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FULL-LENGTH PAPER

Design, synthesis, in vitro cytotoxic activity evaluation, and apoptosis-induction study of new 9(10*H*)-acridinone-1,2,3-triazoles

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Abstract A new series of 9(10*H*)-acridinone-1,2,3-triazole derivatives were designed, synthesized and evaluated for their cytotoxic activity against human breast cancer cell lines. The acridone skeleton was prepared through the Ullman condensation of 2-bromobenzoic acid and anilines. Subsequently, it was functionalized with propargyl bromide. Then, a click reaction of the latter compound and in situ prepared 1-(azidomethyl)-4-methoxybenzene derivatives led to the formation of the desired triazole products. Finally, all products were investigated for their capability to cause cytotoxicity against MCF-7, T-47D, and MDA-MB-231 cell lines. Among them, 2-methoxy-10-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acridin-9(10*H*)-one **8c** exhibited the most potency ($IC_{50} = 11.0 \pm 4.8\ \mu M$) against MCF-7 cells, being more potent than etoposide ($IC_{50} = 12.4 \pm 4.7\ \mu M$). Also, apoptosis induced by compound **8c** was confirmed via acridine orange/ethidium bromide and Annexin V-FITC/propidium iodide (PI) double staining.

Keywords Acridone-1,2,3-triazoles · Cytotoxic activity · Click chemistry · Breast cancer

Introduction

Cancer is characterized by uncontrolled cell growth leading to 15 % of human deaths worldwide [1]. Apoptosis is a programmed cell death process by which the body eliminates damaged or unnecessary cells. This is a biologically important phenomenon playing a vital role in cancer development and tumor cell survival [2]. It has been proven that dysregulation of apoptosis leads to cancer and tumor progression [3,4]. Killing tumor cells using chemotherapeutic methods is usually accomplished through the induction of apoptosis which does not destroy the organism. Hence, apoptosis inducers have been widely studied as a versatile platform for cancer therapy in medicinal chemistry [5–9].

Acridone is an important heterocyclic scaffold and both synthetic and naturally occurring derivatives have shown various valuable biological properties [10]. Acridones can be considered as 10-aza-analogs of anthrones [11] or xanthones [12] from a structural point of view. A wide range of acridone derivatives has been evaluated in vitro and in vivo to explore novel anticancer agents [13–16]. Recently, acridone-based anticancer agents that target DNA [17], topoisomerases [18], telomerase [19], multidrug resistance [13], and apoptotic inducer [20] have been reported in the literature. Furthermore, the 1,2,3-triazole ring has received much attention in both chemistry and biology due to its high dipole moment [21], metabolic stability, and capability to form hydrogen bonds [22]. Also, it can be considered an amide bioisos-

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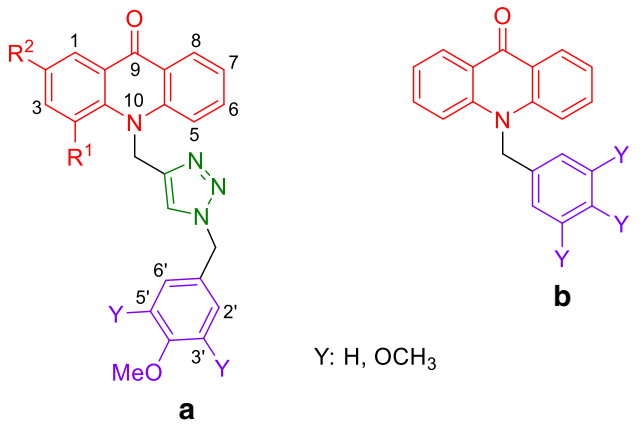


Fig. 1 Structure of designed (**a**) and reported (**b**) anticancer agents

teric replacement [23]. The introduction of click chemistry by Sharpless and co-workers provided an efficient method for the construction of the 1,2,3-triazole ring and drug discovery investigations [24,25]. Their antibacterial [26], antifungal [27], antitubercular [28], and anti-HIV [29] activities have been well documented. Also, extensive attention has been devoted to their anticancer activities [30,31].

