NJC

PAPER

View Article Online
View Journal

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.

Check for updates

Cite this: DOI: 10.1039/c8nj01713a

New bow-tie cationic carbosilane dendritic system with a curcumin core as an anti-breast cancer agent†

Tania Lozano-Cruz,[ab] Rafael Gómez,[abc] F. Javier de la Mata[abc] and Paula Ortega *[abc]

Received 9th April 2018,
Accepted 31st May 2018

DOI: 10.1039/c8nj01713a

rsc.li/njc

Though curcumin has been demonstrated as being highly cytotoxic towards various cancer cell lines, the major drawbacks for its use are the insolubility and instability in water, provoking low bioavailability. On the other hand, carbosilane dendritic systems with a cationic charge on the surface have been studied in different biomedical applications such as antibacterial, anticancer or as non-viral vehicles for the delivery of nucleic acids. In this study, we combine both systems by synthesizing a new "bow-tie" cationic carbosilane dendrimer where curcumin is located at the core. The resulting new system is completely water-soluble, maintains antioxidant properties and induces higher cytotoxicity against MCF-7 cancer cells compared with free curcumin. In addition, the effects on the cell cycle and apoptosis induction are evaluated by using flow cytometry analysis to obtain insights of the cytotoxic mechanism of action.

1. Introduction

Breast cancer is the most frequent malignancy among women worldwide and affects 1 in 8 women over the course of their lives.[1] The main treatments for breast cancer are surgery, radiotherapy, chemotherapy, hormone therapy and targeted therapy.[2,3] Due to the development of drug resistance, severe side effects, relapses and recurrences of cancer, there is a need to develop new anticancer drugs, which are less toxic and more comfortable to the patient with potent activity, tissue selectivity and novel modes of action.[4,5]

Curcumin is a hydrophobic natural polyphenol, known for decades, that could act to modulate numerous molecular targets and exerts antioxidant, anti-inflammatory, anticancer, and neuroprotective activities.[6–8] In the literature, several studies concerning the effect of curcumin against breast cancer showed excellent results, inducing cellular apoptosis for both *in vitro* and *in vivo* experiments.[9,10] However, curcumin has several limitations such as nonspecific biodistribution and targeting, lack of water solubility and poor oral bioavailability and adsorption. Although curcumin could be a potent anticancer agent with a remarkable safety profile, all these disadvantages are important parameters for rendering a compound unsuitable as a therapeutic agent. For that reason, it would be desirable to develop carrier platforms that could improve the efficacy and reduce such limitations by specific delivery of the therapeutic agent to the tumor sites as well as to increase its solubility and bioavailability.

Advances in nanotechnology and materials science have offered new nanostructured systems such as polymers,[11–13] microparticles,[14] microcapsules,[15] nanoparticles,[16] liposomes,[17] micelles[18] and dendrimers[19–23] that could allow a decrease of the dosing frequency, lower drug concentration in blood, major bioavailability and reduction of side effects.[24,25] Most nanoformulations of curcumin with some of these drug delivery systems have been tested successfully and are promising for reaching clinical application.[26–30] In this approach, dendrimers play an important role because they are highly branched macromolecules, ideally monodisperse, that provide many exciting opportunities for the design of novel drug-carriers. They hold promise in tissue targeting applications or controlled drug release and, moreover, their interesting nanoscopic architecture might allow easier passage across biological barriers.[31–33]

Our research interest is focused on carbosilane dendrimers, which are interesting systems not only because of their chemical and thermal stability, but also for their biocompatibility. We have studied their activity in different biomedical applications such as antiviral, antibacterial, anticancer agents, delivery of nucleic material in gene therapy or in neurodegenerative diseases.[34–40] There are only a few studies combining dendrimers and curcumin, where the dendrimers used are PAMAM or PPI[41–44] to encapsulate curcumin or its analogues. In both cases, the solubility and

[a] Departamento de Química Orgánica y Química Inorgánica. Instituto de Investigación Química "Andrés M. del Río" (IQAR), Universidad de Alcalá, Campus Universitario, E-28871 Alcalá de Henares, Spain. E-mail: paula.ortega@uah.es
[b] Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Spain
[c] Instituto Ramón y Cajal de Investigación Sanitaria. (IRYCIS). Ctra. Colmenar Viejo, km. 9,100 28034, Madrid, Spain

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c8nj01713a

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.

bioavailability were improved compared to free curcumin and showed higher anti-proliferative activity against different cell lines. Another approach is the covalent attachment of curcumin to dendritic systems. Only one example has been described by modification of curcumin phenol groups with second-generation polyester dendrons and its antioxidant and cytotoxic capacity were measured with respect to free curcumin.[45] However, to the best of our knowledge, no studies concerning carbosilane dendritic systems have been published so far.

