European Journal of Medicinal Chemistry 53 (2012) 356–363

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Convenient synthesis of novel geiparvarin analogs with potential anti-cancer activity via *click chemistry*

Yikai Zhang [a], Zhiliang Lv [a], Hanyu Zhong [a], Dongping Geng [a], Mingfeng Zhang [a], Tao Zhang [a], Yongmei Li [b,*], Ke Li [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Second Military Medical University, No. 325, Guohe Road, Shanghai 200433, China
[b] Department of Oncology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China

ARTICLE INFO

Article history:
Received 4 December 2011
Received in revised form
15 April 2012
Accepted 18 April 2012
Available online 26 April 2012

Keywords:
Benzopyran-4-one derivative
Bioisosteric transformation
Synthesis
Click chemistry
Anti-cancer activity

ABSTRACT

Based on the advantages of natural products in new anti-cancer drug development, we synthesized a series of novel benzopyran-4-one derivatives and evaluated their *in vitro* anti-cancer activities. The bioassays showed that the majority of the resultant compounds exerted anti-tumor effect against six human cancer cell lines to various extents, which supported the rationale of the design. Compound **5s** exhibited highest potency of all the synthesized compounds.

1. Introduction

Natural products have become a leading category of compounds in improving the rational drug design for novel anti-cancer therapeutics. Geiparvarin is one of the naturally occurring compounds with remarkable bioactivities against various human tumors albeit the mechanisms underlying these effects are still unclear [1,2].

As shown in Fig. 1, geiparvarin carries a benzopyran-2-one (Fig. 2[A]) residue and a 5-membered ring, which are linked by a long chain containing an ether functional group. Total synthesis of geiparvarin needs at least 7 steps according to Kang and its yield was only about 20% [3]. A series of geiparvarin analogs with the 3′-methyl group of the unsaturated alkenyloxy bridge being removed have been evaluated against a panel of human tumor cell lines, and the results demonstrated that these analogs gained strongly increased anti-tumor activities comparing with the lead compound [4,5]. Furthermore, geiparvarin analogs incorporating benzopyran-2-one and 5-membered heterocyclic residues via $-OCH_2-$ bridge into a single molecule also showed good anti-cancer activities [6].

Drug design is highly dependent on the available structure–activity relationships reported from previous studies, which could help us to determine the essential structural features of the designed compounds with certain biological activities. The benzopyrones can be subdivided into the benzopyran-2-one (Fig. 2[A]) to which the coumarins belong and the benzopyran-4-one (Fig. 2[B]), of which the flavonoids are principal members. Thereby, it is considered that benzopyran-2-one and benzopyran-4-one are bioisosteres [7]. Also, The derivatives of benzopyran-4-one, also known as chromone, have shown promising anti-cancer activities [8–10]. The most prevailing hypothesis is that the chromone acts as a mimetic of the adenine moiety of ATP, the receptor co-factor, which enables the compound the ability to bind to the ATP-binding site of several kinases [11]. In these reasons, we altered benzopyran-2-one residue of the geiparvarin into benzopyran-4-one.

On the other hand, 1, 2, 3-Triazole is considered as a useful component and presents in many drugs [12,13]. Compounds with 1, 2, 3-triazole could actively participate in hydrogen bonding and dipole–dipole interactions due to their strong dipole moments; moreover, it is extremely hard to be hydrolyzed and keeps stable in oxidative or reductive conditions [14]. In particular, 1, 2, 3-triazole has been of interest to medicinal chemists since Sharpless first introduced the concept of -*click chemistry*, by which the Huisgen's [2 + 3] cycloaddition reaction of organic azides and terminal alkynes can be efficiently catalyzed by copper (I) ions and hence be performed at room temperature, that results in the exclusive formation of 1, 4-regioisomers of 1, 2, 3-triazole [15–18]. Therefore,

* Corresponding authors. Tel.: +86 21 81871237.
E-mail addresses: liyongmei@sina.com (Y. Li), proflike@sina.com (K. Li).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2012.04.026

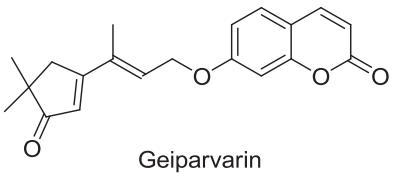


Fig. 1. Chemical structure of geiparvarin.

the diversity of chemical structures of the 1, 2, 3-triazole family members and their biological activities enable us to choose them as the 5-membered heterocyclic residues [4,5] as mentioned above.

Inspired by these reports, our group applied the principle of bioisosteric transformation and the "*click chemistry*" approach to synthesize a series of geiparvarin analogs (chromone linked with 1, 2, 3-triazole residues via $-OCH_2-$ bridge, Fig. 3) and then investigated the anti-cancer activities of the designed compounds. Compared with geiparvarin, especially, the synthetic route of our geiparvarin analogs only needs 4 steps from starting material and the yield could be 50–60%.

2. Chemistry

The chemical synthesis of benzopyran-4-one derivatives was achieved by a 5-step protocol, as illustrated in Scheme 1. As a key intermediate of our designed compounds, compound **3** was produced with a 70% yield by reacting 1-(2, 4-dihydroxyphenyl) ethanone (**1**) with triethyl orthoformate and 70% perchloric acid, and being followed by aqueous hydrolysis of the intermediate perchlorate salt (**2**) [19,20]. Compound **3** was then heated at reflux with propargyl bromide and K_2CO_3 in acetone under N_2 protection for 8 h to afford compound **4** with a 91% yield. According to Huisgen's [2 + 3] cycloaddition reaction [18], intermediate **4** was reacted with substituted benzyl bromides, NaN_3, sodium ascorbate and $CuSO_45H_2O$ in DMSO at room temperature overnight to afford the target compounds **5a**–**5t**. The chemical structures of these compounds were confirmed by ESI-MS, NMR spectroscopic analysis and elemental analyses. The purity of final compounds was assessed on the basis of analytical HPLC (≥95%). (see Supplementary Data). In order to determine the configuration of the 1, 2, 3-triazole, the crystal structure of compound **5p** was determined and its crystal data are presented in Table 1. Fig. 4 shows the ORTEP drawing of compound **5p**, indicating that the Huisgen's [2 + 3] cycloaddition was trans-addition.

3. Pharmacology

Twenty-one Compounds (**4**, **5a**–**5t**) were evaluated for their *in vitro* cytotoxic activities against one human normal cell line and six human cancer cell lines via MTT method, including L02 (normal human liver cell line), A-549 (human lung adenocarcinoma cell line), HeLa (human cervical carcinoma cell line) and QGY-7701 (human hepatoma cell line), SW480 (human colon carcinoma cell line), SGC7901 (human gastric adenocarcinoma cell line) SGC7901 (human gastric carcinoma cell line), MDA-MB-231 (human breast carcinoma cell line). Both geiparvarin and 5-Fluorouracil (5-Fu) were used as positive controls.

