Synthesis and in vitro antitumor activity of \(1E,4E\)-1-aryl-5-(2-((quinazolin-4-yl)oxy)phenyl)-1,4-pentadien-3-one derivatives

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Abstract

Background: Cancer is one of the leading causes of death and only second to heart diseases. Recently, preclinical studies have demonstrated that curcumin had a number of anticancer properties. Thus, we planned to synthesize a series of curcumin analogs to assess their antiproliferation efficacy.

Results: A series of \(1E,4E\)-1-aryl-5-(2-((quinazolin-4-yl)oxy)phenyl)-1,4-pentadien-3-one derivatives (curcumin analogs) were synthesized and characterized by IR, NMR, and elemental analysis techniques. All of the prepared compounds were screened for antitumor activities against MGC-803, PC3, and Bcap-37 cancer cell lines. A significant inhibition for cancer cells were observed with compound \(5f\) and also less toxic on NIH3T3 normal cells. The mechanism of cell death induced by compound \(5f\) was further investigated by acridine orange/ethidium bromide staining, Hoechst 33,258 staining, TUNEL assay, and flow cytometry, which revealed that the compound can induce cell apoptosis in MGC-803 cells.

Conclusions: This study suggests that most of the derivatives could inhibit the growth of human cancer cell lines. In addition, compound \(5f\) could induce apoptosis of cancer cells, and it should be subjected to further investigation as a potential anticancer drug candidate.

Keywords: Synthesis, Asymmetric curcumin analogs, Quinazoline derivatives of curcumin, Antitumor activity, MGC-803, Apoptosis

Background

Cancer is one of the leading causes of death and only second to heart diseases \([1, 2]\). The efficacy of current chemotherapeutics is low and undesirable side effects are still unacceptably high \([3–5]\). Hence, the development of novel, and less toxic and anti-cancer agents remains an important and challenging goal of medicinal chemist worldwide, and much attention has recently been paid to the discovery and development of new, more selective anticancer agents \([3, 6–8]\).

Natural products have become a leading category of compounds in improving the rational drug design for novel anti-cancer therapeutics \([9, 10]\). Curcumin is a natural phenolic compound originally isolated from turmeric, a rhizome used in India for centuries as a spice and medicinal agent \([11]\). A literature survey reveals that curcumin, and its derivatives (analogs) have various pharmacological activities and medicinal applications such as antioxidant \([12, 13]\), anti-inflammatory \([12, 14]\), anti-HIV \([15, 16]\), anti-angiogenesis and so on \([12]\). Recently, preclinical studies have demonstrated that curcumin had a number of anticancer properties, such as growth inhibition and induction of apoptosis in a variety of cancer cell lines \([17–19]\). Its mechanisms of action include inhibition of transcriptional factor NF-\(\kappa\)B, HSP90 and epigenetic modulation related to direct inhibition of the catalytic site of DNMT-1 \([20]\). Moreover, the latest research shows that curcumin can effectively suppress NF-\(\kappa\)B activity and COX-2 expression, as well as cell proliferation/survival in...
the setting of NSCLC [21]. Consequently, analogues of curcumin with similar safety profiles but increased anticancer activity have been developed in recent years [22]. Chandru et al. synthesized four novel dienone cyclopropoxy curcumin analogs by nucleophilic substitution reaction with cyclopropyl bromide, and found that the tumor growth inhibitory effects of synthetic dienone cyclopropoxy curcumin analogs could be mediated by promoting apoptosis and inhibiting tumor angiogenesis [23]. New 1,5-diaryl-1,4-pentadien-3-one derivatives (curcumin analogs), which can effectively inhibit proliferation of cancer cells at very low concentrations, were synthesized [24, 25], and we also found that curcumin analogs exhibited promising ex vivo antiviral bioactivities against tobacco mosaic virus and cucumber mosaic virus [26].

In order to discover more potent and selective anticancer agents based on curcumin scaffold, we have synthesized a series of (1E,4E)-1-aryl-5-(2-((quinazolin-4-yl)oxy)phenyl)-1,4-pentadien-3-one derivatives (eleven novel compounds 5a–5n) (Fig. 1). In our present study, all the target compounds were evaluated for their activity against MGC-803, PC3, and Bcap-37 cancer cell lines. Furthermore, the possible mechanism of MGC-803 cell growth inhibition by compound 5f was also investigated in this paper.

