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Discovery of novel AHLs as potent antiproliferative agents

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ABSTRACT

Three series of novel AHL analogs were synthesized and evaluated for their *in vitro* cytotoxic activity against four human cancer cell lines. The SARs investigation indicated that AHLs with a terminal phenyl group, especially those with the chalcone scaffold had remarkably enhanced cytotoxicity than those with the hydrophobic side chains. Besides, some of these compounds were much more potent than 5-Fu and natural OdDHL. Through the detailed SARs discussions, we found that compounds **10a-k** and **14** with the 4-amino chalcone scaffold showed excellent inhibition against all the tested cancer cell lines and were much more potent than 5-Fu and AHLs. Such scaffold may act as a template for further lead optimization. Compound **10i** with a 3, 4, 5-trimethoxy group was the most potent one against all the tested cancer cell lines. Flow cytometry analysis indicated that analog **11e** induced the cellular apoptosis and cell cycle arrest of MCF-7 cells at G2/M phase in a concentration-and time-dependent manner.

1. Introduction

N-Acyl homoserine lactones (AHLs) have been identified to be responsible for the largest proportion of quorum sensing signals of Gram negative bacteria [1]. *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, Fig. 1), as the well-known quorum sensing signal of *Pseudomonas aeruginosa*, was reported to play a critical role in the infection caused by *P. aeruginosa* [2] and to be able to interfere the eukaryotic system. Besides, numerous studies have showed that OdDHL can induce apoptosis of human breast cancer cell lines through ablating the activity of the signal transducer and activator of transcription protein 3 (STAT3) with little effect on normal breast cells and serve as *r*TSβ mimics down-regulating thymidylate synthase, inhibiting the growth of human colorectal cancer cells (H630) and enhancing the anti-tumor activity of 5-fluorouracil, taxol and tomudex [3,4]. In immunity, OdDHL has been proved to be capable of stimulating the production of IL-8 and inducing apoptosis in specific host immune cells [5,6]. OdDHL and its analogs, as the anticancer agents, have been extensively studied. For example, phenacylhomoserine lactones presented potent anticancer activity and minimum quorum sensing activation [7], and acridine-based AHL analogs showed excellent antiproliferative activity against human oral squamous carcinoma cell lines and even induced radiation-sensitizing effects on Ca9-22 cells and polyploidy in SAS at the concentration of 21.2 μM [8,9].

Dithiocarbamates have received considerable attention for their excellent biological activities and abundance in nature. The natural Brassinin (Fig. 1) has been indentified as potent antifungal agent and moderate inhibitor of cancer immunosuppression target Indoleamine 2, 3-dioxygenase (IDO). The dithiocarbamate group was found to be crucial for the cytotoxic activity [10]. Besides, the dithiocarbamate motif has always been used as a linkage to combine different biologically active scaffold to design new chemical entities [11–14]. Very recently, our group have reported a series of selective LSD1 inhibitors, which can inhibit gastric cancer cell growth, invasion and migration (Fig. 1) [12].

Chalcones and their derivatives have been reported to possess various biological activities [15–22], the anticancer activity, in particular, has received significant attention in last decades [16–18,20,21,23]. In addition to the α, β-unsaturated carbonyl scaffold [20], the amino group of 4′-amino chalcone was also found as the promising site for further modifications to obtain more biologically promising chalcone derivatives with high efficacy and selectivity [18,21].

Inspired by the above-mentioned findings and in continuation with our previously efforts toward finding novel anticancer agents

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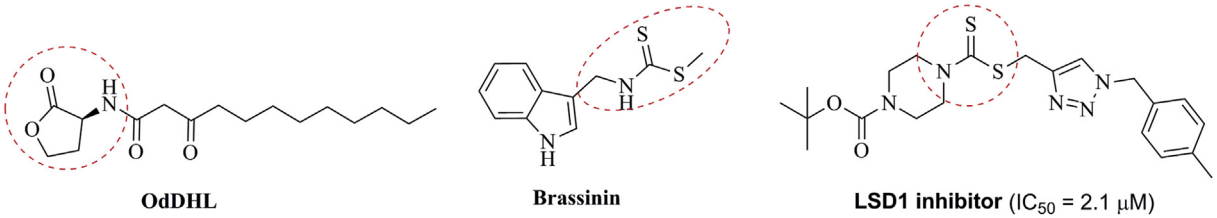


Fig. 1. Structures of OdDHL, Brassinin and LSD1 inhibitor previously reported.

[12–14], we herein report the identification of novel antiproliferative AHL analogs with the chalcone and homoserine lactone scaffold linked by the dithiocarbamate group through extensive SARs investigations. Besides, the promising 4′-amino chalcone scaffold with potent cytotoxicity was first discovered.

2. Results and discussion

2.1. Chemistry

L-homoserine lactone hydrochloride salt **1** was prepared from L-methionine following the literature reported method [24]. Compounds **3a-r** were obtained from the corresponding anilines through the well established acylation reaction. AHL analogs **4a-r** and **6a-g** were then synthesized from compound **1** and corresponding intermediates **3a-r** and **2a-g** via a three-component one-pot reaction following our previously reported method [23] (Scheme 1).

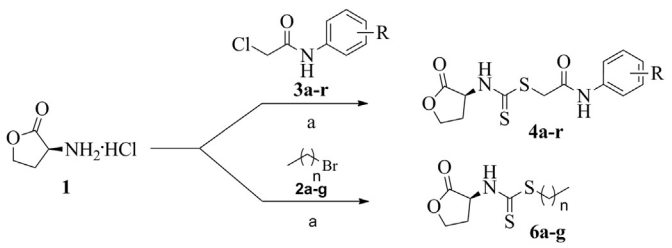
The condensation of 4-amino acetophenone and **8a-k** in the presence of NaOH efficiently gave 4'-amino chalcones **9a-k**, which then reacted with chloroacetyl chloride, generating acylated 4'-amino chalcones **10a-k**. With these compounds in hand, analogs **11a-g** were efficiently synthesized via a three-component one-pot reaction (Scheme 2). To further explore the SARs, compound **14** with an additional double bond was also synthesized from cinnamaldehyde **12**.

Natural OdDHL was synthesized according to the literature reported method [25]. Meldrum's acid **17** was easily formed via the reaction of malonic acid **15** with acetone **16**, followed by the coupling reaction with *n*-decanoic acid in the presence of DCC and DMAP, giving compound **19**, which reacted with compound **1** to give OdDHL in the presence of Et_3N (Scheme 3).

2.2. Biological evaluation

2.2.1. Cytotoxic activity

The IC_{50} values (concentration required to inhibit the proliferation of cancer cells by 50%) for all obtained AHL analogs **4a-r**, **6a-g**, **11a-g**, **14** and substituted amino chalcones **10a-k** against four human cancer cell lines including MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line), EC-9706 (human esophageal cancer cell line) and SMMC-7721 (human hepatocellular carcinoma cell line) were determined using MTT assay [26], and OdDHL and 5-Fluorouracil were both used as positive controls.

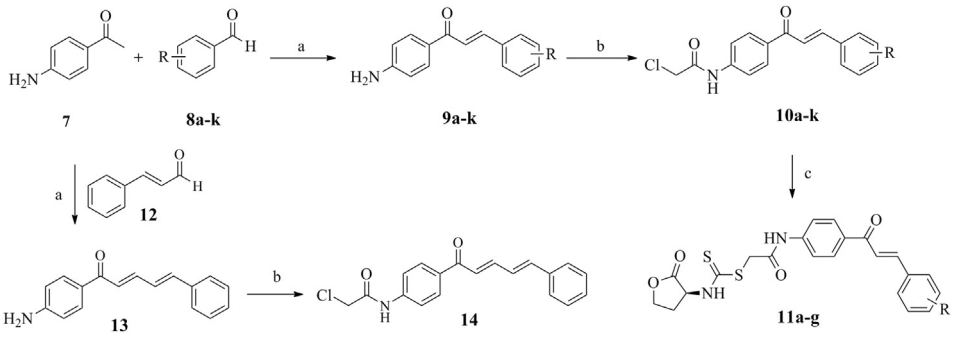


Scheme 1. Synthesis of AHL analogs **4a-r** and **6a-g**. Reagents and conditions: (a) CS_2, $Na_3PO_4 \cdot 12H_2O$, acetone, rt.