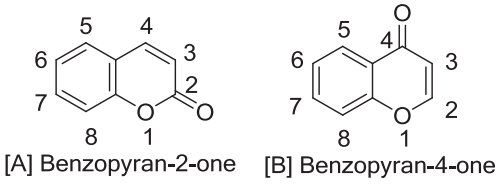


Fig. 2. Chemical structures benzopyrones.

To further illuminate drug-induced apoptosis, geiparvarin, compounds **5m** and **5t** were evaluated for A-549 cell apoptosis. In the early stage of apoptosis, the membrane phospholipid PS is exported from the intracellular leaflet to the extracellular leaflet. The exposed PS binds to the phospholipid-binding protein annexin V in the presence of calcium, providing a reliable method for quantifying the percentage of cells undergoing apoptosis using flow cytometry. The membrane impermeable DNA-staining PI was used to detect cell membrane destruction in necrotic cells [21,22]. Different labeling patterns in this assay enable us to identify different cell populations: intact living cells (both annexin V and PI negative), early apoptotic cells (annexin V positive but PI negative), and late/secondary apoptotic cells (both annexin V and PI positive) [23,24].

4. Results and discussion

4.1. In vitro cytotoxic activity

The results of the assay are summarized in Table 2 (where the IC_{50} value is defined as the concentration of a compound that corresponds to 50% growth inhibition). Data in the table showed the majority of the synthesized compounds displayed certain anti-tumor activities against the five human cancer cell lines and low toxicity to human normal cell line, which could support the rationale of the drug design.

An attempt was made to draw some considerations concerning structure-activity relationship (SAR). Compound **5a** with no substitution on the benzene ring exhibited poor anti-tumor effect. Nevertheless, the presence of an electron-donating group on the benzene (compounds **5b**–**5f**) was associated with a noticeable increase in the growth inhibitory effect, especially compound **5d** showing the comparable inhibitory against SW480 cell line ($IC_{50} = 10.40$ μM) with both 5-Fu ($IC_{50} = 15.71$ μM) and geiparvarin ($IC_{50} = 20.34$ μM). This could suggest the importance of the substituted benzene of this molecular modification for the bioactivity.

Introduction of halogen groups on the benzene ring (**5g**–**5p**) led to remarkable progress in the growth inhibitory effect once more when compared with compounds **5b**–**5f**. For example, the 4-OCH_3 analog **5e** was less potent than the 4-F analog **5i**. Therefore, the driver behind increased cytotoxicity appears to be electron-withdrawing substituents and this could imply that the halogen-disubstituted benzene directly linked to 1, 2, 3-triazole is necessary for inhibiting cancer cell lines. Moreover, the sequence of dominant substituent position is "meta-" ≈ "para-" > "ortho".

According to the hypothesis above, further synthesis was done with other powerful electron-withdrawing groups replacing the halogen (compounds **5q**–**5t**). The electron-withdrawing ability of nitrile group is similar to the fluorine radical and the result of the two compounds with nitrile group (**5q** and **5r**) displayed moderate and no effects against tested cancer cell lines, respectively. We proposed the explanation is that the triple-bond of the nitrile group could offer conjugation effect which might weaken the electron-withdrawing ability. On the contrary, compound **5s** with a stronger electron-withdrawing group (NO_2) at position-3 of the benzyl moiety displayed the best anti-tumor activity against three cell lines with IC_{50}s values of 9.45 (A-549), 17.29 (HeLa) and 14.07 (QGY-7701) μM, which exhibited similar inhibitory effect when compared with parent compound geiparvarin and identified itself as the most promising candidate. However, when nitro-group was replaced at para-position (**5t**), the result was inferior to the compound **5s**, which could also be explained via the conjugation effect. The modification above implied us that the stronger ability of

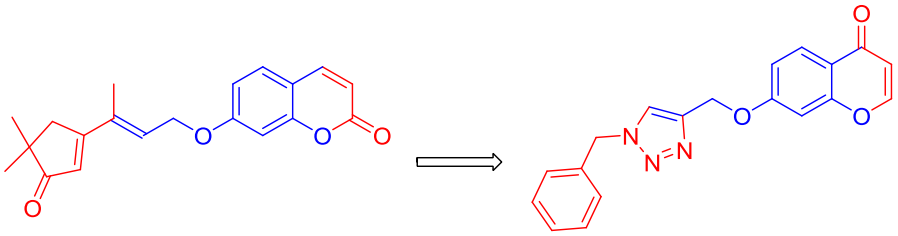


Fig. 3. Bioisosteric transformation.

the electron-withdrawing group on the benzyl moiety would be positive to the effects against cancer cell line.

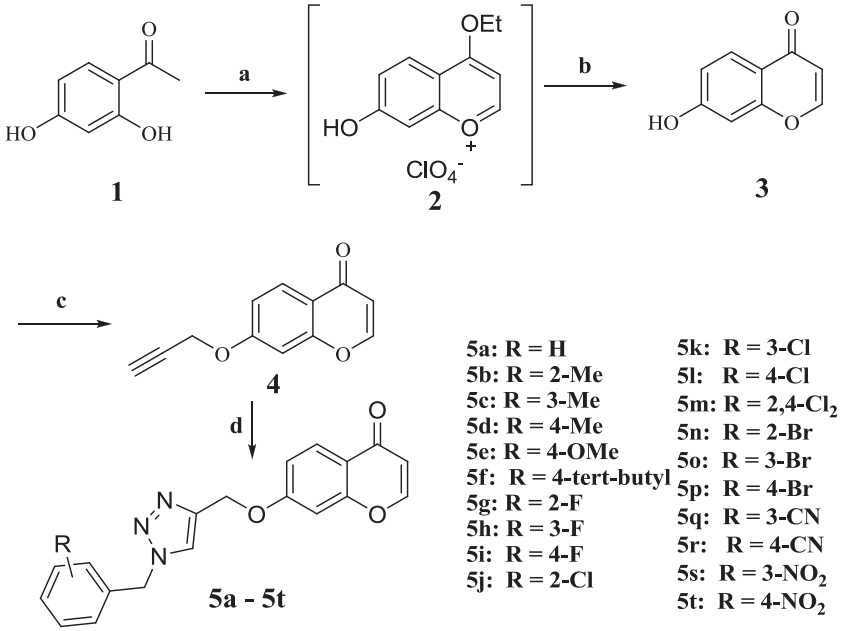
Our reference drug 5-Fu has the similar effective dose and toxic dose, which results in that they could kill both cancer cells and normal cells. It is notable that all the synthesized compounds exhibited low toxicity on human normal liver cell.

