Med Chem Res (2015) 24:430–441
DOI 10.1007/s00044-014-1137-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Long-chain alkyltriazoles as antitumor agents: synthesis, physicochemical properties, and biological and computational evaluation

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Received: 27 March 2014 / Accepted: 25 June 2014 / Published online: 15 July 2014

Abstract A series of novel long-chain alkyltriazoles were prepared from commercial diols in a rapid process with good yields. The compounds were evaluated in vitro for their anticancer potential against two human cancer cell lines: colon carcinoma (RKO) and uterine carcinoma (HeLa). The results of colorimetric MTT assays showed that six of fourteen compounds tested decreased cell viability in these cell lines. Compounds **5e** and **6a** were the most active against RKO cells, with IC_{50} values of 16.70 and 14.57 μM, respectively. The same compounds, **5e** and **6a**, were the most active in HeLa cells as well, with IC_{50} values of 11.05 and 12.77 μM, respectively. In addition, compound **5e** was found to induce apoptosis in RKO cells, as assessed by TUNEL assay. The results suggest that compound **5e** may be a promising prototype anticancer agent.

Electronic supplementary material The online version of this article (doi:10.1007/s00044-014-1137-3) contains supplementary material, which is available to authorized users.

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Keywords Alkyltriazoles · Antitumoral · Click chemistry · Heterocycles

Introduction

Cancer is the second leading cause of death in the world. Most of the anticancer drugs currently available for cancer treatment have well-established shortcomings, such as poor efficiency and selectivity, and high toxicity. Therefore, the identification of potent, selective, and less toxic anticancer agents remains an important and challenging goal of medicinal chemistry (Hilário *et al*., 2011; Correale *et al*., 2011).

Single long-chain alkylphospholipids (APLs) are a relatively new class of structurally related antitumor agents that, unlike conventional chemotherapeutic drugs, induce apoptosis in tumor cells by acting on cell membranes rather than on DNA. APLs accumulate in the cell and interfere with lipid-dependent survival signaling pathways, notably the PI3K-Akt and Raf-Erk1/2 pathways, and de novo phospholipid biosynthesis (Blitterswijk and Verheij, 2012). Alkyllysophospholipids and alkylphosphocholines (APCs) are two classes of APL ether lipids that could be potential anticancer agents. Miltefosine, perifosine, erucylphosphocholine, and erufosine (Fig. 1) are APCs, which are derived from alkyllysophospholipids by the removal of the glycerol group. In the APC edelfosine, however, the glycerol backbone is maintained. Hexadecylphosphocholine (**1**) is a lipid analog that exerts antiproliferative activity against a broad spectrum of established tumor cell lines (Blitterswijk and Verheij, 2012; Wieder *et al*., 1998). Studies on cytotoxic APLs revealed that a long alkyl chain and a polar group are essential for antitumor activity (Rakotomanga *et al*., 2007).

In the last decade, a large number of studies have reported the synthesis and biological screening of several

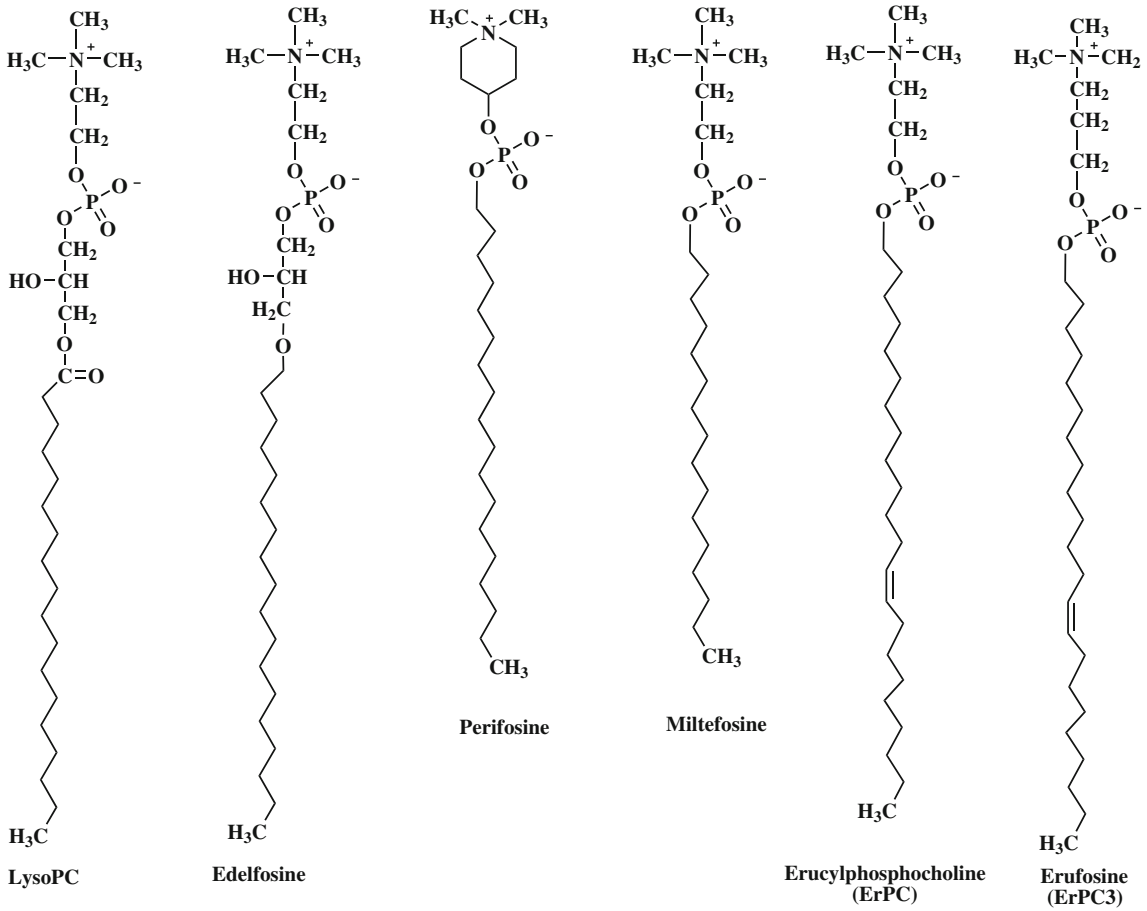
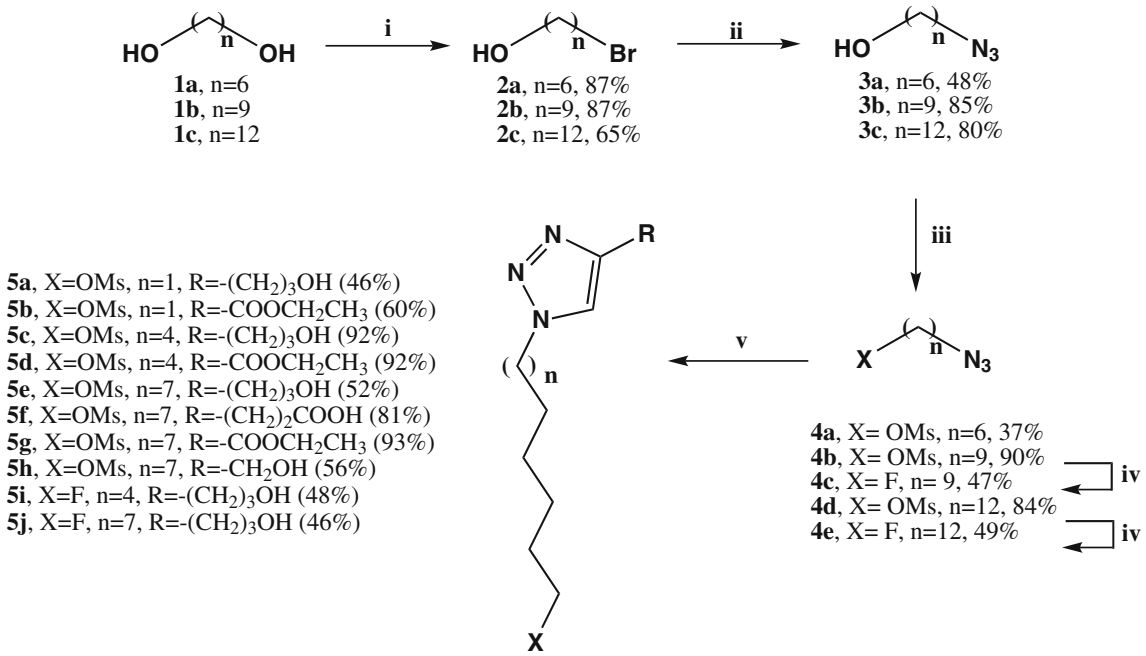