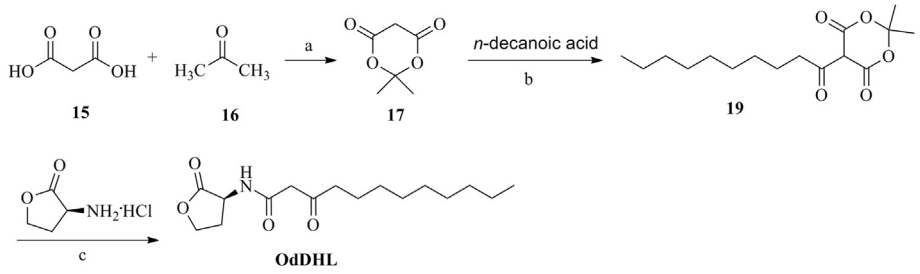
The SARs were fully explored by changing the substituents attached to the dithiocarbamate group. The *in vitro* inhibitory activity was summarized in Table 1. It is evident that compounds **6a-g** with hydrophobic carbon chains showed weak or no inhibition against the tested cancer cells. Specifically, no cytotoxicity was observed for compounds **6a-e** (n < 8), compounds **6f-g** with relatively prolonged side chains (n = 11 and 13) showed slightly improved inhibitory activity. This indicated that the hydrophobic chain may be not beneficial for the activity.

Compounds **4a-r** with different terminal phenyl groups were then synthesized and evaluated for their cytotoxicity. Interestingly, most of these compounds showed improved inhibitory effect against the tested cell lines compared to compounds **6a-g**. Compounds **4a-r** had weak or no activity against EC-9706 and SMMC-7721 cells. However, an increased inhibition against MGC-803 and MCF-7 cells was observed. It is worth noting that compounds **4l-n** showed excellent inhibition against MCF-7 cells (IC_{50} = 3.13, 5.46 and 5.83 μM, respectively) and were much more potent than OdDHL and 5-Fu (IC_{50} = 37.31 and 12.24 μM, respectively). For MCF-7 cells, analogs with electron-withdrawing groups showed better cytotoxicity than those with electron-donating groups. In particular, compounds with electron-withdrawing substituents at *meta*-position of the phenyl ring contributed more to the cytotoxicity, such as analogs **4l** (3-CF_3) and **4m** (3-NO_2). Analogs with the same electron-withdrawing group at *meta*-position showed better cytotoxicity against MCF-7, while weaker cytotoxicity was observed when the group was replaced at the *ortho*-position, like **4f** (3-Cl) >**4b** (4-Cl) >**4h** (2-Cl). Analogs substituted by halogen atoms F, Cl and Br at the same position on the phenyl ring exerted an activity sequence of **4n** (4-F) >**4b** (4-Cl) >**4c** (4-Br). For MGC-803 cells, compounds **4a-r** showed moderated inhibition with the IC_{50} values ranging from 17.88 μM to >128 μM. However, most of them were much more potent than OdDHL (IC_{50} = 101.83 μM). From the biological data of compounds **6a-g** and **4a-r** against MGC-803 cells, we can conclude that compounds with a terminal phenyl group have better cytotoxicity than those with a hydrophobic side chain regardless of the length.

This interesting finding promoted us to further explore the SARs by incorporating the chalcone scaffold into our molecules (compounds **11a-g**). A significant enhanced cytotoxicity against the tested cancer cell lines was observed, especially for EC-9706 cells, compared to compounds **6a-g** and **4a-r**. Specifically, compounds **11e** and **11g** represented excellent inhibitory effect against MCF-7 cells (IC_{50} = 5.57 and 2.67 μM, respectively) and were much more potent than OdDHL and 5-Fu. Besides, compound **11a** also had excellent inhibition against MGC-803 cells with the IC_{50} value of 6.90 μM. This finding indicated that the chalcone scaffold played an



Scheme 2. Synthesis of compounds **10a-k**, **11a-g** and **14**. Reagents and conditions: (a) NaOH, EtOH/H_2O; (b) $ClCH_2COCl$, anhydrous K_2CO_3, acetone, rt; (c) **1**, CS_2, $Na_3PO_4{\cdot}12H_2O$, acetone, rt.



Scheme 3. Synthesis of natural OdDHL. Reagents and conditions: (a) Ac_2O, H_2SO_4, 0 °C-rt. (b) DCC, DMAP, rt. (c) Et_3N, CH_3CN, reflux.

important role in the activity.

Based on the above findings, we further tested the cytotoxicity of 4′-amino chalcone intermediates **10a-k**. To compare the activity, compound **14** with an additional double bond was also synthesized. To our surprise, almost all these compounds showed excellent cytotoxic activity with the IC_{50} values less than 10 μM regardless of the position and nature of the substituents on the phenyl group. And these compounds were remarkably much more potent than 5-Fu and OdDHL. This may explain why compounds **11a-g** with a chalcone scaffold had improved cytotoxicity. Compound **14** had a comparable inhibition against the tested cell lines. Among them, compound **10i** with a 3, 4, 5-trimethoxy group showed the best inhibitory effect toward the tested cancer cell lines with the IC_{50} values of 1.72, 1.32, 2.73 and 3.00 μM against MGC-803, MCF-7, EC-9706 and SMMC-7721, respectively. This scaffold may serve as a starting point for further lead optimization.

OdDHL, the quorum sensing signal molecule of *P. aeruginosa*, only inhibited the proliferation of MCF-7 cells moderately with an IC_{50} value of 37.31 μM.

Next, we evaluated the cytotoxicity of compound **11e** against human normal cell lines GES-1 and HEK293. However, certain cytotoxicity of compound **11e** against these two normal cell lines was observed with IC_{50} values of 23.49 ± 1.41 and 21.12 ± 1.00 μM, respectively.

2.2.2. Apoptosis assay

Analog **11e** was chosen to further explore the mechanism of action for its excellent cytotoxic activity against human breast cancer cell line MCF-7. A biparametric cytofluorimetric analysis using propidium iodide (PI) and annexin-V-FITC in MCF-7 cells was performed to characterize the mode of cell death induced by compound **11e**. After treatment with compound **11e** at different concentrations (0, 2.0, 4.0, 8.0 μM) for 12 h and 24 h, respectively, MCF-7 cells were labeled with the two dyes, and the resulting red (in web version) (PI) and green (in web version) (FITC) fluorescence was monitored by flow cytometry. As shown in Fig. 2A, after treatment for 12 h, compound **11e** caused early apoptosis with the percentage increased from 7.2% (DMSO control) to 19.0%. After 24 h, the extent of early apoptosis increased significantly from 4.5% (DMSO control) to 43.2% as shown in Fig. 2B. The results showed that compound **11e** effectively induced the cellular apoptosis in a concentration-and time-dependent manner.

2.2.3. Cell cycle analysis

To better characterize the cytotoxic activity of analog **11e**, a cell cycle progression was performed by treating MCF-7 cells at different concentrations of compound **11e** (0, 2.0, 4.0, 8 μM). Treatment of MCF-7 cells for 12 h, the percentage of cells at G2/M phase increased to 33.6% for the high concentration group from 16.5% for the control group (Fig. 3A), whereas when treated for 24 h, the proportion of cells at G2/M phase was added up to 63.1% from 16.27% (Fig. 3B). These data indicated that **11e** caused an obvious G2/M arrest in a concentration-and time-dependent manner.

