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Synthesis and preliminary antiproliferative activity of new pteridin-7(8*H*)-one derivatives

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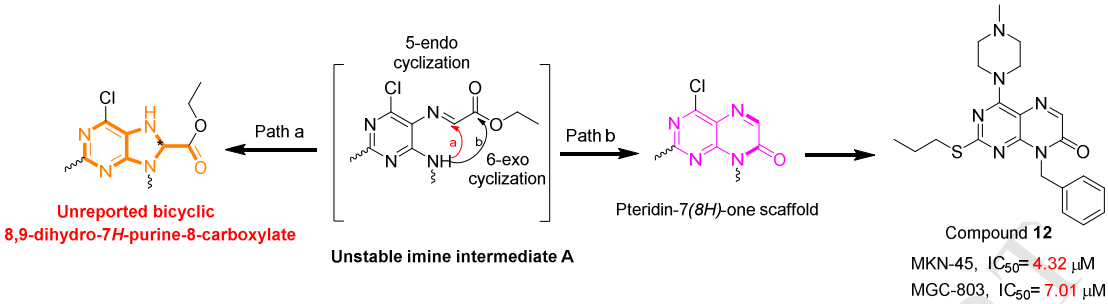
Graphic abstract



Pteridin-7(8*H*)-ones were synthesized and evaluated for their antiproliferative activity. Compound **12** displayed acceptable growth inhibition, induced apoptosis of MKN-45 cells. Besides, a novel bicyclic 8,9-dihydro-7*H*-purine-8-carboxylate scaffold was first prepared.

Synthesis and preliminary antiproliferative activity of new pteridin-7(8*H*)-one derivatives

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ABSTRACT: Pteridines are an important class of heterocyclic compounds with diverse biological activities. Here, we report a series of pteridin-7(8*H*)-one derivatives and their antiproliferative activities toward MKN-45, MGC-803, EC-109, and H1650. Structure-activity relationship studies showed that compound **12** exerted the most potent antiproliferative activity against MKN-45 and MGC-803 with the IC_{50} values of 4.32 and 7.01 μM, respectively. Besides, compound **12** induced morphological changes and apoptosis of MKN-45 cells, increased expression of Bax, down-regulated expression of Bcl-2 and caused cleavage of caspase-3/9. Additionally, we first reported the construction of the novel bicyclic 8,9-dihydro-7*H*-purine-8-carboxylate scaffold through the competitive 5-endo cyclization reaction with two C-N bonds and a chiral carbon center established.

Keywords: Pteridin-7(8*H*)-one, Antiproliferative activity, Apoptosis, Cell cycle arrest

1. Introduction

Pteridines are an important class of bicyclic *N*-heteroarenes, which have received considerable attention from medicinal community due to their diverse biological activities, such as antimicrobial [1], antiallergic [2], immunosuppressive [3], anti-inflammatory [4, 5], antibacterial [6, 7], and anticancer activities [8, 9]. Some biologically active natural products (e.g. folic acid, etc.) are also found to possess pteridine scaffolds. As shown in Fig. 1, folic acid (also named as vitamin B9 or vitamin M) is necessary for human health and is involved in DNA synthesis, DNA repair and DNA methylation [10]. Antifolate methotrexate (MTX) is a chemotherapy agent and immune system suppressant for treatment of cancer and autoimmune diseases . Moreover, pteridine analogs are well known to act upon a wide range of targets for therapeutic potential [4]. Compound **A**, as a selective PI3K/mTOR dual inhibitor, is highly efficacious in mouse xenograft model bearing glioma cell line U87 [11]. Compound **B** has been proved to be able to inhibit $EGFR^{wt}$ and $EGFR^{T790M/L858R}$ potently and exerts remarkable *in vivo* growth inhibition of human non-small cell lung cancers (NSCLC) [12]. Compound **C** is a pteridinone-based TLR7 (Toll-like receptor 7) agonist, which is currently in clinical evaluation for the treatment of chronic HBV (hepatitis B) infection [13] (Fig. 1). The diverse bioactivities and natural prevalence make pteridines and their structural analogs promising bicyclic scaffolds to develop new agents for the treatment of different diseases.

Following our previous work on the synthesis of new bicyclic heterocycles with anticancer potentials [14-17], we herein report the synthesis of a series of new pteridin-7(8H)-one derivatives through cascade reactions, their antiproliferative activity and preliminary mechanisms of inducing cancer cell death. Also, we first report the synthesis of new 8,9-dihydro-7*H*-purine-8-carboxylate *via* the 5-endo cyclization.

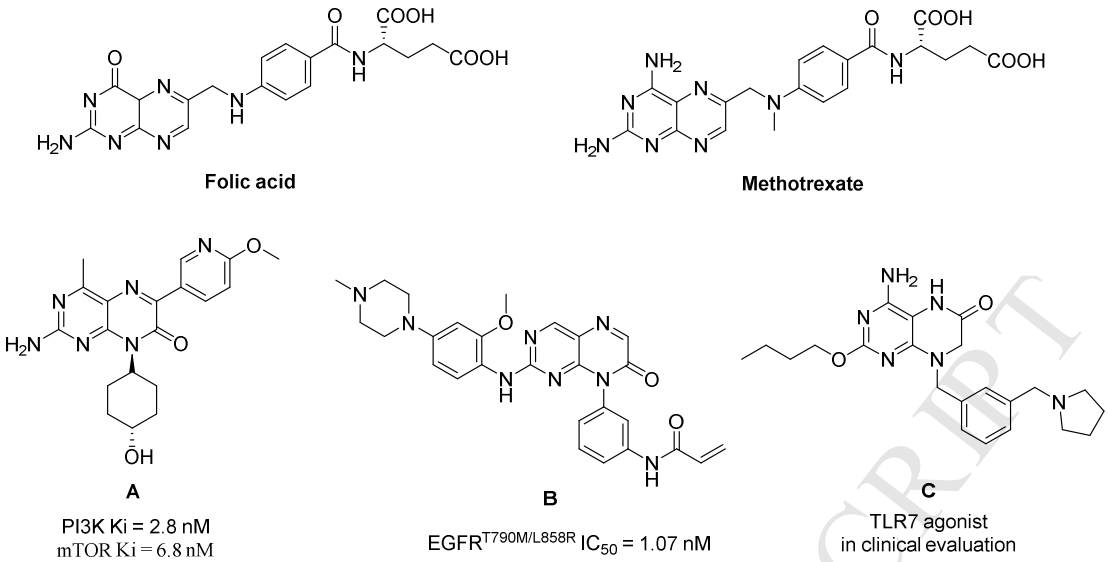
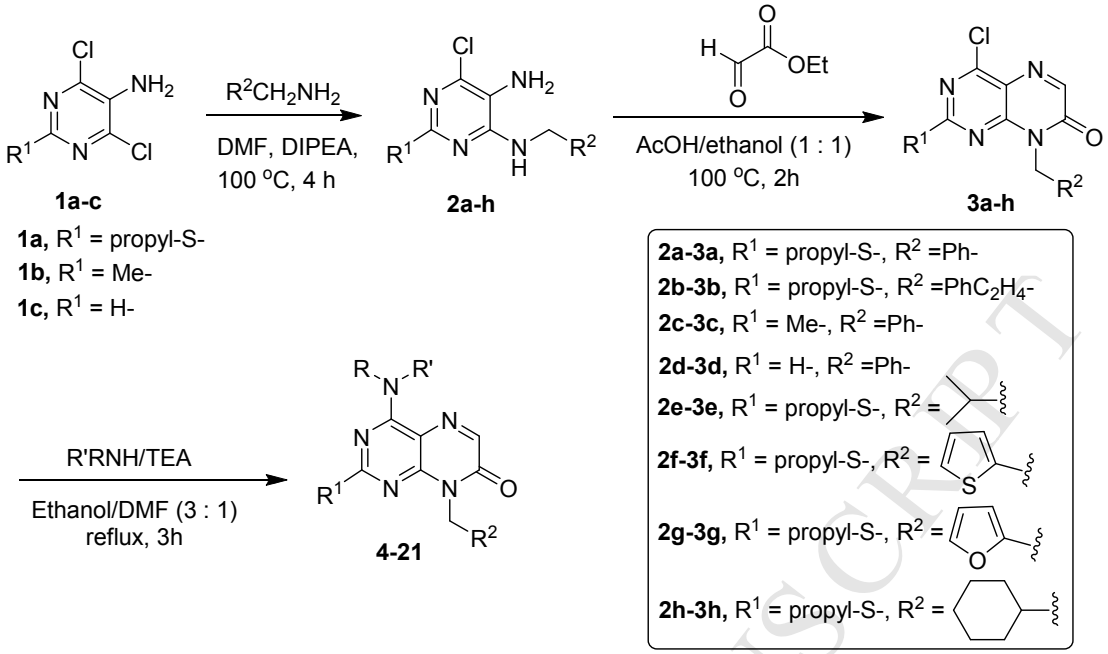