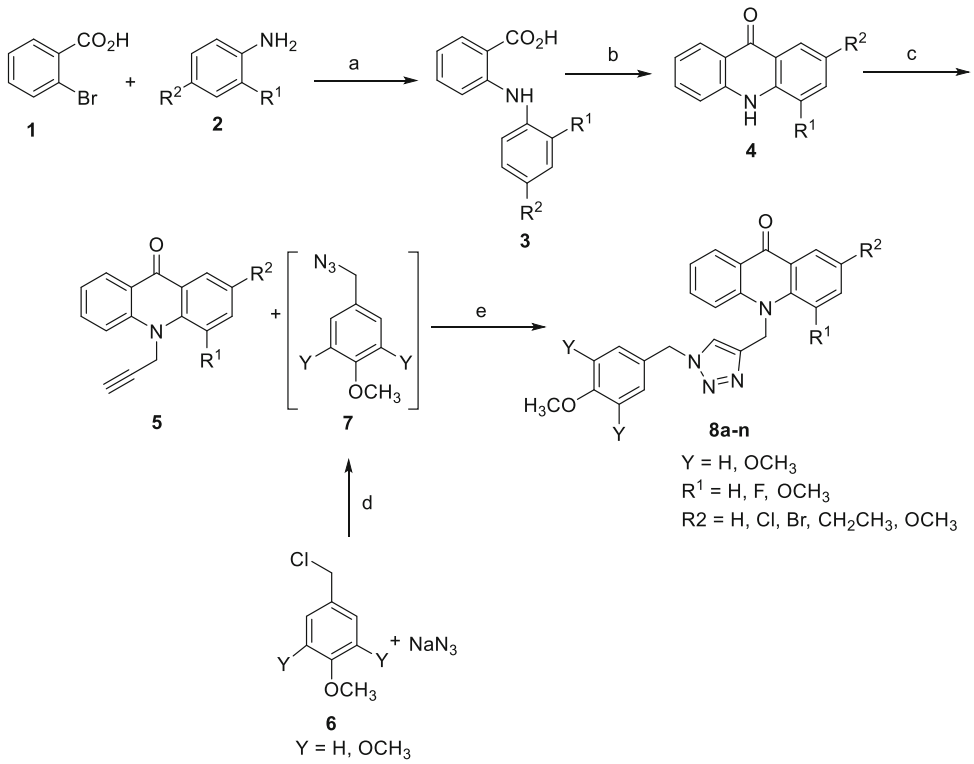
Considering the many adverse effects and development of tumor resistance to a wide variety of anticancer agents [32], there is a big demand for novel and efficient anticancer agents. Herein, in continuation of our studies on the development of new anticancer agents [33–36] and focusing on the versatile biological activity of acridone-1,2,3-triazole system [36], we designed, synthesized, and evaluated novel 9(10*H*)-acridinone-1,2,3-triazole hybrids (**A**, Fig. 1) against human breast cancer cell lines.

Results and discussion

Chemistry

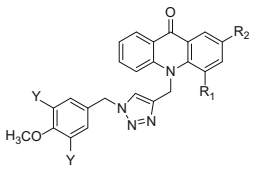
Exploring novel 9(10*H*)-acridinone-1,2,3-triazole hybrids we focused on 10-benzyl-9(10*H*)-acridinone derivatives reported by Gao et al. (**B**, Fig. 1) [20]. Their study revealed that a benzyl group bearing methoxy substituents showed better antileukemic activitiy and, among them, 10-(3,5-dimethoxy)benzyl-9(10*H*)-acridinone was the most potent derivative with an IC_{50} of about 0.7 μM. Considering the anticancer activity of the 1,2,3-triazole skeleton [30,31], we decided to combine the 9(10*H*)-acridinone and 1,2,3-triazole scaffolds and investigate the cytotoxicity profile of the corresponding products **8a–n** (Scheme 1).

The synthesis of 9(10*H*)-acridinone-1,2,3-triazole hybrids **8a–n** is described in Scheme 1. At first, the Ullman condensation reaction of 2-bromobenzoic acid **1** with various aniline derivatives **2** in the presence of potassium carbonate (K_2CO_3) and copper in EtOH under reflux conditions gave 2-arylamino benzoic acids **3** [37]. Then, the cyclization of compound **3** in the presence of polyphosphoric acid (PPA) at 100 °C afforded acridones **4** [38] which were reacted with



Scheme 1 Synthesis of compounds **8a–n**. Reagents and conditions: **a** K_2CO_3, Cu, EtOH, reflux, 7 h; **b** PPA, 100 °C , 3 h; **c** propargyl bromide, KO*t*Bu, DMSO, rt, 3 h; **d** NEt_3, H_2O/*t*-BuOH (1:1), rt **e** CuI, 24–56 h

Table 1 Cytotoxic activity (IC_{50}, μM) of compounds **8a–n**



Entry	R^1	R^2	Y	Cytotoxicity (IC_{50} μM)[a]		
				MCF-7	T-47D	MD-MB-231
8a	H	H	H	>100	48.4 ± 4.3	18.8±5.7
8b	H	Cl	H	>100	>100	>100
8c	H	OCH_3	H	11.0 ± 4.8	14.5 ± 5.2	16.6 ± 5.9
8d	H	Br	H	>100	>100	>100
8e	H	CH_2CH_3	H	22.0 ± 4.2	88.5 ± 4.7	34.4 ± 5.3
8f	F	H	H	>100	>100	>100
8g	OCH_3	H	H	60.0 ± 4.6	50.0 ± 5.5	22.6 ± 4.2
8h	H	H	OCH_3	>100	>100	>100
8i	H	Cl	OCH_3	>100	>100	>100
8j	H	OCH_3	OCH_3	>100	>100	>100
8k	H	Br	OCH_3	>100	>100	>100
8l	H	CH_2CH_3	OCH_3	>100	>100	>100
8m	F	H	OCH_3	>100	>100	>100
8n	OCH_3	H	OCH_3	>100	>100	>100
Etoposide				12.4 ± 4.7	11.8 ± 5.8	15.7 ± 5.4