3. Conclusion

In conclusion, three series of novel AHL analogs were synthesized and evaluated for their *in vitro* cytotoxic activity against four human cancer cell lines. The SARs were fully explored by changing the groups attached to the dithiocarbamate linker. Compounds **6a-g** with the hydrophobic side chains showed no or weak inhibition against the tested cancer cell lines. By contrast, Compounds with the terminal phenyl group (compounds **4a-r**), especially those (compounds **11a-g**), with the 4'-amino-chalcone scaffold had remarkably enhanced inhibition against the tested cancer cell lines, an effect which was probably attributed to the introduction of the biologically active chalcone scaffold. Compounds **10a-k** and **14** showed excellent inhibition against all the tested cancer cell lines and were much more potent than 5-Fu. Such scaffold may act as a starting point for further lead optimization. Compound **10i** with a 3,

Table 1
In vitro cytotoxic activity against four human cancer cell lines.

Compound	R/n	IC_{50} (μM)[a]			
		MGC-803	MCF-7	EC-9706	SMMC-7721
6a	n = 0	>128	>128	>128	>128
6b	n = 1	>128	>128	>128	>128
6c	n = 3	>128	>128	>128	>128
6d	n = 5	>128	>128	>128	>128
6e	n = 7	>128	>128	>128	65.05 ± 6.28
6f	n = 11	33.28 ± 4.01	61.09 ± 2.42	58.30 ± 6.06	50.28 ± 3.96
6g	n = 13	36.60 ± 3.15	30.46 ± 2.03	66.18 ± 3.55	56.79 ± 2.87
4a	2-F	122.19 ± 4.88	28.61 ± 3.02	>128	>128
4b	4-Cl	76.92 ± 4.13	50.34 ± 3.63	>128	>128
4c	4-Br	42.78 ± 7.73	66.48 ± 5.83	67.43 ± 2.13	28.31 ± 2.25
4d	2,3-dimethyl	nd[b]	>128	>128	42.15 ± 2.59
4e	3-CH_3	36.60 ± 3.31	63.23 ± 4.04	>128	>128
4f	3-Cl	21.77 ± 2.54	46.55 ± 3.49	106.63 ± 4.51	>128
4g	H	39.69 ± 3.51	>128	>128	>128
4h	2-Cl	31.95 ± 2.90	90.80 ± 4.33	>128	49.90 ± 2.83
4i	4-CH_3	44.34 ± 2.43	33.52 ± 2.59	93.87 ± 7.42	32.72 ± 3.86
4j	2,4-dichloro	48.52 ± 3.30	29.23 ± 2.80	>128	>128
4k	4-OCH_3	62.06 ± 3.89	>128	>128	>128
4l	3-CF_3	17.88 ± 2.19	3.13 ± 0.20	79.67 ± 3.91	>128
4m	3-NO_2	58.86 ± 3.72	5.46 ± 0.81	>128	>128
4n	4-F	37.61 ± 3.32	5.83 ± 0.86	67.11 ± 4.09	>128
4o	4-OH	80.02 ± 2.69	>128	>128	>128
4p	4-$COCH_3$	99.44 ± 2.36	>128	>128	>128
4q	4-$CO_2C_2H_5$	21.72 ± 1.73	60.25 ± 2.04	112.28 ± 7.39	64.87 ± 2.61
4r	4-C_4H_9	20.52 ± 2.20	40.17 ± 4.35	44.24 ± 4.34	27.34 ± 2.73
11a	4-Cl	6.90 ± 1.08	20.25 ± 2.07	38.91 ± 2.67	>128
11b	H	24.76 ± 2.36	44.31 ± 2.93	44.90 ± 2.94	>128
11c	4-Br	19.64 ± 1.94	18.71 ± 1.91	91.20 ± 3.25	18.04 ± 1.94
11d	4-F	45.69 ± 2.88	83.69 ± 4.21	46.11 ± 3.45	>128
11e	2-Cl	12.35 ± 1.62	5.57 ± 0.89	34.06 ± 2.54	20.44 ± 2.08
11f	3-Cl	15.63 ± 1.83	11.62 ± 1.56	32.54 ± 2.50	39.09 ± 0.91
11g	4-NO_2	29.35 ± 2.38	2.67 ± 0.25	>128	>128
10a	4-Cl	4.96 ± 0.66	4.10 ± 0.41	8.34 ± 1.33	8.16 ± 1.30
10b	H	6.94 ± 1.06	2.48 ± 0.43	6.37 ± 0.94	12.56 ± 1.92
10c	4-Br	6.05 ± 0.95	4.64 ± 0.65	10.11 ± 1.54	13.34 ± 1.86
10d	4-F	4.68 ± 0.54	5.47 ± 0.76	8.60 ± 1.38	15.07 ± 2.14
10e	2-Cl	2.75 ± 0.85	5.26 ± 0.73	6.98 ± 1.10	11.12 ± 1.71
10f	3-Cl	2.01 ± 1.27	5.34 ± 0.75	5.56 ± 0.80	7.76 ± 1.24
10g	4-NO_2	2.35 ± 0.27	3.91 ± 0.38	3.67 ± 0.30	9.06 ± 1.72
10h	3-OCH_3	2.91 ± 0.68	3.02 ± 0.003	5.97 ± 0.90	10.33 ± 1.62
10i	3, 4, 5-trimethoxyl	1.72 ± 0.44	1.32 ± 0.74	2.73 ± 0.07	3.00 ± 0.17
10j	4-CH_3	3.60 ± 0.64	9.18 ± 1.46	7.15 ± 1.12	22.92 ± 2.73
10k	3, 4-difluoro	3.19 ± 0.08	2.29 ± 0.34	4.63 ± 0.57	5.96 ± 0.90
14	–	5.97 ± 0.59	4.59 ± 0.53	10.73 ± 1.67	36.73 ± 3.31
OdDHL	–	101.83 ± 4.98	37.31 ± 3.51	>128	>128
5-Fu	–	10.72 ± 1.51	12.24 ± 0.42	15.67 ± 0.85	20.40 ± 1.31

[a] Inhibitory activity was assayed by exposure for 72 h to substances and expressed as concentration required to inhibit tumor cell proliferation by 50% (IC_{50}). Data are presented as the means ± SDs of three independent experiments, μM = μmol/L.
[b] Not detected.

4, 5-trimethoxy group was the most potent one against all the tested cancer cell lines. Further exploration indicated that analog **11e** induced the cellular apoptosis and cell cycle arrest of MCF-7 cells at G2/M phase in a concentration-and time-dependent manner.

4. Experimental section

4.1. General

All commercial reagents were used without further purification. The reaction process was monitored by TLC with silica gel plates. The target analogs were purified by column chromatography with silica gel (200-300 meshes). The structures reported were identified by ^{1}H NMR and ^{13}C NMR (400 MHz and 100 MHz) in DMSO-d_6 or $CDCl_3$ with TMS as internal standard, high resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-T of Micromass spectrometer. Melting points were determined on an electro thermal melting point apparatus and reported uncorrected.

4.2. Procedure for the synthesis of intermediates *3n-r*

Compounds **3a-m** were synthesized previously in our group [23], so only the spectra data of compounds **3n-r** were given below. To the mixture of corresponding amine (4.2 mmol) and K_2CO_3 (0.691 g, 5.0 mmol) in acetone was added chloroacetyl chloride (0.565 g, 5.0 mmol) dropwise. The reaction mixture was stirred at room temperature for 1–1.5 h. Upon completion of the reaction, the mixture was poured into 20 mL of ice water. The precipitate formed was filtrated and washed with water. The product was dried under vacuum to obtain compounds **3n-r**, which were pure enough without further purification.