Fig. 1 Structures of natural lysophosphatidylcholine and some synthetic alkyl-phospholipid (APL) analogs. (Adapted of Blitterswijk and Verheij, 2012)

APC compounds (Ungera and Eibl, 1991; Van der Luit *et al*., 2007; Vink *et al*., 2007). Although they are structurally simple, the purification of these highly polar compounds is very difficult, time-consuming, expensive, and frequently results in low yields.

1,2,3-Triazoles are a very important class of synthetic heterocycles that have received widespread attention in recent years because of their broad range of pharmacological properties and easy synthetic accessibility by click chemistry. They are found in various bioactive molecules, including antifungal (Aher *et al*., 2009), antibacterial (Demaray *et al*., 2008; Wang *et al*., 2010), antiallergic (Buckle *et al*., 1983), anti-HIV (Whiting *et al*., 2006; Giffin *et al*., 2008), antitubercular (Costa *et al*., 2006; Patpi *et al*., 2012), and anti-inflammatory agents (Simone *et al*., 2011). In addition, the synthesis of different 1,2,3-triazoles with anticancer activity has also been increasingly noted (Alam *et al*., 2013; Praveena *et al*., 2014; Kurumurthy *et al*., 2014). Triazoles are often considered bioisosteres of amide functionalities in bioactive compounds due to similarities in spatial structure and electronic effect. Furthermore, these heterocycles are resistant to metabolic degradation and can interact with biological structures in several noncovalent ways (Deiters *et al*., 2003; Wang *et al*., 2003; Dirks *et al*., 2005; Kosiova *et al*., 2007; Santos *et al*., 2008). Finally, they have a large dipole moment and are capable of hydrogen bonding, which could allow them to act as a polar head group.

Considering the potential anticancer activity of APCs and the speculation that their activity is linked to the presence of a hydrophobic tail attached to a hydrophilic polar head group, we focused our attention on synthesizing simpler potentially antitumoral compounds containing a 1,2,3-triazole ring and alkyl chains with different lengths and functionalizations. These compounds can be prepared using a 1,3-dipolar cycloaddition reaction between an alkyne and an azide (Struthers *et al*., 2010). This cyclization reaction, developed in the early 1960s by Huisgen (1961), became highly popular when Sharpless (Rostovtesv *et al*., 2002) and Meldal (Tornøe *et al*., 2002) separately reported its Cu(I)-catalyzed version (Scheme 1). The reaction is now known as the Cu-catalyzed azide–alkine



Scheme 1 Reagents and conditions: (*i*) HBr (48 %), toluene, 110 °C, 24 h, 65–87 %; (*ii*) NaN_3, DMSO, rt, 24 h, 48–85 %; (*iii*) CH_2Cl_2, mesyl chloride, triethylamine, rt, 24 h, 37–90 %; (*iv*) KF/18-crown-6, DMSO, 110 °C, 24 h, 47–49 %; (*v*) NaAsc (20 mol%), $CuSO_4{\cdot}5H_2O$ (8 mol%), alkyne: pent-4-yn-1-ol, ethyl propiolate, 4-pentynoic acid, or propargyl alcohol, CH_2Cl_2:H_2O (1:1), rt., 24 h, 46–93 %

cycloaddition (CuAAC) (Amblard *et al*., 2009; Meldal and Tornoe, 2008) or click reaction. Sharpless strongly defended its use in drug discovery, reasoning that, in this field, all searches must be restricted to molecules that are easy to make (Kolb *et al*., 2001). In addition to synthesizing alkyltriazoles, we also evaluated the antitumoral activity in vitro of the new compounds against two human cancer cell lines: colon carcinoma (RKO-AS451) cells and uterine carcinoma (HeLa).

Methods and materials

General

Reagents and solvents were purchased as reagent grade and used without further purification. All melting points were measured on Fisher–Jonhs and are uncorrected. IR spectra were recorded on Perkin-Elmer *Spectrum One SP-IR Spectrometer*. ^1H and ^{13}C NMR spectra were recorded on a *Bruker AVANCE DRX* 200 MHz spectrometer using TMS as an internal standard. The results are presented as chemical shift δ in ppm, number of protons, multiplicity, *J* values in Hertz (Hz), proton position, and carbon position. Multiplicities are abbreviated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and qn (quintet). High resolution mass spectra were recorded on ESI-MS—Bruker Daltonics Micro TOF mass spectrometer with electrospray ionization coupled to time-of-flight (Solvent: MeOH). The progress of the reactions was monitored by TLC on Merck silica plates (GF254). Column chromatography was performed over silica gel 60, 70–230 mesh (Merck).

Synthesis

*General procedure for the synthesis of methanesulfonate alkylazides (**4a**, **4b**, and **4d**)*

To a stirred solution of 1,6-hexanodiol **1a** (1.00 equiv.), 1,9-nonanediol **1b** (1.00 equiv.), or 1,12-dodecanediol **1c** (1.00 equiv.), in 30 mL of toluene was added HBr 48 % (2.00 equiv.). The reaction was stirred at 110 °C for 24 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography over silica gel, eluting with hexane/EtOAc 9:1, to yield pure haloalcohol **2a–c**. These compounds were transformed into their corresponding azidoalcohols **3a–c** by S_N2 substitution (Scheme 1). A stock solution of 0.5 M NaN_3 in DMSO was prepared by stirring the solution for 24 h at room temperature. To a 100-mL round-bottom flask equipped with a magnetic stir bar was added a 0.5 M solution of NaN_3 in DMSO at room temperature. To this solution was added the bromoalcohol **2a** (1.00 equiv.), **2b** (1.00 equiv.), or **2c** (1.00 equiv.), and the mixture was stirred for 24 h at room temperature. The reaction was quenched with H_2O (50 mL) and stirred until it cooled to room temperature. The mixture was extracted with Et_2O

(3 × 30 mL), and the resulting extracts were washed with H_2O (3 × 50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4) and filtered, and the residue obtained was purified by column chromatography over silica gel, eluting with hexane/EtOAc 9:1, to yield pure alkyl azidoalcohols **3a**–**c**. A solution of the azidoalcohol **3a** (1.00 equiv.), **3b** (1.00 equiv.), or **3c** (1.00 equiv.) in CH_2Cl_2 (50 mL) was cooled to 0 °C. Et_3N (2.00 equiv.) and methanesulfonyl chloride (2.00 equiv.) was added. The reaction mixture was stirred for 24 h and then allowed to reach room temperature. The reaction mixture was poured into crushed ice (70 mL) and was then extracted with methylene chloride (3 × 30 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue obtained was purified by column chromatography over silica gel, eluting with hexane/EtOAc 9:1, to yield highly purified halo alcohol pure methanesulfonate alkylazides compounds **4a**, **4b**, and **4d**.