[a] Values are the mean ± SD. All experiments were performed at least three times

propargyl bromide using potassium *tert*-butoxide in DMSO at room temperature to obtain 10-(prop-2-yn-1-yl)acridin-9-one derivatives **5**. Using the click methodology described by Sharpless et al. [24], the reaction of compound **5** and in situ prepared 1-(azidomethyl)-4-methoxybenzene derivative **7** led to the formation of 9(10*H*)-acridinone-1,2,3-triazole derivatives **8a–n**. To obtain compound **7**, 4-methoxy-benzyl chloride derivative **6** and sodium azide reacted in the presence of Et_3N in H_2O/*t*-BuOH (1:1) at ambient temperature for 1 h. Then, the mixture of compound **5** and CuI was added to the mixture containing **7** and the reaction was conducted at room temperature for 24–56 h to give the 9(10*H*)-acridinone-1,2,3-triazole **8a–n** in good yields.

Biological study

Cytotoxicity assay

The cytotoxicity of products **8a–n** was evaluated in vitro against three human breast cancer cell lines comprising MCF-7, T-47D, and MDA-MB-231 using the MTT tetrazolium salt assay [39]. The calculated IC_{50} values of compounds **8a–n** as well as etoposide (standard drug) are listed in Table 1. Compounds **8c**, being the most potent compound against MCF-7 (IC_{50} = 11.0 μM), showed slightly better activity than etoposide (IC_{50} = 12.4 ± 4.8 μM). However, IC_{50} values against MDA-MB-231 and T-47D were 16.6 ± 5.9 and 14.5 ± 5.22 μM, respectively.

Compounds **8a–n** could be categorized into two groups considering the number of methoxy substituents on the pendant benzyl group of 1,2,3-triazol ring: (i) mono-methoxy (compounds **8a–g**) and (ii) tri-methoxy (compounds 8**h–n**). According to our results in Table 1, the first group (**8a–g**) exhibited IC_{50} = 11.0 − 60.0 μM while the second group (**8h–n**) did not show activity against breast cancer cell lines (IC_{50} > 100 μM). It may relate to the compounds which were not able to enter the cell.

As can be seen in Table 1, the kind of substituents as well as their positions on the acridone moiety of compounds **8a–g** play important role in the obtained cytotoxicity. Compound **8a** without substituents on the acridone ring showed no activity against MCF-7 (IC_{50} > 100 μM); however, it showed moderate activity against T-47D (IC_{50} = 48.4 ± 4.3 μM) and MB-231(IC_{50} = 18.8 ± 5.7 μM), respectively. Our results revealed that the presence of a halogen group (F, Cl, and Br) at any position on the acridone moiety led to loss of cytotoxic activity as **8b**, **8d**, and **8f** were not active against all cell lines. From the mono-methoxy group (compounds **8a–g**), compound **8c** (methoxy group at 2-postion) showed the best activity in all three cell lines especially MCF-7

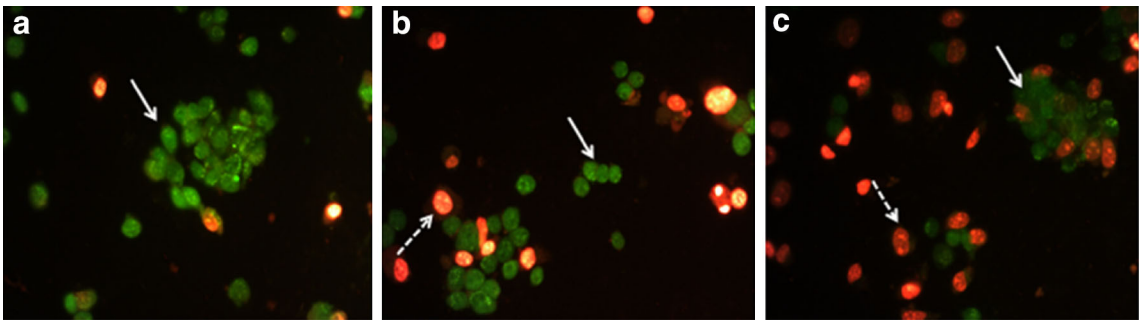


Fig. 2 Morphological analysis of MCF-7 cells by acridine orange/ethidium bromide double staining: **a** control condition, **b** after exposure to etoposide for 24 h, **c** cells treated with compound **8c** for 24 h. *White arrows* indicate live cells, *dashed arrows* show apoptosis