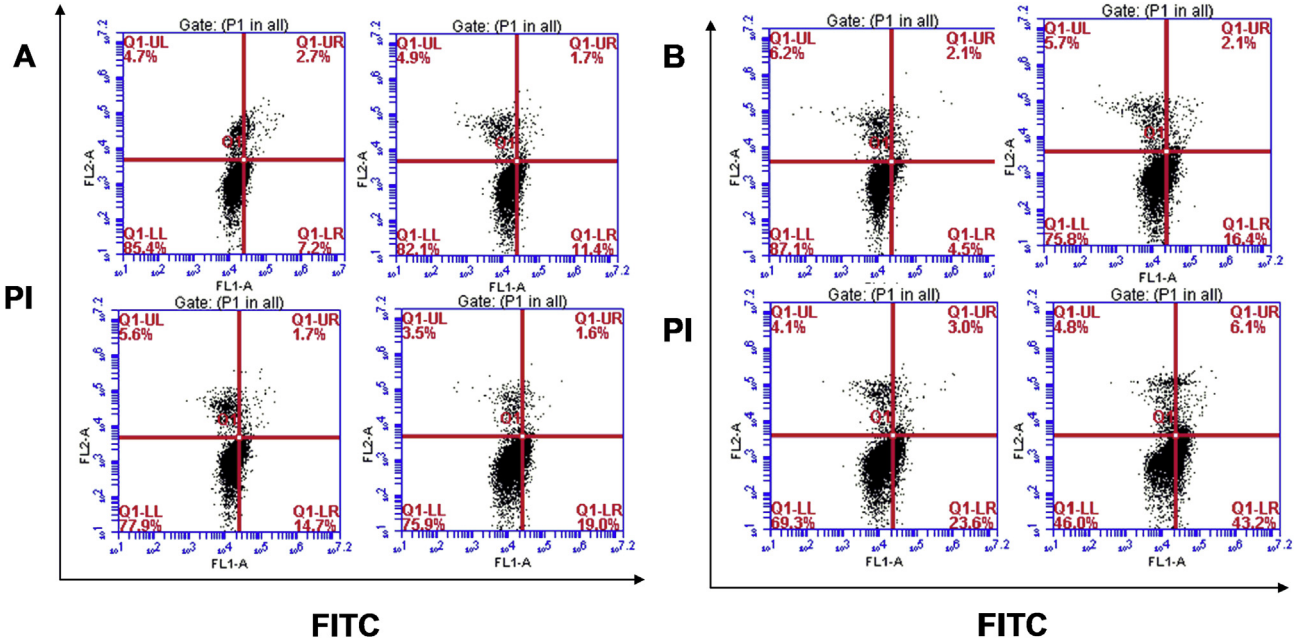


Fig. 2. Apoptotic effect on human MCF-7 cell line induced by compound **11e**. Apoptotic cells were detected with Annexin V-FITC/PI double staining after incubation with compound **11e** (0, 2, 4, 8 μM) for 12 h or 24 h. (A) Incubated for 12 h; (B) incubated for 24 h. The lower left quadrants represent live cells, the lower right quadrants are for early/primary apoptotic cells, upper right quadrants are for late/secondary apoptotic cells, while the upper left quadrants represent cells damaged during the procedure. The experiments were performed three times, and a representative experiment is shown.

*4.2.1. 2-Chloro-N-(4-fluorophenyl) acetamide (**3n**)*

Yield 67%, gray solid; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.23 (s, 1H), 7.71–7.36 (m, 2H), 7.16–6.83 (t, J = 8.6 Hz, 2H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 163.84, 161.14, 158.71, 132.67, 132.64, 122.11, 122.03, 115.99, 115.76, 42.81.

*4.2.2. 2-Chloro-N-(4-hydroxyphenyl) acetamide (**3o**)*

Yield 71%, adobe brown solid; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.06 (s, 1H), 9.30 (s, 1H), 7.37 (d, J = 8.9 Hz, 2H), 6.72 (d, J = 8.9 Hz, 2H), 4.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 164.41, 154.26, 130.51, 121.65, 115.65, 43.97.

*4.2.3. N-(4-acetylphenyl)-2-chloroacetamide (**3p**)*

Yield 73%, yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.49 (s, 1H), 7.99 (d, J = 8.7 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 4.23 (s, 2H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 196.91, 164.11,

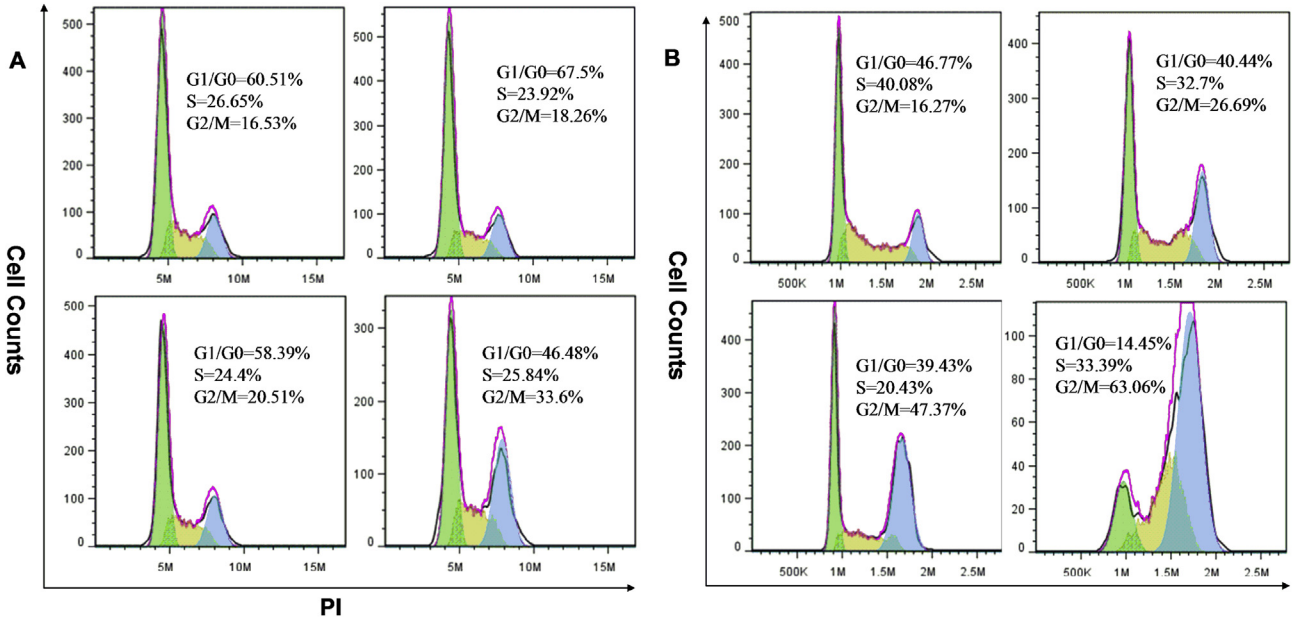


Fig. 3. Effect of compound **11e** on the cell cycle distribution of MCF-7 cells. Cells were treated with different concentrations (0, 2, 4, 8 μM) for 12 h or 24 h. Then the cells were fixed and stained with PI to analyze DNA content by flow cytometry. (A) Incubated for 12 h; (B) incubated for 24 h. The experiments were performed three times, and a representative experiment is shown.

140.97, 133.74, 129.76, 119.31, 42.89, 26.49.