*General procedure for the synthesis of alkylfluoro (**4c** and **4e**)*

To a stirred solution of 9-azidononyl methanesulfonate **4b** (1.00 equiv.) or 12-azidododecyl methanesulfonate **4d** (1.00 equiv.) in 4 mL of anhydrous DMSO was added KF (2.00 equiv.) and 18-crown-6 (2.00 equiv.). The reaction was mainteined at 110 °C for 24 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography over silica gel, eluting with hexane/EtOAc 9:1, to yield pure alkylfluoro **4c** and **4e**.

*1-Azido-9-fluorononane (**4c**)* Yellow oil, 49 %; IR (neat) ν_{max} 2929, 2857, 2091 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.20–1.49 (12H, m, 6× $C\underline{H}_2$), 1.52–1.81 (2H, m, $-C\underline{H}_2CH_2F$), 3.26 (2H, t, J = 6.0 Hz, $-C\underline{H}_2N_3$), 4.43 (2H, td, J = 6.0, 48.0 Hz, $-C\underline{H}_2F$). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 25.2 ($-\underline{C}H_2CH_2N_3$), 26.8 ($-\underline{C}H_2CH_2F$), 29.0, 29.2, 29.3, 29.5 (4× $\underline{C}H_2$), 30.6 (d, J = 19.0, $-\underline{C}H_2CH_2F$), 51.6 ($-\underline{C}H_2N_3$), 84.3 (d, J = 163.0, $-\underline{C}H_2F$).

*1-Azido-12-fluorododecane (**4e**)* Yellow oil, 47 %; IR (neat) ν_{max} 2929, 2857, 2091 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.19–1.44 (18H, m, 9× $C\underline{H}_2$), 1.52–1.80 (2H, m, $-C\underline{H}_2CH_2F$), 3.26 (2H, t, J = 6.0 Hz, $-C\underline{H}_2N_3$,). 4.44 (2H, t, J = 6.0 Hz, $-C\underline{H}_2F$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 25.3 ($-\underline{C}H_2CH_2N_3$), 26.9 ($-\underline{C}H_2CH_2F$), 29.0, 29.3, 29.4, 29.7 (7× $-\underline{C}H_2$), 30.6 (d, J = 19.5 Hz, $-\underline{C}H_2CH_2F$), 51.7 ($-\underline{C}H_2N_3$), 84.5 (d, J = 156.5 Hz, $-\underline{C}H_2F$).

*General procedure for the synthesis of alkyltriazoles (**5a**–**j**)*

The azide compound (**4a**–**e**) (1.00 equiv.) was added to a 10-mL round-bottom flask containing 1 mL of dichloromethane, 1 mL of water, $CuSO_4{\cdot}5H_2O$ (0.08 equiv.), sodium ascorbate (0.20 equiv.), and alkyne (pent-4-yn-1-ol, propargyl alcohol, 4-pentynoic acid, or ethyl propiolate (1.00 equiv.). The reaction mixture was vigorously stirred at room temperature for 24 h. After completion of the reaction, 5 mL of water were added, followed by extraction with dichloromethane (3 × 8 mL). The resulting organic layer was washed three times with a 25 % EDTA solution buffered with NH_4Cl at pH 9.5. The organic layer was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by column chromatography over silica gel, eluting with dichloromethane, dichloromethane:EtOAc (8:2 v/v; 5:5 v/v; 2:8 v/v), EtOAC and EtOAc/MeOH (8:2 v/v; 5:5 v/v; 2:8 v/v), to give pure compounds **5a**–**j**.

*6-(4-(3-Hydroxypropyl)-1H-1,2,3-triazol-1-yl)hexyl methanesulfonate (**5a**)* Yellow oil, 46 %; IR (neat) ν_{max} 3276, 2916, 2850, 1331, 1162, 1052–848 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.24–1.96 (10H, m, 5× $C\underline{H}_2$), 2.79 (2H, t, J = 6.0 Hz, $-C\underline{H}_2-C_{triazole}$), 2.98 (3H, s, $-C\underline{H}_3SO_2$), 3.66 (2H, t, J = 6.0 Hz, $-C\underline{H}_2-N_{triazole}$), 4.18 (2H, t, J = 6.0 Hz, $-C\underline{H}_2OH$), 4.30 (2H, t, J = 6.0 Hz, $-C\underline{H}_2OMs$), 7.33 (1H, s, $-C{=}C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 21.5, 24.4, 25.4, 28.4, 29.6 and 31.8 (6× $\underline{C}H_2$), 36.9 ($-\underline{C}H_3SO_2$), 49.6 ($-\underline{C}H_2-N_{triazole}$), 60.8($-\underline{C}H_2OH$), 69.9 ($-\underline{C}H_2OMs$), 121.1 ($-C{=}\underline{C}H_{triazole}$), 147.2 ($-\underline{C}{=}C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 306.1350 $C_{12}H_{24}N_3O_4S$ (calcd. 306.1487).

*6-(4-(Ethoxycarbonyl)-1H-1,2,3-triazol-1-yl)hexyl methanesulfonate (**5b**)* Yellow–white solid, 60 %; m.p. = 70–72 °C; IR (neat) ν_{max} 2945, 2915, 2869, 1728, 1344, 1197, 1097–957, 1166, 1156, 1097, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.30–1.38 (7H, m, $-OCH_2C\underline{H}_3$ and 2× $C\underline{H}_2$), 1.68 (2H, qn, J = 6.0 Hz, $-C\underline{H}_2CH_2N_{triazole}$), 1.90 (2H, qn, J = 6.0 Hz, $-C\underline{H}_2CH_2OMs$), 2.95 (3H, s, $-C\underline{H}_3SO_2$), 4.15 (2H, t, J = 6.0 Hz, $-C\underline{H}_2OMs$), 4.26–4.42 (4H, m, $-C\underline{H}_2-N_{triazole}$ and $-OC\underline{H}_2CH_3$), 8.08 (1H, s, $-C{=}C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 14.2 ($-OCH_2\underline{C}H_3$), 24.7, 25.6, 28.7 and 29.8 (4× $\underline{C}H_2$), 37.2 ($-\underline{C}H_3SO_2$), 50.3 ($-\underline{C}H_2-N_{triazole}$), 61.1 ($-O\underline{C}H_2CH_3$), 69.7 ($-\underline{C}H_2OMs$), 127.3 ($-C = \underline{C}H_{triazole}$), 140.1 ($-\underline{C}{=}C_{triazole}$), 160.6 ($-\underline{C}O$); HRESIMS *m/z* $[M+H]^+$: 320.1138 $C_{12}H_{22}N_3O_5S$ (calcd. 320.1280).