4.2. Apoptosis assay by annexin V-FITC binding

Compounds **5m** and **5s** were selected to study their preliminary mechanism. We detected compound **5m** on A-549 apoptosis effects by flow cytometry (FCM). (Fig. 5) A-549 cells were treated with 1, 2, 4 and 8 μM of compound **5m** for 48 h, respectively. As shown in Fig. 5, the compound increased the percentage of early apoptosis stage (7.13%, 17.62%, 28.45% and 29.73%, respectively) with the concentration improvement which indicated that compound **5m** induced apoptosis of A-549 in a dose-dependent manner. Further flow cytometric experiments identified the compound **5s** as potent inducers of apoptosis in human lung adenocarcinoma cell line. Likewise, a concentration-dependent induction of apoptotic cells could also be observed in apoptosis assay of compound **5s** (8.07%, 10.87%, 13.55% and 21.54% of early apoptosis stage, respectively) and the results were in good agreement with cytotoxic potency. Moreover, the control data with the lead (geiparvarin) was also detected by apoptosis assay (Fig. 5) and the image displayed similar dose-dependent trend which could also be proved by Chimichi etc. [4]. All the assay images could tell us directly that the apoptosis mainly happened in early stage. An early event in apoptotic cell death is the translocation of phosphatidyl-serine residues to the outer cell membrane. This event precedes nuclear breakdown, DNA fragmentation and appearance of most apoptosis-associated molecules [25], which is readily distinguished from the late apoptotic processes by annexin V/propidium iodide binding assay. Therefore, the basic mechanism of the geiparvarin analogs could be explained and the apoptosis did not reach the high percentage implied that apoptosis might be one of the mechanisms utilized by these compounds to elicit cytotoxicity.

5. Conclusion

Bioisosterism is one of the most useful and effective drug design method to optimize the pharmacological activity, selectivity and pharmacokinetics of the lead compound [26,27]. In the present study, a series of benzopyran-4-one derivatives were designed and synthesized. *In vitro* cytotoxicity screen revealed that the target compounds gained potential anti-cancer activities against five human cancer cell lines. In addition, compounds **5c**, **5e** and **5g** exhibited broad-spectrum activity against four cancer cell lines. Moreover, compound **5s** displayed highest potency of all the synthesized compounds. Compared with the parent compound (geiparvarin), we simplified the synthetic route, increase the total yield and obtained the similar or even better anti-cancer result. It is also notable that all of our resultant compounds have low toxicity to the human normal liver cell. Furthermore, the preliminary



Scheme 1. Synthesis of title compounds. Reagents and conditions: (a) Triethyl orthoformate, 70% $HClO_4$, −20 °C-r.t., 8 h; (b) H_2O, 100 °C, 1 h; (c) Propargyl bromide, K_2CO_3, Acetone, N_2 protection, Reflux, 8 h; (d) Substituted benzyl bromides, NaN_3, Sodium ascorbate, $CuSO_45H_2O$, DMSO, r.t., Overnight.

Table 1
Crystallographical and experimental data for compound **5p**.

Data	Compound **5p**
CCDC deposition no.	777465
Empirical Formula	$C_{19}H_{14}BrN_3O_3$
Formula weight	412.24
Temperature (k)	293(2)
Wavelength (Å)	0.71073
Crystal system	Monoclinic
Space group, *Z*	*P2(1)/c*
a (Å)	8.354(3)
b (Å)	6.932(2)
c (Å)	29.415(9)
α (°)	90
β (°)	93.533(4)
γ (°)	90
$D_{calc.}$ (Mg/m^3)	1.611
F(000)	832
θ range (°)	1.39–26.01
Crystal size (mm^3)	0.15 × 0.10 × 0.08
Reflections collected/unique	7400/3342 [*R*(*int*) = 0.0490]
Max. & min. transmission	0.8285 & 0.7108
Data/restraints/parameters	3342/0/235
R, *Rw*[I > 2σ(I)]	0.0483/0.1265
Goodness-of-fit on F^2	0.954

mechanism of compounds **5m** and **5s** underlying growth inhibitory effect was detected by flow cytometry (FCM), and the compound exerted anti-tumor activity via inducing the early apoptosis of A-549 cell line in a dose-dependent manner. Overall, these data could support the rationale of our design. The novel structure determined in this study represents a new kind of geiparvarin analog with effective anti-tumor activities. Current studies are focused on *in vivo* activity.

6. Experimental protocols

Melting points were measured on an uncorrected X-5 digital melting point apparatus (Gongyi City Yuhua Instrument Co., Ltd.; China). ^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 300 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ (99.8% D, Cambridge Isotope Laboratories) or DMSO-d^6 (99.9% D, Cambridge Isotope Laboratories) as solvents. Chemical shifts (*δ* values) and coupling constants (*J* values) are given in ppm and Hz, respectively. Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within 0.4%. The mass spectra were recorded on an Esquire 3000 LC-MS mass spectrometer. Crystal structure was determined on a Bruker SMART CCD (Bruker Company, Germany). Purity analysis was determined on Agilent 1100 Serial HPLC (Agilent Company, US); A C_{18} column (ZORBAX^{TM} 4.6 × 150 mm, 5 μm, PN: 993967906 SN: USRK042041). TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel thin-layer chromatography was performed with Silica gel 60 G (Qindao Haiyang Chemical, China). All solvents and reagents were analytically pure, and no further purification was needed. All starting materials were commercially available. The starting material **3** was prepared according the reported procedures [19,20].

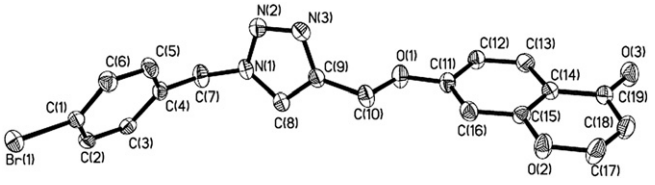


Fig. 4. ORTEP drawing of **5p**.

6.1. General procedure for the synthesis of intermediate **4**

In a mixture of 3 g (18 mmol) of compound **3**, 4.9 g (36 mmol) of K_2CO_3 and 2.8 mL (36 mmol) of propargyl bromide in 80 mL of dry acetone. The reaction mixture was stirred under N_2 protection in refluxing for 8 h. The mixture was poured into 100 mL of ice-water with severe stirring. The solution was extracted with EtOAc and the EtOAc layer was washed with brine, dried with $MgSO_4$ and concentrated to afford compound **4**. Color: White; m.p. 207.0–207.6 °C; Yield: 91%; ^{1}H NMR: (300 MHz, $CDCl_3$) *δ*: 2.60 (m, 1H), 4.80 (d, 2H, *J* = 2.4 Hz), 6.30 (d, 1H, *J* = 2.4 Hz), 6.98 (d, 1H, *J* = 6 Hz), 7.04 (m, 1H), 7.80 (d, 2H, *J* = 6 Hz), 8.15 ppm (d, 1H, *J* = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 56.23, 76.44, 77.36, 100.54, 110.44, 115.31, 118.68, 127.39, 148.53, 160.94, 164.93, 174.35 ppm; ESI-MS: *m/s* (%): 201.15 $(M + 1)^+$. Anal. calcd for $C_{12}H_8O_3$: C 72.00, H 4.03, found: C 72.01, H 4.03.

