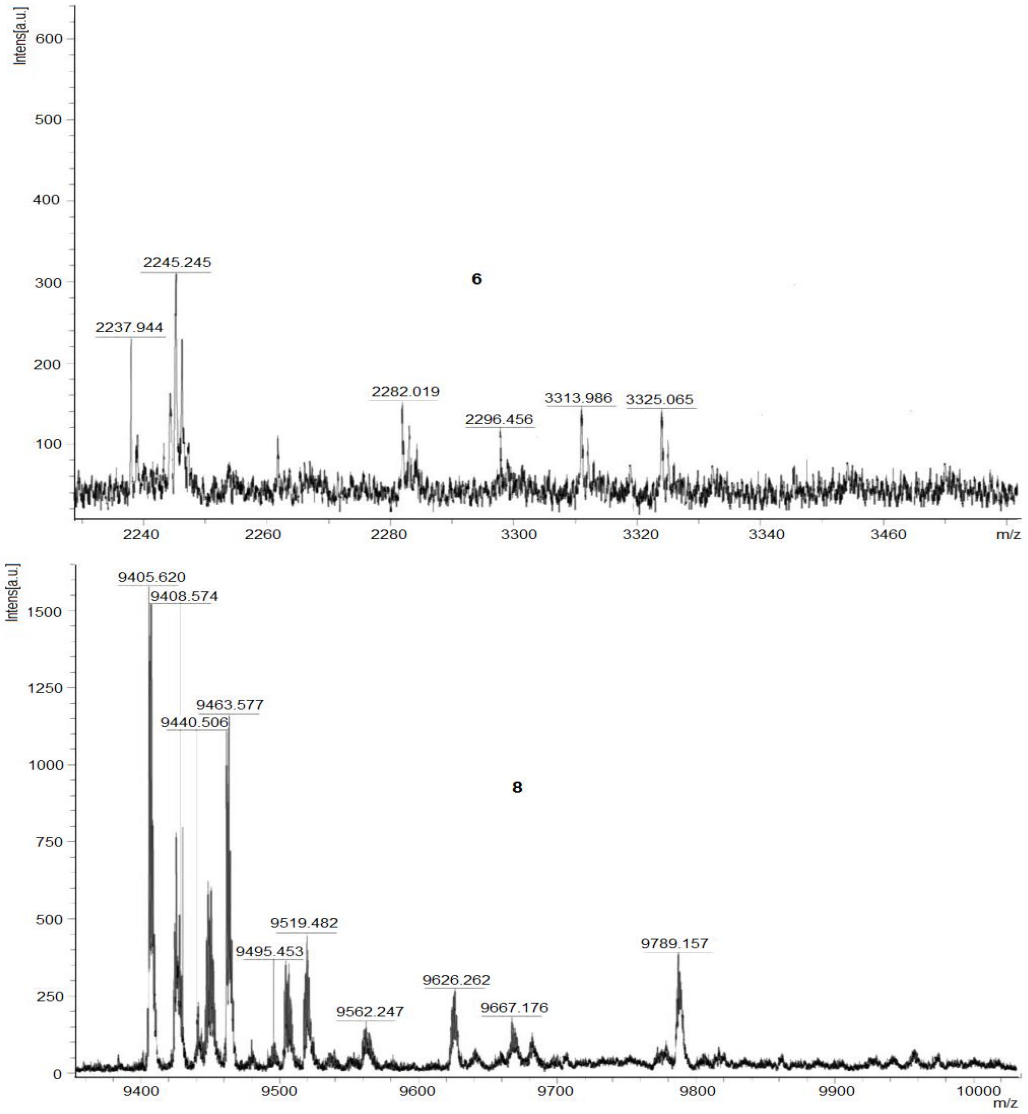

Figure 2. Electrospray mass spectrum of the conjugates 6 and 8.

Table 2. Inhibition on the growth (%) of human tumor cell lines compounds **1-8** at 25 μM in DMSO.