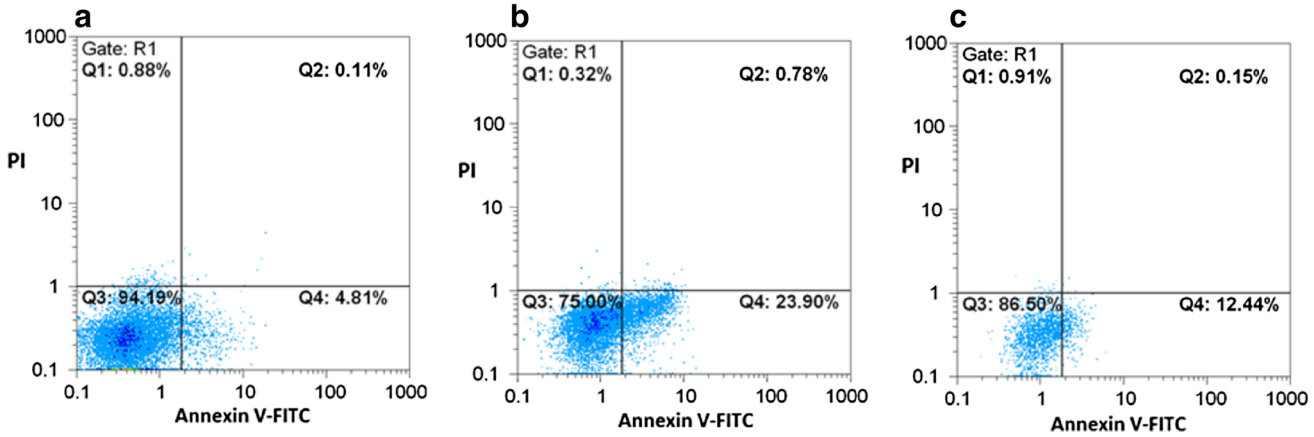


Fig. 3 Flow cytometric analysis of MCF-7 cells treated with synthetic compound **8c**. Cells were stained with Annexin V-FITC/PI and quantitated by flow cytometry. The cells treated with **a** DMSO 1 % (negative control), **b** IC_{50} values of compound **8c** for 12 h, **c** IC_{50} values of etoposide as positive control for 12 h

($IC_{50} = 11.0 \pm 4.8\,\mu M$). However, introducing the methoxy group at the 4-position of acridone and also presence of an ethyl substituent at the 2-position of the acridone decreased anticancer activity (Table 1, **8g** and **8e**, respectively).

Compounds **8c** and **8e** having the methoxy and ethyl group at the 2-position of acridone ring, respectively, exhibited selective activity on MCF-7 cell. Compounds **8a** and **8g** having no substitution at the 2-position of acridone moiety showed selective inhibition of MDA-MB-231 cell growth.

Acridine orange/ethidium bromide double staining

The most active compound **8c** was selected to determine its potential to induce apoptosis in MCF-7 cells morphologically by acridine orange/ethidiumbromide (AO/EB) double staining test [40] (Fig. 2). Living cells have a normal green nucleus, but apoptotic cells show orange-stained nuclei with chromatin condensation or fragmentation. Analysis of the AO/EB staining revealed that compound **8c** clearly can be inducer of apoptosis because the appearance of chromatin condensation and nuclear fragmentation are evident in Fig. 2.

Annexin V-FITC and propidium iodide (PI) double staining

Apoptosis induction for compound **8c** was further confirmed by flow cytometry analysis [41] (Fig. 3). As shown in Fig. 3, compound **8c** induced 12.44 % apoptosis in the cancer cells. Therefore, it is evident that the cytotoxicity of this compound is related to inducing apoptosis in cancer cell lines.

Conclusion

In conclusion, novel 9(10*H*)-acridinone-1,2,3-triazole hybrids were designed, synthesized, and evaluated as cytotoxic and apoptosis-inducing agents. The preliminary in vitro anti-proliferative activity test was done against MCF-7, T-47D, and MDA-MB-231 cells using MTT assay. Among them, 2-methoxy-10-((1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acridin-9(10*H*)-one **8c** with IC_{50} value of $11.0 \pm 4.8\,\mu M$ was more potent than etoposide ($IC_{50} = 12.4 \pm 4.7\,\mu M$) against MCF-7 cells. Also, AO/EB staining and flow cytometry analysis using Annexin V- FITC/PI double staining showed that compound **8c** can induce apoptosis.

Experimental

Chemistry

Melting points are uncorrected and were measured using a Kofler hot stage apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-500, using TMS as an internal standard and DMSO as a solvent. Chemical shifts are expressed as δ (ppm). IR spectra were recorded on a Nicolet Magna FTIR 550 spectrophotometer using KBr disks. Mass spectra were recorded on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis for C, H, and N was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode.

2-Arylamino benzoic acids **3**, acridone derivatives **4**, and 10-(prop-2-yn-1-yl)acridin-9-ones **5** were prepared according to our recent report [36].

General procedure for the synthesis of 9(10H)-acridinone-1,2,3-triazole derivatives **8**

A mixture 4-methoxy benzyl chloride derivative **6** (1.1 mmol), sodium azide (0.9 mmol), and Et_3N (1.3 mmol) in water (4 mL) and *t*-BuOH (4 mL) was stirred at room temperature for 1 h. Then, a mixture of 10-(prop-2-yn-1-yl)acridin-9-one derivative **5** (1 mmol) and CuI (7 mol%) was added to prepared azide derivative **7** and the reaction mixture was stirred at room temperature for 24–56 h. After completion of the reaction (monitored by TLC), the reaction mixture was diluted with water, poured onto ice, and the precipitate was filtered off, washed with cold water, and purified by flash chromatography on silica gel using petroleum ether/ ethyl acetate (4:1) to afford desire product.