Fig. 1. Biologically active pteridine derivatives.

2. Results and discussion

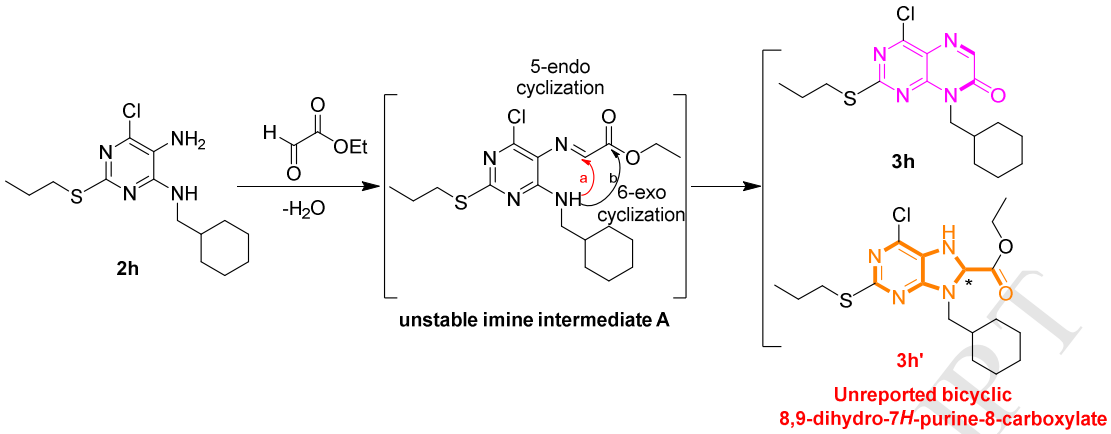
2.1 Chemistry

The general synthetic route is illustrated in Scheme 1. The intermediate derivatives **2a-h** were synthesized by condensation of commercially available pyrimidine analogs **1a-c** with appropriate primary amines in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in DMF. The obtained intermediates **2a-h** reacted with ethyl glyoxalate in AcOH/EtOH under heating to afford pteridin-7(8*H*)-one skeletons **3a-h** [11], which then reacted with various amines in the presence of TEA in a mixed solvent of ethanol and DMF, generating the target compounds **4-21**.



Scheme 1. Synthesis of pteridin-7(8*H*)-one derivatives.

Interestingly, compound **3h'** was also observed in the conversion from compound **2h** to compound **3h**. Compound **3h'** features a novel 8,9-dihydro-7*H*-purine-8-carboxylate scaffold. Both compounds were formed through the unstable imine intermediate **A**. For the synthesis of compound **3h**, it involved the 6-*exo* cyclization (Scheme 2, path b), generating one C-N bond, one C=N bond, and the pteridin-7(8*H*)-onescaffold. While compound **3h'** was competitively formed *via* the 5-*endo* cyclization (Scheme 2, path a), accompanying by the formation of two C-N bonds, a chiral center, and the 8,9-dihydro-7*H*-purine-8-carboxylate architecture. To the best of our knowledge, this is the first report on the rapid construction of the new 8,9-dihydro-7*H*-purine-8-carboxylate framework, which also possesses pluripotent groups (e.g. –COOEt, Cl) for further structural derivations. Besides, the C-N single bond of compound **3h'** could be potentially oxidized to aromatic bicyclic *N*-heteroarene.



Scheme 2. Proposed reaction mechanism for the synthesis of compounds **3h** and **3h'**.

Compound **3h'** was then characterized by 2D NMR spectra (see supporting information for details). Intriguingly, the methylene protons (highlighted in red) appeared at 3.99 and 3.17 ppm, respectively. The unique intramolecular H-bond interactions and the adjacent chiral center may be responsible for the remarkably different chemical shifts of the methylene protons.

2.2 Evaluation of biological activity

2.2.1 Antiproliferative activity

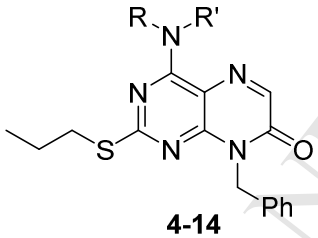
Compounds **4-21** were evaluated for their antiproliferative activity against MKN-45 and MGC803 (human gastric cancer cell lines), EC-109 (human esophageal cancer cell line), and H1650 (human lung cancer cell line), by using the MTT assay, and 5-fluorouracil (5-FU) was employed as the reference drug [18]. The preliminary data are summarized in Tables 1-3.