6.2. General procedure for the synthesis of compounds ***5a*–*5s***

6.2.1. 7-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (***5a***)

In a mixture of 0.6 g (3 mmol) of compound **4**, 0.4 mL (3.3 mmol) of benzyl bromide, 0.24 g (3.6 mmol) of NaN_3, catalytic amount of sodium ascorbate, catalytic amount of $CuSO_4.5H_2O$ in 30 mL of DMSO. The reaction mixture was stirred at room temperature for 10 h. The mixture was poured into 200 mL of ice-water with severe stirring. The solution wad extracted with EtOAc and the EtOAc layer was washed with brine, dried with $MgSO_4$ and concentrated to afford **5a**. Color: Yellow; m.p. 188.1–188.2 °C; Yield: 81%; ^{1}H NMR: (300 MHz, $CDCl_3$) *δ*: 5.27 (s, 2H), 5.56 (s, 2H), 6.29 (d, 1H, *J* = 6 Hz), 7.02 (m, 2H), 7.29 (m, 2H), 7.38 (m, 3H), 7.62 (s, 1H), 7.79 (d, 1H, *J* = 6 Hz), 8.11 ppm (d, 1H, *J* = 9.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 50.32, 62.35, 101.51, 112.97, 114.86, 119.19, 121.67, 124.88, 126.80, 127.33, 128.76, 130.72, 131.18, 132.78, 136.19, 154.89, 158.06, 162.50, 176.92 ppm; ESI-MS: *m/s* (%): 334.02 $(M + 1)^+$. Anal. calcd for $C_{19}H_{15}N_3O_3$: C 68.46, H 4.54, N 12.61, found: C 68.21, H 4.69, N 12.76.

The synthetic methods for the following compounds **5b**–**5s** were similar to the synthesis of compound **5a**.

6.2.2. 7-((1-(2-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (***5b***)

Color: White; m.p. 183.3–183.5 °C; Yield: 76%; ^{1}H NMR: (300 MHz, $CDCl_3$) *δ*: 2.29 (s, 3H), 5.26 (s, 2H), 5.57 (s, 2H), 6.28 (d, 1H, *J* = 6 Hz), 7.01 (m, 2H), 7.20 (m, 1H), 7.24 (m, 2H), 7.31 (m, 1H), 7.46 (s, 1H), 7.79 (d, 1H, *J* = 6 Hz), 8.12 ppm (d, 1H, *J* = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 18.94, 52.51, 62.39, 101.44, 112.86, 114.15, 118.01, 122.70, 124.37, 126.70, 127.18, 128.83, 129.26, 131.02, 132.12, 136.99, 154.87, 158.03, 162.45, 176.90 ppm; ESI-MS: *m/s* (%): 348.13 $(M + 1)^+$. Anal. calcd for $C_{20}H_{17}N_3O_3$: C 69.15, H 4.93, N 12.10, found: C 69.39, H 4.97, N 12.12.

6.2.3. 7-((1-(3-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (***5c***)

Color: White; m.p. 165.3–165.6 °C; Yield: 80%; ^{1}H NMR: (300 MHz, $CDCl_3$) *δ*: 2.35 (s, 3H), 5.27 (s, 2H), 5.51 (s, 2H), 6.28 (d, 1H, *J* = 6 Hz), 7.01 (m, 2H), 7.09 (m, 2H), 7.27 (m, 2H), 7.57 (s, 1H), 7.79 (d, 1H, *J* = 6 Hz), 8.12 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 18.66, 52.51, 62.41, 101.50, 112.91, 114.79, 119.12, 122.87, 124.70, 126.13, 127.05, 129.01, 129.67, 131.10, 132.87, 137.06, 153.88, 157.99, 162.60, 176.90 ppm; ESI-MS: *m/s* (%): 348.13 $(M + 1)^+$. Anal. calcd for $C_{20}H_{17}N_3O_3$: C 69.15, H 4.93, N 12.10, found: C 69.33, H 4.97, N 12.13.

6.2.4. 7-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (***5d***)

Color: Yellow; m.p. 183.7–184.0 °C; Yield: 77%; ^{1}H NMR: (300 MHz, $CDCl_3$) *δ*: 2.36 (s, 3H), 5.26 (s, 2H), 5.51 (s, 2H), 6.29 (d,

Table 2
In vitro anti-cancer activity of target compounds.

Compounds	*R*	Cytotoxicity IC_{50} ± SEM (μM)[a]						
		L02	A-549	HeLa	QGY-7701	SW480	SGC7901	MDA-MB-231
4	–	>300	$>10^2$	64.68 ± 4.76	58.48 ± 3.60	$>10^2$	$>10^2$	$>10^2$
5a	H	>300	$>10^2$	$>10^2$	$>10^2$	42.88 ± 2.36	$>10^2$	$>10^2$
5b	2-Me	>300	$>10^2$	15.30 ± 0.45	$>10^2$	$>10^2$	$>10^2$	$>10^2$
5c	3-Me	>300	20.30 ± 4.00	$>10^2$	22.39 ± 0.13	11.73 ± 3.48	$>10^2$	15.99 ± 1.83
5d	4-Me	>300	50.72 ± 0.49	$>10^2$	68.51 ± 1.13	10.40 ± 0.61	$>10^2$	$>10^2$
5e	4-OMe	>300	16.49 ± 1.42	14.37 ± 2.15	56.63 ± 0.08	20.39 ± 0.73	$>10^2$	$>10^2$
5f	4-tert-butyl	>300	$>10^2$	17.58 ± 0.46	$>10^2$	28.84 ± 0.61	$>10^2$	$>10^2$
5g	2-F	>300	10.76 ± 6.09	$>10^2$	36.87 ± 2,56	11.42 ± 0.94	$>10^2$	29.83 ± 9.81
5h	3-F	>300	$>10^2$	7.55 ± 1.57	$>10^2$	$>10^2$	$>10^2$	$>10^2$
5i	4-F	>300	$>10^2$	11.99 ± 4.59	14.37 ± 9.93	11.18 ± 2.16	$>10^2$	$>10^2$
5j	2-Cl	>300	$>10^2$	$>10^2$	19.73 ± 5.23	23.53 ± 6.33	$>10^2$	15.08 ± 0.44
5k	3-Cl	>300	13.68 ± 5.32	$>10^2$	28.42 ± 0.63	12.93 ± 0.54	$>10^2$	$>10^2$
5l	4-Cl	>300	$>10^2$	37.63 ± 0.63	41.68 ± 5.22	$>10^2$	$>10^2$	$>10^2$
5m	2,4-Cl_2	>300	5.26 ± 0.66	80.64 ± 2.63	13.31 ± 0.23	$>10^2$	$>10^2$	$>10^2$
5n	2-Br	>300	$>10^2$	14.53 ± 4.46	$>10^2$	$>10^2$	$>10^2$	$>10^2$
5o	3-Br	>300	$>10^2$	$>10^2$	17.58 ± 0.43	$>10^2$	$>10^2$	$>10^2$
5p	4-Br	>300	$>10^2$	$>10^2$	96.42 ± 0.60	37.51 ± 0.99	$>10^2$	$>10^2$
5q	3-CN	>300	14.34 ± 8.34	$>10^2$	24.38 ± 0.43	$>10^2$	$>10^2$	$>10^2$
5r	4-CN	>300	$>10^2$	$>10^2$	$>10^2$	$>10^2$	$>10^2$	$>10^2$
5s	3-NO_2	>300	9.45 ± 0.53	17.29 ± 2.94	14.07 ± 1.46	$>10^2$	$>10^2$	$>10^2$
5t	4-NO_2	>300	$>10^2$	25.69 ± 0.15	$>10^2$	19.33 ± 2.54	$>10^2$	71.33 ± 1.11
Geiparvarin[b]	–	>300	18.02 ± 0.20	9.09 ± 0.75	17.68 ± 0.40	20.34 ± 0.75	7.59 ± 0.48	9.88 ± 0.84
5-Fu[b]	–	46.57 ± 0.68	5.64 ± 0.55	16.32 ± 0.36	14.30 ± 0.055	15.71 ± 0.86	6.04 ± 0.83	13.65 ± 0.82