Results and discussion

Chemistry

Target compounds 5a–5n were synthesized as shown in Scheme 1. The starting material 2-aminobenzoic acid was conveniently cyclized to intermediate 1 by heating it with formamide at 140–145 °C as described in the literature. Upon refluxing with freshly distilled phosphorus oxychloride and pentachlorophosphorane, intermediate 1 yielded the corresponding 4-chloro derivative 2. Treatment of salicylaldehyde with acetone in the presence of sodium hydride at room temperature got intermediate 3. The key intermediates 4 were synthesized by reacting intermediate 3 with substituted 4-chloroquiazoline 2 in the present of K2CO3 in CH3CN at 30–50 °C for 6 h. And then, the target compounds 5a–5n were synthesized by reacting the substituted aldehydes with 4 in the present of anhydrous alcohol at room temperature. The structures of the final products were confirmed by their IR, 1H NMR, 13C NMR, and elemental analysis techniques.

Evaluation of anti-tumor bioactivity of synthetic compounds

The in vitro antitumor activity of the newly synthesized compounds 5a–5n were evaluated against a panel of three human cancer cell lines, including human gastric cancer cell line MGC-803, human prostate cancer cell line PC3, and human breast cancer cell line Bcap-37, and one normal cell line NIH3T3 (mouse embryo fibroblast cell line) by MTT method. Adriamycin (ADM) was chosen as a reference drug due to its availability and widespread use. Each experiment was repeated at least three times. The results are presented in Table 1.

As depicted in Table 1, the title compounds suppressed proliferation of the above three cancer cell lines in different extents (IC50 values of 0.85–15.64 μM), and exhibited broad spectrum antitumor activity. Among these studied compounds, the inhibitory ratios of 5d–5g, and 5m against MGC-803 cells at 10 μM were 87.5, 87.0, 90.7, 85.9, and 81.1%, respectively, and their IC50 values were 1.72, 1.89, 0.85, 2.02, and 2.05 μM, respectively, similar to that of ADM (0.74 μM). Compounds 5d, 5f, 5g, and 5m displayed higher inhibitory activities against PC3 cells at 10 μM than that of the rest compounds, with inhibitory ratios of 86.3, 93.0, 83.1, and 81.2%, respectively, which were similar to or higher than that of ADM (91.2%). The inhibitory ratios of 5f and 5g against Bcap-37 cells at 10 μM, were 76.5 and 74.9% (IC50 values of 4.98 and 5.61 μM), respectively, which were higher than that of the rest compounds. Also noteworthy is that the potency of the compounds was generally more pronounced against the MGC-803 cells than against PC3 and Bcap-37 cells. Moreover, the antiproliferation activities of the title compounds against NIH3T3 normal cell line were also evaluated. Most of the title compounds showed stronger antiproliferative activities against the cancer cell lines.
than NIH3T3 lines. Compound 5f, which showed excellent levels of inhibition against MGC-803, PC3, and Bcap-37 cancer cells, have no significant activity against NIH3T3 cells, with inhibitory ratio of 21.5% at 10 μM. That is to say that the compound was less toxic on normal fibroblasts than on the investigated cancer cell lines and more selective to cancer cells.

Subsequently structure–activity relationships (SAR) studies were performed to determine how the substituents affected the anticancer activity. To examine SAR, different substituent groups were introduced into R1 and R2 in the quiazoline ring. Based on the activity values indicated in Table 1, the relationships of the activities with different R1 and R2 (type, position, and number of substituents) were deduced. Two main conclusions were drawn. On the one hand, compared with the same substituents on quiazoline, the corresponding molecules containing a 6-methyl group always had higher inhibitory rates than the compound containing a 8-methyl group. For example, the IC50 values of 5f (R1: 6-methyl, R2: 2,6-dichlorophenyl) and 5m (R1: 8-methyl, R2: 2,6-dichlorophenyl) on MGC-803 cells were 0.85 and 2.05 μM, respectively. By contrast, the inhibition rates of 5c (R1: 6-methyl, R2: p-chlorophenyl) and 5j (R1: 8-methyl, R2: p-chlorophenyl) at 10 μM were 79.2 and 76.3% on MGC-803 cells, 76.5 and 71.9% on PC3 cells, and 54.2 and 45.4% on Bcap-37 cells, respectively. On the other hand, when R2 was o-flurophenyl-fixed, the compounds always showed weak activity. For example, the inhibition rates of 5a (R1: 6-methyl, R2: o-flurophenyl) at 10 μM were 71.9, 68.5, and 44.1% on the three cancer cells, respectively, which suggested the weaker activity than that of the rest compounds.