*9-(4-3-Hydroxypropyl)-1H-1,2,3-triazol-1-yl)nonyl methanesulfonate (**5c**)* White solid, 39 %; m.p. = 64–66 °C; IR (neat) ν_{max} 3276, 2919, 2851, 1332, 1164, 1059–848 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ = 1.02–1.45 (10H, m, 5× CH_2), 1.57–1.71 (m, $-C\underline{H}_2CH_2N_{triazole}$), 1.70–1.99 (4H, m, $-C\underline{H}_2CH_2OMs$ and $-C\underline{H}_2CH_2OH$), 2.76 (2H, t, J = 6.0 Hz, $-C\underline{H}_2-C_{triazole}$), 2.94 (3H, s, $-C\underline{H}_3SO_2$), 3.63 (2H, t, J = 6.0 Hz, $-C\underline{H}_2OH$), 4.15 (2H, t, J = 6.0 Hz, $-C\underline{H}_2OMs$), 4.24 (2H, t, J = 6.0 Hz, $-C\underline{H}_2-N_{triazole}$), 7.24 (1H, s, $-C{=}C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ =

21.8, 24.9, 25.9, 28.5, 28.9, 29.8 and 31.9 (9× $\underline{C}H_2$), 36.9 (–$\underline{C}H_3SO_2$), 49.5 (–$\underline{C}H_2$–$N_{triazole}$), 61.4 (–$\underline{C}H_2OH$), 70.1 (–$\underline{C}H_2OMs$), 120.7 (–$\underline{C}$=$C_{triazole}$), 147.4 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 348.1834 $C_{15}H_{30}N_3O_4S$ (calcd. 348.1957).

*9-(4-(Ethoxycarbonyl)-1H-1,2,3-triazol-1-yl)nonyl methanesulfonate (**5d**)* Yellow solid, 92 %; m.p. = 72–74 °C; IR (neat) ν_{max} 2936, 2912, 2852, 1713, 1352, 1215, 1168, 1051–979 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.14–1.33 (10H, m, 5× CH_2), 1.39 (2H, t, J = 6.0 Hz, –$OCH_2\underline{C}H_3$), 1.71 (2H, qn, J = 6.0, –$\underline{C}H_2CH_2OMs$,), 1.81–1.99 (2H, m, –$\underline{C}H_2CH_2N_{triazole}$), 2.90 (3H, s, –$\underline{C}H_3SO_2$), 4.19 (2H, t, J = 6.0 Hz, –$\underline{C}H_2OMs$), 4.30–4.50 (2H, m, –$\underline{C}H_2$–$N_{triazole}$), 8.08 (1H, s, –C=$\underline{C}H_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 13.7 (–$OCH_2\underline{C}H_3$), 24.9, 26.3, 28.6, 29.0 and 29.8 (5× $\underline{C}H_2$), 37.4 (–$\underline{C}H_3OMs$), 49.9 (–$\underline{C}H_2$–$N_{triazole}$), 60.9 (–$O\underline{C}H_2CH_3$), 69.7 (–$\underline{C}H_2OMs$), 127.3 (–C=$\underline{C}H_{triazole}$), 140.2 (–$\underline{C}$=$C_{triazole}$), 160.6 (–$\underline{C}O$); HRESIMS *m/z* $[M+H]^+$: 362.1861 $C_{15}H_{28}N_3O_5S$ (calcd. 362.1749).

*12-(4-(3-Hydroxypropyl)-1H-1,2,3-triazol-1-yl)dodecyl methanesulfonate (**5e**)* White solid, 52 %; m.p. = 80–82 °C; IR (neat) ν_{max} 3389, 2916, 2850, 1332, 1163, 1045, 956 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.25–1.44 (16H, m, 2× $C\underline{H}_2$), 1.75 (2H, qn, J = 6.0 Hz, –$C\underline{H}_2CH_2OMs$), 1.84–1.99 (4H, –$C\underline{H}_2CH_2N_{triazole}$ and –$C\underline{H}_2CH_2OH$), 2.84 (2H, t, J = 6.0 Hz, –$C\underline{H}_2$–$C_{triazole}$), 3.01 (3H, s, –$C\underline{H}_3OMs$,), 3.71 (2H, t, J = 6.0 Hz, –$C\underline{H}_2OH$), 4.22 (2H, t, J = 6.0 Hz, –$C\underline{H}_2$–$N_{triazole}$), 4.31 (2H, t, J = 6.0 Hz, –$C\underline{H}_2OMs$), 7.74 (1H, s, –C=$C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 21.2 (–$\underline{C}H_2(CH_2)_2OMs$), 24.8, 25.9, 28.5, 28.6, 28.8, 29.0, 29.8 and 31.9 (11× $\underline{C}H_2$), 35.5 (–$\underline{C}H_3OMs$), 49.8 (–$\underline{C}H_2$–$N_{triazole}$), 60.5(–$\underline{C}H_2OH$), 70.2 (–$\underline{C}H_2OMs$), 121.7 (–C=$\underline{C}H_{triazole}$), 150.1 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 390.2572 $C_{18}H_{34}N_3O_5S$ (calcd. 390.2426).

*3-(1-(12-Methanesulfonoyldodecyl)-1H-1,2,3-triazol-1-yl) propanoic acid (**5f**)* Yellow solid, 81 %; IR (neat) ν_{max} 2916, 2850, 1729, 1331, 1163, 1052–952, 850 cm^{-1}; ^{1}H ($CDCl_3$, 200 MHz): δ = 1.18–1.26 (16H, m, 8× $C\underline{H}_2$), 1.50–1.69 (4H, m, –$C\underline{H}_2CH_2N_{triazole}$ and –$C\underline{H}_2CH_2OMs$), 2.19–2.50 (4H, m, $C\underline{H}_2C\underline{H}_2COOH$), 3.19 (2H, t, J = 6.0 Hz, –$C\underline{H}_2$–$N_{triazole}$), 4.15 (2H, t, J = 6.0 Hz, –CH_2OMs), 7.98 (1H, s, –C=$C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 25.3 (–$\underline{C}H_2(CH_2)_2OMs$), 26.6, 28.5, 28.7, 28.9, 29.0 and 29.4 (11× $\underline{C}H_2$), 33.2 (–$\underline{C}H_2COOH$), 37.1 (–$\underline{C}H_3OMs$), 51.0 (–$\underline{C}H_2$–$N_{triazole}$), 70.3 (–$\underline{C}H_2OMs$), 115.6 (–C=$\underline{C}H_{triazole}$), 136.3 (–$\underline{C}$=$C_{triazole}$), 178.9 (–$\underline{C}O$); HRESIMS *m/z* $[M+H]^+$: 404.2464 $C_{18}H_{34}N_3O_5S$ (calcd. 404.2219).

*12-(4-(Ethoxycarbonyl)-1H-1,2,3-triazol-1-yl)dodecyl methanesulfonate (**5g**)* White solid, 93 %; m.p. = 78–80 °C; IR (neat) ν_{max} 2916, 2850, 1717, 1341, 1226, 1207, 1168, 1046–943, 848 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.20–1.30 (16H, m, 8× $C\underline{H}_2$), 1.41 (2H, t, J = 6.0 Hz, –$OCH_2C\underline{H}_3$), 1.74 (2H, qn, J = 6.0 Hz, –$C\underline{H}_2CH_2OMs$), 1.87–1.98 (2H, m, –$C\underline{H}_2CH_2N_{triazole}$), 2.97 (3H, s, –$CH_3OMs$), 4.18 (2H, t, J = 6.0 Hz, –$C\underline{H}_2$–$N_{triazole}$), 4.39–4.45 (4H, m, –CH_2OMs and –$OC\underline{H}_2CH_3$), 8.05 (1H, s, –C=$C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 14.2 (–$OCH_2\underline{C}H_3$), 25.2, 26.2, 29.0, 29.2, 29.5, 29.8 and 30.3 (8× $\underline{C}H_2$), 37.0 (–$\underline{C}H_3OMs$), 50.8 (–$\underline{C}H_2$–$N_{triazole}$), 61.5 (–$OCH_2\underline{C}H_3$), 70.4 (–$\underline{C}H_2OMs$), 127.2 (–C=$\underline{C}H_{triazole}$), 140.1 (–$\underline{C}$=$C_{triazole}$), 160.7 (–$\underline{C}O$); HRESIMS *m/z* $[M+H]^+$: 404.2464 $C_{18}H_{34}N_3O_5S$ (calcd. 404.2219).