Abbreviations: L02: normal human liver cell line; 293T HEKC: human embryonic kidney 293 cells; A-549: human lung adenocarcinoma cell line; HeLa: human cervical carcinoma cell line; QGY-7701: human hepatoma cell line; SW480: human colon carcinoma cell line; SGC7901: human gastric adenocarcinoma cell line; MDA-MB-231: human breast carcinoma cell line.

[a] The IC_{50} value was defined as the concentration at which 50% survival of cells was observed after 48 h incubation.

[b] Used as a positive control.

1H, J = 6 Hz), 7.00 (m, 2H), 7.25 (m, 4H), 7.55 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.11 ppm (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.12, 52.51, 62.44, 100.54, 112.94, 114.31, 118.98, 122.39, 124.53, 126.40, 127.28, 128.35, 129.30, 131.01, 132.00, 136.35, 153.54, 158.01, 162.32, 176.91 ppm; ESI-MS: *m/s* (%): 348.13 $(M + 1)^+$. Anal. calcd for $C_{20}H_{17}N_3O_3$: C 69.15, H 4.93, N 12.10, found: C 69.38, H 4.96, N 12.12.

6.2.5. 7-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5e**)

Color: Yellow; m.p. 162.1–163.0 °C; Yield: 61%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 3.82 (s, 3H), 5.26 (s, 2H), 5.49 (s, 2H), 6.29 (d, 1H, J = 6 Hz), 6.92 (m, 2H), 7.01 (dd, 2H, J_1 = 2.4 Hz, J_2 = 1.8 Hz), 7.25 (m, 2H), 7.54 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.11 ppm (d, 1H, J = 8.4 Hz); ESI-MS: *m/s* (%): 364.00 $(M + 1)^+$. Anal. calcd for $C_{20}H_{17}N_3O_4$: C 66.11, H 4.72, N 11.56, found: C 66.05, H 4.63, N 11.57.

6.2.6. 7-((1-(4-tert-butylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5f**)

Color: Yellow; m.p. 182.2–183 °C; Yield: 53%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 1.31 (s, 9H), 5.27 (s, 2H), 5.52 (s, 2H), 6.29 (d, 1H, J = 6 Hz), 7.01 (m, 2H), 7.25 (m, 2H), 7.41 (m, 2H), 7.58 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.12 ppm (m, 1H); ESI-MS: *m/s* (%): 390.08 $(M + 1)^+$. Anal. calcd for $C_{23}H_{23}N_3O_3$: C 70.93, H 5.95, N 10.79, found: C 70.92, H 5.99, N 10.74.

6.2.7. 7-((1-(2-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5g**)

Color: Pink; m.p. 165.0–165.1 °C; Yield: 85%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.28 (s, 2H), 5.62 (s, 2H), 6.29 (d, 1H, J = 6.3 Hz), 7.02 (m, 2H), 7.14 (m, 2H), 7.35 (m, 2H), 7.69 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.11 ppm (d, 1H, J = 8.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.91, 62.35, 101.51, 112.97, 114.86, 119.19, 121.67, 124.88, 126.80, 127.33, 128.76, 130.72, 131.18, 132.78, 136.19, 154.89, 158.06, 162.50, 176.92 ppm; ESI-MS: *m/s* (%): 352.14 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}FN_3O_3$: C 64.95, H 4.02, N 11.96, found: C 64.93, H 4.02, N 11.90.

6.2.8. 7-((1-(3-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5h**)

Color: White; m.p. 175.5–175.7 °C; Yield: 76%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.29 (s, 2H), 5.56 (s, 2H), 6.29 (d, 1H, J = 6 Hz), 7.02 (m, 2H), 7.10 (d, 2H, J = 2.4 Hz), 7.36 (m, 2H), 7.64 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.12 ppm (d, 1H, J = 8.4 Hz); ESI-MS: *m/s* (%): 352.14 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}FN_3O_3$: C 64.95, H 4.02, N 11.96, found: C 64.98, H 4.01, N 11.92.

6.2.9. 7-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5i**)

Color: Pink; m.p. 194.6–194.9 °C; Yield: 86%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.28 (s, 2H), 5.53 (s, 2H), 6.30 (d, 1H, J = 6.3 Hz), 7.06 (m, 4H), 7.20 (m, 2H), 7.60 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.12 ppm (d, 1H, J = 8.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.91, 62.35, 101.46, 112.36, 114.43, 119.20, 121.70, 124.88, 126.79, 127.20, 128.78, 130.72, 131.19, 132.78, 136.18, 154.89, 158.00, 162.54, 176.90 ppm; ESI-MS: *m/s* (%): 352.14 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}FN_3O_3$: C 64.95, H 4.02, N 11.96, found: C 64.94, H 4.04, N 11.98.

6.2.10. 7-((1-(2-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5j**)

Color: White; m.p. 154.6–154.8 °C; Yield: 75%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.28 (s, 2H), 5.70 (s, 2H), 6.28 (d, 1H, J = 6 Hz), 7.02 (d, 2H, J = 8.4 Hz), 7.25 (d, 2H, J = 7.2 Hz), 7.34 (m, 1H), 7.45 (d, 1H, J = 7.2 Hz), 7.72 (s, 1H), 7.79 (d, 1H, J = 5.7 Hz), 8.11 ppm (d, 1H, J = 8.7 Hz); ESI-MS: *m/s* (%): 368.16 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}ClN_3O_3$: C 62.05, H 3.84, N 11.43, found: C 62.09, H 3.87, N 11.51.

