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Synthesis and antiproliferative activity of novel steroidal dendrimer conjugates

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ABSTRACT

We describe the synthesis of steroidal dendrimer conjugates of first and second generation with tetramethylene core and 5-hydroxy-isophtalic acid dimethyl ester as branching unit modified to incorporate ethynylestradiol or 17α-estradiol as terminal units. The steroidal dendrimer conjugates, the free drug (steroids) and dendrimer were tested against a panel of cancer cell lines (CEM, MCF7, HeLa) and normal human fibroblast (BJ). The steroidal dendrimer conjugates of first generation exhibited cytotoxic activity and induced apoptosis in chronic leukemia (CEM) as resultant activation of caspase cascade which is mainly provoked in G2/M arrested cells.

1. Introduction

More than 11 million people are diagnosed with cancer each year and the incidence of this disease is projected to rise continuously to 16 million by 2020 [1]. For this reason, the discovery of new active drugs and the development of delivery devices capable of improving the therapeutic index of biologically active molecules and decreasing unwanted side effects [2] are of crucial importance.

Dendrimers are synthetic macromolecules possessing well defined branching architectures in nanometric size that can be easily tailored to endow specific properties [3–7]. They have attracted great attention due to their potential in the delivery of anticancer drugs because their high multivalency enhances cellular interactions and promotes a faster endocytosis [8–11]. As drug delivery systems, three strategies [12–14] have been employed: (i) formation of dendrimer networks around the drug; (ii) drug encapsulation inside the dendrimer mediated by electrostatic and van der Walls interactions; (iii) drug "conjugation" by covalent attachment or electrostatic binding at the periphery. However, several reports suggest [13,14] that, when a drug is encapsulated or electrostatically attached to a dendrimer, it may be released prematurely in the body as a result of a small pH change. Consequently, the observed therapeutic effect is lower than one would expect. On the contrary, the covalent conjugation, with or without the assistance of target moieties, improves the selective drug accumulation, increases the circulation time in the body, favoring a sustained liberation [2,12,14] and inducing apoptosis [15–17].

Synthetic estrogens, such as 17α-ethynylestradiol and 17α-estradiol have been used to prevent or reduce menopause symptoms, as oral contraceptives, in the treatment of alopecia, and in neurodegenerative disorders such as Alzheimer and Parkinson diseases [18]. Interestingly steroidal compounds have also shown antiproliferative activity and the ability to induce apoptosis [19]. In particular, the cytotoxicity of 17α-estradiol toward human leukemia Jurkat T cells has been attributed to apoptosis, mainly induced in G2/M-arrested cells [20], while 17α-ethynylestradiol has been evaluated with promising results as inhibitor of human prostate [21] and colon cancer [22]. The combination of dendritic compounds with steroidal derivatives has given rise to new architectures with a wide-range of biological effects when used as drug delivery systems [23–26], including the treatment of malaria [27] and in vitro in the lung inflammatory process [28].

With the aim to exploit the high drug payload of dendrimers without loss of monodispersity and well defined structure, to explore the possibility to enhance the cytotoxic activity of 17α-ethynylestradiol and 17α-estradiol, and bearing in mind the advantages of covalent conjugation in drug delivery, four new steroidal dendrimer conjugates were synthetized in this work. The dendrimers were prepared using a flexible tetramethylene core, and 5-hydroxy-isophtalic acid dimethyl ester as branching unit modified to incorporate 4 units of 17α-ethynylestradiol or

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17α-estradiol for first generation (**8** and **9**) and 8 units for the second (**10** and **11**). Their preliminary biological assays show that all new dendrimers and dendrimeric conjugates of G1 are noncytotoxic against normal fibroblastic cells (BJ), but the conjugates of first generation are able to induce apoptosis in leukemia cancer cells (CEM) displaying a higher cytotoxic activity than the free drug.

2. Experimental

2.1. General

The ^{1}H and ^{13}C NMR spectra were recorded on JEOL 400 and 500, and Bruker 300 using $CDCl_3$, DMSO-d_6 as solvent. Chemical shifts are reported in parts per million (ppm) relative to internal TMS. Mass spectra were recorded with an Agilent Technologies MS TOF using the ESI(+) technique. IR spectra were recorded using a Perkin–Elmer Spectrum GX FTIR spectrometer.

All reagents were commercially available. THF was refluxed over sodium/benzophenone and distilled under reduced pressure in a nitrogen atmosphere prior to use. Column chromatography was carried out with silica gel (70–230 mesh). 5-Hydroxy-isophthalic acid dimethyl ester (**1**) was synthesized following the literature [29]. Compounds **2** and **4** have been reported in the literature, however, using the method described herein the yields were optimized [30,31]. Unambiguous NMR spectral assignment was attained using one and two dimensional spectra.