As shown in Table 1, compounds **4-6** with different aniline substituents exerted moderate antiproliferative activity against the tested cancer cell lines, the substituents attached had certain effect on the activity, especially for MKN-45 and MGC-803 cells. Compound **6** with the 3,4,5-trimethoxyl group was more potent than compound **4** with the IC_{50} values of 14.25 and 15.03 μM, respectively against MKN-45 and MGC-803 cells. Compounds **7**, **8**, **13** and **14** with the aliphatic amine substituents showed

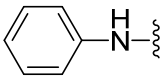
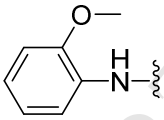
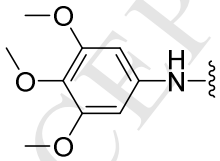
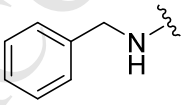
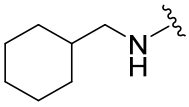
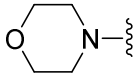
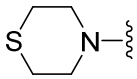
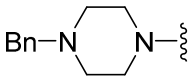
decreased inhibitory activity against the tested cancer cell lines. Compound **12** was significantly more potent than compounds **9**-**10**, highlighting the importance of nitrogen atom of cyclic amine groups for the activity. Compound **11** with a benzyl group attached to the piperazine nitrogen atom showed decreased activity compared to compound **12**. Compound **12** displayed good and broad-spectrum antiproliferative activities with IC_{50} values less than 10 μM, comparable to that of 5-FU.

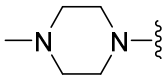
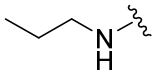
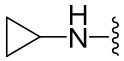
Table 1

Inhibitory activity of compounds **4**-**14** against the tested cancer cell lines



4-14

Compound	R'RN-	IC_{50} (μM)[a]			
		MKN-45	MGC-803	EC-109	H1650
4		38.60±3.75	36.60±4.69	19.03±1.38	>64
5		14.65±1.80	19.35±0.56	>64	51.84±3.86
6		14.25±1.42	15.03±1.86	38.67±1.56	36.94±0.96
7		>64	46.64±2.56	>64	>64
8		21.02±1.16	30.40±2.54	36.04±0.98	>64
9		>64	49.64±0.86	>64	>64
10		>64	34.71±1.43	>64	54.80±2.43
11		15.43±2.47	18.71±5.69	>64	>64

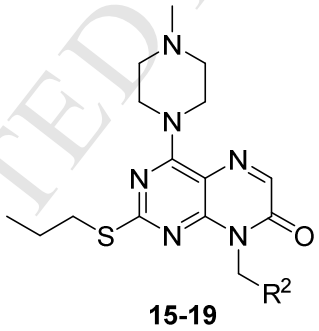
12		4.32±1.87	7.01±3.61	9.85±2.81	9.92±1.41
13		39.23±4.72	>64	>64	>64
14		23.26±1.86	43.74±1.80	29.71±1.65	>64
5-Fu	-	8.89±1.65	7.52±0.98	6.21±0.75	14.25±2.73

[a] Inhibitory activity was assayed by exposure for 48 h to substance 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.

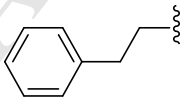
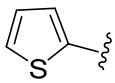
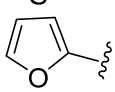
Next, the influence of R^2 substituents on the activity was also investigated. As shown in Table 2, compounds **15-19** generally displayed decreased or comparable activities toward the tested cancer cells, regardless of the patterns of R^2 group.

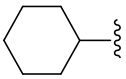
Table 2

Inhibitory activity of compounds **15-19** against the tested cancer cell lines



15-19

Compound	R^2	IC_{50} (μM)[a]			
		MKN-45	MGC-803	EC-109	H1650
15		9.97±0.97	13.75±3.81	18.28±2.95	>64
16		11.32±1.57	16.41±3.16	19.69±6.07	>64
17		11.56±6.50	>64	24.04±4.88	54.91±6.08

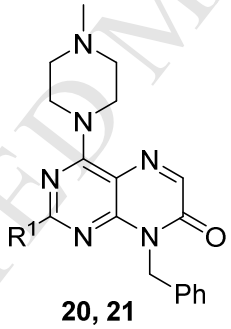
18		17.92±2.82	53.67±5.66	>64	51.81±3.21
19		16.83±5.65	17.17±2.35	37.48±4.62	45.43±2.90
5-Fu	-	8.89±1.65	7.52±0.98	6.21±0.75	14.25±2.73

[a] Inhibitory activity was assayed by exposure for 48 h to substance 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.

However, the replacement of propylthio group with methyl (compound **20**) or hydrogen (compound **21**) led to the loss of antiproliferative activity against the tested cancer cell lines, significantly less potent than 5-FU and compound **12** (Table 3).

Table 3

Inhibitory activity of compounds **20-21** against the tested cancer cell lines



20, 21

Compound	R^1	IC_{50} (μM)[a]			
		MKN-45	MGC-803	EC-109	H1650
12	propyl-S-	4.32±1.87	7.01±3.61	9.85±2.81	24.91±4.41
20	Me-	>64	>64	38.98±0.99	>64
21	H	>64	>64	41.66±5.02	>64
5-Fu	-	8.89±1.65	7.52±0.98	6.21±0.75	14.25±2.73

[a] Inhibitory activity was assayed by exposure for 48 h to substance 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.

2.2.2 Cell apoptosis of MKN-45 and possible mechanism involved

The acceptable antiproliferative activity of compound **12** promoted us to investigate its effect on the apoptosis and morphological changes. Hochest 33258 staining was performed to investigate morphological changes of MKN-45 cells [19]. After 24 h incubation with compound **12** at indicated concentrations, characteristic apoptotic morphological changes were observed, including cell rounding, chromatin shrinkage and formation of apoptotic bodies (Fig. 2A). Notably, this phenomena were more remarkable at higher concentrations. To further explore the effect of compound **12** on cell apoptosis, the apoptotic analysis was also performed with Annexin V-FITC/PI double staining and analyzed with flow-cytometry calculation [20]. Treatment of MKN-45 cells with compound **12** resulted in a concentration-dependent apoptosis increase (Fig. 2B/2C).