6.2.11. 7-((1-(3-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5k**)

Color: White; m.p. 178.3–178.8 °C; Yield: 84%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.29 (s, 2H), 5.54 (s, 2H), 6.29 (d, 1H, J = 6.3 Hz), 7.02 (m, 2H), 7.18 (m, 2H), 7.32 (m, 2H), 7.62 (s, 1H), 7.79 (d, 1H, J = 6 Hz), 8.12 ppm (d, 1H, J = 6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 53.67,

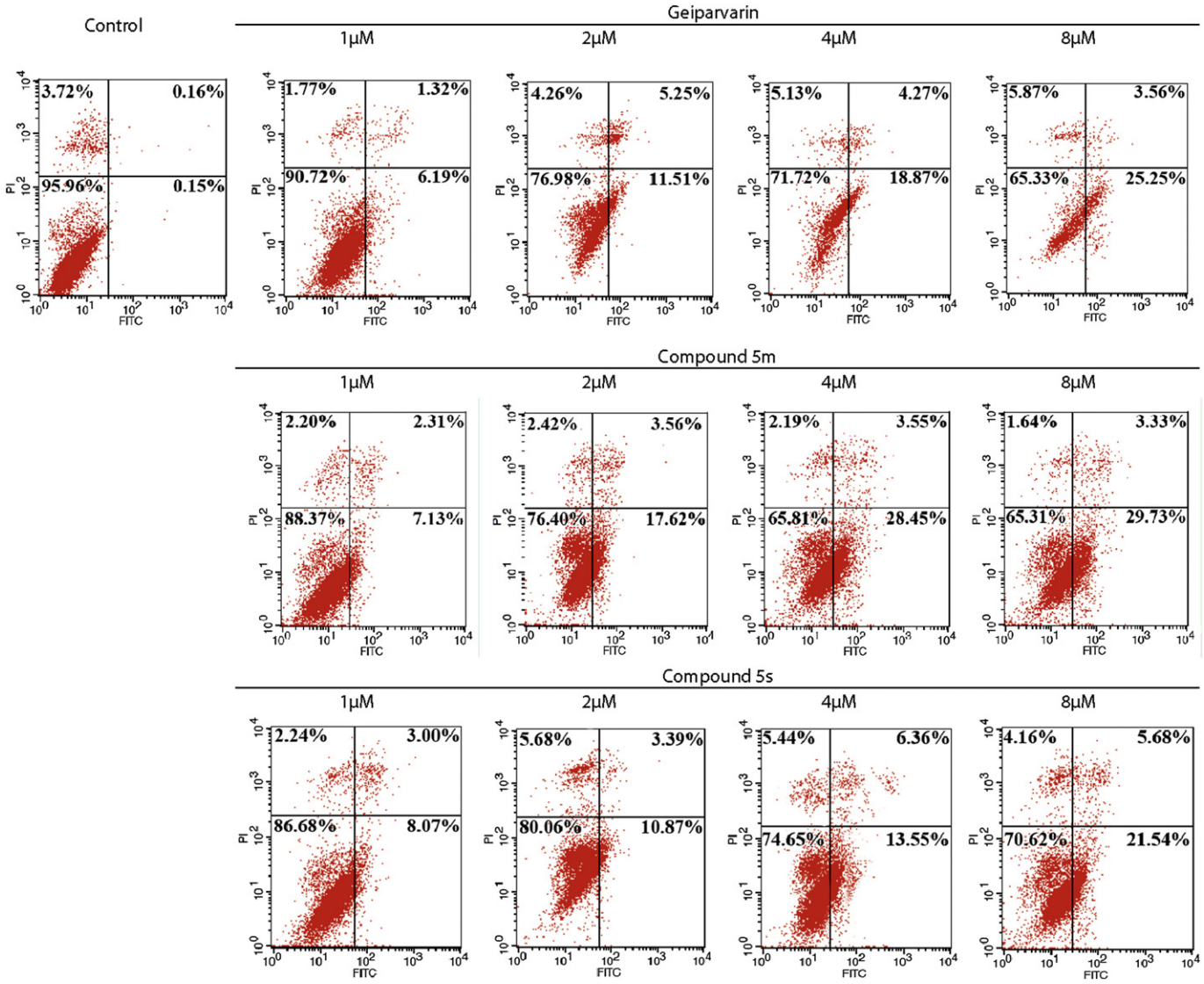


Fig. 5. Geiparvarin, compounds **5m** and **5s** induce apoptosis. A-549 cells were treated with 1, 2, 4 and 8 μM of geiparvarin, compound **5m** and **5s** for 48 h, respectively, stained with annexin V/propidium iodide, and analyzed by flow cytometry. Untreated control cells (48 h) were also included in the analysis. Cells in the lower left quadrant (annexin V-negative, propidium iodide-negative) are viable, whereas the upper right represents late apoptosis as detected by annexin V/Pi staining; the lower right represents early apoptosis as detected by only annexin V staining; the total percentage from upper right and lower right was referred as the percentage of apoptosis.

62.36, 101.51, 112.99, 114.84, 119.24, 121.84, 124.63, 126.14, 127.36, 128.16, 129.15, 130.49, 135.10, 136.17, 154.90, 158.06, 162.44, 176.90 ppm; ESI-MS: *m/s* (%): 368.16 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}ClN_3O_3$: C 62.05, H 3.84, N 11.43, found: C 62.02, H 3.85, N 11.49.

*6.2.12. 7-((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5l**)*

Color: White; m.p. 185.5–185.8 °C; Yield: 76%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.28 (s, 2H), 5.53 (s, 2H), 6.29 (d, 1H, $J = 6$ Hz), 7.02 (m, 2H), 7.24 (d, 2H, $J = 8.4$ Hz), 7.37 (d, 2H, $J = 8.4$ Hz), 7.58 (s, 1H), 7.79 (d, 1H, $J = 6$ Hz), 8.11 ppm (d, 1H, $J = 8.4$ Hz); ESI-MS: *m/s* (%): 368.16 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}ClN_3O_3$: C 62.05, H 3.84, N 11.43, found: C 62.08, H 3.87, N 11.41.

*6.2.13. 7-((1-(2,4-dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5m**)*

Color: White; m.p. 195.0–195.2 °C; Yield: 85%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.29 (s, 2H), 5.66 (s, 2H), 6.29 (d, 1H, $J = 6$ Hz), 7.02 (m, 2H), 7.19 (d, 1H, $J = 8.1$ Hz), 7.29 (d, 1H, $J = 2.1$ Hz), 7.47 (d, 1H, $J = 2.1$ Hz), 7.70 (s, 1H), 7.79 (d, 1H, $J = 6$ Hz), 8.12 ppm (d, 1H, $J = 8.4$ Hz); ESI-MS: *m/s* (%): 402.07 $(M + 1)^+$. Anal. calcd for $C_{19}H_{13}Cl_2N_3O_3$: C56.73, H 3.26, N 10.45, found: C 56.74, H 3.26, N 10.42.