Apoptosis is one of the major pathways that lead to the process of cell death [27]. Most cancer cells retain their sensitivity to some apoptotic stimuli from chemotherapeutic agent [28]. In the present study, compound 5f was selected and its mechanism of growth inhibition
of MGC-803 cells was evaluated. To determine whether antiproliferation and cell death are associated with apoptosis, MGC-803 cells were stained with acridine orange (AO)/ethidium bromide (EB) staining and Hoechst 33,258 staining after exposure to compound 5f and observed under fluorescence microscopy.

It is well known that AO can pass through cell membranes, but EB cannot. Under the fluorescence microscope, living cells appear green. Necrotic cells stain red but have a nuclear morphology resembling that of viable cells. Apoptotic cells appear green, and morphological changes such as cell blebbing and formation of apoptotic bodies will be observed [29]. Representative images of the cells treated with 10 μM of HCPT (used as positive control) and 1, 5, 10 μM of compound 5f for 12 h are shown in Fig. 2a. While treatment of cells with HCPT and compound 5f, the apoptotic cells with typical apoptotic features, such as staining brightly, condense chromatin and fragment nuclei were observed. These results suggested that the proliferative inhibition and the death of target cells upon treatment with compound 5f were consequent to the induction of apoptosis.

Membrane-permeable Hoechst 33,258 was a blue fluorescent dye and stained the cell nucleus. When cells were treated with Hoechst 33,258, live cells with uniformly

Table 1 Effect of title compounds against cell viability of different cell lines

| Comps | Inhibition rates against different cells (%) | IC<sub>50</sub> (μM) |
|-------|-------------------------------------------|-----------------|
|       | MGC-803 | PC3 | Bcap-37 | NIH3T3 | MGC-803 | PC3 | Bcap-37 |
| 5a    | 71.9 ± 0.9 | 68.5 ± 2.3 | 44.1 ± 2.6 | 65.7 ± 1.3 | 7.69 ± 0.37 | 9.24 ± 0.46 | 11.71 ± 0.39 |
| 5b    | 83.5 ± 1.5 | 78.7 ± 2.4 | 57.3 ± 2.0 | 15.4 ± 1.5 | 5.20 ± 0.51 | 8.12 ± 0.46 | 10.05 ± 0.67 |
| 5c    | 79.2 ± 2.8 | 76.5 ± 1.7 | 54.2 ± 1.1 | 16.1 ± 2.7 | 5.44 ± 0.45 | 7.85 ± 0.32 | 9.34 ± 0.52 |
| 5d    | 87.0 ± 0.2 | 86.3 ± 0.8 | 58.5 ± 1.3 | 71.9 ± 1.4 | 1.72 ± 0.22 | 2.68 ± 0.43 | 7.52 ± 0.36 |
| 5e    | 90.7 ± 0.6 | 93.0 ± 1.8 | 76.5 ± 1.9 | 21.5 ± 2.7 | 0.85 ± 0.43 | 1.37 ± 0.22 | 4.98 ± 0.39 |
| 5f    | 90.7 ± 0.6 | 93.0 ± 1.8 | 76.5 ± 1.9 | 21.5 ± 2.7 | 0.85 ± 0.43 | 1.37 ± 0.22 | 4.98 ± 0.39 |
| 5g    | 85.9 ± 1.2 | 83.1 ± 2.8 | 74.9 ± 2.0 | 74.3 ± 0.9 | 2.02 ± 0.37 | 2.18 ± 0.31 | 5.61 ± 0.50 |
| 5h    | 62.3 ± 1.4 | 43.7 ± 12.1 | 36.6 ± 2.7 | 13.4 ± 2.2 | 9.06 ± 0.40 | 11.68 ± 0.48 | 15.64 ± 0.51 |
| 5i    | 79.4 ± 2.4 | 65.1 ± 2.6 | 47.1 ± 2.6 | 14.4 ± 2.6 | 7.78 ± 0.48 | 9.36 ± 0.52 | 10.73 ± 0.62 |
| 5j    | 76.3 ± 0.8 | 71.9 ± 3.1 | 45.4 ± 2.2 | 41.0 ± 2.1 | 6.42 ± 0.44 | 8.64 ± 0.47 | 12.11 ± 0.31 |
| 5k    | 82.1 ± 3.0 | 76.3 ± 2.5 | 60.2 ± 1.4 | 30.0 ± 2.3 | 2.94 ± 0.32 | 8.12 ± 0.46 | 8.92 ± 0.58 |
| 5l    | 61.2 ± 1.3 | 55.7 ± 1.5 | 54.6 ± 1.0 | 0.8 ± 1.6 | 4.33 ± 0.35 | 9.08 ± 0.52 | 9.83 ± 0.46 |
| 5m    | 81.1 ± 1.7 | 81.2 ± 2.8 | 66.4 ± 1.5 | 12.4 ± 2.5 | 2.05 ± 0.39 | 3.79 ± 0.47 | 7.82 ± 0.39 |
| 5n    | 78.7 ± 2.7 | 69.0 ± 1.7 | 55.8 ± 1.1 | 29.6 ± 2.4 | 3.33 ± 0.42 | 4.16 ± 0.43 | 8.70 ± 0.54 |
| ADM<sup>c</sup> | 97.5 ± 1.2 | 91.2 ± 0.4 | 94.5 ± 0.9 | 100.0 ± 0.7 | 0.74 ± 0.24 | 1.01 ± 0.20 | 1.90 ± 0.25 |