4.2.4. Ethyl 4-(2-chloroacetamido) benzoate (**3q**)

Yield 68%, white solid; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.44 (s, 1H), 8.04 (d, J = 8.7 Hz, 2H), 7.65 (d, J = 8.8 Hz, 2H), 4.37 (q, J = 7.1 Hz, 2H), 4.21 (s, 2H), 1.39 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.99, 164.08, 140.71, 130.83, 126.96, 119.17, 61.03, 42.88, 14.33.

4.2.5. N-(4-butylphenyl)-2-chloroacetamide (**3r**)

Yield 69%, light blue solid; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.21 (s, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 4.17 (s, 2H), 2.59 (t, J = 7.8 Hz, 2H), 1.67–1.49 (m, 2H), 1.48–1.23 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 162.75, 139.08, 133.23, 127.99, 119.23, 41.86, 34.05, 32.56, 21.24, 12.90.

4.3. Procedure for the synthesis of analogs **4n-r**

Compounds **4a-m** were synthesized previously in our group [23], so only the spectra data of compounds **4n-r** were given below. To the mixture of compound **1** (0.4 g, 2.91 mmol) and $Na_3PO_4{\cdot}12H_2O$ (1.104 g, 2.91 mmol) in acetone (10 mL) was added CS_2 (0.22 mL, 3.64 mmol). 30 min later, acylated aniline (**3n-r**, 2.91 mmol) was added to the mixture slowly, the mixture was stirred at room temperature for another 0.5 h. Upon completion (monitored by TLC), the solvent was removed under reduced pressure, the residue was extracted with dichloromethane, washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the residue, which was purified using column chromatography to obtain analogs **4n-r**.

4.3.1. (S)-2-((4-fluorophenyl) amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**4n**)

Yield 69%, yellow solid, m.p.: 137–138 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.47 (d, J = 7.9 Hz, 1H), 10.35 (s, 1H), 7.68–7.55 (m, 2H), 7.21–7.10 (m, 2H), 5.52 (dt, J = 11.1, 8.7 Hz, 1H), 4.41 (t, J = 8.3 Hz, 1H), 4.35–4.24 (m, 1H), 4.21 (s, 2H), 2.64–2.53 (m, 1H), 2.28–2.18 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.06, 174.06, 165.89, 159.70, 157.32, 135.80, 135.78, 121.35, 121.27, 115.94, 115.72, 65.98, 54.98, 40.02, 28.36; HRMS (ESI) Calcd for $C_{13}H_{13}FN_2NaO_3S_2$ $[M+Na]^+$: 351.0249, found: 351.0251.

4.3.2. (S)-2-((4-hydroxyphenyl) amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl) carbamodithioate (**4o**)

Yield 64%, brown solid, m.p.: 86–88 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.46 (d, J = 8.0 Hz, 1H), 10.03 (s, 1H), 9.22 (s, 1H), 7.35 (d, J = 8.9 Hz, 2H), 6.69 (d, J = 8.9 Hz, 2H), 5.51 (dt, J = 11.2, 8.7 Hz, 1H), 4.40 (t, J = 8.3 Hz, 1H), 4.33–4.27 (m, 1H), 4.15 (s, 2H), 2.67–2.52 (m, 1H), 2.33–2.13 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.12, 174.06, 165.24, 153.95, 131.02, 121.35, 115.56, 65.98, 54.94, 40.19, 28.37; HRMS (ESI) Calcd for $C_{13}H_{14}N_2NaO_4S_2$ $[M+Na]^+$: 349.0293, found: 349.0296.

4.3.3. (S)-2-((4-acetylphenyl) amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl) carbamodithioate (**4p**)

Yield 70%, yellow solid, m.p.: 157–158 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.64 (s, 1H), 10.49 (d, J = 8.0 Hz, 1H), 7.94 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H), 5.53 (dt, J = 11.1, 8.7 Hz, 1H), 4.41 (t, J = 8.4 Hz, 1H), 4.34–4.31 (m, 1H), 4.28 (s, 2H), 2.67–2.55 (m, 1H), 2.53 (s, 3H), 2.36–2.15 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.02, 196.98, 174.05, 166.60, 143.70, 132.29, 130.01, 118.79, 65.99, 55.03, 40.18, 28.37, 26.91; HRMS (ESI) Calcd for $C_{15}H_{16}N_2NaO_4S_2$ $[M+Na]^+$: 375.0449, found: 375.0452.

4.3.4. (S)-ethyl 4-(2-(((2-oxotetrahydrofuran-3-yl) carbamothioyl) thio) acetamido) benzoate (**4q**)

Yield 60%, dark yellow solid, m.p.: 141–142 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.62 (s, 1H), 10.47 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 8.8 Hz, 2H), 5.51 (dt, J = 10.9, 8.6 Hz, 1H), 4.40 (t, J = 8.3 Hz, 1H), 4.35–4.27 (m, 1H), 4.26 (s, 2H), 2.65–2.53 (m, 1H), 2.50 (m, 2H), 2.30–2.13 (m, 1H), 1.31 (t, J = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.01, 174.04, 166.59, 165.75, 143.71, 130.77, 124.82, 118.91, 65.98, 60.92, 55.02, 40.18, 28.36, 14.68; HRMS (ESI) Calcd for $C_{16}H_{18}N_2NaO_5S_2$ $[M+Na]^+$: 405.0555, found: 405.0552.

4.3.5. (S)-2-((4-butylphenyl) amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl) carbamodithioate (**4r**)

Yield 71%, yellow solid, m.p.: 69-70108-109 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.43 (d, J = 7.0 Hz, 1H), 10.17 (s, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.09 (d, J = 8.3 Hz, 2H), 5.49 (dt, J = 11.1, 8.7 Hz, 1H), 4.37 (t, J = 8.6 Hz, 1H), 4.31–4.22 (m, 1H), 4.17 (s, 2H), 2.61–2.52 (m, 1H), 2.51–2.47 (m, 2H), 2.26–2.15 (m, 1H), 1.57–1.39 (m, 2H), 1.36–1.17 (m, 2H), 0.85 (t, J = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.10, 174.06, 165.71, 137.86, 137.06, 128.95, 119.59, 65.98, 54.97, 40.13, 34.70, 33.68, 28.37, 22.15, 14.26; HRMS (ESI) Calcd for $C_{17}H_{22}N_2NaO_3S_2$ $[M+Na]^+$: 389.0970, found: 389.0972.

4.4. Procedure for the synthesis of compounds **10a-k** and **14**

The solution of compounds **7** (0.5 g, 3.699 mmol) and **8a-k** or **12** (3.792 mmol) in EtOH (25 mL) was added to the aqueous NaOH solution (0.185 g NaOH, 25 mL H_2O) dropwise. The reaction mixture was stirred at room temperature for 4–12 h. Upon completion of the reaction, the mixture was poured into 25 mL ice water, filtrated and washed with water and dried over $MgSO_4$ to obtain compounds **9a-k** and **13** without further purification. To the mixture of **9a-k** or **13** (3.399 mmol) and K_2CO_3 (0.564 g, 4.079 mmol) in acetone (10 mL) was added chloroacetyl chloride (0.461 g, 4.079 mmol) dropwise. The resulting solution was stirred at room temperature for 1–1.5 h. Upon completion, the mixture was poured into 20 mL of ice water. The precipitate formed was filtrated, washed with water and dried under vacuum to obtain **10a-k** and **14** without further purification.

4.4.1. (E)-2-chloro-N-(4-(3-(4-chlorophenyl) acryloyl) phenyl) acetamide (**10a**)

Yield 77%, yellow solid, m.p.: 199–200 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.43 (s, 1H), 8.06 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 15.7 Hz, 1H), 7.73 (d, J = 8.7 Hz, 2H), 7.58 (d, J = 8.5 Hz, 2H), 7.50 (d, J = 15.7 Hz, 1H), 7.40 (d, J = 8.5 Hz, 2H), 4.23 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 188.60, 164.02, 143.32, 140.81, 136.50, 134.57, 133.37, 129.98, 129.62, 129.28, 122.07, 119.41, 42.88; HRMS (ESI) Calcd for $C_{17}H_{23}N_2O_3S_2$ $[M+H]^+$: 334.0402, found: 334.0397.