10-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl) acridin-9(10H)-one **(8a)**

Yellow crystals; yield: 84 %, mp 164–166 °C. IR (KBr): 3080, 2956, 2853, 1743, 1632, 1598cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.37 (d, J = 8.0 Hz, 2H, H_1, H_8), 8.17 (s, 1H, triazole), 7.78 (t, J = 8.0 Hz, 2H, H_3, H_6), 7.69 (d, J = 8.0 Hz, 2H, H_4, H_5), 7.35 (t, J = 8.0 Hz, 2H, H_2, H_7), 7.14 (d, J = 8.4 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.91 (d, J = 8.4 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.75 (s, 2H, CH_2), 5.55 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3). Anal. Calcd for $C_{24}H_{20}N_4O_2$: C, 72.71; H, 5.09; N, 14.13. Found: C, 72.93; H, 4.96; N, 13.96.

2-Chloro-10-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8b)**

Yellow crystals; yield: 79 %, mp 234–235 °C. IR (KBr): 3080, 2959, 2843, 1742, 1629, 1595, 1489 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.34 (d, J = 7.5 Hz, 1H, H_8), 8.25 (s, 1H, H_1), 8.17 (s, 1H, triazole), 8.00 (d, J = 8.7 Hz, 1H, H_3), 7.94 (d, J = 8.7 Hz, 1H, H_4), 7.83–7.82 (m, 2H, H_5, H_6), 7.36 (t, J = 7.5 Hz, 1H, H_7), 7.22 (d, J = 7.8 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.89 (d, J = 7.8 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.79 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 175.6, 159.1, 142.4, 141.6, 140.4, 134.5, 133.8, 129.5, 127.7, 126.6, 126.1, 125.2, 123.1, 122.5, 121.9, 121.5, 119.1, 116.4, 114.1, 55.1, 52.4, 41.8. MS m/z (%) 432 ([$M^{+\cdot}$ + 2], 2), 430 ($M^{+\cdot}$6), 228 (9), 200 (10), 174 (12), 121 (100), 78 (11). Anal. Calcd for $C_{24}H_{19}ClN_4O_2$: C, 66.90; H, 4.44; N, 13.00. Found: C, 67.11; H, 4.23; N, 12.91.

2-Methoxy-10-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8c)**

Yellow crystals; yield: 88 %, mp 177–179 °C. IR (KBr): 2934, 2834, 1742, 1617, 1595, 1498 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.36 (dd, J = 8.0, 1.1 Hz, 1H, H_8), 8.14 (s, 1H, triazole), 7.93–7.91 (m, 2H, H_4, H_5), 7.78–7.75 (m, 2H, H_1, H_6), 7.45 (dd, J = 9.4, 3.3 Hz, 1H, H_3), 7.31 (t, J = 8.0 Hz, 1H, H_7), 7.24 (d, J = 8.6 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.88(d, J = 8.6 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.77 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3).^{13}C NMR (125 MHz, DMSO-d_6): 176.0, 159.1, 154.1, 142.8, 141.6, 136.5, 133.8, 129.5, 127.8, 126.6, 123.9, 123.0, 122.4, 121.1, 120.9, 118.2, 116.0, 114.0, 106.1, 55.4, 55.0, 52.3, 41.6. Anal. Calcd for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.21; N, 13.14. Found: C, 70.59; H, 5.08; N, 13.02.

2-Bromo-10-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8d)**

Yellow crystals; yield: 76 %, mp 237–239 °C. IR (KBr): 2922, 2849, 1735, 1632, 1598 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.41 (d, J = 2.0 Hz, 1H, H_1), 8.35 (d, J = 7.5 Hz, 1H, H_8), 8.26 (s, 1H, triazole), 8.92 (dd, J = 9.0, 2.0 Hz, 1H, H_3), 7.81 (t, J = 7.5 Hz, 1H, H_6), 7.72 (d, J = 9.0 Hz, 1H, H_4), 7.68 (d, J = 7.5 Hz, 1H, H_5), 7.37 (t, J = 7.5 Hz, 1H, H_7), 7.22 (d, J = 8.4 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.89 (d, J = 8.4 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.77 (s, 2H, CH_2), 5.54 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 175.6, 159.0, 142.1, 141.3, 140.3, 134.4, 133.8, 128.9, 128.4, 127.0, 126.6, 125.5, 123.4, 123.0, 122.0, 121.6, 119.4, 116.5, 114.1, 55.1, 52.7, 42.8. Anal. Calcd for $C_{24}H_{19}BrN_4O_2$: C, 60.64; H, 4.03; N, 11.79. Found: C, 60.81; H, 4.14; N, 11.63.