<sup>a</sup> Inhibitory percentages of cells treated with 10 μM concentration of each compound for 72 h

<sup>b</sup> Agent concentration (μM) that inhibited cell growth by 50% at 72 h after treatment

<sup>c</sup> Adriamycin, positive control
light blue nuclei were observed under fluorescence microscope, while apoptotic cells exhibited bright blue because of karyopyknosis and chromatin condensation, and the nuclei of dead cells could not be stained [30]. MGC-803 cells treated with compound 5f at concentrations of 1, 5, and 10 μM for 12 h were stained with Hoechst 33,258, with HCPT as positive control at 10 μM for 12 h. The results are illustrated in Fig. 2b.

Figure 2b shows that MGC-803 cells treated with the negative control DMSO were normally blue. Compared with the negative control, a part of cells with smaller nuclei and condensed staining appeared in the positive control group. After treated with compound 5f, the cells exhibited strong blue fluorescence and revealed typical apoptotic morphology. These findings demonstrate that compound 5f induced apoptosis against MGC-803 cell lines, consistent with the results for AO/EB double staining.

To further verify AO/EB and Hoechst 33,258 staining results, TUNEL assay was also carried out. TUNEL (Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling) is a very popular assay for identifying apoptotic cells. The assay identifies apoptotic cells in situ by using terminal deoxynucleotidyl transferase (TdT) to transfer biotin-dUTP to these strand breaks of cleaved DNA. The biotin-labeled cleavage sites are then detected by reaction with HRP conjugated streptavidin and visualized by DAB showing brown color [24]. MGC-803 cells treated with compound 5f at 5 μM for 6, 12, and 18 h were stained with TUNEL, with HCPT as positive control at 5 μM for 18 h. As shown in Fig. 3, cells in control group (DMSO treatment) did not appear as brown precipitates. However, the cells treated with compound 5f and HCPT appeared as brown precipitate. We further concluded that compound 5f induced apoptosis against MGC-803.