*6.2.14. 7-((1-(2-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5n**)*

Color: Yellow; m.p. 159.6–159.9 °C; Yield: 76%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.29 (s, 2H), 5.70 (s, 2H), 6.28 (d, 1H, $J = 6$ Hz), 7.02 (d, 2H, $J = 8.1$ Hz), 7.23 (m, 3H), 7.33 (m, 1H), 7.63 (m, 1H), 7.78 (d, 1H, $J = 6$ Hz), 8.11 ppm (d, 1H, $J = 8.7$ Hz); ESI-MS: *m/s* (%): 412.24 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}BrN_3O_3$: C 55.36, H 3.42, N 10.19, found: C 55.30, H 3.47, N 10.18.

*6.2.15. 7-((1-(3-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5o**)*

Color: White; m.p. 196.9–197.3 °C; Yield: 53%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.29 (s, 2H), 5.53 (s, 2H), 6.28 (d, 1H, $J = 6$ Hz), 7.24 (m, 1H), 7.44 (s, 1H), 7.51 (m, 2H), 7.61 (s, 1H), 7.79 (d, 1H, $J = 6$ Hz), 8.12 ppm (d, 1H, $J = 8.4$ Hz); ESI-MS: *m/s* (%): 412.23

$(M + 1)^+$. Anal. calcd for $C_{19}H_{14}BrN_3O_3$: C 55.36, H 3.42, N 10.19, found: C 55.38, H 3.44, N 10.16.

*6.2.16. 7-((1-(4-bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5p**)*

Color: Yellow; m.p. 188.0–188.2 °C; Yield: 86%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.27 (s, 2H), 5.51 (s, 2H), 6.28 (d, 1H, $J = 6.3$ Hz), 7.01 (m, 2H), 7.16 (m, 2H), 7.51 (m, 2H), 7.60 (s, 1H), 7.78 (d, 1H, $J = 6$ Hz), 8.11 ppm (d, 1H, $J = 8.4$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 53.01, 62.46, 101.51, 112.28, 114.97, 120.01, 121.98, 124.74, 126.15, 127.97, 128.15, 129.77, 129.84, 135.07, 136.17, 155.11, 158.10, 162.45, 176.90 ppm; ESI-MS: *m/s* (%): 412.24 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}BrN_3O_3$: C 55.36, H 3.42, N 10.19, found: C 55.30, H 3.43, N 10.18.

*6.2.17. 3-((4-((4-Oxo-4H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (**5q**)*

Color: White; m.p. 186.0–186.9 °C; Yield: 46%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.31 (s, 2H), 5.61 (s, 2H), 6.29 (d, 1H, $J = 6.3$ Hz), 7.02 (m, 2H), 7.53 (m, 2H), 7.58 (s, 1H), 7.68 (m, 2H), 7.79 (d, 1H, $J = 6$ Hz), 8.12 ppm (d, 1H, $J = 8.7$ Hz); ESI-MS: *m/s* (%): 359.63 $(M + 1)^+$. Anal. calcd for $C_{20}H_{14}N_4O_3$: C 67.03, H 3.94, N 15.63, found: C 67.02, H 3.98, N 15.67.

*6.2.18. 4-((4-((4-Oxo-4H-chromen-7-yloxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (**5r**)*

Color: White; m.p. 218.0–218.9 °C; Yield: 69%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.30 (s, 2H), 5.63 (s, 2H), 6.29 (d, 1H, $J = 5.7$ Hz), 7.01 (d, 2H, $J = 9.6$ Hz), 7.38 (d, 2H, $J = 8.1$ Hz), 7.68 (m, 3H), 7.79 (d, 1H, $J = 6$ Hz), 8.12 ppm (d, 1H, $J = 8.7$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 47.21, 62.29, 101.32, 112.64, 114.00, 117.56, 119.19, 121.67, 124.89, 126.81, 127.43, 128.76, 130.31, 131.20, 132.78, 136.29, 154.65, 158.05, 162.54, 176.90 ppm; ESI-MS: *m/s* (%): 359.63 $(M + 1)^+$. Anal. calcd for $C_{20}H_{14}N_4O_3$: C 67.03, H 3.94, N 15.63, found: C 67.02, H 3.91, N 15.61.

*6.2.19. 7-((1-(3-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5s**)*

Color: White; m.p. 184.1–184.7 °C; Yield: 64%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.31 (s, 2H), 5.68 (s, 2H), 6.29 (d, 1H, $J = 6$ Hz), 7.01 (m, 2H), 7.62 (m, 2H), 7.70 (s, 1H), 7.79 (d, 1H, $J = 6$ Hz), 8.11 (m, 1H), 8.18 (m, 1H), 8.25 ppm (d, 1H, $J = 2.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 46.85, 61.98, 101.51, 112.97, 114.96, 119.11, 121.67, 124.45, 126.80, 127.13, 128.46, 130.73, 131.18, 132.78, 136.05, 154.43, 158.05, 162.50, 176.91 ppm; ESI-MS: *m/s* (%): 379.11 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}N_4O_5$: C 60.32, H 3.73, N 14.81, found: C 60.30, H 3.71, N 14.86.

*6.2.20. 7-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)-4H-chromen-4-one (**5t**)*

Color: White; m.p. 178.5–179.6 °C; Yield: 70%; ^{1}H NMR: (300 MHz, $CDCl_3$) δ: 5.31 (s, 2H), 5.69 (s, 2H), 6.29 (d, 1H, $J = 6$ Hz), 7.01 (m, 2H), 7.20 (m, 2H), 7.40 (d, 1H, $J = 8.7$ Hz), 8.11 (m, 1H), 7.60 (s, 1H), 7.79 (d, 1H, $J = 6$ Hz), 8.12 ppm (d, 1H, $J = 8.7$ Hz); ESI-MS: *m/s* (%): 379.11 $(M + 1)^+$. Anal. calcd for $C_{19}H_{14}N_4O_5$: C 60.32, H 3.73, N 14.81, found: C 60.30, H 3.72, N 14.86.