Based on this background, the main goal of the work here presented is the functionalization of curcumin with cationic carbosilane dendrons by covalent attachment that permits combining the unique properties of both systems. The hydrophobic character of the carbosilane skeleton on the one hand would facilitate the passage of the system to the cell through the lipid bilayer and, on the other hand, the cationic terminal groups of the surface may promote its solubilization in aqueous medium, increasing its bioavailability. In addition, both curcumin and carbosilane dendritic systems containing alcohol or ammonium groups (*vide infra*) have been shown to have anticancer activity, and for this reason, a cooperative effect between them may be possible.[6,7,36]

2. Experimental section

2.1 Chemistry: general considerations

In general, the employed nomenclature is of the type $XG_n(SY)_m$ for dendrons and $(YS)_mG_nXG_n(SY)_m$ for bow-tie dendrimers. G_n stands for the dendritic generation, $(Y)_m$ stands for the peripheral function and its number, and S indicates the procedure used for functionalization of the periphery by hydrothiolation, Finally, X refers to the nature of the focal point in the dendrons or the core (curcumin) in the bow-tie structure.

All reactions were carried out under an inert atmosphere and solvents were purified from the appropriate drying agents when necessary. Compounds, 3-methoxy-4-(2-propyloxy)-benzaldehyde and azide-terminated carbosilane wedge $N_3G_2(SNMe_3I)_4$ (**II**) were prepared by previously reported methods[46,47] and the other reagents were obtained from commercial sources and used as received.

2.2 Methods

NMR spectra were recorded on a Varian Unity VXR-300 or on a Bruker AV400 (300.13 (^{1}H), 75.47 (^{13}C) MHz). Chemical shifts (δ) are given in ppm. ^{1}H and ^{13}C resonances were measured relative to internal deuterated solvent peaks considering TMS = 0 ppm. When necessary, assignment of resonances was done from HSQC, HMBC, and DOSY NMR experiments. Elemental analyses were performed on a LECO CHNS-932 instrument.

2.3 Synthesis of di-propargyl-curcumin (I)

A solution of 0.08 mL of acetylacetone (0.79 mmol) and 0.04 g of boron oxide (0.55 mmol) was added over another solution of 3-methoxy-4-(2-propyloxy)-benzaldehyde (0.30 g, 1.58 mmol) and tributylborate (0.85 mL, 3.16 mmol) after stirring each one for 30 min at 50 °C. The combined mixture was heated at 50 °C for another 15 min. Subsequently, *n*-butylamine in EtOAc (1 mL) was added dropwise for a period of 10 min at 50 °C and additionally stirred at 80 °C for 18 h. After cooling to 50 °C, preheated (50 °C) aqueous HCl solution (3 mL, 0.4 M) was added and then stirred for another 60 min at the same temperature. The mixture was extracted several times with EtOAc and the combined organic layers were washed acid-free, dried over magnesium sulfate, filtrated and concentrated under reduced pressure. The residue was recrystallized from methanol (−20 °C) to give the corresponding orange product (0.24 g, 69%). The ^{1}H and ^{13}C-NMR signals are consistent with the proposed structure and in accord with the literature.

2.4 Synthesis of $(IMe_3NS)_4G_2$[curcumin]$G_2(SNMe_3I)_4$ (1)

Over a solution of 0.03 g (0.06 mmol) of compound $(CH{\equiv}CCH_2O)$-curcumin$(OCH_2C{\equiv}CH)$[46] (**I**) in DMSO was added 2.2 equivalents (0.17 g, 0.12 mmol) of $N_3G_2(SNMe_3I)_4$ (**II**), 2.2 equivalents of NEt_3 (0002 mL, 0011 mmol) and 5.3 mg (0.003 mmol) of CuI. The resulting mixture was stirred at room temperature for 24 h. Then, the solvent was removed under vacuum and the solid obtained was dissolved in water and purified by dialysis for one week using a dialysis membrane with a molecular weight cut off of 2000 Da to give a brown solid corresponding to compound **1** (0.11 g, 61%). ^{1}H-NMR (DMSO-d_6): δ (ppm) −0.08 (s, 6H, SiCH_3), 0.08 (s, 12H, SiCH_3), 0.60 (m, 20H, $NCH_2CH_2CH_2CH_2Si$, $SiCH_2CH_2CH_2Si$), 0.86 (m, 16H, $SiCH_2CH_2S$), 1.27 (m, 12H, $NCH_2CH_2CH_2CH_2Si$, $SiCH_2CH_2CH_2Si$), 1.82 (m, 4H, $NCH_2CH_2CH_2CH_2Si$), 2.63 (m, 16H, $SiCH_2CH_2S$), 2.90 (m, 16H, SCH_2CH_2N), 3.08 (s, 72H, $N(CH_3)_3$), 3.53 (m, 16H, SCH_2CH_2N), 3.80 (m, 6H, OCH_3), 4,35 (m, 4H, $NCH_2CH_2CH_2CH_2Si$), 5,16 (m, 4H, OCH_2), 7.24–8.00 (m, 11H, C_6H_3, $CH{=}CH$, $COCH{=}COH$), 8.26 (m, 2H, $C{=}CH$–N). ^{29}Si-NMR (DMSO-d_6): δ (ppm) 1.6 ($Si_{int}CH_3$), 2.3 ($Si_{ext}CH_3$). Elemental analysis $C_{109}H_{218}I_8N_{14}O_6S_8Si_6$ (3261.26 g mol^{-1}) calcd (%): C, 40.14; H, 6.74; N, 6.01; S, 7.86. Found: C, 40.25; H, 6.75; N, 6.00; S, 7.84.