In addition, the apoptosis ratios induced by compound 5f in MGC-803 cells were determined by flow cytometry, using Annexin V/PI double staining. Flow cytometry was performed on the total cell population (including both adherent and detached cells) and apoptosis detection was carried out as mentioned above. This double staining procedure discriminated necrotic cells (Q1, Annexin−/PI−), late apoptotic cells (Q2, Annexin+/PI+), intact cells (Q3, Annexin−/PI−) and early apoptotic cells (Q4, Annexin+/PI−) [31, 32]. As shown in Fig. 4, compound 5f could induce apoptosis of MGC-803 cells, and the highest apoptosis ratio (26.4%) was obtained after 24 h of treatment at a concentration of 10 μM. For the positive control HCPT, the apoptosis ratio was only 22.3% after 24 h of treatment at a concentration of 10 μM. In addition, as shown in Fig. 5, the apoptosis of MGC-803 cells treated with compound 5f gradually increased in a time-dependent manner.

**Conclusions**

As a development of our previous studies, we have synthesized and evaluated in vitro a series of (1E,4E)-1-aryl-5-(2-((quinazolin-4-yl)oxy)phenyl)-1,4-pentadien-3-one derivatives as potential antitumor agents. Most of the derivatives exhibited equivalent inhibitory activities against MGC-803, PC3, and Bcap-37 cancer cells. Compound 5f appeared to be more effective than other compounds against the three cells, with IC50 values of 0.85, 1.37, and 4.98 μM, respectively. And compounds 5f was found to exhibit a good degree of selectivity towards...
cancer cells than normal cells. In addition, the apoptosis-inducing activity of compound 5f in MGC-803 cells was investigated by AO/EB staining, Hoechst 33,258 staining, TUNEL assay, and flow cytometry. The results revealed that the compound may inhibit cell growth by inducing apoptosis, with apoptosis ratio of 26.4% at 10 μM for 24 h, which was higher than that of HCPT (22.3% at 10 μM for 24 h). Further studies on the specific mechanisms of compound 5f in MGC-803 cells are currently underway.

**Experimental**

**Reagents and chemicals**

Melting points were determined by using an XT-4 binocular microscope (Beijing Tech Instrument Co., China) without correction. IR spectra were recorded on a Bruker VECTOR 22 spectrometer. NMR spectra were recorded in a CDCl3 solvent using a JEOL-ECX 500 NMR spectrometer operating at 500 MHz for 1H, and at 125 MHz for 13C by using TMS as internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Silica gel (200–300 mesh) and TLC plates (Qingdao Marine Chemistry Co., Qingdao, China) were used for chromatography. All solvents (Yuda Chemistry Co., Guiyang, China) were analytical grade, and used without further purification unless otherwise noted.

**Synthetic procedures**

6-methyl-quinazolin-4(3H)-one, 8-methyl-quinazolin-4(3H)-one, 6-methyl-4-chloroquiazoline, and 8-methyl-4-chloroquiazoline were prepared according to a previously described method [33]. Intermediate (E)-4-(2-hydroxyphenyl)-3-butylene-2-one was prepared according to a previously reported [34].

**General synthetic procedures for the preparation of compounds 5a–5n**

Compounds 2 (10 mmol), 3 (10 mmol) and K2CO3 (70 mmol) in 20 mL of acetonitrile was stirred at 30–40 °C for 3.5 h. The reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, and recrystallized with ethanol to afford the desired solid compound 4a or 4b, respectively. To the mixture of compound 4a or 4b (0.5 mmol) and sodium hydroxide (1%) in 20 mL of 75 vol% ethanol/water solution was added substituted aldehydes (0.5 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated and suspended in water (20 mL), adjusted with 5% HCl to pH 7, and filtered. Recrystallization with ethanol afforded the desired solid compounds 5a–5n.

(1E,4E)-1-(2-fluorophenyl)-5-(2-((6-methylquinazolin-4-yloxy)phenyl)penta-1,4-dien-3-one (5a) Yield: 52.6%; yellow powder; mp: 121–123 °C; IR (KBr, cm−1): v: 3442, 1657, 1622, 1596, 1465, 1398, 1356, 1221, 983; 1H NMR (CDCl3, 500 MHz) δ: 8.70 (s, 1H, Qu-2-H), 8.23 (d, J = 12.00 Hz, 1H, F–Ar–CH=), 7.93 (d, J = 8.6 Hz, 1H, Ar–CH=), 7.78–7.85 (m, 3H, Qu-5,7,8-H), 7.47–7.50 (m, 3H, F–Ar-4,6-H, Ar-3-H), 7.30–7.39 (m, 5H, F–Ar-3,5-H, Ar-4,5-H, F–Ar–C=CH), 7.10 (d, J = 16.0 Hz, 1H, Ar–C=CH), 6.81 (d, J = 14.8 Hz, 1H, Ar-6-H), 2.61 (s, 3H, CH3);