4.4.2. (E)-2-chloro-N-(4-cinnamoylphenyl) acetamide (**10b**)

Yield 72%, yellow solid, m.p.: 157–158 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.47 (s, 1H), 8.06 (d, J = 8.7 Hz, 2H), 7.82 (d, J = 15.7 Hz, 1H), 7.73 (d, J = 8.7 Hz, 2H), 7.65 (dd, J = 6.7, 2.8 Hz, 2H), 7.53 (d, J = 15.7 Hz, 1H), 7.42 (dd, J = 4.9, 1.7 Hz, 3H), 4.22 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 188.97, 164.06, 144.87, 140.75, 134.86, 134.73, 130.61, 129.97, 128.99, 128.48, 121.68, 119.41, 42.90; HRMS (ESI) Calcd for $C_{17}H_{14}ClNNaO_2$ $[M+Na]^+$: 322.0611, found: 322.0614.

4.4.3. (E)-N-(4-(3-(4-bromophenyl)acryloyl)phenyl)-2-chloroacetamide (**10c**)

Yield 75%, yellow solid, m.p.: 208–210 °C; ^{1}H NMR (400 MHz,

DMSO-d_6, δ, ppm): 10.70 (s, 1H), 8.19 (d, J = 8.6 Hz, 2H), 7.99 (d, J = 15.6 Hz, 1H), 7.86 (d, J = 8.3 Hz, 2H), 7.80 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 16.0 Hz, 1H), 7.67 (d, J = 8.8 Hz, 2H), 4.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 187.95, 165.71, 143.42, 142.59, 134.55, 133.18, 132.34, 131.20, 130.49, 124.34, 123.22, 119.23, 44.10; HRMS (ESI) Calcd for $C_{17}H_{14}BrClNO_2$ $[M+H]^+$: 377.9896, found: 377.9897.

4.4.4. (E)-2-chloro-N-(4-(3-(4-fluorophenyl)acryloyl)phenyl) acetamide (**10d**)

Yield 70%, yellow solid, m.p.: 162–164 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (s, 1H), 8.19 (d, J = 8.7 Hz, 2H), 7.98 (dd, J = 7.6, 5.9 Hz, 2H), 7.92 (d, J = 15.7 Hz, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 15.6 Hz, 1H), 7.31 (t, J = 8.3 Hz, 2H), 4.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 187.98, 165.71, 165.08, 162.60, 143.35, 142.74, 133.27, 131.94, 131.91, 131.68, 131.60, 130.43, 122.32, 119.23, 116.50, 116.28, 44.09; HRMS (ESI) Calcd for $C_{17}H_{14}ClFNO_2$ $[M+H]^+$: 318.0697, found: 318.0699.

4.4.5. (E)-2-chloro-N-(4-(3-(2-chlorophenyl)acryloyl)phenyl) acetamide (**10e**)

Yield 65%, yellow solid, m.p.: 167–168 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.71 (s, 1H), 8.21 (dd, J = 9.1, 5.1 Hz, 3H), 8.02 (d, J = 5.0 Hz, 2H), 7.81 (d, J = 8.8 Hz, 2H), 7.63–7.55 (m, 1H), 7.52–7.42 (m, 2H), 4.34 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 187.84, 165.74, 143.56, 138.53, 134.79, 132.99, 132.85, 132.37, 130.60, 130.49, 129.02, 128.15, 125.17, 119.26, 44.10; HRMS (ESI) Calcd for $C_{17}H_{14}Cl_2NO_2$ $[M+H]^+$: 334.0402, found: 334.0406.

4.4.6. (E)-2-chloro-N-(4-(3-(3-chlorophenyl)acryloyl)phenyl) acetamide (**10f**)

Yield 68%, yellow solid, m.p.: 143–145 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.46 (s, 1H), 8.06 (d, J = 8.7 Hz, 2H), 7.82–7.68 (m, 3H), 7.64 (s, 1H), 7.58–7.46 (m, 2H), 7.42–7.32 (m, 2H), 4.22 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 187.46, 163.05, 142.05, 139.90, 135.69, 133.99, 133.40, 129.37, 129.21, 129.00, 126.91, 125.80, 121.81, 118.42, 41.87; HRMS (ESI) Calcd for $C_{17}H_{14}Cl_2NO_2$ $[M+H]^+$: 334.0402, found: 334.0403.

4.4.7. (E)-2-chloro-N-(4-(3-(4-nitrophenyl)acryloyl)phenyl) acetamide (**10g**)

Yield 71%, yellow solid, m.p.: 200–202 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.71 (s, 1H), 8.28 (d, J = 8.7 Hz, 2H), 8.22 (d, J = 8.7 Hz, 2H), 8.19–8.08 (m, 3H), 7.87–7.76 (m, 3H), 4.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 187.81, 165.74, 148.47, 143.64, 141.73, 141.08, 132.89, 130.65, 130.25, 126.44, 124.37, 119.24, 44.10; HRMS (ESI) Calcd for $C_{17}H_{14}ClN_2O_4$ $[M+H]^+$: 345.0642, found: 345.0641.

4.4.8. (E)-2-chloro-N-(4-(3-(3-methoxyphenyl)acryloyl)phenyl) acetamide (**10h**)

Yield 73%, yellow solid, m.p.: 124–126 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.51 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.77 (d, J = 15.7 Hz, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 15.7 Hz, 1H), 7.34 (t, J = 7.9 Hz, 1H), 7.24 (d, J = 7.7 Hz, 1H), 7.18–7.12 (m, 1H), 6.97 (dd, J = 8.1, 1.9 Hz, 1H), 4.22 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 189.00, 164.14, 159.97, 144.79, 140.82, 136.22, 134.65, 129.98, 121.98, 121.12, 119.44, 116.37, 113.52, 55.38, 42.92; HRMS (ESI) Calcd for $C_{18}H_{17}ClNO_3$ $[M+H]^+$: 330.0897, found: 330.0898.

4.4.9. (E)-2-chloro-N-(4-(3-(3, 4, 5-trimethoxyphenyl)acryloyl) phenyl)acetamide (**10i**)

Yield 66%, yellow solid, m.p.: 174–175 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.49 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.73 (dd, J = 12.1, 3.4 Hz, 3H), 7.40 (d, J = 15.6 Hz, 1H), 6.87 (s, 2H), 4.23 (s, 2H), 3.93 (s, 6H), 3.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 189.03, 164.13, 153.51, 145.07, 140.74, 140.53, 134.77, 130.34, 129.94, 121.08, 119.44, 105.75, 61.02, 56.27, 42.91; HRMS (ESI) Calcd for $C_{20}H_{21}ClNO_5$ $[M+H]^+$: 390.1108, found: 390.1111.

4.4.10. (E)-2-chloro-N-(4-(3-(p-tolyl)acryloyl)phenyl)acetamide (**10j**)

Yield 69%, yellow solid, m.p.: 185–187 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.68 (s, 1H), 8.18 (d, J = 8.7 Hz, 2H), 7.89 (d, J = 15.6 Hz, 1H), 7.80 (d, J = 2.8 Hz, 2H), 7.77 (d, J = 2.2 Hz, 2H), 7.71 (d, J = 15.6 Hz, 1H), 7.28 (d, J = 8.0 Hz, 2H), 4.33 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 188.03, 165.69, 144.06, 143.25, 141.07, 133.41, 132.52, 130.37, 130.01, 129.33, 121.35, 119.23, 44.10, 21.56; HRMS (ESI) Calcd for $C_{18}H_{17}ClNO_2$ $[M+H]^+$: 314.0948, found: 314.0950.