*12-(4-(Hydroxymethyl)-1H-1,2,3-triazol-1-yl)dodecyl methanesulfonate (**5h**)* Yellow solid, 56 %; m.p. = 86–88 °C; IR (neat) ν_{max} 3117, 3023, 2916, 2850, 1331, 1162, 1120, 1052–951 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.16–1.38 (16H, m, 8× $C\underline{H}_2$), 1.62–1.88 (4H, m, $C\underline{H}_2CH_2OMs$ and $C\underline{H}_2CH_2N_{triazole}$), 2.95 (3H, s, –$CH_3OMs$), 4.16 (2H, t, J = 6.0 Hz, –$C\underline{H}_2OMs$), 4.27 (2H, t, J = 6.0 Hz, –$C\underline{H}_2$–$N_{triazole}$), 4.70 (2H, s, –$C\underline{H}_2OH$), 7.54 (1H, s, –C=$C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 25.4, 26.3, 28.8, 28.9, 29.2, 30.1 and 31.2 (10× $\underline{C}H_2$), 37.2 (–$\underline{C}H_3OMs$), 50.3 (–$\underline{C}H_2$–$N_{triazole}$), 55.9 (–$\underline{C}H_2OH$), 70.3 (–$\underline{C}H_2OMs$), 121.8 (–C=$\underline{C}H_{triazole}$), 147.8 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 362.2047 $C_{16}H_{32}N_3O_4S$ (calcd. 362.2113).

*3-(1-(9-Fluorononyl)-1H-1,2,3-triazol-1-yl)propan-1-ol (**5i**)* White solid, 48 %; m.p. = 62–64 °C; IR (neat) ν_{max} 3312, 2913, 2848, 1050 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.29–1.40 (10H, m, 5× $C\underline{H}_2$), 1.68 (2H, qnd, J = 2.0 Hz, J = 24.0 Hz, –$C\underline{H}_2CH_2F$,), 1.83–1.98 (4H, m, –$C\underline{H}_2CH_2OH$ and $C\underline{H}_2CH_2N_{triazole}$), 2.83 (2H, t, J = 8.0 Hz, –$C\underline{H}_2$–$C_{triazole}$), 3.66–3.74 (2H, m, –$C\underline{H}_2OH$), 4.31 (2H, t, J = 8.0 Hz, –$C\underline{H}_2$–$N_{triazole}$), 4.43 (2H, td, J = 8.0 Hz, J = 48.0 Hz, –$C\underline{H}_2F$), 7.54 (1H, s, –C=$C\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 21.85 (–$\underline{C}H_2(CH_2)_2F$), 24.9, 26.3, 28.7, 28.9, 29.0 and 30.1 (6× $\underline{C}H_2$), 30.22 (d, J = 13.5 Hz, –$\underline{C}H_2F$), 33.3 (–$HOCH_2\underline{C}H_2$–$C_{triazole}$), 51.5 (–$\underline{C}H_2$–$N_{triazole}$), 62.8 (–$\underline{C}H_2OH$), 85.4 (d, J = 163.0 Hz, –$\underline{C}H_2F$), 122.0 (–C=$\underline{C}H_{triazole}$), 148.0 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 272.1973 $C_{14}H_{27}FN_3O$ (calcd. 272.2138).

*3-(1-(12-Fluorododecyl)-1H-1,2,3-triazol-1-yl)propano-1-ol (**5j**)* White solid, 46 %; m.p. = 88–90 °C; IR (neat) ν_{max} 3321, 2917, 2846, 1006 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.21–1.44 (16H, m, 2× $C\underline{H}_2$), 1.56–2.05 (6H, 3× CH_2), 2.86 (2H, t, J = 6.0 Hz, –$C\underline{H}_2$–$C_{triazole}$), 3.70 (2H, t, J = 6.0 Hz, –$C\underline{H}_2OH$), 4.32 (2H, t, J =

6.0 Hz, –C$\underline{H}_2$–$N_{triazole}$), 4.55 (2H, td, $J = 6.0$ Hz, $J = 46.0$ Hz, –C$\underline{H}_2$F), 7.34 (1H, s, –C=C$\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 21.7$ (–$\underline{C}H_2(CH_2)_2$F), 25.2, 26.6, 29.6, 29.0 and 30.4 (9× $\underline{C}H_2$), 30.5 (d, $J = 19.0$ Hz, –$\underline{C}H_2$F), 32.2 (HOCH$_2\underline{C}H_2CH_{2triazole}$), 49.8 (–$\underline{C}H_2$–$N_{triazole}$), 61.1 (–$\underline{C}H_2$OH), 84.2 (d, $J = 179.5$ Hz, –$\underline{C}H_2$F), 121.0 (–C=$\underline{C}H_{triazole}$), 147.3 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 314.2458 $C_{17}H_{33}FN_3O$ (calcd. 314.2608).

General procedure for the synthesis of ***6a–d***

To a solution of compounds **5c**, **5d**, **5e,** or **5g** (1.00 equiv.) in acetone (5 mL), was added sodium iodide (2.00 equiv.). The mixture was heated at reflux for 24 h. Afterward the reaction mixture was diluted with water and extracted with dichloromethane (3 × 15 mL). The organic extracts were combined and dried (Na_2SO_4), and the solvent was removed under reduced pressure. The residue obtained was purified by column chromatography over silica gel, eluting with mixtures of EtOAc/MeOH (100:0; 80:20, and 0:100), to give pure compounds **6a**–**d**.

3-(1-(9-Iodononyl)-1H-1,2,3-triazol-4-yl)propan-1-ol ***(6a)*** Yellow solid, 84 %; m.p. = 88–90 °C; IR (neat) ν_{max} 3350, 2924, 2851 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 1.24$–1.34 (10H, m, 5× C$\underline{H}_2$), 1.74–1.94 (6H, m, –C$\underline{H}_2$CH$_2$N$_{triazole}$, –C$\underline{H}_2$CH$_2$OH and –C$\underline{H}_2$CH$_2$I), 2.80 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$C$_{triazole}$), 3.17 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$I), 3.67 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$OH), 4.29 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$N$_{triazole}$), 7.31 (1H, s, –C=C$\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 7.5$ (–$\underline{C}H_2$I), 22.0 (–$\underline{C}H_2$(CH$_2$)$_3$I), 26.6, 28.5, 29.00, 29.3, 30.4, 32.2, 33.6 (8× $\underline{C}H_2$), 50.7 (–$\underline{C}H_2$–$N_{triazole}$), 61.6 (–$\underline{C}H_2$OH), 121.0 (–C=$\underline{C}H_{triazole}$), 147.6 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 380.1170 $C_{14}H_{27}IN_3O$ (calcd. 380.1199).

3-(1-(12-Iododododecyl)-1H-1,2,3-triazol-4-yl)propan-1-ol ***(6b)*** Yellow solid, 73 %; m.p. = 84–86 °C; IR (neat) ν_{max} 3308, 2917, 2848 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 1.12$–1.46 (16H, m, 8× C$\underline{H}_2$), 1.62–2.04 (6H, m, –C$\underline{H}_2$CH$_2$N$_{triazole}$, –C$\underline{H}_2$CH$_2$OH and –C$\underline{H}_2$CH$_2$I), 2.83 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$–C$_{triazole}$), 3.18 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$I), 3.70 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$OH), 4.30 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$–$N_{triazole}$), 7.31 (1H, s, –C=C$\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 7.34$ (–$\underline{C}H_2$I), 21.91 (–$\underline{C}H_2$(CH$_2$)$_3$I), 26.3, 28.3, 28.8, 29.2, 29.3, 30.2, 30.3, 31.9 and 34.0 (9× $\underline{C}H_2$), 49.9 (–$\underline{C}H_2$–$N_{triazole}$), 61.3 (–$\underline{C}H_2$OH), 120.8 (–C=$\underline{C}H_{triazole}$), 147.4 (–$\underline{C}$=$C_{triazole}$); HRESIMS *m/z* $[M+H]^+$: 422.1637 $C_{17}H_{33}IN_3O$ (calcd. 422.1668).