	% of inhibition						
Sample	**U251**	**PC-3**	**K562**	**HCT-15**	**MFC-7**	**SLKU-1**	**HGF**
1	Nc	23.12±0.3	11.76±0.1	9.23±0.1	6.43±0.4	Nc	Nc
2	Nc	24.09±0.3	Nc	Nc	5.00±0.5	Nc	Nc
3	Nc	24.09±0.2	Nc	Nc	27.00±0.4	Nc	Nc
4	Nc	28.03±0.2	Nc	Nc	8.43±0.3	Nc	Nc
5	ND	75.9±3.4	ND	ND	85.8±3.6	ND	ND
6	ND	92.5±7.1	ND	ND	94.5±4.2	ND	ND
7	ND	77.7±4.1	ND	ND	98.2±6.1	ND	ND
8	ND	95.9±3.1	ND	ND	88.7±2.1	ND	ND
Ibuprofen	14.91±2.3	12.36±1.7	Nc	4.73±1.4	3.53±3.1	Nc	Nc
Cisplatin	87.41±0.1	42.65±0.1	79.15±0.1	32.42±0.1	52.58±0.1	81.35±0.1	45.27±0.1

Nc= not cytotoxic; ND= non determinate

The antiproliferative experiments were carried out in order to gain insight into the anticancer properties that are displayed in vitro by the synthesized compounds. In the experiment shown in Figure 3, the PC-3 and MCF-7 cell lines were exposed during five days to five concentrations (5, 10, 15, 20 and 25 μM) of the **5-8** ibuprofen-conjugates. As seen in Figure 3, a marked dose-dependent inhibition of cell growth was consistently observed in the two cancer cell lines. The four conjugates **5-8** inhibited the growth of the PC-3 and MCF-7 cell lines.

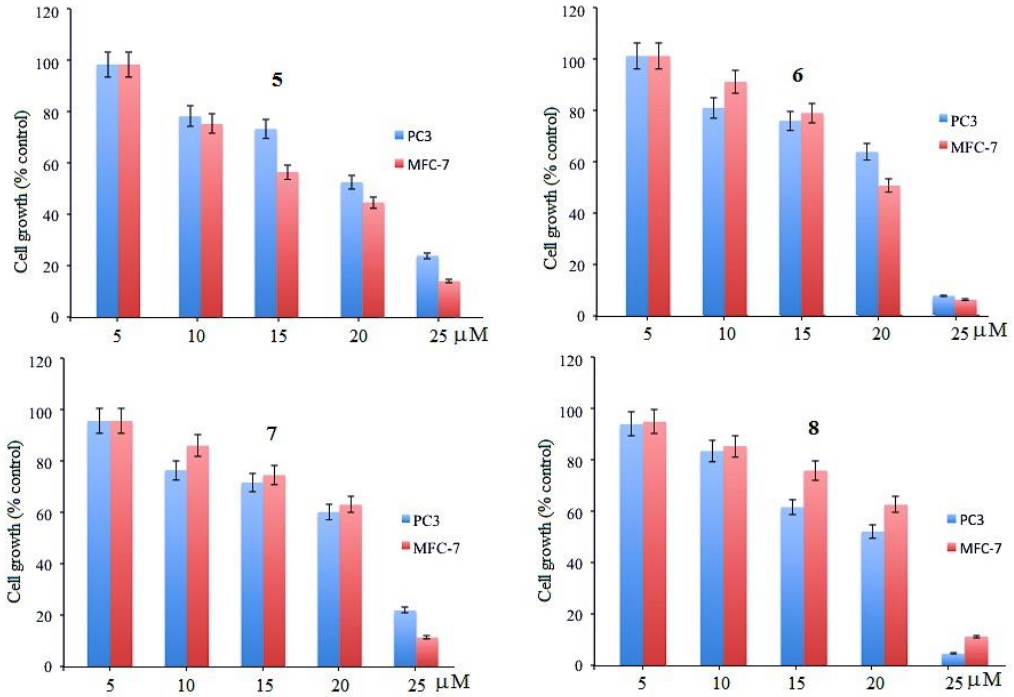

Figure 3. Dose–response curves obtained from PC-3, MCF-7 cells exposed to compounds **5**, **6**, **7** and **8**. Points represent mean values ± SD of seven independent experiments.