2.5 ABTS radical scavenging capacity (%)

The capacity to scavenge $ABTS^{\bullet+}$ (pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) was measured according to Wiriyaphan *et al.*[48] Briefly, an $ABTS^{\bullet+}$ stock solution was prepared by mixing a 7.4 mM solution of $ABTS^{\bullet+}$ and a 2.6 mM solution of potassium persulfate in water. This solution was kept in the dark for 16 h at room temperature. An $ABTS^{\bullet+}$ working solution in DMSO was prepared daily, just before measuring, by dilution of the $ABTS^{\bullet+}$ stock solution and adjustment of the absorbance at 0.70 ± 0.01 UA. Next, 1.66 μL of a solution of the sample (3 mM in DMSO) was mixed with 98.33 μL of the $ABTS^{\bullet+}$ working solution, incubated in the dark for 6 min and the absorbance corresponding to ABTS radicals was measured at 734 nm. The scavenging capacity of the samples was determined as per eqn (1):

$$\mathrm{ABTS}^{\bullet} + \text{radical scavenging capacity (\%)} = \frac{\mathrm{Abs_{blank}} - \mathrm{Abs_{sample}}}{\mathrm{Abs_{blank}}} \times 100 \quad (1)$$

This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2018

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.

where Abs_{sample} is the absorbance of 1.66 μL of sample with 98.33 μL of $ABTS^{\bullet+}$ working solution and Abs_{blank} is the absorbance of 1.66 μL of solvent (DMSO) with 98.33 μL of $ABTS^{\bullet+}$ working solution (without samples).

2.6 Cell culture and MTT assays for MCF-7 cells

The human breast adenocarcinoma cell line MCF-7 utilized in this assay was kindly donated by Unidad de Cultivos at the University of Alcalá. Cells were grown routinely in DMEM (Dulbecco's modified Eagle Medium) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/amphotericin B (all from Sigma-Aldrich) at 37 °C and 5% CO_2. The cells were seeded in 24-well plates (Nunclon Delta Surface, Thermo Fischer Scientific) as monolayers (20 000 cells per well) and grown for 48 h in complete medium (500 μL). Solutions of compounds were prepared by diluting a freshly prepared stock solution in DMSO of the corresponding compound in aqueous medium (DMEM). Afterward, the intermediate dilutions of the compounds were serially diluted to the appropriate concentration (ranging from 0 to 100 μM) and the cells that were incubated for another 24 h in DMSO at comparable concentrations did not show any effects on cell cytotoxicity. Cytotoxicity was determined using the MTT assay (MTT 3-(4,5dimethyl 2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). Subsequently, MTT (1 : 10 of 5.0 mg mL^{-1} solution) was added to the cells and the plates were incubated for a further 3.5 h. Then, the culture medium was removed and the purple formazan crystals formed by the mitochondrial dehydrogenase and reductase activity of live cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 570 nm (background correction at 690 nm) using a multiwell plate reader (ELX800 Bio-Teck instrument) and the fraction of surviving cells was calculated from the absorbance of the untreated control cells. The IC_{50} value was calculated as the concentration reducing the proliferation of the cells by 50% and is presented as a mean (±SE) from three independent experiments, each comprising three microcultures per concentration level.

2.7 Determination of intracellular ROS using the DCFH assay

The fluorogenic dye 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Invitrogen D-399) is deacetylated by cellular esterases to the 2′,7′-dichlorodihydrofluorescein (DCFH) anion, which is retained in the cell and later oxidized by ROS into the fluorescent 2′,7′-dichlorofluorescein (DCF) product. To detect intracellular ROS production, *tert*-butyl hydroperoxide (TBHP) and *N*-acetyl-cysteine (NAC) were used as the oxidizing and antioxidant control agents, respectively. MCF-7 cells were seeded in 6-well plates (2×10^5) and cultured at 37 °C in 5% CO_2 for 48 h. Solutions of compounds were prepared in DMSO, added to the cell to a final concentration of 2.75 and 0.55 mM and incubated for another 24 h. On the day of the experiment, the negative control sample was prepared by incubating the cells with NAC (final concentration: 375 mM) for 1 h. Afterwards, the cells were washed, resuspended in complete growth media, and treated with DCFH-DA (stock: 5 mM; staining solution: 8 mL + 10 μL of the stock solution) for 30 min/37 °C. After DCFH-DA was removed, the control and treated cells were exposed to TBHP (690 μM) for 10 min. Then, the fluorescence was measured in the presence of propidium iodide (for exclusion of dead cells) (final concentration: 10 mg mL^{-1}) using a FACS calibur Flow cytometer (Becton Dickinson, San Jose, CA) and analysed by Flow Jo software (Tree Star, Ashland, OR) Cyflogic v 1.2.1 program. Three different wells were analysed for each concentration of oxidative stress-inducing agent or antioxidant, and three replicates were performed for each experiment.