4.4.11. (E)-2-chloro-N-(4-(3-(3, 4-difluorophenyl)acryloyl)phenyl) acetamide (**10k**)

Yield 57%, yellow solid, m.p.: 187–189 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.70 (s, 1H), 8.29 (s, 1H), 8.21 (d, J = 8.7 Hz, 2H), 8.06 (d, J = 15.6 Hz, 1H), 7.88 (d, J = 8.4 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 8.6 Hz, 1H), 7.70 (d, J = 15.7 Hz, 1H), 4.33 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 187.81, 165.73, 143.53, 141.16, 136.15, 133.09, 133.04, 132.29, 131.46, 130.60, 130.56, 129.59, 124.49, 119.22, 44.10; HRMS (ESI) Calcd for $C_{17}H_{13}C_{l3}NO_2$ $[M+H]^+$: 336.0603, found: 336.0606.

4.4.12. 2-Chloro-N-(4-((2E, 4E)-5-phenylpenta-2, 4-dienoyl) phenyl)acetamide (**14**)

Yield 56%, earthy yellow solid, m.p.: 165–168 °C; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 8.50 (s, 1H), 8.01 (d, J = 8.7 Hz, 2H), 7.71 (d, J = 8.6 Hz, 2H), 7.61 (ddd, J = 14.9, 7.0, 3.3 Hz, 1H), 7.50 (d, J = 7.0 Hz, 2H), 7.41–7.30 (m, 3H), 7.09 (d, J = 14.8 Hz, 1H), 7.05–7.00 (m, 2H), 4.22 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 187.93, 163.13, 143.86, 141.07, 139.70, 135.07, 133.69, 128.80, 128.26, 127.85, 126.31, 125.88, 123.98, 118.39, 41.91; HRMS (ESI) Calcd for $C_{19}H_{17}ClNO_2$ $[M+H]^+$: 326.0948, found: 326.0951.

4.5. General procedure for the synthesis of compounds **11a-g**

To the mixture of compound **1** (0.2 g, 1.454 mmol) and $Na_3PO_4{\cdot}12H_2O$ (0.454 g, 1.454 mmol) in acetone (10 mL) was added CS_2 (110 μL, 1.82 mmol). The mixture was stirred at room temperature for 0.5 h. Then compounds **10a-g** (1.454 mmol) were added to the mixture slowly, and stirred at room temperature for another 0.5 h. Upon completion, the solvent was removed under reduced pressure, the residue was extracted with dichloromethane, washed with water, brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified using column chromatography to give analogs **11a-g**.

4.5.1. (S, E)-2-((4-(3-(4-chlorophenyl)acryloyl)phenyl)amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11a**)

Yield 50%, yellow solid, m.p.: 110–111 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.68 (s, 1H), 10.49 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 15.6 Hz, 1H), 7.94 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 15.6 Hz, 1H), 7.53 (d, J = 8.5 Hz, 2H), 5.53 (dt, J = 11.0, 8.7 Hz, 1H), 4.41 (t, J = 8.3 Hz, 1H), 4.35–4.26 (m, 3H), 2.65–2.54 (m, 1H), 2.31–2.18 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.03, 187.89, 174.03, 166.66, 143.89, 142.40, 135.44, 134.25, 132.81, 130.99, 130.50, 129.41, 123.20, 118.94, 65.99, 55.04, 40.21, 28.37; HRMS (ESI) Calcd for $C_{22}H_{19}ClN_2NaO_4S_2$ $[M+Na]^+$: 497.0372, found: 497.0374.

*4.5.2. (S, E)-2-((4-cinnamoylphenyl) amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11b**)*

Yield 46%, yellow solid, m.p.: 96–97 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.69 (s, 1H), 10.50 (d, J = 7.9 Hz, 1H), 8.17 (d, J = 8.8 Hz, 2H), 7.95 (d, J = 15.5 Hz, 1H), 7.89 (dd, J = 6.5, 2.8 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 15.6 Hz, 1H), 7.54–7.42 (m, 3H), 5.52 (dt, J = 11.0, 8.6 Hz, 1H), 4.41 (t, J = 8.4 Hz, 2H), 4.35–4.31 (m, 1H), 4.29 (s, 2H), 2.62–2.56 (m, 1H), 2.30–2.19 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.04, 188.06, 174.05, 166.67, 143.92, 143.79, 135.22, 132.93, 130.99, 130.44, 130.37, 129.39, 129.27, 122.44, 119.11, 118.99, 66.01, 55.04, 40.16, 28.34; HRMS (ESI) Calcd for $C_{22}H_{20}N_2NaO_4S_2$ $[M+Na]^+$: 463.0762, found: 63.0764.

*4.5.3. (S, E)-2-((4-(3-(4-bromophenyl)acryloyl)phenyl)amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11c**)*

Yield 50%, yellow solid, m.p.: 167–168 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.67 (s, 1H), 10.48 (d, J = 7.9 Hz, 1H), 8.17 (d, J = 8.8 Hz, 2H), 7.99 (d, J = 15.6 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 8.8 Hz, 2H), 7.71–7.66 (m, 3H), 5.52 (dt, J = 11.0, 8.7 Hz, 1H), 4.41 (t, J = 8.3 Hz, 1H), 4.36–4.23 (m, 3H), 2.63–2.54 (m, 1H), 2.30–2.17 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.03, 187.90, 174.03, 166.67, 143.89, 142.50, 134.57, 132.80, 132.34, 131.20, 130.50, 124.31, 123.25, 118.95, 65.99, 55.04, 40.21, 28.36; HRMS (ESI) Calcd for $C_{22}H_{19}BrN_2NaO_4S_2$ $[M+Na]^+$: 540.9867, found: 540.9871.

*4.5.4. (S, E)-2-((4-(3-(4-fluorophenyl)acryloyl)phenyl)amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11d**)*

Yield 32%, yellow solid, m.p.: 124–125 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.66 (s, 1H), 10.48 (d, J = 7.8 Hz, 1H), 8.17 (d, J = 8.8 Hz, 2H), 7.97 (dd, J = 8.7, 5.7 Hz, 2H), 7.91 (d, J = 15.6 Hz, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.73 (d, J = 15.7 Hz, 1H), 7.31 (t, J = 8.8 Hz, 2H), 5.52 (dt, J = 10.6, 8.7 Hz, 1H), 4.41 (t, J = 8.3 Hz, 1H), 4.35–4.31 (m, 1H), 4.29 (s, 2H), 2.64–2.54 (m, 1H), 2.30–2.19 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.04, 187.95, 174.03, 166.66, 165.06, 162.58, 143.81, 142.65, 132.91, 131.95, 131.92, 131.65, 131.57, 130.43, 122.35, 118.96, 116.49, 116.27, 66.00, 55.05, 40.20, 28.35; HRMS (ESI) Calcd for $C_{22}H_{19}FN_2NaO_4S_2$ $[M+Na]^+$: 481.0668, found: 481.0670.

*4.5.5. (S, E)-2-((4-(3-(2-chlorophenyl)acryloyl)phenyl)amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11e**)*

Yield 41%, yellow solid, m.p.: 95–96 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.71 (s, 1H), 10.50 (d, J = 7.9 Hz, 1H), 8.25–8.20 (m, 2H), 8.19 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 4.0 Hz, 2H), 7.79 (d, J = 8.8 Hz, 2H), 7.60–7.55 (m, 1H), 7.52–7.42 (m, 2H), 5.52 (dt, J = 10.9, 8.7 Hz, 1H), 4.41 (t, J = 8.4 Hz, 1H), 4.35–4.27 (m, 3H), 2.66–2.54 (m, 1H), 2.30–2.19 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.04, 187.82, 174.05, 166.72, 144.01, 138.48, 134.77, 132.84, 132.62, 132.35, 130.59, 130.47, 128.99, 128.14, 125.15, 119.00, 66.01, 55.05, 40.21, 28.33; HRMS (ESI) Calcd for $C_{22}H_{19}ClN_2NaO_4S_2$ $[M+Na]^+$: 497.0372, found: 497.0374.