Compounds **6** and **7** were equally selective against both PC-3 and MCF-7 cell lines. Compound **8** was more active against the PC-3 cell line. The dose-response behavior of the conjugates **5**, **6**, **7** and **8** showed that the activity was dependent on the amount of ibuprofen in the dendrimer. For instance, the IC50 values obtained with conjugates **6** and **7**, both containing eight ibuprofen molecules, were very similar for both PC-3 and MCF-7 cell lines (14-18 μM); whereas the IC50 values obtained with compound **8** that contains thirty-two ibuprofen molecules were lower, ranging from 5-8 μM.

The most interesting result was obtained with compound **8**, which was found to be the best inhibitor of this series against PC-3 and MCF-7 cell lines. Moreover, compound **8** showed much higher selectivity against cancer cells and no activity against healthy cells. The compounds **6** and **7** contain eight ibuprofen moieties in the structure, but the length of the dendritic arms is different: the length of the dendritic arms of compound **6** is larger than that of compound **7**. This difference in the length of the dendritic arms did not have any effect on the antiproliferative growth of the cancer cells.

In the case of compound **8** with thirty-two ibuprofen moieties in the structure, the differences are larger. Compound **8** showed better results in the inhibition of cancer cell proliferation than compounds **5**, **6** and **7**. The results of these experiments indicate that the conjugate **8** with thirty-two ibuprofen moieties inhibited the growth of both, the PC-3 and MCF-7 cell lines tested. The rank order of potency was: **8** > **7** > **6** > **5** at the concentrations assayed.

Table 3. *In vitro* results of conjugates of ibuprofen against PC-3 and MCF-7 tumor cell lines and normal fibroblasts HGF expressed as IC50 in μM.

Cell line/(M ± S.D.)	5	6	7	8	Cisplatin
PC-3	19.9±1.5	15.9±1.4	14.4±0.3	5.4±0.2	17.5±1.4
MCF-7	14.2±1.3	17.9±1.7	15.5±0.5	8.3±0.6	17.4±0.8
HGF	NA	NA	NA	NA	12.2±0.9

IC50 values are presented as the mean ±SD (standard deviation) from five separate experiments, NA: no activity

Fluorescence microscopy. PC-3 cells were incubated during 6 hours with 10 μM of the ibuprofen-conjugate **8**. Cells were then examined under a fluorescence microscope (10X and 40X). Photographs of the observed cells are shown in Figure 4. Figure 4a shows the PC-3 control cells as seen under bright field illumination. The PC-3 cells that were untreated (control) did not show any fluorescence during excitation with UV light (Figure 4b). Figure 4c shows that the PC-3 cells treated with ibuprofen-

conjugate **8** resulted in cells becoming rounder and more detached. Figure 4d shows that after incubation of the PC-3 cells with the ibuprofen-conjugate **8**, the cells exhibited blue-fluorescence indicating the uptake of **8**. The difference in the number of cells per plate was due to the inhibitory effect of the conjugates on the proliferation rate. The PC-3 cells were incubated during 48 hours with 5 μM of ibuprofen-conjugate **8** and without ibuprofen-conjugate **8**. Subsequently, the cells were irradiated with UV light for 10 minutes and then observed under an optical microscope. Interestingly, after the cell line PC-3 was treated with the conjugate **8**, the cells also showed strong internal movements (see video recording in Supplementary Material). The damage to the cells occurs from within. The UV damage in PC-3 cells after incorporating **8**, likely results in both apoptosis and necrosis. In the control PC-3 cells that were also irradiated with UV light but not exposed to conjugate **8**, the widespread internal movements were not observed.