**Fig. 4** The apoptosis ratios of MGC-803 cells treated with compound 5f and HCPT.

**Fig. 5** Annexin V/PI dual staining of MGC-803 cell lines. a Negative control; b treated with HCPT at 10 μM for 24 h; c–e treated with compound 5f at 10 μM for 6, 12, and 24 h, respectively.
153.4, 151.7, 150.4, 136.9, 136.7, 136.5, 131.7, 129.4, 128.2, 127.9, 127.7, 127.2, 127.1, 126.6, 126.5, 123.6, 123.5, 122.3, 116.4, 21.9; Anal. Calcd for C_{26}H_{18}ClN_{2}O_{2}: C 55.2%; yellow powder; mp: 187–189 °C; IR (KBr, cm\(^{-1}\))\(\nu\): 3443, 1655, 1618, 1582, 1499, 1333, 1225, 986; 1H NMR (CDCl\(_3\), 500 MHz)\(\delta\): 8.68 (s, 1H, Qu-2-H), 8.20 (s, 1H, Qu-5-H), 7.70–7.92 (m, 4H, Cl–Ar=CH=, Cl–Ar–CH=), 7.38–7.43 (m, 4H, Cl–Ar–CH=, Ar-3-H, Cl–Ar–5-H), 7.26–7.31 (m, 3H, Ar–CH=, Cl–Ar–C=CH), 7.12 (d, \(J = 16.5\) Hz, 1H, Ar–C=CH), 6.80 (d, \(J = 16.1\) Hz, 1H, Ar-6-H), 2.61 (s, 3H, CH\(_3\)); 13C NMR (CDCl\(_3\), 125 MHz)\(\delta\): 188.5, 161.7, 151.3, 151.0, 142.6, 142.1, 136.5, 134.3, 133.3, 132.0, 130.0, 129.6, 129.4, 127.4, 125.8, 122.9, 122.7, 121.2, 116.3, 17.7; Anal. Calcd for C_{26}H_{18}ClN_{2}O_{2}: C 76.7%; H 4.56; N 6.42.

(1E,4E)-1-(2,6-dichlorophenyl)-5-(2-((6-methylquinazolin-4-yl)oxy)phenyl)penta-1,4-dien-3-one (5f) Yield: 52.2%; yellow powder; mp: 122–123 °C; IR (KBr, cm\(^{-1}\))\(\nu\): 3443, 1655, 1618, 1582, 1499, 1333, 1225, 986; 1H NMR (CDCl\(_3\), 500 MHz)\(\delta\): 8.68 (s, 1H, Qu-2-H), 8.20 (s, 1H, Qu-5-H), 7.70–7.92 (m, 4H, Cl–Ar=CH=, Cl–Ar–CH=), 7.38–7.43 (m, 4H, Cl–Ar–CH=, Ar-3-H, Cl–Ar–5-H), 7.26–7.31 (m, 3H, Ar–CH=, Cl–Ar–C=CH), 7.12 (d, \(J = 16.5\) Hz, 1H, Ar–C=CH), 6.80 (d, \(J = 16.1\) Hz, 1H, Ar-6-H), 2.61 (s, 3H, CH\(_3\)); 13C NMR (CDCl\(_3\), 125 MHz)\(\delta\): 188.5, 161.7, 151.3, 151.0, 142.6, 142.1, 136.5, 134.3, 133.3, 132.0, 130.0, 129.6, 129.4, 127.4, 125.8, 122.9, 122.7, 121.2, 116.3, 17.7; Anal. Calcd for C_{26}H_{18}ClN_{2}O_{2}: C 76.7%; H 4.56; N 6.42.
\( (1E,4E)-1-(2\text{-}chlorophenyl)-5-(2\text{-}((8\text{-}methylquinazolin-4\text{-}yl)oxy)phenyl)penta-1,4-dien-3\text{-}one\) \((5i)\) Yield: 58.6%; yellow powder; mp: 161–163 °C; IR (KBr, cm\(^{-1}\)) \(\nu: 3445, 1647, 1616, 1576, 1481, 1406, 1358, 1229, 979;\) 
\(1^H\) NMR (CDCl\(_3\), 500 MHz) \(\delta: 8.79\) (s, 1H, Qu-2-H), 8.30 (d, \(J = 8.5\) Hz, 1H, Ar–CH=), 7.80–7.85 (m, 3H, 4H, Ar–CH=), 7.72–7.78 (m, 3H, 4H, Ar–CH=), 7.58–7.64 (m, 4H, Cl–Ar–C=C=, Ar–CH=), 7.41–7.42 (m, 1H, Ar–5-H), 7.30–7.32 (m, 1H, Ar–4-H), 7.11–7.14 (d, \(J = 15.0\) Hz, 1H, Ar–6-H), 6.93–6.96 (d, \(J = 15.0\) Hz, 1H, Ar–C=C=), 2.77 (s, 3H, CH\(_3\)); \(1^3C\) NMR (CDCl\(_3\), 125 MHz) \(\delta: 188.6, 167.1, 153.2, 153.1, 151.7, 141.9, 136.9, 136.7, 134.6, 131.7, 129.5, 129.2, 128.4, 128.1, 127.6, 127.2, 126.7, 125.8, 123.6, 121.1, 17.8;\) Anal. Calcd for C\(_{26}\)H\(_{19}\)ClN\(_2\)O\(_2\): C 73.15; H 4.49; N 6.56; Found: C 73.04; H 4.74; N 6.67%.