Ethyl-1-(9-iodononyl)-1H-1,2,3-triazole-4-carboxylate ***(6c)*** White solid, 97 %; m.p. = 90–92 °C; IR (neat) ν_{max} 2919, 2848, 1723, 1216, 1197, 1164 cm^{-1};^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 1.22$–1.28 (10H, m, 5× C$\underline{H}_2$), 1.41 (3H, t, $J = 6.0$ Hz, –OCH$_2\underline{C}H_3$), 1.71–1.96 (4H, m, –C$\underline{H}_2$CH$_2$N$_{triazole}$ and –C$\underline{H}_2$CH$_2$I), 3.18 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$I), 4.30–4.51 (4H, m, –C$\underline{H}_2$–$N_{triazole}$ and –OC$\underline{H}_2$CH$_3$), 8.07 (1H, s, –C=C$\underline{H}_{triazole}$); ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 7.37$ (–$\underline{C}H_2$I), 14.5 (–OCH$_2\underline{C}H_3$), 26.5, 28.7, 29.1, 29.6, 30.3, 30.6 and 33.3 (7× $\underline{C}H_2$), 50.6 (–$\underline{C}H_2$–$N_{triazole}$), 61.8 (–$\underline{C}H_2$OH), 127.4 (–C=$\underline{C}H_{triazole}$), 140.5 (–$\underline{C}$=$C_{triazole}$), 160.8 (–CO); HRESIMS *m/z* $[M+H]^+$: 394.1123 $C_{14}H_{25}IN_3O_2$ (calcd. 394.0991).

Ethyl-1-(12-iododododecyl)-1H-1,2,3-triazole-4-carboxylate ***(6d)*** Yellow solid, 90 %; m.p. = 80–82 °C. IR (neat) ν_{max} 2919, 2848, 1723, 1224, 1207, 1164, 777 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 1.15$–1.39 (16H, m, 8× C$\underline{H}_2$), 1.41 (3H, t, $J = 6.0$ Hz, –OCH$_2\underline{C}H_3$), 1.81 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$CH$_2$I), 1.95 (2H, qn, $J = 6.0$ Hz, –C$\underline{H}_2$CH$_2$N$_{triazole}$), 3.18 (2H, t, $J = 6.0$ Hz, –C$\underline{H}_2$I), 4.47 (4H, m, –C$\underline{H}_2$–$N_{triazole}$ and –OC$\underline{H}_2$CH$_3$), 8.06 (1H, s, –C=C$\underline{H}_{triazole}$); ^{13}C–NMR (50 MHz, $CDCl_3$): 6.9 (–$\underline{C}H_2$I), 14.3 (–OCH$_2\underline{C}H_3$), 26.3, 28.3, 28.8, 29.1, 30.1, 30.4 and 33.4 (6× $\underline{C}H_2$), 50.9 (–$\underline{C}H_2$–$N_{triazole}$), 61.1 (–$\underline{C}H_2$OH), 127.2 (–C=$\underline{C}H_{triazole}$), 140.09 (–$\underline{C}$=$C_{triazole}$), 160.8 (CO); HRESIMS *m/z* $[M+H]^+$: 436.1419 $C_{17}H_{31}IN_3O_2$ (calcd. 436.1461).

Biological assays

Cytotoxicity assay

The cytotoxicity of the compounds was assessed with the human cell lines RKO (colon carcinoma ATCC# CRL-2577), uterine carcinoma (HeLa), and lung fibroblast (WI-26VA4) cells, using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, St. Louis, MO, USA) colorimetric method. Briefly, the cells were plated in 96-well plates (1 × 105 cells/well) and incubated for 24 h at 37 °C in a humid atmosphere with 5 % CO_2 to adhesion. After this period, the wells were washed with culture medium (EMEM + 10 % inactivated fetal calf serum + 2 mM L-glutamine) and incubated with the compounds at different concentrations (0.01–500 μM). Control with etoposide (Sigma-Aldrich, St. Louis, MO) used as reference anticancer drug, was performed in parallel. After the incubation, the plates were treated with MTT. The reading was performed using a SpectraMax M5e microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 550 nm. Cytotoxicity was scored as the percentage reduction in absorbance versus untreated control cultures (Hilário *et al.*, 2011). All experiments were performed in triplicate. The

results were expressed as the mean of the IC_{50} (the lethal drug concentration that reduced cell viability by 50 %).

DNA Nick-End labeling by the TUNEL method and immunofluorescence

Apoptotic cell death was measured using the APO-BrdU TUNEL assay kit (Invitrogen, CA, USA). Briefly, the effect of the compound **5e** and etoposide on DNA fragmentation was determined using RKO cells. The cells were incubated with the compounds during 48 h as described above, and fixed using a solution of ice-cold 70 % ethanol. The cells were then counterstained with 5-Bromo-2′-deoxyuridine 5′-triphosphate (BrdUTP) in the presence of the terminal deoxynucleotidyl transferase (TdT) and stained with Anti-BrdU monoclonal antibody PRB-1 Alexa Fluor 488 conjugate, as previous described (Pereira *et al*., 2012). The loaded cells were visualized by fluorescence microscopy using a Zeiss Axiovert 200.

Virtual screening

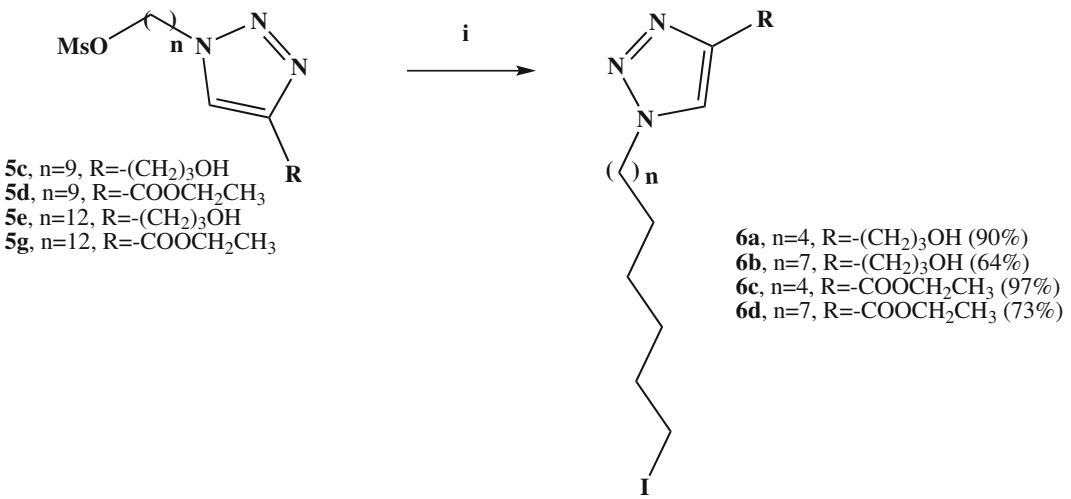
All ligands were built and optimized using GaussView (Frisch *et al*., 2009) and Parametric Method 6 (PM6) (Stewart, 2007) implemented in Gaussian 09W (Carregal *et al*., 2012), respectively. Next, the inverse virtual screening approach was used to identify the molecular target of each ligand present in Our Own Molecular Targets Data Bank (OOMT) (Carregal *et al*., 2013), OOMT is a data bank with 34 molecular targets from Protein Data Bank (PDB) (Berman *et al*., 2013), and built by comparative homology modeling, which was parameterized for screening studies by redocked of respective crystallographic ligand (Nunes *et al*., 2013). The parameterization of OOMT includes the construction of grid box, which was defined as a cube with the geometric center in the crystallographic ligand sufficiently to accommodate the whole binding site and with spaced points of 1 Å. Hence, all ligands were docked against molecular target using AutoDock Vina 1.1.2. The search algorithm used was Iterated Local Search Global Optimizer for global optimization. In this process, a succession of steps with a mutation and local optimization (the method of Broyden–Fletcher–Goldfarb–Shanno [BFGS]) were conducted, and each step followed the Metropolis criterion (Trott and Olson, 2010).