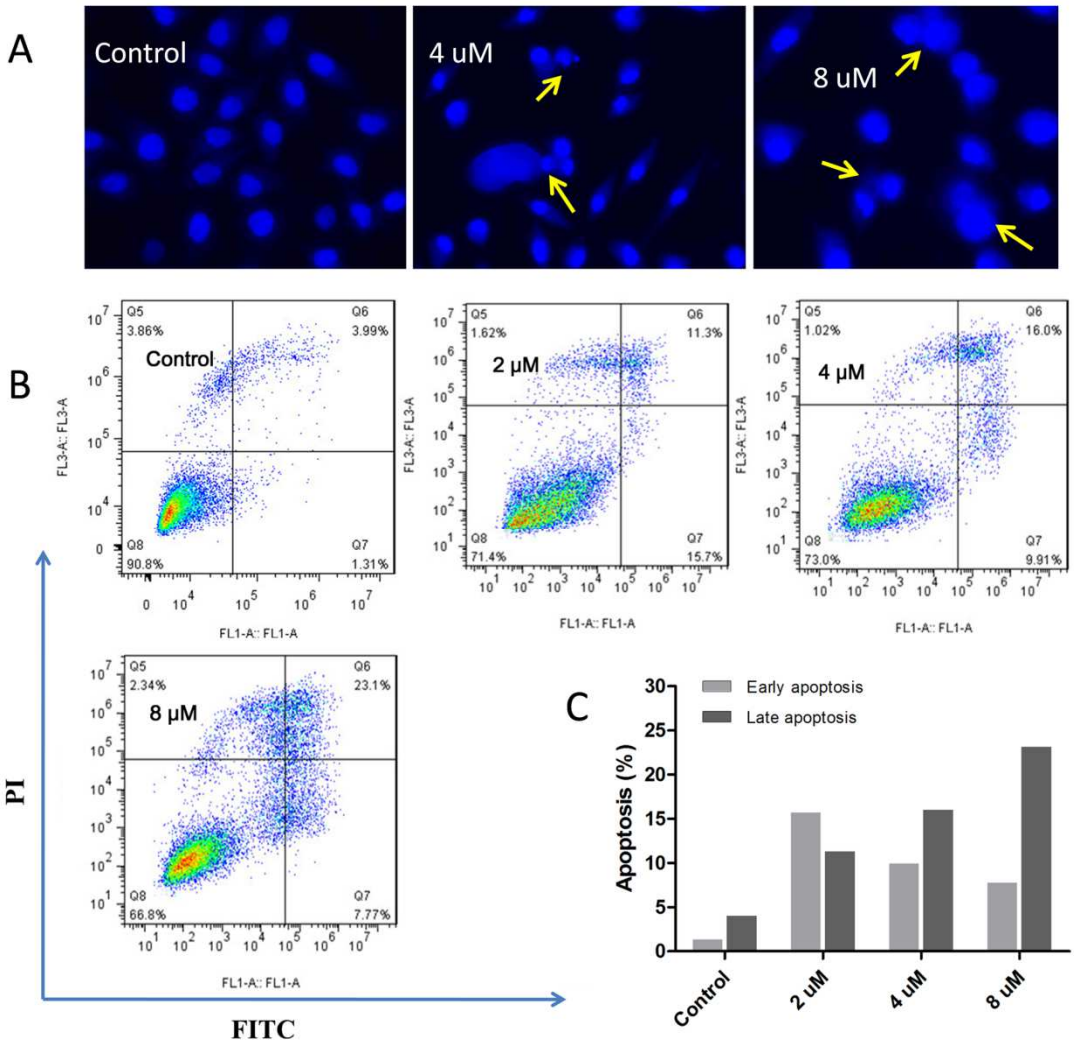


Fig. 2. Compound **12** induced apoptosis of MKN-45 cells. (A) Apoptosis analysis with Hoechst 33258 staining; (B) Apoptosis effect on MKN-45 cell line induced by compound **12** for 24 h using Annexin V-FITC/PI double staining and flow-cytometry calculation. The lower left quadrant represents live cells, the lower right is for early/primary apoptotic cells, upper right is for late/secondary apoptotic cells, and the upper left represents cells damaged during the procedure; (C) Quantitative analysis of apoptotic cells. The experiments were performed three times, and a representative experiment is shown.

Next, the western blot analysis was performed to examine the expression of apoptosis-related proteins. As shown in Fig. 3A, treatment of MKN-45 cells with compound **12** increased expression of Bax in a concentration-dependent manner (Fig. 3B). Bax was able to activate the caspases, and promoted the release of cytochrome c

and other pro-apoptotic factors from the mitochondria [21]. Meanwhile, the expression of anti-apoptotic protein Bcl-2 decreased accordingly (Fig. 3C). As shown in Fig. 3D and 3E, treatment of compound **12** also concentration-dependently caused the cleavage of caspases-3 and caspases-9, which then inducing cell apoptosis. These results indicated that compound **12** may induce MKN-45 apoptosis through the intrinsic apoptotic pathway.

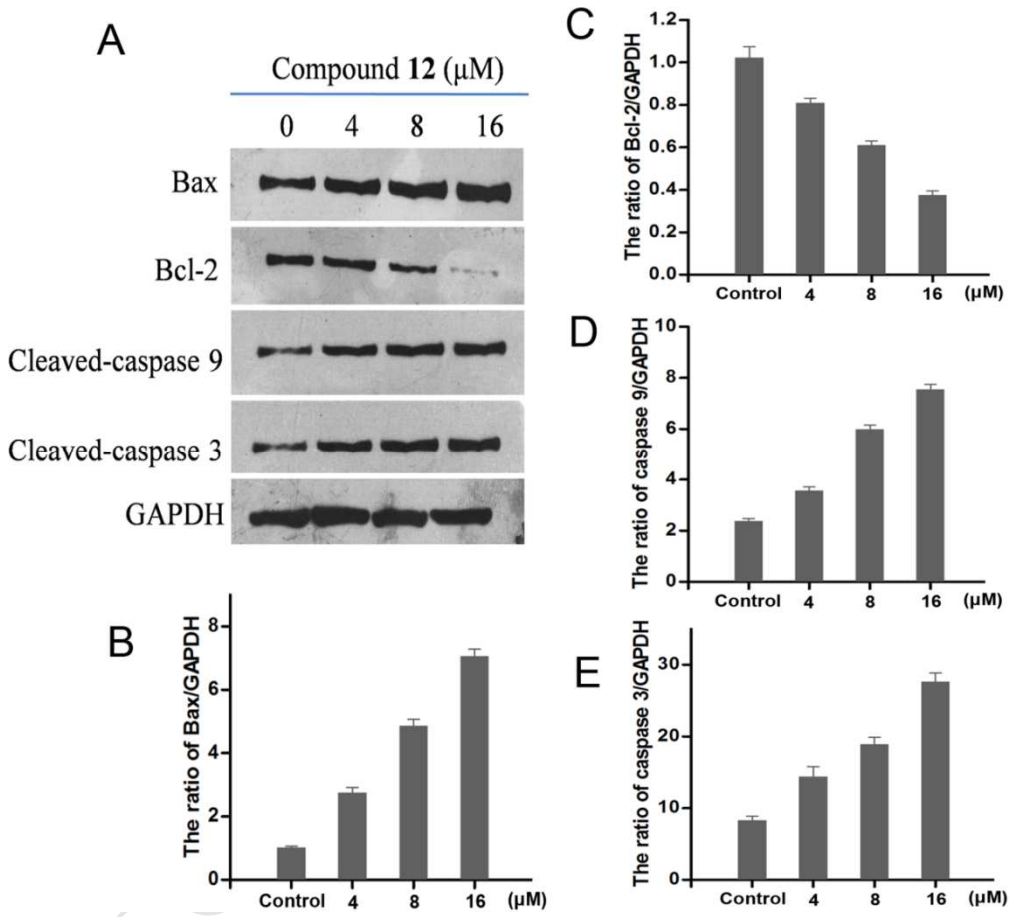


Fig. 3. Expression changes of apoptosis-related proteins induced by compound **12**. (A) Compound **12** induced expression changes of Bax, Bcl-2 and caspase family members in MKN-45 cells; (B-E) Statistical analysis of expression levels of Bax, Bcl-2 and cleaved-caspased 9/3.