2.8 Cell cycle assays

MCF-7 cells (2×10^5) were grown in 6-well plates for 48 h, treated with the dendrimer and incubated for another 24 h. Then, the cells were washed with PBS and detached with trypsin. Cells were centrifuged at 1300 rpm for 5 min at 4 °C and the pellets were mixed with ice-cold 70% ethanol and then kept at −20 °C for 30 min. After removing the ethanol by centrifugation, the pellets were washed with PBS and centrifuged again. The supernatants were discarded and the pellets were suspended in PBS, 0.2 mg mL^{-1} RNase A and 20 μg mL^{-1} PI before flow cytometry analysis with the cytometer mentioned above. The results obtained were analyzed with the Cyflogic v 1.2.1 program.

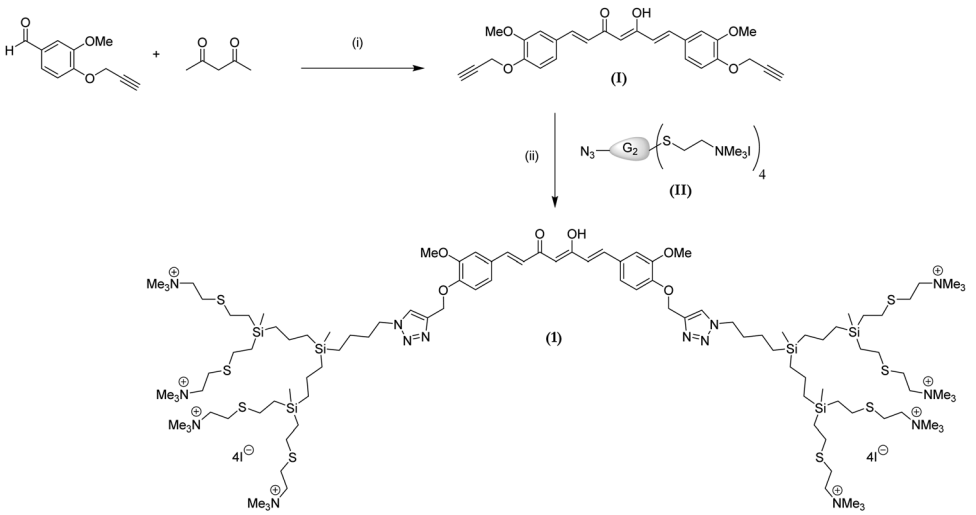
3. Results and discussion

3.1 Synthesis and characterization

In order to evaluate the possibility of introducing curcumin into the dendritic macromolecule by covalent linkage, from the synthetic point of view, the simplest possibility was to employ a ''bow-tie'' topology system where curcumin was used as a core through dendronization of the curcumin phenol groups. The coupling of the dendritic wedges to the curcumin molecule was performed through Huisgen cycloaddition (''click chemistry'' reaction)[49] of the azide-terminated carbosilane wedge (**II**) previously reported in our research group[47] with a curcumin derivative where the hydroxy substituents on two aromatic rings were replaced by the propargyl group (**I**). The propargylic derivative of curcumin (**I**) has been previously described,[50] but a different methodology was necessary for its use due to its low yield. The new synthesis was carried out according to a slightly modified version of Pabon's method,[51] as described in Scheme 1, starting from 3-methoxy-4-(2-propyloxy)-benzaldehyde[46] and acetylacetone.

The reaction was performed using anhydrous EtOAc as solvent by addition of a solution of acetylacetone and boron oxide over another solution of 3-methoxy-4-(2-propyloxy)-benzaldehyde and tributylborate, both solutions were pre-heated at 50 °C for 30 minutes. Then, the resulting mixture was maintained for another 15 minutes at the same temperature. Subsequently, a solution of *n*-butylamine in EtOAc was prepared and slowly added to the above mixture and kept at 80 °C for 18 h. Afterwards, the temperature was lowered to 50 °C, hot HCl (50 °C) was added and stirred for 60 minutes at this temperature to afford the corresponding system **I** as an orange solid in good yield. Representative signals in the ^{1}H-NMR spectrum for compound **I** are the disappearance of the singlet corresponding to the

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.



Scheme 1 Synthesis of $(IMe_3NS)_4G_2[curcumin]G_2(SNMe_3I)_4$ (**1**). Reaction conditions: (i) B_2O_3, $B(OBu)_3$, $^nBuNH_2$ in EtOAc: (ii) CuI, NEt_3 in DMSO.

aldehyde group of the vanillin analogue precursor and the appearance of new resonances at 7.56 and 6.83 ppm for the –CH– groups of the double bonds formed. Furthermore, the proton of the unsaturated bond characteristic of the enol tautomer, which confirms the existence of only the enolic species of the two possible tautomers of curcumin, was observed at 6.10 ppm. The ^{13}C-NMR data are in agreement with the ^{1}H-NMR spectrum, and the most significant signals are those corresponding to the carbon of the central methine group at 100.5 ppm, olefinic groups located at 121.9 and 139.6 ppm and the resonances for the propargyl fragment at 78.0 and 55.5 ppm.