2-Ethyl-10-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8e)**

Pale yellow crystals; yield: 79 %, mp 193–195 °C. IR (KBr): 3071, 2928, 1735, 1635, 1598 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.35 (d, J = 7.5 Hz, 1H, H_8), 8.17 (m, 2H, H_1, triazole), 7.91 (d, J = 7.5 Hz, 1H, H_5), 7.86 (d, J = 8.8 Hz,

1H, H_4), 7.78 (t, J = 7.5 Hz, 1H, H_6), 7.67 (dd, J = 8.8, 1.8 Hz, 1H, H_3), 7.32 (t, J = 7.5 Hz, 1H, H_7), 7.25 (d, J = 7.7 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.86 (d, J = 7.7 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.75 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 3.72 (s, 3H, OCH_3), 2.73 (q, J = 7.5 Hz, 2H, CH_2), 1.30 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 176.4, 159.1, 142.8, 141.6, 140.1, 136.9, 134.4, 133.9, 131.2, 129.5, 127.8, 126.6, 124.5, 123.0, 121.6, 121.2, 116.4, 116.1, 114.0, 55.1, 52.3, 41.5, 27.3, 15.5. Anal. Calcd for $C_{26}H_{24}N_4O_2$: C, 73.56; H, 5.70; N, 13.20. Found: C, 73.74; H, 5.58; N, 13.03.

4-Fluoro-10-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8f)**

White crystals; yield: 71 %, mp 132–133 °C. IR (KBr): 3150, 2937, 1738, 1638, 1608 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.29 (d, J = 7.3 Hz, 1H, H_8), 8.18–8.16 (m, 2H, H_1, triazole), 7.81–7.76 (m, 2H, H_5, H_6), 7.67 (ddd, J = 15.2, 7.6, 1.3'Hz, 1H, H_3), 7.36–7.29 (m, 2H, H_2, H_7), 7.24 (d, J = 8.6 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.89 (d, J = 8.6 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.69 (s, 2H, CH_2), 5.47(s, 2H, CH_2), 3.66 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 176.0, 159.0, 151.3 (d, J_{C-F} = 243.9 Hz), 144.2, 143.5, 134.5, 132.2, 129.4, 127.8, 126.4, 124.9, 122.9, 122.7, 122.2, 121.9 (d, J_{C-F} = 7.6 Hz), 121.8, 121.1 (d, J_{C-F} = 23.4 Hz), 116.6, 114.0, 55.1, 52.3, 47.1 (d, J_{C-F} = 14.8 Hz). Anal. Calcd for $C_{24}H_{19}FN_4O_2$: C, 69.55; H, 4.62; N, 13.52. Found: C, 69.73; H, 4.51; N, 13.35.

4-Methoxy-10-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8g)**

Pale yellow crystals; yield: 69 %, mp 134–135 °C. IR (KBr): 3120, 2922, 1735, 1629, 1598 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.24 (dd, J = 8.0, 1.4 Hz, 1H, H_8), 8.03 (s, 1H, triazole), 7.92 (dd, J = 7.6, 1.0 Hz, 1H, H_1), 7.79–7.72 (m, 2H, H_5, H_6), 7.43 (dd, J = 7.6, 1.0 Hz, 1H, H_3), 7.32–7.29 (m, 2H, H_2, H_7), 7.18 (d, J = 7.8 Hz, 2H, $H_{2'}$, $H_{6'}$), 6.80 (d, J = 7.8 Hz, 2H, $H_{3'}$, $H_{5'}$), 5.73 (s, 2H, CH_2), 5.51 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3). Anal. Calcd for $C_{25}H_{22}N_4O_3$: C, 70.41; H, 5.20; N, 13.14. Found: C, 70.68; H, 5.04; N, 12.94.

10-((1-(3,4,5-Trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one ***(8h)***

White crystals; yield: 68 %, mp 232–234 °C . IR (KBr): 3114, 2937, 1745, 1631, 1603 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.24 (dd, J = 7.5 Hz, 1.3 Hz, 2H, H_1, H_8), 8.22 (s, 1H, triazole), 7.94 (d, J = 7.5 Hz, 2H, H_4, H_5), 7.82–7.78 (t, J = 7.5 Hz, 2H, 2H, H_3, H_6), 7.35 (t, J = 7.5 Hz, 2H, H_2, H_7), 6.56 (s, 2H, $H_{2'}$, $H_{6'}$), 5.81 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 3.70 (s, 6H, OCH_3), 3.69 (s, 3H, OCH_3). Anal. Calcd for $C_{26}H_{24}N_4O_4$: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.59; H, 5.18; N, 12.13.