2.2.3 Cell cycle analysis

The effect of compound **12** on the cell cycle was also evaluated. After treatment of MKN-45 cells with compound **12** for 24 h at indicated concentrations (0, 2, 4, 8 μM),

the percentage of cells in G2/M phase were 1.62%, 16.6%, 29.2% and 40.8%, respectively (Fig. 4), suggesting that compound **12** caused an obvious G2/M arrest in a concentration-dependent manner with concomitant decrease in terms of the number of cells in other phases of the cell cycle.

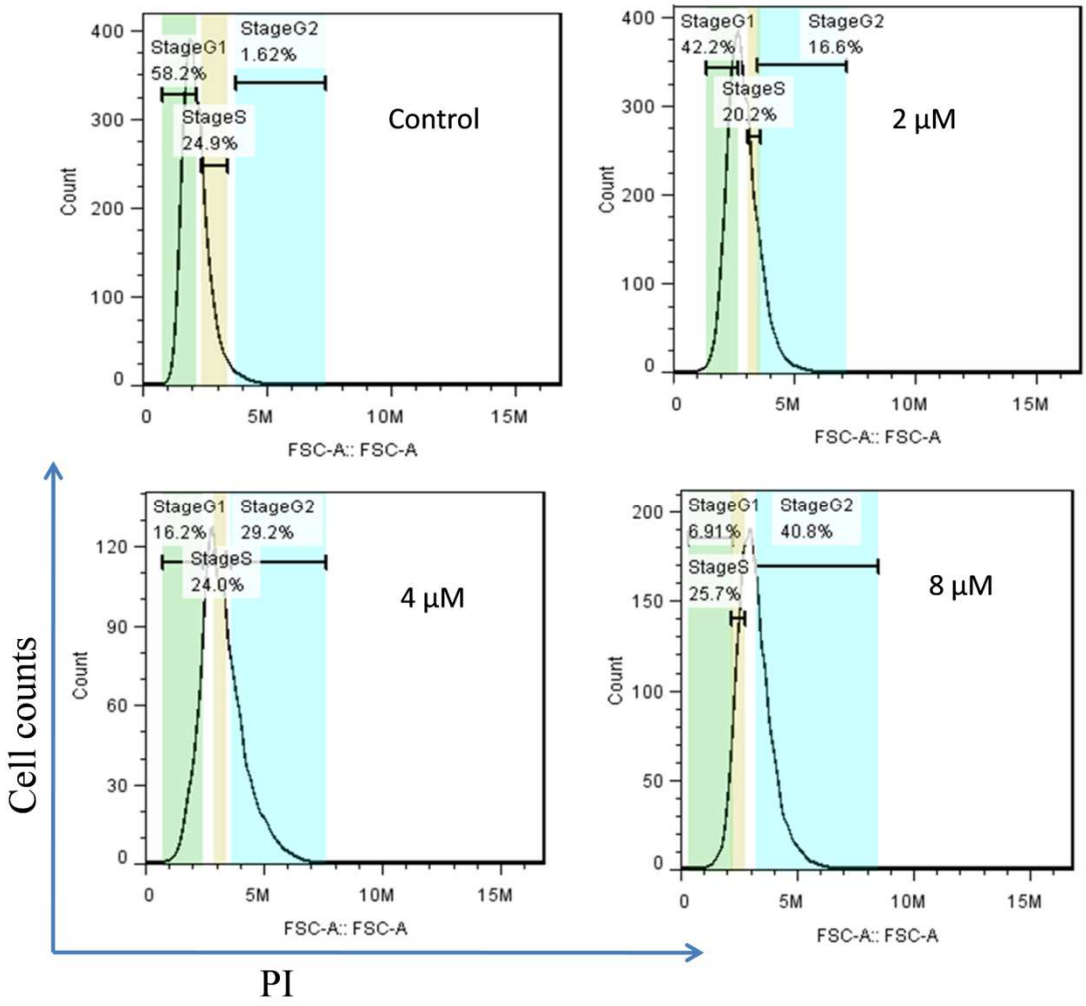


Fig. 4. Effect of compound **12** on the cell cycle distribution of MKN-45 cells. Cells were treated with indicated concentrations of compound **12** (0, 2, 4, 8 μM) for 24 h. Then the cells were fixed and stained with PI to analyze DNA content by flow cytometry. The experiments were performed three times and a representative experiment is shown.

3. Conclusions

In summary, a new series of pteridin-7(8*H*)-one derivatives were prepared and evaluated for their antiproliferative activity. Among them, compound **12** exhibited the

most potent and broad-spectrum inhibition against the tested cancer cells (MKN-45, MGC803, EC-109 and H1650) and displayed comparable activity with the anticancer drug 5-FU. Besides, compound **12** induced morphological changes and apoptosis of MKN-45 cells in a concentration-dependent manner, increased expression of pro-apoptotic protein Bax, down-regulated expression of anti-apoptotic protein Bcl-2 and caused cleavage of caspase-3/9, indicating that compound **12** induced apoptosis *via* the intrinsic apoptotic pathway. The G2/M phase arrest induced by compound **12** was also observed. Additionally, we first reported the construction of the novel bicyclic 8,9-dihydro-7*H*-purine-8-carboxylate scaffold through the competitive 5-endo cyclization reaction with two C-N bonds and a chiral carbon center established. This scaffold contains several pluripotent handles, which allows for further structural modifications for identifying potential bioactive molecules.

4. Experimental section

4.1 General

Reagents and solvents were purchased from commercial sources and were used without further purification. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker (DPX-400) spectrometer, operating at 400 and 100 MHz, respectively. High resolution mass spectra (HRMS) were recorded on a Waters Micromass Q-Tof of Micromass spectrometer by electrospray ionizaton (ESI).