The CuAAC reaction of propargyl curcumin with azide functionalized carbosilane dendron $N_3G_2(SNMe_3I)_4$ (**II**) leads to $(IMe_3NS)_4G_2[curcumin]G_2(SNMe_3I)_4$ (**1**), through a Huisgen cycloaddition using a Cu(I) catalyst in DMSO as solvent, as a water-soluble brown solid in moderate yields. The new compound was characterized by elemental analysis and NMR (see ESI† in Fig. S1 and S2). Also, the DOSY experiment showed only the presence of one compound in solution, indicating the connectivity between the fragments. These spectra show the disappearance of the alkyne units and the appearance of the triazole ring by a singlet at 8.26 ppm corresponding to the proton of the triazole (c) and two multiplets located at 4.35 (d) and 5.16 pm (b) corresponding to methylene groups adjacent to the triazole unit. The signals corresponding to the outer chain $S(CH_2)_2NMe_3I$ of the carbosilane wedge showed two multiplets at 2.90 and 3.53 ppm for the protons of the SCH_2 and the CH_2N groups, respectively, whereas a singlet at 3.08 ppm confirmed the presence of the trimethylammonium groups.

3.2 Determination of anticancer and antioxidant activity

Since curcumin has been demonstrated to possess multifaceted therapeutic and pharmacologic effects such as being non-toxic, highly antioxidant, and having anti-inflammatory and anti-cancer activity, there are several studies concerning its use as a therapeutic agent. Among them, we focused on its antitumor and antioxidant properties. The potential of **1** as an anticancer agent was investigated by measuring the *in vitro* cytotoxicity in terms of the half-maximal inhibitory concentration (IC_{50}) towards the breast adenocarcinoma cell line (MCF-7 cells). Several studies have also reported the cytotoxic effects of curcumin on almost all types of cancer,[6] however the specific mechanism of curcumin-induced cytotoxicity still remains unclear due to the different evidence that involves anti- and pro-apoptotic signaling pathways in different cell types.[52,53] In particular, recent studies have shown that curcumin, either alone or in combination with other anticancer agents, can efficiently induce an apoptotic mechanism in MCF-7 cells.[54,55] The cytotoxic activity of the new curcumin derivative **1** was assayed by monitoring its ability to inhibit cell growth using the MTT assay. The result for **1** shows a growth-inhibitory activity with an $IC_{50} = 2.75 \pm 0.11$ μM lower than that of free curcumin ($IC_{50} = 14.09 \pm 0.01$ μM) and the analogous dendron $N_3G_2(SNMe_3I)_4$ (**II**) (9.83 ± 0.53 μM), which surprisingly also shows anticancer activity. These data suggest either the existence of a cooperative effect between curcumin and the cationic dendrons or an increase of curcumin bioavailability as result of its solubilization in water. To investigate the cellular death mechanism induced by **1**, the cell cycle perturbation was analyzed by flow cytometry after treatment of MCF-7 cells for 24 h with different concentrations (0.08, 0.55 and 2.75 μM) of compound **1**. The results obtained in the cell cycle did not show any significant number of cells in the SubG0 phase (see Fig. 1), indicating a non-apoptotic pathway. Similar results have

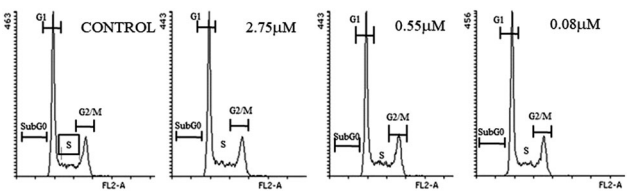


Fig. 1 Histograms of cell cycle of MCF-7 cells after treatment with the derivative $(IMe_3NS)_4G_2[curcumin]G_2(SNMe_3I)_4$ (**1**) at different concentrations (2.75, 0.55 and 0.08 μM).

 This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2018

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.

been observed when curcumin was dendronized with polyester dendrons of second generation where the mechanism of cellular death was not apoptotic in the C6 glioma cell line.[45] Analogously, other synthetic derivatives of curcumin like hydrazinobenzoylcurcumin induced A549 cell autophagy.[56] Interestingly, the dendron $N_3G_2(SNMe_3I)_4$ (**II**) also did not afford any apoptotic pathway (see Fig. S4 in ESI†). This fact indicates that **1** drives cellular death *via* a non-apoptotic mechanism acting by a different mechanism than free curcumin and suggesting that the presence of dendritic carbosilane wedges could be implicated in the mechanism of death.

In relation to the antioxidant activity, although the exact mechanism by which curcumin is able to trap radicals remains under investigation, many theoretical and experimental studies have agreed on the importance of the phenolic group. However, this seems to depend on the type of ROS radical or species studied and not exclusively on the nature of the substituents located on the aromatic rings.[57] The antioxidant activity of the dendritic wedge **II**, free curcumin and compound **1** containing cationic carbosilane dendrons was evaluated by the ABTS radical scavenging test. For compound **1**, the percentage of inhibition obtained was 94.8%, practically the same percentage shown by free curcumin (96.1%) under the same conditions (see Table 1).