Statistical analysis

The average of IC_{50} was compared using Tukey's test. Differences between the values were evaluated with Origin 6.0. A p value of 0.05 was considered to be statistically significant.

Results and discussion

The synthesis of new long-chain alkyltriazole compounds is depicted in Scheme 1. The mesylate compounds (**4a**–**e**) were prepared using 1,6-hexanodiol (**1a**), 1,9-nonanediol (**1b**), or 1,12-dodecanediol (**1c**) as starting materials (Hilário *et al*., 2011; Chong *et al*., 2000). These diols were converted in their monobrominated derivatives by treatment with hydrobromic acid. The haloalcohols obtained were transformed into azido alcohols by S_N2 substitution. The azide compounds were transformed in their corresponding mesylates by reaction with mesyl chloride in alkaline conditions. Compounds **4b** and **4d** were treated with KF/18-crown-6 in dimethylsulfoxide to yield **4c** and **4e**. To obtain the alkyltriazoles **5a**–**j**, a solution of commercially available alkynes (pent-4-yn-1-ol, propargyl alcohol, 4-pentynoic acid, or



Scheme 2 Reagents and conditions: (*i*) NaI, acetone, reflux, 24 h

ethyl propiolate) and alkylazides (**4a–e**) in dichloromethane was treated with a solution of copper sulfate pentahydrate (8 mol%) and sodium ascorbate (20 mol%) in water. The reaction mixture was stirred for 24 h at room temperature, exclusively producing high yields of the 1,4-disubstituted 1,2,3-triazoles (**5a–j**).

We synthesized four additional novel long-chain alkyltriazoles that contained an iodine atom (Scheme 2).

All synthesized compounds were evaluated in vitro for their anticancer potential against two human cancer cell lines (colon carcinoma RKO and uterine carcinoma HeLa). The compounds were also tested on a non-cancerous human cell line (lung fibroblast WI-26VA4) to determine the selectivity index. Colorimetric MTT assays determined that compounds **5e** and **6a** presented potent antitumor activity in vitro (Table 1). Against RKO cells, compounds **5e** and **6a** showed IC_{50} values of 16.70 and 14.57 μM, respectively. These same compounds presented similar IC_{50} values (11.05 and 12.77 μM, respectively) against the HeLa cell line. The cytotoxicity of compounds **5e** and **6a** was comparable to that of etoposide, an anticancer agent (Correale *et al*., 2011).

The physicochemical properties of miltefosine and all the alkyltriazole compounds synthesized in this study are shown in Table 2. All new alkyltriazoles showed a desirable profile for an oral drug (Leeson and Springthorpe, 2007). Based on IC_{50} values and physicochemical properties, one of the most active compounds, i.e., **5e**, was selected for further investigation of its cytotoxic mode of action.

Apoptosis via cytotoxicity is considered an efficient strategy for the identification of potential antitumor drugs

Table 2 Physicochemical properties of miltefosine and the alkyltriazole compounds **5a–j** and **6a–d**

Compounds	cLog *P*	MW	HBD	HBA
Miltefosine	6.0	407.56	0	4
5a	0.59	305.39	1	4
5b	1.24	319.37	0	5
5c	1.67	347.47	1	4
5d	2.32	361.45	0	5
5e	2.74	389.55	1	4
5f	2.36	403.53	1	5
5g	3.57	403.53	0	5
5h	2.12	361.5	1	4
5i	2.70	271.37	1	2
5j	3.77	313.45	1	2
6a	3.37	379.28	1	1
6b	4.45	421.36	1	1
6c	3.86	393.26	0	2
6d	4.93	435.34	0	2

MW molecular weight, *HDB* hydrogen-bond donors, *HBA* hydrogen-bond acceptors

Table 1 In vitro cytotoxic activity of the alkyltriazole compounds against human colon carcinoma (RKO) cells, uterine carcinoma (HeLa) cells, and lung fibroblast (WI-26VA4) cells

Compounds	IC_{50} (μM) ± SD[a]			SI	
	HeLa	RKO	WI	WI/HeLa	WI/RKO
Miltefosine	13.80 ± 4.2	>100	ND	ND	ND
5a	210.10 ± 5.18	397.90 ± 6.54	489.15 ± 4.02	2.33	1.23
5b	202.18 ± 0.31	198.23 ± 18.02	339.34 ± 27.86	1.67	1.71
5c	24.48 ± 5.20	182.03 ± 28.4	ND	ND	ND
5d	37.10 ± 3.90	20.49 ± 5.80	ND	ND	ND
5e	11.05 ± 3.70	16.70 ± 3.40	ND	ND	ND
5f	35.95 ± 7.38	99.94 ± 0.89	39.45 ± 10.42	1.09	0.39
5g	208.17 ± 25.12	180.16 ± 5.92	490.41 ± 4.03	2.35	2.72
5h	21.87 ± 0.78	19.87 ± 4.22	36.54 ± 3.21	1.67	1.84
5i	84.25 ± 9.92	307.21 ± 8.77	184.43 ± 1.51	2.19	0.60
5j	25.82 ± 9.19	90.82 ± 0.83	80.48 ± 12.66	3.11	1.13
6a	12.77 ± 1.16	14.57 ± 2.18	21.40 ± 3.36	1.67	1.47
6b	145.33 ± 13.22	138.16 ± 10.10	27.68 ± 4.08	0.19	0.20
6c	52.22 ± 12.74	199.01 ± 26.30	253.82 ± 59.95	4.87	2.27
6d	26.61 ± 2.20	62.23 ± 2.55	71.84 ± 14.10	2.70	1.15
Etoposide	11.35 ± 2.73	10.66 ± 2.23	4.30 ± 1.34	0.39	0.40