*4.5.6. (S, E)-2-((4-(3-(3-chlorophenyl)acryloyl)phenyl)amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11f**)*

Yield 42%, yellow solid, m.p.: 168–169 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.68 (s, 1H), 10.48 (d, J = 7.8 Hz, 1H), 8.20 (d, J = 8.8 Hz, 2H), 8.09 (s, 1H), 8.04 (d, J = 15.6 Hz, 1H), 7.84–7.80 (m, 1H), 7.78 (d, J = 8.8 Hz, 2H), 7.70 (d, J = 15.6 Hz, 1H), 7.54–7.44 (m, 2H), 5.52 (dt, J = 10.5, 8.7 Hz, 1H), 4.41 (t, J = 8.3 Hz, 1H), 4.35–4.26 (m, 3H), 2.64–2.54 (m, 1H), 2.30–2.16 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.03, 187.87, 174.03, 166.68, 143.95, 142.15, 137.53, 134.28, 132.73, 131.14, 130.58, 130.49, 128.34, 128.32, 123.98, 118.94, 66.00, 55.05, 40.21, 28.36; HRMS (ESI) Calcd for $C_{22}H_{19}ClN_2NaO_4S_2$ $[M+Na]^+$: 497.0372, found: 497.0372.

*4.5.7. (S, E)-2-((4-(3-(4-nitrophenyl)acryloyl)phenyl)amino)-2-oxoethyl (2-oxotetrahydrofuran-3-yl)carbamodithioate (**11g**)*

Yield 30%, yellow solid, m.p.: 126–128 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.71 (s, 1H), 10.50 (d, J = 8.0 Hz, 1H), 8.29 (d, J = 8.8 Hz, 2H), 8.20 (d, J = 8.8 Hz, 2H), 8.17 (d, J = 8.7 Hz, 2H), 8.14 (d, J = 15.6 Hz, 1H), 7.82–7.78 (m, 3H), 5.52 (dt, J = 11.0, 8.7 Hz, 1H), 4.41 (t, J = 8.4 Hz, 1H), 4.35–4.27 (m, 3H), 2.64–2.54 (m, 1H), 2.32–2.17 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 198.04, 187.81, 174.05, 166.73, 148.46, 144.10, 141.74, 141.02, 132.52, 130.65, 130.24, 126.47, 124.38, 119.00, 66.01, 55.04, 40.14, 28.32; HRMS (ESI) Calcd for $C_{22}H_{19}N_3NaO_6S_2$ $[M+Na]^+$: 508.0613, found: 508.0614.

4.6. Procedure for the synthesis of OdDHL

To a mixture of malonic acid **15** (4.20 g, 40.36 mmol) and acetic anhydride (4.96 mL, 52.47 mmol) was added H_2SO_4 (0.17 mL, 3.23 mmol), the mixture was then stirred for about 0.5 h in an ice-water bath, followed by addition of acetone **16** (4.16 mL, 56.51 mmol). After stirring for another 4 h, the resulting solution was then allowed to warm to room temperature and kept overnight at the same temperature. Upon completion of the reaction, the precipitate was filtrated, washed with ice water and dried to gain Meldrum's acid **17**. To a solution of *n*-decanoic acid (1.376 g, 8 mmol) in dry CH_2Cl_2 were added DMAP (1.024 g, 8.4 mmol), DCC (1.816 g, 8.8 mmol) and **17** (1.152 g, 8 mmol) sequentially, and the reaction mixture was stirred overnight at room temperature. *N*, *N*'-dicyclohexylurea formed was removed by filtration, and the filtrate was concentrated under vacuo to give the residue, which was then dissolved in ethyl acetate, washed with 2 N hydrochloric acid, dried over $MgSO_4$ and concentrated to give the crude mixture of **19**. To a solution of **1** (0.461 g, 3.35 mmol) and **19** (1.001 g) in acetonitrile (50 mL) was added Et_3N (0.407 g, 4.02 mmol). The reaction mixture was stirred at room temperature for 4 h and then stirred under reflux. Upon completion of the reaction, the reaction mixture was concentrated under vacuo to give the residue, which was then subjected to column chromatography (petroleum ether: acetone = 10: 1) to give OdDHL.

*4.6.1. (S)-3-oxo-N-(2-oxotetrahydrofuran-3-yl) dodecanamide (**OdDHL**)*

Yield 30%, white solid; ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.71 (d, J = 5.2 Hz, 1H), 4.61 (ddd, J = 11.4, 8.7, 6.8 Hz, 1H), 4.48 (t, J = 8.7 Hz, 1H), 4.31–4.25 (m, 1H), 3.48 (s, 2H), 2.83–2.65 (m, 1H), 2.53 (t, J = 7.4 Hz, 1H), 2.35–2.11 (m, 1H), 1.70–1.46 (m, 2H), 1.26 (s, 12H), 0.88 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 206.58, 174.94, 166.48, 65.92, 49.02, 48.20, 43.88, 31.85, 29.71, 29.39, 29.35, 29.24, 28.99, 23.35, 22.66, 14.11.

4.7. Cell culturing

Human cancer cell lines were maintained in minimal essential medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cancer cells were maintained in RPMI1640 medium. All cell lines were purchased from the China Center for Type Culture Collection (CCTCC, Shanghai, China). For pharmacological investigations, 10 mM stock solutions of the tested compounds were prepared with DMSO. The highest DMSO concentration of the medium (0.1%) did not have any substantial effect on the determined cellular functions.

4.8. Cytotoxic activity assays

Exponentially growing cells were seeded into 96-well plates at a concentration of 5 × 10^3 cells per well. After 24 h incubation at

37 °C, the culture medium was removed and replaced with fresh medium containing the tested compounds at different concentrations. The cells were incubated for another 72 h. Afterwards 20 μL of MTT solution (5 mg/mL) was added to all wells and incubated for 4 h at 37 °C. Discarded the suspension and added 150 mL of DMSO to each well and shook the plates to dissolve the dark blue crystals (formazan); the absorbance was measured using a microplate reader at a wavelength of 570 nm. Each concentration was analyzed in triplicate and the experiment was repeated for three times. The average 50% inhibitory concentration (IC_{50}, μM) was determined from the dose–response curves according to the inhibition ratio for each concentration.

4.9. Analysis of cellular apoptosis

MCF-7 cells were plated in 6-well plates (5.0 × 106 cells/mL) and incubated at 37 °C for 12 or 24 h. Exponentially growing cells were then incubated for 12 or 24 h with complete medium (blank) or with the compound **11e**. Cells were then harvested and the Annexin-V-FITC/PI apoptosis kit (Biovision) was used according to the manufacturer's instructions to detect apoptotic cells. Ten thousand events were collected for each sample and analyzed by Accuri C6 flow cytometer.

4.10. Flow cytometric analysis of cell cycle distribution

For flow cytometric analysis of DNA content, 5.0 × 106 MCF-7 cells in exponential growth were treated with different concentrations of the test compounds for 12 or 24 h. After an incubation period, the cells were collected, centrifuged and fixed with ice-cold ethanol (70%). The cells were then treated with buffer containing RNAse A and 0.1% Triton X-100 and then stained with PI. Samples were analyzed on Accuri C6 flow cytometer (Becton, Dickinson). Data obtained from the flow cytometer was analyzed using the FlowJo software (Tree Star, Inc., Ashland, OR, USA).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.02.026.

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