2.2. Synthesis of compounds

2.2.1. 1,4-Bis(3,5-bis(carboxymethyl)phenoxy)butane (**2**)

5-Hydroxy-isophthalic acid dimethyl ester (**1**) (4.29 g, 20.41 mmol) and potassium carbonate (8.29 g, 59.98 mmol) were stirred in acetonitrile, followed by slow addition of 1,4-dibromobutane (1.1 mL, 9.26 mmol) and 18-crown-6 (1 mg). The mixture was heated at 60 °C during 48 h, filtered and rinsed with CH_2Cl_2. The solvent was removed under reduced pressure to obtain 4.30 g (9.06 mmol, 98%) of compound **2** as beige solid. M.p. 152–153 °C, in agreement with the literature [24].

^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.24 (2H, t, J = 1.5 Hz, H-6), 7.71 (4H, d, J = 1.5 Hz, H-4), 4.11 (4H, m, H-2), 3.91 (12H, s, H-8), 2.01 (4H, m, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm): 166.2 (C-7), 159.0 (C-3), 131.8 (C-5), 123.0 (C-6), 119.8 (C-4), 68.0 (C-2), 52.4 (C-8), 25.8 (C-1). IR (ν_{max}/cm^{-1}): 2951, 2852, 2360, 1725 (O–C=O), 1596, 1453, 1433, 1337, 1312, 1238 (C–O), 1111 (C-O), 1050, 1018, 1000, 902, 875, 753, 671, 628. HR-ESI-TOF-MS (m/z): calcd for $C_{24}H_{26}O_{10}$[M+H]$^+$: 475.1604; found: 475.1583.

2.2.2. 1,4-Bis(3,5-bis(hydroxymethyl)phenoxy)butane (**3**)

A solution of tetraester **2** (1.52 g, 40.00 mmol) in dry THF was added dropwise to a magnetically stirred suspension of $LiAlH_4$ (3.00 g, 6.32 mmol) in dry THF under N_2. The reaction was stirred 24 h at room temperature, and quenched by the slow addition of a solution of ammonium chloride (10 mL) and ethyl acetate (40 mL) with cooling. The aluminum salts were filtered and the solvents were then removed under reduced pressure to give 1.52 g (4.19 mmol, 56%) of compound **3** as a white solid. M.p. 147–149 °C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ(ppm): 6.82 (2H, s, H-6), 6.73 (4H, s, H-4), 4.42 (8H, s, H-7), 3.99 (4H, brs, H-2), 3.35 (OH), 1.84 (4H, brs, H-1).^{13}C NMR (DMSO-d_6, 75 MHz) δ(ppm): 159.0 (C-3), 144.3 (C-5), 116.9 (C-6), 111.0 (C-4), 67.4 (C-2), 63.2 (C-7), 25.9 (C-1). IR (ν_{max}/cm^{-1}): 3291, 3170, 2943, 2873, 2853, 1595, 1449, 1297, 1265, 1168, 1064, 1027, 979, 918, 841, 701, 677, 607. HR-ESI-MS (m/z) calcd for $C_{20}H_{26}O_6$[M+Na]$^+$: 385.1627; found: 385.1609.

2.2.3. 1,4-Bis(3,5-bis(bromomethyl)phenoxy)butane (**4**)

Compound **3** (1.28 g, 3.53 mmol) was dissolved in a 2:1 solution of HBr: H_2SO_4 (100 mL), The mixture was heated at 100–110 °C for 1 h, allowed to cool and diluted with H_2O (100 mL). The aqueous solution was extracted with CH_2Cl_2 (3 × 150 mL) and the combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (100 mL), dried ($MgSO_4$), filtered and concentrated to yield a brown solid. The crude product was purified by column chromatography using hexane to obtain 1.84 g (3.01 mmol, 84%) of **4** as a white crystalline solid. M.p. 126–128 °C.

^{1}H NMR ($CDCl_3$, 300 MHz) δ(ppm): 6.99 (2H, brs, H-6), 6.85 (4H, brs,H-4), 4.42 (8H, s, H-7), 4.04 (4H, m, H-2), 1.98 (4H, m, H-1).^{13}C NMR ($CDCl_3$, 75 MHz) δ(ppm): 159.3 (C-3), 139.6 (C-5), 121.8 (C-6), 115.2 (C-4), 67.5 (C-2), 32.9 (C-7), 25.8 (C-1). IR (ν_{max}/cm^{-1}): 2928, 2871, 1593, 1452, 1388, 1328, 1296 (C-O), 1166 (C–O–C), 1055, 1019, 940, 870, 848, 732, 697, 671, 648 y 633 (C–Br).