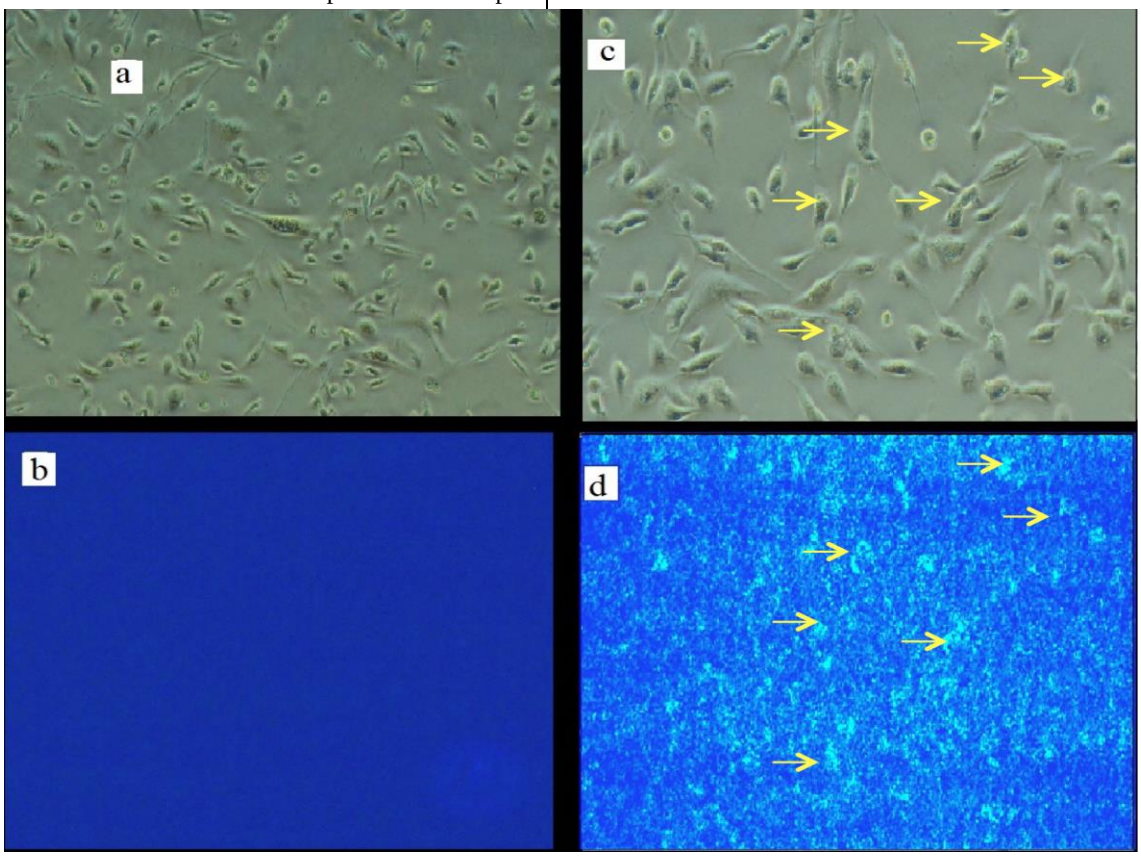

Figure 4. Microscopy photographs of prostate cancer cells. a) Control PC-3 cells without treatment seen under bright field (10X), b) PC-3 cells without treatment exposed to UV light (4X), c) PC-3 cells incubated with porphyrin-PAMAM-ibuprofen dendrimer **8** (40X) and d) PC-3 cells incubated with porphyrin-PAMAM-ibuprofen conjugate **8** exposed to UV light (4X amplification) (the arrows show the incidence with the fluorescence).

4. CONCLUSIONS

Four porphyrin-dendrimer-conjugates of ibuprofen **5**-**8** were synthesized in this work. Compound **5** had four ibuprofen-moieties in the structure. Compounds **6** and **7**, both contained eight ibuprofen-moieties, but the arm-length differed between the two. Compound **8** contained thirty-two ibuprofen-moieties in the structure. Good synthesis yields were obtained of the conjugates of ibuprofen with porphyrin-dendrimers held by polyamidoamine dendritic arms. The cellular internalization of porphyrin-PAMAM-ibuprofen dendrimers is occurring. The biological activity results obtained in this work indicate that the porphyrin-PAMAM-ibuprofen dendrimers have high potential activity against PC-3 and MCF-7 cancer cell lines. All the compounds synthesized in the present work had higher activity against cancer cells compared to free ibuprofen and were not toxic for healthy HGF cells. A synergism effect was observed due to the conjugation between the ibuprofen and the dendrimers. When the PC-3 cells were incubated with the ibuprofen-conjugate **8** and irradiated with UV light, the cells became larger and more round and strong internal movements inside the cells were observed. Porphyrin conjugates of ibuprofen are very attractive candidates to be further developed as drug therapies against cancer instead of the free compounds.

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