It is worth stating that the free dendron $N_3G_2(SNMe_3I)_4$ (**II**) also showed an antioxidant behavior with an inhibition value of 95.8%, probably as a consequence of the presence of iodide counter-anions, which are known to have an important role as an antioxidant.[58] Although probably the antioxidant capacity of **1** comes from the existence of ammonium terminated dendrons containing iodide as counter-anion, it cannot be ruled out that such radical scavenging activity can be also produced by the curcumin moiety or a combination of both. Recently, the dendronization of curcumin by ester dendrons making the two phenol groups unavailable enables antioxidant properties, suggesting a different redox mechanism compared to the free curcumin.[45]

In addition, the intracellular ROS protection efficiency and free radical-scavenging activity of curcumin derivative **1** were also determined from an *in vitro* experiment on the MCF-7 cell line by flow cytometry using the oxidant-sensing fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). It is possible to determine the levels of endogenous ROS formation when the oxidative stress is induced by *tert*-butyl hydroperoxide (TBHP). To evaluate the antioxidant activity, it is necessary to perform a pre-treatment of the MCF-7 cells with **1**, **II** and free curcumin in order to ensure the internalization of the compounds inside the cells. The concentrations used are below the IC_{50} of **1** (IC_{50} = 2.75 ± 0.11 μM) and acetylcysteine (NAC) was used as a positive control for the antioxidant activity. As expected, compound **1** showed a free radical-scavenging activity, provoking a significant reduction of ROS levels, being more active than free curcumin and dendritic wedge **II** independently (Fig. 2 and Fig. S4 in ESI†). Again, the existence of a possible cooperative effect between curcumin and the cationic dendrons is noteworthy.

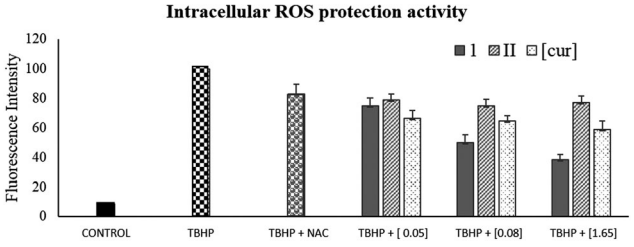


Fig. 2 DCFH-DA fluorescence assay used to measure intracellular ROS by flow cytometry in MCF-7 cells. All samples were first incubated (24 h) with different concentrations of free curcumin, **1** and **II** (0.05, 0.08 and 1.65 μM) prior to the addition of the ROS stimulating agent (TBHP). NAC is an antioxidant positive control.

Table 1 Antioxidant[1] and anticancer[2] activities of free curcumin, dendronized curcumin **1** and dendron (**II**) derivatives

Compound	Radical scavenging (%)[1] ABTS	IC_{50} μM ± SD MCF-7[2]
1	94.8	2.75 ± 0.11
Curcumin [CUR]	96.1	14.01 ± 0.01
Dendron II	95.8	9.83 ± 0.53

4. Conclusions

To summarize, this work reports on the synthesis and characterization of a new bow-tie cationic carbosilane system with curcumin at the core, $(IMe_3NS)_4G_2$[curcumin]$G_2(SNMe_3I)_4$ (**1**). The new derivative is soluble in water, increasing the bioavailability of curcumin in physiological medium.

In vitro cytotoxicity of **1** causes a potent inhibitory activity against breast cancer cells that is higher than those of the free curcumin and the dendritic wedge **II** alone. The study of the cell cycle of MCF-7 cells reveals non-apoptotic pathways for the cell death mechanism both for **1** and dendron **II**, different from the apoptotic one of the free curcumin. Probably, the presence of the dendritic wedges in the curcumin molecule modifies the mode of action. In addition, derivative **1** presents an efficient antioxidant property that is more pronounced than those of curcumin and dendron **II** separately. These two facts strongly indicate a cooperative effect between both fragments where the elimination of the phenolic groups of curcumin does not prevent its antioxidant capacity. Therefore, the presence of dendritic wedges in the curcumin structure plays an important role in the anticancer and antioxidant properties. A possible relationship between antioxidant and anticancer activities of compound **1** can be inferred since one of the causes of death by apoptosis is the excess of ROS. The reduction or elimination of free radicals by **1** may help to modify the mechanism of action toward a non-apoptotic one. Hence, compound **1** can be considered as a new and promising anti-breast cancer agent. Future studies are in progress to deeply understand the cell death mechanism induced by this compound.

Conflicts of interest

There are no conflicts of interest to declare.

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.

Acknowledgements

This work has been supported by grants CTQ2017-86224-P (from MINECO), Consortium NANODENDMED-II-CM (B2017/BMD-3703) and IMMUNOTHERCAN-CM (B2017/BMD3733). CIBER-BBN as an initiative funded by the VI National R&D&i Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