\( (1E,4E)-1-(2\text{-}chlorophenyl)-5-(2\text{-}((8\text{-}methylquinazolin-4\text{-}yl)oxy)phenyl)penta-1,4-dien-3\text{-}one\) \((5j)\) Yield: 56.1%; yellow powder; mp: 161–163 °C; IR (KBr, cm\(^{-1}\)) \(\nu: 3445, 1647, 1616, 1587, 1481, 1400, 1359, 1225, 984;\) 
\(1^H\) NMR (CDCl\(_3\), 500 MHz) \(\delta: 8.76\) (s, 1H, Qu-2-H), 8.28 (d, \(J = 8.5\) Hz, 1H, Ar–CH=), 7.73–7.85 (m, 3H, 4H, Ar–CH=), 7.52–7.59 (m, 3H, 4H, Ar–CH=), 7.29–7.41 (m, 4H, Ar–3-H, Cl–Ar–C=C=, Cl–Ar–C=C=), 7.16 (m, 1H, Ar–4-H), 7.07 (d, \(J = 16.0\) Hz, 1H, Ar–C=C=), 6.98 (d, \(J = 17.0\) Hz, 1H, Ar–6-H), 2.75 (s, 3H, CH\(_3\)); \(1^3C\) NMR (CDCl\(_3\), 125 MHz) \(\delta: 188.96, 167.10, 153.15, 151.70, 150.99, 137.59, 136.75, 135.68, 135.17, 134.57, 132.97, 131.85, 129.88, 128.5, 128.12, 127.88, 127.65, 127.45, 126.72, 123.60, 121.04, 116.02, 17.79;\) Anal. Calcd for C\(_{26}\)H\(_{19}\)ClN\(_2\)O\(_2\) (461): C, 67.69; H, 3.93; N, 6.07; Found: C 67.27; H 4.03; N 5.96%.
(m, 1H, Ar-4-H), 7.15–7.29 (m, 2H, CH₃O–Ar–C=CH, Ar–C=CH), 6.98 (s, 1H, CH₂O–Ar–6-H), 6.89–6.93 (m, 2H, Ar-6-H, CH₂O–Ar–3-H), 6.81 (d, J = 8.6 Hz, 1H, CH₂O–Ar–4-H), 3.77 (s, 6H, 2CH₃O), 2.76 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 125 MHz) δ: 189.25, 167.14, 153.57, 153.18, 151.66, 151.03, 138.78, 136.58, 136.25, 134.51, 131.45, 128.41, 128.23, 127.55, 127.24, 126.58, 124.21, 123.54, 121.16, 120.94, 117.61, 116.16, 113.22, 112.47, 56.08, 55.85, 17.75. Anal. Calcd for C₂₈H₂₄N₂O₄ (453): C, 74.32; H, 5.35; N, 6.19; %. Found: C, 74.55; H, 5.68; N, 5.95%.