4.2 General procedure for the synthesis of compounds 3h', 4-21.

A solution of **2a** (1eq) with ethyl glyoxalate (50 wt. % in toluene, 1.2 eq) in a mixed solvent of AcOH/ethanol (1 : 1) was heated to 100 ^{o}C for 2 h. After the completion of the reaction monitored by TLC (PE/EA = 4 : 1), the purification was conducted by flash column chromatography on silica gel using PE/EA (4 : 1) as eluent to afford **3a**. As a representative example, the new compound **3h'** was separated and characterized by NMR. Compounds **4** can be readily obtained by refluxing **3a** (1eq)**,** aniline (1.2 eq) and triethylamine (1.2 eq) in the mixed solvent of ethanol/DMF (3 : 1) for 3 h,

following by purification on flash column chromatography on silica gel. This procedure was also applied to preparation of compounds **5-21**.

*4.2.1 Ethyl (R)-6-chloro-9-(cyclohexylmethyl)-2-(propylthio)-8,9-dihydro-7H-purine-8-carboxylate (**3h'**)*

Pale yellow solid, yield 40%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.90 (s, 1H), 5.10 (s, 1H), 3.96-4.01 (m, 1H), 3.60-3.66 (m, 2H), 3.5-3.20 (m, 1H), 3.04-3.11 (m, 1H), 2.93-3.00 (m, 1H), 1.61-1.73 (m, 8H), 1.14-1.20 (m, 3H), 1.09 (t, *J* = 7.0 Hz, 3H), 0.98 (t, *J* = 7.3 Hz, 3H), 0.90-0.96 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.3, 160.2, 150.0, 141.1, 112.2, 85.9, 64.4, 52.2, 36.1, 32.3, 30.1, 30.1, 26.0, 25.3, 25.3, 22.6, 14.9, 13.3. HR-MS (ESI): Calcd. $C_{18}H_{27}ClN_4O_2S$, $[M+H]^+$m/z: 399.1621, found: 399.1623.

*4.2.2 8-Benzyl-4-(phenylamino)-2-(propylthio)pteridin-7(8H)-one (**4**)*

Pink solid, yield 75%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.42 (s, 1H), 7.98 (s, 1H), 7.75 (m, 1H), 7.49-7.51 (m, 2H), 7.36-7.40 (m, 2H), 7.23-7.34 (m, 3H), 7.12-7.16 (m, 1H), 5.51 (s, 2H), 3.13 (t, *J* = 7.2 Hz, 2H), 1.73-1.83 (m, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.50, 156.99, 155.61, 147.37, 144.76, 137.85, 135.89, 129.01, 128.51, 127.85, 124.11, 120.41, 110.37, 43.77, 33.43, 22.99, 13.61.HR-MS (ESI): Calcd. $C_{22}H_{21}N_5OS$, $[M+K]^+$m/z: 442.1104, found: 442.1102.

*4.2.3 8-Benzyl-4-((2-methoxyphenyl)amino)-2-(propylthio)pteridin-7(8H)-one (**5**)*

Yellow solid, yield 81%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.15 (s, 1H), 8.62-8.64 (m, 1H), 8.02 (s, 1H), 7.49 (m, 2H), 7.27-7.33 (m, 2H), 6.93-7.10 (m, 3H), 5.52 (s, 2H), 3.97 (s, 3H), 3.15 (t, *J* = 7.4 Hz, 2H), 1.75-1.83 (m, 2H), 1.05 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.36, 157.03, 155.39, 148.79, 147.26, 144.77, 135.96, 128.97, 128.49, 127.80, 127.67, 123.54, 120.78, 119.99, 110.99, 110.12, 55.90, 43.74, 33.43, 23.00, 13.63.HR-MS (ESI): Calcd. $C_{23}H_{23}N_5O_2S$, $[M+Na]^+$m/z: 456.1470, found: 456.1472.

*4.2.4 8-Benzyl-2-(propylthio)-4-((3,4,5-trimethoxyphenyl)amino)pteridin-7(8H)-one (**6**)*

Yellow solid, yield 86%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.34 (s, 1H), 7.99 (s, 1H), 7.47-7.49 (m, 2H), 7.28-7.32 (m, 3H), 7.07 (s, 2H), 5.53 (s, 2H), 3.90 (s, 6H), 3.85 (s, 3H), 3.15 (t, *J* = 7.3 Hz, 2H), 1.72-1.77 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.46, 156.97, 155.48, 153.36, 147.30, 144.76, 135.84, 134.77, 133.88, 128.80, 128.52, 127.85, 110.35, 98.32, 61.02, 56.21, 43.83, 33.40, 22.56, 13.49. HR-MS (ESI): Calcd. $C_{25}H_{27}N_5O_4S$, $[M+Na]^+$m/z: 516.1681, found: 516.1682.

*4.2.5 8-Benzyl-4-(benzylamino)-2-(propylthio)pteridin-7(8H)-one (**7**)*

Yellow solid, yield 68%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.48 (m, 2H), 7.35 (m, 4H), 7.26-7.31 (m, 4H), 6.78 (t, *J* = 5.6 Hz, 1H), 5.49 (s, 2H), 4.77 (d, *J* = 5.9 Hz, 2H), 3.09 (t, *J* = 7.3 Hz, 2H), 1.69-1.78 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.22, 158.01, 157.14, 147.19, 144.21, 137.88, 136.05, 128.92, 128.78, 128.46, 127.76, 127.70, 110.33, 44.80, 43.75, 33.39, 22.88, 13.58. HR-MS (ESI): Calcd. $C_{23}H_{23}N_5OS$, $[M+Na]^+$m/z: 440.1521, found: 440.1524.

*4.2.6 8-Benzyl-4-((cyclohexylmethyl)amino)-2-(propylthio)pteridin-7(8H)-one (**8**)*

White solid, yield 76%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.46-7.49 (m, 2H), 7.27-7.31 (m, 2H), 7.22-7.25 (m, 1H), 6.57 (t, *J* = 6.1 Hz, 1H), 5.48 (s, 2H), 3.42 (t, *J* = 6.5 Hz, 2H), 3.09 (t, *J* = 7.4 Hz, 2H), 1.62-1.80 (m, 8H), 1.16-1.30 (m, 3H), 0.96-1.05 (m, 5H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 173.05, 158.35, 157.20, 147.03, 143.84, 136.12, 128.94, 128.43, 127.71, 110.35, 47.04, 43.69, 37.99, 33.37, 30.88, 26.37, 25.83, 23.01, 13.62. HR-MS (ESI): Calcd. $C_{23}H_{29}N_5OS$, $[M+Na]^+$m/z: 446.1991, found: 446.1992.