2.2.4. 1,4-Bis(3,5-bis(3,5-bis(carboxymethyl)phenoxymethyl) phenoxy) butane (**5**)

5-Hydroxy-isophthalic acid dimethyl ester (**1**) (0.13 g, 0.62 mmol) and potassium carbonate (0.30 g, 2.17 mmol) were stirred in acetonitrile, followed by slow addition of 1,4-bis(3,5-bis(bromomethyl)phenoxy)butane (0.10 g, 0.16 mmol) and tetrabutylammonium fluoride (1 mg) of. The mixture was refluxed 72 h, filtered and rinsed with CH_2Cl_2. The solvent was removed under reduced pressure to obtain 0.15 g (0.13 mmol, 82%) of compound **5** as a beige solid. M.p. 176–177 °C.

^{1}H NMR ($CDCl_3$, 400 MHz) δ(ppm): 8.27 (4H, s, H-11), 7.81 (8H, s, H-9), 7.08 (8H, s, H-7), 6.96 (4H, s, H-4), 5.10 (2H, s, H-6), 4.08 (4H, brs, H-2), 3.92 (24H, s, COOMe), 2.00 (4H, brs, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz) δ(ppm): 166.1 (O=COMe), 159.7 (C-3), 158.7 (C-8), 138.1 (C-5), 131.9 (C-10), 123.4 (C-11), 120.1 (C-9), 118.5 (C-6), 113.3 (C-4), 70.2 (C-7), 67.6 (C-2), 52.5 (COOMe), 26.0 (C-1). IR (ν_{max}/cm^{-1}): 2954, 1721 (O–C=O), 1597, 1431, 1339, 1311, 1239 (C-O), 1170, 1113, 1064, 1043, 1000, 907, 875, 836, 792, 752, 721, 682, 564. HR-ESI-TOF-MS (m/z): calcd for $C_{60}H_{58}O_{22}$[M+Na]$^+$: 1153.3317; found: 1153.3315.

2.2.5. 1,4-Bis(3,5-bis(3,5-bis(hydroxymethyl)phenoxymethyl) phenoxybutane (**6**)

A solution of **5** (0.10 g, 0.09 mmol) in dry THF was added dropwise to a magnetically stirred suspension of $LiAlH_4$ (0.04 g, 1.05 mmol) in dry THF under N_2. The reaction was stirred 24 h at room temperature, and quenched by the slow addition of a solution of ammonium chloride (10 mL) and ethyl acetate (40 mL) with cooling. The aluminum salts were filtered and the solvents were then removed under reduced pressure to give 0.04 g (0.05 mmol, 53%) of compound **6** as a white solid. M.p. 146–147 °C.

^{1}H NMR (DMSO-d_6, 300 MHz) δ(ppm): 7.18 (2H, brs, H-6), 7.08 (4H, brs, H-11), 6.85 (4H, brs, H-4), 6.82 (8H, brs, H-9), 5.04 (8H, brs, H-7), 4.44 (16H, brs, H-12), 4.05 (4H, brs, H-2), 2.48 (8H, brs, OH), 1.87 (4H, brs, H-1). ^{13}C NMR (DMSO-d_6, 75 MHz) δ(ppm): 159.2 (C-3), 158.7 (C-8), 144.4 (C-10), 139.4 (C-5), 118.9 (C-6), 117.3 (C-11), 113.2 (C-4), 111.3 (C-9), 69.3 (C-7), 67.6 (C-2), 63.3 (C-12), 25.8 (C-1). IR (ν_{max}/cm^{-1}): 3317 (OH), 2918, 2850, 2359, 1720, 1595, 1451, 1375, 1294, 1256, 1154, 1110, 1021 (C-O), 843, 801, 752, 684. HR-APCI-TOF-MS (m/z): calcd for $C_{52}H_{58}O_{14}$[M+Na]$^+$: 929.3724; found: 929.3720.

2.2.6. 1,4-Bis(3,5-bis(3,5-bis(chloromethyl)phenoxymethyl) phenoxybutane (**7**)

To a magnetically stirred solution of **6** (0.75 g, 0.83 mmol) in dry CH_2Cl_2 was added dry pyridine (0.59 mL, 7.28 mmol), followed by dropwise addition of $SOCl_2$ (0.52 mL, 7.28 mmol) under N_2. The reaction was stirred 48 h at room temperature, and quenched by the slow addition of water. The organic phase was extracted with