6.3. Antitumor screening

6.3.1. Methodology of the in vitro cancer screen

In vitro anti-cancer activity was measured by means of the IC_{50} using the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylte trazolium bromide] assay method [28–30]. Test cancer cell lines were obtained from the Shanghai Institute of Biological Sciences, CAS. The IC_{50} determination was performed according to the National Committee for Clinical Laboratory Standards (NCCLS) recommendations. All compounds were dissolved in DMSO with the stock concentration of 10 g/L (stored at 4 °C), and diluted with medium freshly before drug administration. Cell lines were seeded into 96-well plates at density of 8000 cells/well. 24 h after seeding, each compound dilution was added in duplicate and incubation continued at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h, add 20 μL MTT (Sigma) at 5 mg/mL in PBS (filter sterilized, light protected, and stored at 4 °C) per well, and after 4 h of incubation at 37 °C, MTT is converted to a blue formazan product by mitochondrial succinate dehydrogenase. This product was eluted from cells by addition of 150 mL of DMSO. The absorbance at 570 nm was determined by an ELISA using a ELX800 microplate spectrophotometer. The IC_{50} value was defined as the concentration at which 50% of the cells could survive.

6.3.2. Methodology of the apoptosis assay by annexin V-FITC binding

After staining with annexin V-FITC and PI using the Annexin V-FITC apoptosis detection kit (Beyotime, China), cells were detected by flow cytometry to assess the membrane and nuclear events during apoptosis. Briefly, A-549 cells (1×10^5/2 mL per well) were seeded in six-well plates and treated with compound **5m** at concentrations of 1, 2, 4 and 8 μM, respectively and incubation continued at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h, cells were collected, washed once with PBS, and resuspended in 195 μL of annexin V-FITC binding buffer which was then added to 5 μL of annexin V-FITC and incubated at room temperature in the dark for 10 min. Subsequently, the buffer was added to 10 μL of PI and incubated at 4 °C in the dark for 5 min. The samples were analyzed by a FACScan flow cytometer (Becton Dickinson, USA).

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 21172259) and Shanghai Rising-Star Program (Grant No. 09QA1407000). We also thanks to the Key laboratory of drug research for special environments, PLA.

Appendix A. Supporting information

CCDC-777465 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via the URL http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

The purities of resultant compounds determined by HPLC with two quite different systems can be obtained from the Supplementary Data.

Appendix B. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2012.04.026.

References

[1] K. Padmawinata, Acta Pharm. 4 (1973) 1–9.
[2] P.J. Jerris, A.B. Smith, J. Org. Chem. 46 (1981) 577–585.
[3] S.H. Kang, C.Y. Hong, Tetrahedron Lett. 28 (1987) 675–678.
[4] S. Chimichi, M. Boccalini, A. Salvador, F. Dall'Acqua, G. Basso, G. Viola, Chem. Med. Chem. 4 (2009) 769–779.
[5] Y.A. Al-Soud, I.A. Al-Masoudi, B. Saeed, U. Beifuß, N.A. Al-Masoudi, Chem. Heterocycl. Compd. 42 (5) (2006) 583–890.
[6] M.M.F. Ismail, H.S. Rateb, M.M.M. Hussein, Eur. J. Med. Chem. 45 (2010) 3950–3959.
[7] A. Lacy, R. O'Kennedy, Curr. Pharm. Des. 10 (2004) 3797–3811.
[8] S. Semba, N. Itoh, M. Ito, M. Harada, M. Yamakawa, Clin. Cancer Res. 8 (2002) 1957–1963.
[9] J. Lee, T. Park, S. Jeong, K.-H. Kim, C. Hong, Bioorg. Med. Chem. Lett. 17 (2007) 1284–1287.

[10] L. Pobst, M. Ames, Cancer Chemother. Pharmacol. 57 (2006) 569–576.
[11] F. Teillet, A. Boumendjel, J. Boutonnat, X. Ronot, Med. Res. Rev. 28 (2008) 715–745.
[12] R. Alvarez, S. Velazquez, A. San-Felix, S. Aquaro, E.D. Clercq, C.-F. Perno, A. Karlsson, J. Balzarini, M.J. Camarasa, J. Med. Chem. 37 (1994) 4185–4194.
[13] M.J. Genin, D.A. Allwine, D.J. Anderson, M.R. Barbachyn, D.E. Emmert, S.A. Garmon, D.R. Graber, K.C. Grega, J.B. Hester, D.K. Hutchinson, J. Morris, R.J. Reischer, C.W. Ford, G.E. Zurenko, J.C. Hamel, R.D. Schaadt, D. Stapert, B.H. Yagi, J. Med. Chem. 43 (2000) 953–970.
[14] S.B. Ferreira, A.C.R. Sodero, M.F.C. Cardoso, E.S. Lima, C.R. Kaiser, F.P. Silva, V.F. Ferreira, J. Med. Chem. 53 (2010) 2364–2375.
[15] H.C. Kolb, M.G. Finn, K.B. Sharpless, Angew. Chem. Int. Ed. Engl. 40 (2001) 2004–2021.
[16] C.W. Tornøe, C. Christensen, M. Meldal, J. Org. Chem. 67 (2002) 3057–3064.
[17] V.V. Rostovtsev, L.G. Green, V.V. Fokin, K.B. Sharpless, Angew. Chem. Int. Ed. Engl. 41 (2002) 2596–2599.
[18] D. Baraniak, K. Kacprzak, L. Celewicz, Bioorg. Med. Chem. Lett. 21 (2011) 723–726.
[19] G.N. Dorofeenko, V.V. Tkachenko, Chem. Heterocycl. Compd. 8 (1972) 935–938.
[20] J.C. Jaen, L.D. Wise, T.G. Heffner, T.A. Pugsley, L.T. Meltzer, J. Med. Chem. 34 (1991) 248–256.
[21] I. Vermes, C. Haanen, H. Steffensnakken, J. Immunol. Methods 184 (1995) 39–51.
[22] C. Riccardi, I. Nicoletti, Nat. Protocol 1 (2006) 1458–1461.
[23] W.T. Zhang, J.L. Ruan, P.F. Wu, F.C. Jiang, L.N. Zhang, W. Fang, X.L. Chen, Y. Wang, B.S. Cao, G.Y. Chen, Y.J. Zhu, J. Gu, J.G. Chen, J. Med. Chem. 52 (2009) 718–725.
[24] S.S. Joo, Y.M. Yoo, J. Pineal Res. 47 (2009) 8–14.
[25] R. Yamada, X. Cao, A.N. Butkevich, M. Millard, S. Odde, N. Mordwinkin, R. Gundla, E. Zandi, S.G. Louie, N.A. Petasis, N. Neamati, J. Med. Chem. 54 (2011) 2902–2914.
[26] L.M. Lima, E.J. Barreiro, Curr. Med. Chem. 12 (2005) 23–49.
[27] G.A. Patani, E.J. LaVoie, Chem. Rev. 96 (1996) 3147–3176.
[28] T. Mosmann, J. Immunol. Methods 65 (1983) 55–63.
[29] J. Carmichael, W.G. DeGraff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Cancer Res. 47 (1987) 936–942.
[30] D.-K. Kim, D.H. Ryu, J.Y. Lee, N. Lee, Y.-W. Kim, J.-S. Kim, K. Chang, G.-J. Im, T.-K. Kim, W.-S. Choi, J. Med. Chem. 44 (2001) 1594–1602.