*4.2.7 8-Benzyl-4-morpholino-2-(propylthio)pteridin-7(8H)-one (**9**)*

Yellow solid, yield 79%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 (s, 1H), 7.45 (m, 2H), 7.29 (m, 2H), 7.23 (m, 1H), 5.53 (s, 2H), 4.27-4.30 (m, 4H), 3.80 (t, *J* = 4.7 Hz, 4H), 3.05 (t, *J* = 7.3 Hz, 2H), 1.68-1.77 (m, 2H), 1.01 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.09, 157.27, 156.17, 149.70, 141.83, 136.15, 128.73, 128.43, 127.66, 112.36, 67.09, 47.94, 44.09, 33.29, 22.85, 13.56. HR-MS (ESI): Calcd. $C_{20}H_{24}N_5O_2S$, $[M+Na]^+$m/z: 420.1470, found: 420.1471.

*4.2.8 8-Benzyl-2-(propylthio)-4-thiomorpholinopteridin-7(8H)-one (**10**)*

White solid, yield 81%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.91 (s, 1H), 7.46 (m, 2H), 7.24-7.31 (m, 3H), 5.52 (s, 2H), 4.49 (t, *J* = 4.9 Hz, 4H), 3.05 (t, *J* = 7.3 Hz, 2H), 2.76 (t, *J* = 5.0 Hz, 4H), 1.70-1.76 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.06, 157.23, 156.13, 149.80, 141.94, 136.17, 128.82, 128.42, 127.68, 112.34, 50.70, 44.09, 33.31, 27.62, 22.91, 13.58. HR-MS (ESI): Calcd. $C_{20}H_{23}N_5OS_2$, $[M+Na]^+$m/z: 436.1242, found: 436.1245.

*4.2.9 8-Benzyl-4-(4-benzylpiperazin-1-yl)-2-(propylthio)pteridin-7(8H)-one (**11**)*

White solid, yield 72%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.45 (m, 2H), 7.23-7.34 (m, 8H), 5.51 (s, 2H), 4.28 (m, 4H), 3.54 (s, 2H), 3.04 (t, *J* = 7.3 Hz, 2H), 2.56 (t, *J* = 5.0 Hz, 4H), 1.67-1.76 (m, 2H), 1.00 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.88, 157.15, 156.22, 149.68, 141.48, 137.60, 136.25, 129.21, 128.79, 128.40, 128.33, 127.62, 127.27, 112.35, 62.96, 53.31, 47.44, 44.04, 33.28, 22.89, 13.56. HR-MS (ESI): Calcd. $C_{27}H_{30}N_6OS$, $[M+H]^+$m/z: 487.2280, found:487.2281.

*4.2.10 8-Benzyl-4-(4-methylpiperazin-1-yl)-2-(propylthio)pteridin-7(8H)-one (**12**)*

White solid, yield 82%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.91 (s, 1H), 7.44-7.46 (m, 2H), 7.28-7.31 (m, 1H), 7.22-7.26 (m, 2H), 5.52 (s, 2H), 4.30 (m, 4H), 3.05 (t, *J* = 7.2 Hz, 2H), 2.54 (t, *J* = 4.9 Hz, 4H), 2.34 (s, 3H), 1.68-1.77 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.64, 156.60, 155.33, 149.36, 141.68,

136.17, 128.19, 127.33, 127.06, 111.54, 54.65, 46.89, 45.50, 43.49, 32.45, 22.48, 13.18. HR-MS (ESI): Calcd. $C_{21}H_{26}N_6OS$, $[M+H]^+$m/z: 411.1967, found: 411.1968.

*4.2.11 8-Benzyl-4-(propylamino)-2-(propylthio)pteridin-7(8H)-one (**13**)*

Yellow solid, yield 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (t, J = 6.0 Hz, 1H), 7.96 (s, 1H), 7.24-7.31 (m, 5H), 5.35 (s, 2H), 3.38-3.43 (m, 2H), 2.99 (t, J = 7.2 Hz, 2H), 1.54-1.66 (m, 2H), 0.86-0.92 (m, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.85, 153.02, 151.93, 141.78, 138.66, 130.86, 123.64, 123.17, 122.44, 105.10, 38.44, 37.37, 28.12, 17.71, 17.54, 8.32, 6.16. HR-MS (ESI): Calcd. $C_{19}H_{23}N_5OS$, $[M+Na]^+$m/z: 392.1521, found:392.1522.

*4.2.12 8-Benzyl-4-(cyclopropylamino)-2-(propylthio)pteridin-7(8H)-one (**14**)*

Yellow solid, yield 68%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (d, J = 4.5 Hz, 1H), 7.95 (s, 1H), 7.22-7.32 (m, 5H), 5.36 (s, 2H), 3.02 (t, J = 7.3 Hz, 2H), 2.96 (m, 1H), 1.58-1.67 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H), 0.68-0.74 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.37, 158.96, 156.27, 146.85, 143.68, 136.24, 128.30, 127.30, 127.13, 110.00, 43.14, 32.51, 24.04, 22.63, 13.24, 5.98. HR-MS (ESI): Calcd. $C_{19}H_{21}N_5OS$, $[M+Na]^+$m/z: 390.1365, found: 390.1366.

*4.2.13 4-(4-Methylpiperazin-1-yl)-8-(3-phenylpropyl)-2-(propylthio)pteridin-7(8H)-one (**15**)*

Yellow solid, yield 63%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.86 (s, 1H), 7.28 (m, 1H), 7.25 (m, 1H), 7.15-7.21 (m, 3H), 4.35-4.39 (m, 2H), 4.31-4.32 (m, 4H), 3.03 (t, J = 7.2 Hz, 2H), 2.73 (t, J = 7.8 Hz, 2H), 2.60 (t, J = 5.1 Hz, 4H), 2.36 (s, 3H), 2.03-2.08 (m, 2H), 1.74-1.79 (m, 2H), 1.05 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.96, 157.26, 156.20, 149.64, 141.53, 141.08, 128.35, 128.23, 125.95, 112.34, 54.96, 46.94, 45.58, 41.08, 33.34, 33.32, 28.72, 22.92, 13.56. HR-MS (ESI): Calcd. $C_{23}H_{30}N_6OS$, $[M+H]^+$m/z: 439.2280, found: 439.2281.

4.2.14 4-(4-Methylpiperazin-1-yl)-2-(propylthio)-8-(thiophen-2-ylmethyl)pteridin-

*7(8H)-one (**16**)*

Yellow waxy solid, yield 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 7.34-7.36 (m, 1H), 7.14 (d, *J* = 3.5 Hz, 1H), 6.92-6.94 (m, 1H), 5.53 (s, 2H), 4.21-4.23 (m, 4H), 3.12 (t, *J* = 7.2 Hz, 2H), 2.45 (t, *J* = 5.0 Hz, 4H), 2.23 (s, 3H), 1.70-1.77 (m, 2H), 1.01 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.97, 157.15, 155.63, 149.19, 141.53, 137.29, 128.87, 126.39, 126.10, 112.28, 55.14, 47.15, 45.81, 38.42, 33.34, 22.88, 13.62. HR-MS (ESI): Calcd. $C_{19}H_{24}N_6OS_2$, $[M+H]^+$m/z: 417.1531, found: 417.1532.