References

1 C. Printz, *Cancer*, 2017, **123**, 1085.
2 K. C. Aalders, K. Tryfonidis, E. Senkus and F. Cardoso, *Cancer Treat. Rev.*, 2017, **53**, 98.
3 K. Athreya and S. Ali, *Transl. Cancer Res.*, 2017, **6**, 30.
4 V. L. Maruthanila, R. Elancheran, A. B. Kunnumakkara, S. Kabilan and J. Kotoky, *Breast Cancer*, 2017, **24**, 191.
5 S. Nagini, *Anticancer Agents Med. Chem.*, 2017, **17**, 152.
6 A. Allegra, V. Innao, S. Russo, D. Gerace, A. Alonci and C. Musolino, *Cancer Invest.*, 2017, **35**, 1.
7 D. Lelli, C. Pedone and A. Sahebkar, *Biomed. Pharmacother.*, 2017, **88**, 832.
8 S. Samarghandian, M. Azimi-Nezhad, T. Farkhondeh and F. Samini, *Biomed. Pharmacother.*, 2017, **87**, 223.
9 Z.-D. Lv, X.-P. Liu, W.-J. Zhao, Q. Dong, F.-N. Li, H.-B. Wang and B. Kong, *Int. J. Clin. Exp. Pathol.*, 2014, **7**, 2818.
10 Y. W. Wang, J. Y. Yu, R. Cui, J. J. Lin and X. T. Ding, *JALA*, 2016, **21**, 723.
11 X. Wei, Q. Luo, L. Sun, X. Li, H. Zhu, P. Guan, M. Wu, K. Luo and Q. Gong, *ACS Appl. Mater. Interfaces*, 2016, **8**, 11765.
12 Z. Duan, Y. Zhang, H. Zhu, L. Sun, H. Cai, B. Li, Q. Gong, Z. Gu and K. Luo, *ACS Appl. Mater. Interfaces*, 2017, **9**, 3474.
13 H. Cai, X. Wang, H. Zhang, L. Sun, D. Pan, Q. Gong, Z. Gu and K. Luo, *Applied Materials Today*, 2018, **11**, 207.
14 S. Sounik and D. A. Kr, *Pharmacol. Res. Perspect.*, 2015, **3**, e00188.
15 L. Song, S. He and Q. Ping, *Exp. Ther. Med.*, 2017, **13**, 2435.
16 P. K. Prathipati, S. Mandal, G. Pon, R. Vivekanandan and C. J. Destache, *Pharm. Res.*, 2017, **34**, 2749.
17 L. Sercombe, T. Veerati, F. Moheimani, S. Y. Wu, A. K. Sood and S. Hua, *Front. Pharmacol.*, 2015, **6**, 286.
18 Y. Zhang, Y. Huang and S. Li, *AAPS PharmSciTech*, 2014, **15**, 862.
19 C. Zhang, D. Pan, J. Li, J. Hu, A. Bains, N. Guys, H. Zhu, X. Li, K. Luo, Q. Gong and Z. Gu, *Acta Biomater.*, 2017, **55**, 153.
20 W. She, D. Pan, K. Luo, B. He, G. Cheng, C. Zhang and Z. Gu, *J. Biomed. Nanotechnol.*, 2015, **11**, 964.
21 N. Li, C. Guo, Z. Duan, L. Yu, K. Luo, J. Lu and Z. Gu, *J. Mater. Chem. B*, 2016, **4**, 3760.
22 N. Li, H. Cai, L. Jiang, J. Hu, A. Bains, J. Hu, Q. Gong, K. Luo and Z. Gu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 6865.
23 D. Pan, W. She, C. Guo, K. Luo, Q. Yi and Z. Gu, *Biomaterials*, 2014, **35**, 10080.
24 E. I. Medina-Reyes, D. Garcia-Viacobo, F. A. Carrero-Martinez and Y. I. Chirino, *Crit. Rev. Ther. Drug Carrier Syst.*, 2017, **34**, 35.
25 N. Asadi, S. Davaran, Y. Panahi, A. Hasanzadeh, J. Malakootikhah, H. F. Moafi and A. Akbarzadeh, *Artif. Cells, Nanomed., Biotechnol.*, 2017, **45**, 18.
26 R. I. Mahran, M. M. Hagras, D. Sun and D. E. Brenner, *AAPS J.*, 2017, **19**, 54.
27 D. H. Yang, H. J. Kim, J. K. Kim, H. J. Chun and K. Park, *J. Ind. Eng. Chem.*, 2017, **45**, 156.
28 E. Marin, M. I. Briceno, A. Torres and C. Caballero-George, *Planta Med.*, 2017, **83**, 877.
29 Z. Hussain, H. E. Thu, S.-F. Ng, S. Khan and H. Katas, *Colloids Surf., B*, 2017, **150**, 223.
30 M. Z. Lin, L. L. Teng, Y. Wang, J. X. Zhang and X. L. Sun, *Drug Delivery*, 2016, **23**, 1420.
31 J. Singh, K. Jain, N. K. Mehra and N. K. Jain, *Artif. Cells, Nanomed., Biotechnol.*, 2016, **44**, 1626.
32 M. Selin, L. Peltonen, J. Hirvonen and L. M. Bimbo, *J. Drug Delivery Sci. Technol.*, 2016, **34**, 10.
33 L.-p. Wu, M. Ficker, J. B. Christensen, P. N. Trohopoulos and S. M. Moghimi, *Bioconjugate Chem.*, 2015, **26**, 1198.
34 D. Sepulveda-Crespo, J. Luis Jimenez, R. Gomez, F. Javier De La Mata, P. L. Majano, M. Angeles Munoz-Fernandez and P. Gastaminza, *Nanomed. Nanotechnol.*, 2017, **13**, 49.
35 E. Vacas-Cordoba, M. Maly, F. J. De la Mata, R. Gomez, M. Pion and M. Angeles Munoz-Fernandez, *Int. J. Nanomed.*, 2016, **11**, 1281.
36 G. Mencia, N. Sanz del Olmo, L. Munoz-Moreno, M. Maroto-Diaz, R. Gomez, P. Ortega, M. Jose Carmena and F. Javier de la Mata, *New J. Chem.*, 2016, **40**, 10488.
37 I. Heredero-Bermejo, J. Sanchez-Nieves, J. Soliveri, R. Gomez, F. J. de la Mata, J. L. Copa-Patino and J. Perez-Serrano, *Int. J. Pharm.*, 2016, **509**, 1.
38 I. Bravo-Osuna, M. Vicario-de-la-Torre, V. Andres-Guerrero, J. Sanchez-Nieves, M. Guzman-Navarro, F. J. de la Mata, R. Gomez, B. de las Heras, P. Argueso, G. Ponchel, R. Herrero-Vanrell and I. T. Molina-Martinez, *Mol. Pharmaceutics*, 2016, **13**, 2966.
39 K. Milowska, A. Szwed, M. Mutrynowska, R. Gomez-Ramirez, F. Javier de la Mata, T. Gabryelak and M. Bryszewska, *Int. J. Pharm.*, 2015, **484**, 268.
40 D. Marson, E. Laurini, P. Posocco, M. Fermeglia and S. Pricl, *Nanoscale*, 2015, **7**, 3876.
41 L. Wang, X. Xu, Y. Zhang, Y. Zhang, Y. Zhu, J. Shi, Y. Sun and Q. Huang, *J. Mater. Sci.: Mater. Med.*, 2013, **24**, 2137.
42 M. Mollazade, K. Nejati-Koshki, A. Akbarzadeh, N. Zarghami, M. Nasiri, R. Jahanban-Esfahlan and A. Alibakhshi, *Asian Pac. J. Cancer Prev.*, 2013, **14**, 6925.
43 P. Kesharwani, L. Xie, S. Banerjee, G. Mao, S. Padhye, F. H. Sarkar and A. K. Iyer, *Colloids Surf., B*, 2015, **136**, 413.
44 D. Shawon, S. Darin, D. Sukanta, S. Chong, A. Saadyah, R. Krishnaswami and E. F. Jimmie, *Anticancer Agents Med. Chem.*, 2013, **13**, 1531.
45 J. M. Landeros, F. Belmont-Bernal, A. T. Pérez-González, M. I. Pérez-Padrón, P. Guevara-Salazar, I. G. González-Herrera and P. Guadarrama, *Mater. Sci. Eng., C*, 2017, **71**, 351.
46 R. Pingaew, P. Mandi, C. Nantasenamat, S. Prachayasittikul, S. Ruchirawat and V. Prachayasittikul, *Eur. J. Med. Chem.*, 2014, **81**, 192.