Cell culture
Human gastric cancer cell line MGC-803, human prostate cancer cell line PC3, and human breast cancer cell line Bcap-37 and one normal cell line NIH3T3 were obtained from Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). NIH3T3 was routinely maintained in a DMEM medium, while all the other cell lines were cultured in a 1640 medium. All the cells were grown in the medium supplemented with 10% FBS at 37 °C with 5% CO₂.

MTT assay
The growth-inhibitory effects of the test compounds were determined on MGC-803, PC3, Bcap-37, and NIH3T3 cells. All cell types were seeded into 96-well plates at a density of 2 × 10³ cells/well 100 μL of the proper culture medium and incubated with increasing concentrations of the compounds at 37 °C under cell culturing conditions. An MTT assay (Roche Molecular Biochemicals, 1465-007) was performed 72 h later according to the instructions provided by Roche. The precipitated formazan crystals were dissolved in SDS, and the absorbance was read at 595 nm with a microplate reader (BIO-RAD, model 680), which is directly proportional to the number of living cells in culture. The experiment was performed in triplicate. The percentage cytotoxicity was calculated using the formula.

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\% \text{Cytotoxicity} = \frac{(\text{Control}_{\text{abs}} - \text{Blank}_{\text{abs}})}{(\text{Test}_{\text{abs}} - \text{Blank}_{\text{abs}})} \times 100
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AO/EB staining
Cells were seeded in 6-well culture plates at a density of 5 × 10⁴ cells/mL in 0.6 mL of medium and allowed to adhere to the plates overnight. The cells were incubated with different concentrations of compounds or vehicle solution (0.1% DMSO) in a medium containing 10% FBS for 12 h. After the treatment, the cover slip with monolayer cells was inverted on the glass slide with 20 μL of AO/EB stain (100 μg/mL), and finally analyzed for morphological characteristics of cell apoptosis under a fluorescence microscope (Olympus Co., Japan).

Hoechst 33,258 staining
Cells were seeded in 6-well culture plates at a density of 5 × 10⁴ cells/mL in 0.6 mL of medium and allowed to adhere to the plates overnight. The cells were incubated with different concentrations of compounds or vehicle solution (0.1% DMSO) in a medium containing 10% FBS for 12 h. After the treatment, the cells were fixed with 4% paraformaldehyde for 10 min, followed by incubation with Hoechst 33,258 staining solution (Beyotime) for 5 min and finally analyzed for morphological characteristics of cell apoptosis under a fluorescence microscope (Olympus Co., Japan).

Flow cytometry analysis
To further quantitative analysis of apoptosis, the cells were washed with PBS, stained with annexinV-FITC and propidium iodide (PI) using the AnnexinV-FITC kit (KeyGEN BioTECH). The cells were then subjected to flow cytometry according to manufacturer’s instructions and the stained cells were analyzed by FACS can flow cytometer (Becton–Dickinson, CA, USA).

Statistical analysis
All statistical analysis was performed with SPSS Version 19.0. Data was analyzed by one-way ANOVA. Mean separations were performed using the least significant difference method. Each experiment was replicated thrice, and all experiments yielded similar results. Measurements from all the replicates were combined, and treatment effects were analyzed.

Abbreviations
ADM: adriamycin; AO/EB: acridine orange/ethidium bromide; ¹³C NMR: ¹³C nuclear magnetic resonance; DMSO: dimethyl sulfoxide; FCM: flow cytometry; HCPT: 10-hydroxyl camptothecine; ¹H NMR: proton nuclear magnetic resonance; IR: infra-red; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TUNEL: terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling.

Authors’ contributions
HL and SY synthesized the compounds and carried out most of the bioassay experiments. DH took part in the compound structural elucidation and bioassay experiments. WX carried out some structure elucidation experiments. PX assisted in structural elucidation experiments. All authors read and approved the final manuscript.

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Competing interests
All authors declare that they have no competing interests.

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