ND not determined, *SI* selectivity index

[a] Values are average ± Standard Deviation

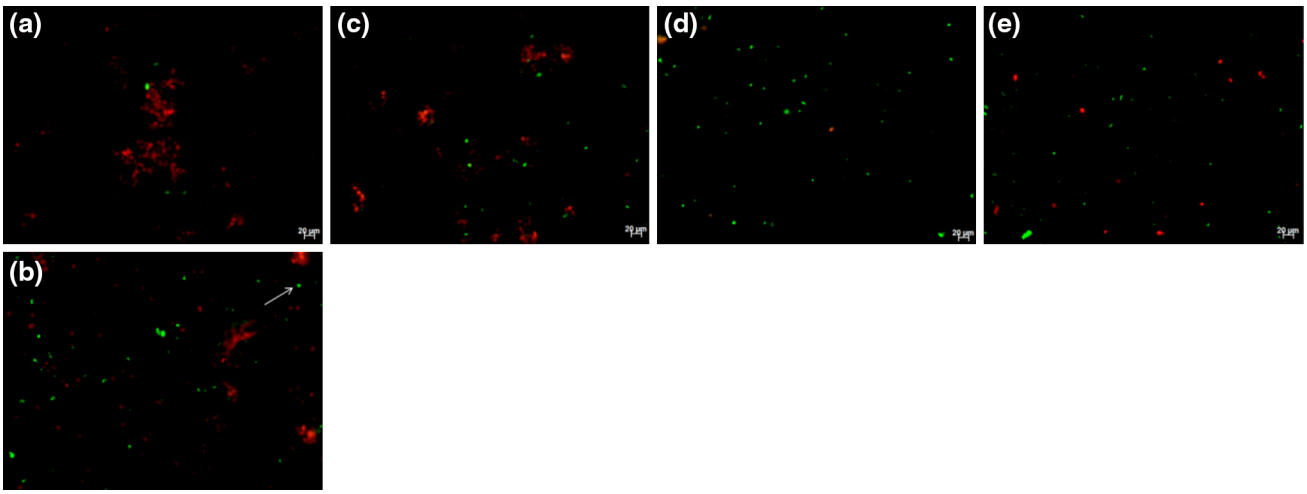


Fig. 2 Apoptosis in human colon carcinoma (RKO) cells. Cells were incubated (48 h) with compound **5e** in different concentrations. (**a**) control of life, (**b**) etoposide at 1 μM, and **5e** (**c**) 1 μM, (**d**) 10 μM, and (**e**) 100 μM. The *arrow* indicates cell death by apoptosis (*green*). Viable cells are stained *red*. *Scale bar:* 20 μm (Color figure online)

(Essack *et al*., 2011). To investigate the possible apoptosis-inducing action of these compounds, specific DNA fragments on RKO cells were detected by a TUNEL assay using compound **5e** and etoposide, an apoptosis-inducing drug (Liu *et al*., 2011). The results presented here indicate that compound **5e** promoted apoptosis in RKO cells (Fig. 2), reducing the number of viable cells in a concentration-dependent manner (Fig. 2a–e). The mechanism by which long-chain APLs trigger apoptosis is, as yet, unclear.

APLs interact with signaling proteins, membrane lipids, or lipid microdomains (Strassheim *et al*., 2000; Samadder *et al*., 2003; Kondapaka *et al*., 2003). The ability of cells to proliferate or initiate apoptosis relies on signaling pathways that produce anti- or pro-apoptotic signals (Ruiter *et al*., 1999; Dineva *et al*., 2012). Anti-apoptotic pathways can comprise the Ras–Raf–MAPK/ERK proliferative pathway (Ruiter *et al*., 2002). Other targets, such as transmembrane proteins, are also involved in the apoptosis process. In this context, galectins (galactoside-binding glycoproteins) were shown to modulate many functions in cell survival, including proliferation and metastasis (Vladoiu *et al*., 2014). In an attempt to understand the mode of action of the compounds synthesized in this work, we performed a virtual screening against 34 potential antitumor targets. All synthesized compounds exhibited the best docking scores against galectin-1 (PDB: 1W6M) and ERK1 (PDB: 2ZOQ) (Table 3). The compound **5e**, an apoptosis inducer, showed a better binding energy for galectin-1 (-4.4 kcal mol^{-1}) and ERK-1 (-6.2 kcal mol^{-1}) than miltefosine, used as reference compound. Figure 3 shows 3D and 2D diagrams of the intermolecular interactions between **5e** and galectin-1 and ERK1.

Table 3 Docking results between miltefosine and alkyltriazoles alkyltriazole compounds **5a–j** and **6a–d** against the molecular targets Galectin-1 and ERK1 carried out by Autodock Vina

Compounds	Binding Energy (kcal mol^{-1})	
	Galectin-1 (PDB: 1W6M)	ERK1 (PDB: 2ZOQ)
Miltefosine	−3.3	−5.7
5a	−4.3	−5.7
5b	−4.4	−6.0
5c	−4.4	−6.2
5d	−4.5	−6.0
5e	−4.1	−5.7
5f	−3.7	−5.6
5g	−3.8	−5.7
5h	−4.0	−6.2
5i	−4.7	−6.4
5j	−4.5	−6.3
6a	−4.7	−5.9
6b	−4.5	−6.3
6c	−4.0	−6.1
6d	−4.7	−5.9

Conclusions

In conclusion, this preliminary investigation demonstrates that very simple long-chain alkyltriazoles may be a promising class of substances with cytotoxic activity, and that this activity can be modified significantly by classical chemical modifications. Among the synthesized compounds, **5e** was the most active, could represent a promising template for developing a new class of antitumor

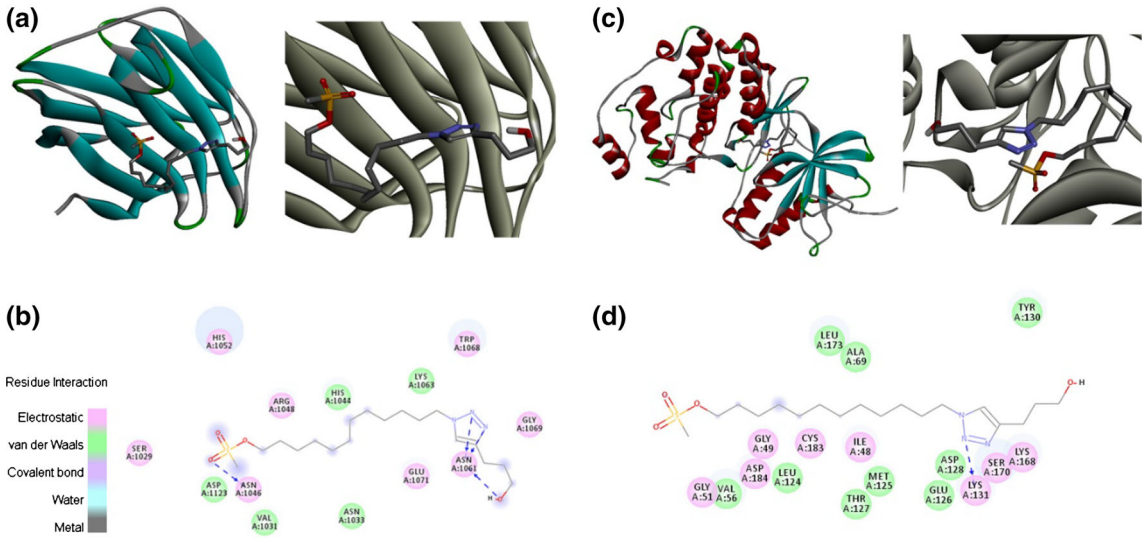


Fig. 3 3D and 2D diagrams of the intermolecular interactions between **5e** and galectin-1 and ERK1. **a** 3D diagram of **5e** and galectin-1 (PDB: 1W6M). **b** Intermolecular interactions between **5e** and galectin-1 (PDB: 1W6M): electrostatic interactions, hydrogen bonds (ASN A:1046), and van der Waals interactions (ASP A:1123) with the mesyl group. The triazole ring and hydroxyl group form electrostatic interactions and hydrogen bonds (ASN A:1061). **c** 3D diagram of **5e** and ERK1 (PDB: 2ZOQ). **d** Intermolecular interactions between **5e** and ERK1 (PDB: 2ZOQ): the triazole ring forms electrostatic interactions and hydrogen bonds (LYS A:131), electrostatic interactions (SER A:170 e LYS A:168), and van der Waals interactions (ASP A:128)

agents, and deserves further investigation of derived scaffolds.

Acknowledgments The authors thank Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support. We further thank Pró-Reitoria de Pesquisa (UFMG) for the financial aid to publish the results.

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