2-Chloro-10-((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8i)**

Yellow crystals; yield: 63 %, mp 244–245 °C. IR (KBr): 3114, 2953, 1787, 16031, 1594 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.35 (d, J = 7.5 Hz, 1H, H_8), 8.26 (d, J = 2.3 Hz, 1H, H_1), 8.22 (s, 1H, triazole), 8.01 (d, J = 9.0 Hz, 1H, H_3), 7.96 (d, J = 9.0 Hz, 1H, H_4), 7.83 (m, 2H, H_5, H_6), 7.37 (t, J = 7.5 Hz, 1H, H_7), 6.56 (s, 2H, $H_{2'}$, $H_{6'}$), 5.82 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 3.66 (s, 6H, OCH_3), 3.61(s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 175.6, 152.9, 142.5, 141.6, 140.4, 137.2, 134.5, 133.8, 131.3, 126.5, 126.1, 125.2, 123.5, 122.6, 121.9, 121.6, 119.1, 116.4, 105.3, 59.9, 55.7, 53.0, 41.7. Anal. Calcd for $C_{26}H_{23}ClN_4O_4$: C, 63.61; H, 4.72; N, 11.41. Found: C, 63.77; H, 4.58; N, 11.58.

2-Methoxy-10-((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8j)**

Yellow crystals; yield: 64 %, mp 193–194 °C. IR (KBr): 3112, 2931, 1735, 1631, 1594 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.36 (d, J = 7.5 Hz, 1H, H_8), 8.19 (s, 1H, triazole), 7.96–7.93 (m, 2H, H_4, H_5), 7.79–7.77 (m, 2H, H_1, H_6), 7.46 (dd, J = 9.2, 3.0 Hz, 1H, H_3), 7.32 (t, J = 7.5 Hz, 1H, H_7), 6.59 (s, 2H, $H_{2'}$, $H_{6'}$), 5.82 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3), 3.65 (s, 6H, OCH_3), 3.61 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 176.0, 154.1, 152.9, 142.8, 141.3, 137.1, 136.5, 133.8, 131.4, 126.6, 123.9, 123.4, 122.4, 121.1, 120.9, 118.3, 116.1, 106.2, 105.2, 59.9, 55.7, 55.4, 52.9, 41.6. Anal. Calcd for $C_{27}H_{26}N_4O_5$: C, 66.66; H, 5.39; N, 11.52. Found: C, 66.83; H, 5.21; N, 11.35.

2-Bromo-10-((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8k)**

Pale yellow crystals; yield: 62 %, mp 246–248 °C . IR (KBr): 3115, 2931, 1751, 1627, 1595 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 8.41 (s, 1H, H_1), 8.35 (d, J = 7.8 Hz, 1H, H_8), 8.22 (s, 1H, triazole), 7.97–7.95 (m, 3H, H_3, H_4, H_5), 7.84–7.81 (t, J = 7.8 Hz, 1H, H_6), 7.38 (t, J = 7.8 Hz, 1H, H_7), 6.56 (s, 2H, $H_{2'}$, $H_{6'}$), 5.82 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 3.66 (s, 6H, OCH_3), 3.61 (s, 3H, OCH_3). MS m/z (%) 537 ([$M^{+\cdot}$ + 2], 10), 535 ($M^{+\cdot}$10), 534 (29), 274 (13), 244 (25), 228 (32), 203 (19), 181 (100), 165 (27), 148 (29), 135 (26), 121 (50), 106 (94), 91 (51), 77 (46), 51 (23). Anal. Calcd for $C_{26}H_{23}$ Br N_4O_4: C, 58.33; H, 4.33; N, 10.46. Found: C, 58.51; H, 4.18; N, 10.29.

2-Ethyl-10-((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8l)**

Yellow crystals; yield: 67 %, mp 197–198 °C. IR (KBr): 3111, 2925, 1735, 1639, 1594 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.36 (dd, J = 8.0, 1.8 Hz, 1H, H_8), 8.18–8.17 (m, 2H, H_1, triazole), 7.93 (d,J = 8.0 Hz, 1H, H_5), 7.88 (d,J = 9.0 Hz, 1H, H_4), 7.80–7.76 (td, J = 8.0, 1.8 Hz, 1H, H_6), 7.67 (dd, J = 9.0, 2.2 Hz, 1H, H_3), 7.33 (t, J = 8.0 Hz, 1H, H_7), 6.55 (s, 2H, $H_{2'}$, $H_{6'}$), 5.80 (s, 2H, CH_2), 5.44 (s, 2H, CH_2), 3.65 (s, 6H, OCH_3), 3.60 (s, 3H, OCH_3), 2.73 (q, J = 7.5 Hz, 2H, CH_2), 1.24 (t, J = 7.5 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 176.7, 152.9, 142.8, 141.6, 140.1, 136.9, 134.4, 133.9, 131.4, 126.6, 125.4, 124.5, 123.4, 122.8, 121.6, 121.2, 116.4, 116.1, 105.2, 59.9, 55.7, 52.9, 41.5, 27.3, 15.5. Anal. Calcd for $C_{28}H_{28}N_4O_4$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.31; H, 5.91; N, 11.32.