 This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2018

Published on 18 June 2018. Downloaded by University of California - Santa Barbara on 6/18/2018 12:49:48 PM.

47 E. Fuentes-Paniagua, C. E. Pena-Gonzalez, M. Galan, R. Gomez, F. J. de la Mata and J. Sanchez-Nieves, *Organometallics*, 2013, **32**, 1789.
48 C. Wiriyaphan, B. Chitsomboon and J. Yongsawadigul, *Food Chem.*, 2012, **132**, 104.
49 K. Rajavelu, P. Rajakumar, M. Sudip and R. Kothandaraman, *New J. Chem.*, 2016, 40.
50 D. d. C. F. Gomes, L. V. Alegrio, M. E. F. d. Lim, L. L. Leon and C. A. C. Araújo, *Arzneimittelforschung*, 2002, **52**, 120.
51 H. Y. Y. Pabon, *Recl. Trav. Chim. Pays-Bas*, 1964, **83**, 379.
52 H.-O. Pae, S.-O. Jeong, G.-S. Jeong, K. M. Kim, H. S. Kim, S.-A. Kim, Y.-C. Kim, S.-D. Kang, B.-N. Kim and H.-T. Chung, *Biochem. Biophys. Res. Commun.*, 2007, **353**, 1040.
53 S. Reuter, S. Eifes, M. Dicato, B. B. Aggarwal and M. Diederich, *Biochem. Pharmacol.*, 2008, **76**, 1340.
54 Y. Akkoc, O. Berrak, E. D. Arisan, P. Obakan, A. Coker-Gurkan and N. Palavan-Unsal, *Biomed. Pharmacother.*, 2015, **71**, 161.
55 K. Mohankumar, S. Pajaniradje, S. Sridharan, V. K. Singh, L. Ronsard, A. C. Banerjea, C. S. Benson, M. S. Coumar and R. Rajagopalan, *Chem.-Biol. Interact.*, 2014, **210**, 51.
56 G.-Z. Zhou, S.-N. Zhang, L. Zhang, G.-C. Sun and X.-B. Chen, *Pharm. Biol.*, 2014, **52**, 111.
57 U. Singh, A. Barik, B. G. Singh and K. I. Priyadarsini, *Free Radical Res.*, 2011, **45**, 317.
58 R. Winkler, *Nat. Sci.*, 2015, **07**, 10.