*4-Fluoro-10-((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one (***8m)**

Yellow crystals; yield: 62 %, mp >250 °C . IR (KBr): 3120, 2938, 1742, 1639, 1597 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.31 (d, J = 7.7 Hz, 1H, H_8), 8.23 (s, 1H, triazole), 8.19 (d, J = 7.6 Hz, 1H, H_1), 7.79–7.78 (m, 2H, H_5, H_6), 7.68 (ddd, J = 15.3, 7.6, 1.1 Hz, 1H, H_3), 7.38–7.31 (m, 2H, H_2, H_7), 6.52 (s, 2H, $H_{2'}$, $H_{6'}$), 5.73 (s, 2H, CH_2), 5.47(s, 2H, CH_2), 3.64 (s, 6H, OCH_3), 3.61 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 179.1, 152.9, 151.4 (d, J_{C-F} = 254.2 Hz), 144.4, 143.5, 134.5, 132.2, 131.5, 126.4, 124.9, 123.3, 122.7, 122.2, 121.9 (d, J_{C-F} = 18.9 Hz), 121.8, 121.2, 121.0, 116.6, 105.1, 59.9, 55.7, 52.9, 47.1 (d, J_{C-F} = 14.8 Hz). Anal. Calcd for $C_{26}H_{23}FN_4O_4$: C, 65.81; H, 4.89; N, 11.81. Found: C, 65.64; H, 5.07; N, 11.98.

4-Methoxy-10-((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)acridin-9(10H)-one **(8n)**

Yellow crystals; yield: 61 %, mp 182–184 °C . IR (KBr): 3113, 2945, 1732, 1623, 1598 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 8.23 (d, J = 7.5 Hz, 1H, H_8), 8.05 (s, 1H, triazole), 7.92 (d, J = 7.5 Hz, 1H, H_1), 7.79–7.72 (m, 2H, H_5, H_6), 7.42 (d, J = 7.5 Hz, 1H, H_3), 7.29 (t, J = 7.5 Hz, 2H, H_2, H_7), 6.57 (s, 2H, $H_{2'}$, $H_{6'}$), 5.74 (s, 2H, CH_2), 5.45 (s, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.68 (s, 6H, OCH_3), 3.62 (s, 3H, OCH_3). ^{13}C NMR (125 MHz, DMSO-d_6): 176.9, 152.9, 149.7, 145.5, 144.8, 137.2, 134.4, 133.9, 131.6, 126.1, 124.8, 123.4, 122.4, 122.2, 121.7, 118.1, 117.3, 116.3, 105.2, 59.94, 56.4, 55.8, 52.9, 48.4. Anal. Calcd for $C_{27}H_{26}N_4O_5$: C, 66.65; H, 5.39; N, 11.52. Found: C, 66.76; H, 5.22; N, 11.39.

Biological assays

Cell culture

MCF-7, T-47D and MDA-MB-231 were purchased from the National Cell Bank of Iran (NCBI). The cells were cultured in RPMI 1640 medium supplemented with 10 % heat-inactivated fetal calf serum (from GibcoeBRL, UK) and 100 mg/mL streptomycin and 100 U/mL penicillin at 37 °C in 5 % CO_2-humidified atmosphere.

In vitro cytotoxicity assay

Three different breast cancer cell lines (MCF-7, T-47D and MDA-MB-23) (5×10^4 cells/mL in 96-well culture plates) were incubated for 48 h with different concentrations of compounds **8a–n** dissolved in DMSO (the final volume of DMSO/medium was less than 1 % in all experiments). Etoposide and DMSO were used as positive and negative controls, respectively. After treatment, the medium was removed and 200 μL of a phenol red-free medium containing MTT (1 mg/mL, final concentration) was added to all wells. After 4 h of incubation, the culture medium was replaced with 100 μL of DMSO to each well. The absorbance was measured at 492 nm with a multi-well plate reader (Gen5, Power wave xs2, BioTek, America). All experiments were performed at least three times and the IC_{50} values for all compounds were calculated by nonlinear regression analysis and expressed in mean ± SD compared with the control [39].

AO/EB staining method

MCF-7 cell grown in 6-well plates (3×10^5 cells/well) were treated with and without compound **8**c for 24 h. Plates were washed three times by phosphate buffered saline (PBS) and 9 μL of cell suspension was stained with 1 μL of dye mixture (100 mg/mL AO and 100 mg/mL EB in PBS). 10 μL of stained cell suspension were placed on a clean microscope slide and covered with a coverslip and examined by fluorescence microscope (Axoscope 2 plus, Zeiss, Germany) [40].

Flow cytometric analysis of apoptosis

Annexin V-FITC/propidium iodide (PI) double staining test was performed using an Annexin-V- FITC kit (Biovision) as described in protocol. The MCF-7 cells were treated with IC_{50} concentrations of the compound **8c**, etoposide and DMSO 1 %. After 24 h incubation, the cells (5×10^5 cells) were collected and washed twice with cold PBS and resuspended in 500 μl of 1X binding buffer. Then, the cells were double stained with 5 μL of Annexin V-FITC and 5 μL of PI solution. Finally, the samples were incubated for 5 min at room temperature and then analyzed by flow cytometry [41].

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