*4.2.15 8-(Furan-2-ylmethyl)-4-(4-methylpiperazin-1-yl)-2-(propylthio)pteridin-7(8H)-one (**17**)*

Yellow waxy solid, yield 73%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.55 (d, *J* = 1.7 Hz, 1H), 6.39 (m, 1H), 6.29 (d, *J* = 3.2 Hz, 1H), 5.38 (s, 2H), 4.18 (m, 4H), 3.04 (t, *J* = 7.2 Hz, 2H), 2.43 (t, *J* = 5.0 Hz, 4H), 2.20 (s, 3H), 1.63-1.73 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 171.04, 157.20, 155.80, 149.37, 142.06, 141.56, 112.27, 110.41, 109.42, 55.06, 47.08, 45.72, 37.04, 33.33, 22.94, 13.58. HR-MS (ESI): Calcd. $C_{19}H_{24}N_6O_2S$, $[M+H]^+$m/z: 401.1760, found: 401.1762.

*4.2.16 8-(Cyclohexylmethyl)-4-(4-methylpiperazin-1-yl)-2-(propylthio)pteridin-7(8H)-one (**18**)*

White solid, m. p. 146-147 ^{o}C, yield 72%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 4.29 (m, 4H), 4.19 (d, *J* = 7.3 Hz, 2H), 3.07 (t, *J* = 7.4 Hz, 2H), 2.53 (t, *J* = 5.1 Hz, 4H), 2.34 (s, 3H), 1.93-1.97 (m, 1H), 1.78-1.84 (m, 2H), 1.69-1.71 (m, 2H), 1.60 (m, 3H), 1.11-1.17 (m, 5H), 1.06 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 170.64, 165.81, 157.41, 156.62, 150.00, 141.57, 112.36, 55.30, 47.35, 46.85, 45.99, 36.46, 33.32, 30.80, 26.29, 25.84, 23.13, 13.63. HR-MS (ESI): Calcd. $C_{21}H_{32}N_6OS$, $[M+H]^+$m/z: 417.2437, found: 417.2438.

*4.2.17 8-Isobutyl-4-(4-methylpiperazin-1-yl)-2-(propylthio)pteridin-7(8H)-one (**19**)*

Yellow waxy solid, yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 4.17 (m, 4H), 4.03 (d, *J* = 3.6 Hz, 2H), 3.04 (t, *J* = 7.4 Hz, 2H), 2.44 (t, *J* = 5.0 Hz, 4H), 2.15-2.21 (m, 4H), 1.67-1.74 (m, 2H), 1.00 (t, *J* = 7.3 Hz, 3H), 0.87 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 174.65, 170.70, 157.39, 156.56, 149.96, 141.69, 127.83, 125.77, 112.31, 55.01, 47.90, 47.03, 45.65, 33.29, 27.14, 23.05, 20.14, 13.60. HR-MS (ESI): Calcd. $C_{18}H_{28}N_6OS$, $[M+H]^+$m/z: 377.2124, found: 377.2125.

*4.2.18 8-Benzyl-2-methyl-4-(4-methylpiperazin-1-yl)pteridin-7(8H)-one (**20**)*

Yellow solid, m. p. 142-143 ^{o}C, yield 61%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (s, 1H), 7.53-7.55 (m, 2H), 7.29 (m, 2H), 7.23 (m, 1H), 5.56 (s, 2H), 4.30 (m, 4H), 2.52 (m, 7H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.86, 157.55, 155.35, 149.54, 142.96, 136.41, 128.18, 127.99, 127.13, 111.94, 54.72, 46.82, 45.52, 43.20, 26.18. HR-MS (ESI): Calcd. $C_{19}H_{22}N_6O$, $[M+H]^+$m/z: 351.1933, found: 351.1932.

*4.2.19 8-Benzyl-4-(4-methylpiperazin-1-yl)pteridin-7(8H)-one (**21**)*

White solid, m. p. 141-142 ^{o}C, yield 73%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.39 (s, 1H), 8.04 (s, 1H), 7.50-7.52 (m, 2H), 7.29 (m,2H), 7.23 (m, 1H), 5.57 (s, 2H), 4.32 (m, 4H), 2.55 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.62, 156.52, 155.30, 149.49, 144.21, 136.22, 128.22, 127.63, 127.12, 113.55, 54.69, 46.99, 45.48, 43.40. HR-MS (ESI): Calcd. $C_{18}H_{20}N_6O$, $[M+H]^+$m/z: 337.1777, found: 337.1777.

4.3 Antiproliferative activity assays

Exponentially growing cells were seeded into 96-well plates at a concentration of 3,000 cells per well. After 24 h of incubation, the culture medium was removed and fresh medium containing various concentrations of the candidate compounds was added to each well. The cells were then incubated for 48 h, thereafter MTT assays were performed and cell viability was assessed at 570 nm by a microplate reader (Biotech, Shanghai, China).

4.4 Hoechst 33258 staining

MKN-45 cells were seeded into a 6-well plate (2×10^5/well) and incubated overnight for adherent and treated with compound **12** at different concentration for 24 h, and underwent Hoechst 33258 staining for 30 min in the dark. The cells were observed under a Nikon Eclipse TE 2000-S fluorescence microscope (Nikon, Japan).

4.5 Cell apoptosis assay

MKN-45 cells were seeded into a 6-well plate (2×10^5/well) and incubated for 24 h. Then the cells were treated with different concentrations of the tested compound **12** for 24 h. Thereafter, the cells were collected and the Annexin-V-FITC/PI apoptosis kit (Biovision) was used according to the manufacturer's protocol. The cells were analyzed by high content screening system (ArrayScan XTI, Thermo Fisher Scientific, MA).

4.6 Western blot analysis

MKN-45 cells were treated with different concentrations of compound **12** for 24 h, the cells were collected, lysed in RIPA buffer contained a protease inhibitor cocktail for 30 min, followed by centrifugation at 12,000 rpm for 10 min at 4 ^{o}C. After the collection of supernatant, the protein concentration was detected using a bicinchoninic acid assay kit (Beyotmie Biotechnology, Haimen, China). After added with loading buffer, cell lyses were boiled for 10 min at 100 ^{o}C for SDS- polyacrylamide gel electrophoresis (PAGE). Proteins were transferred to nitrocellulose (NC) membranes. Then the membranes were blocked with 5% skim milk at room temperature for 2 h, and then incubated overnight at 4^{o} C with primary antibodies. After washing the membrane with the secondary antibody (1: 5000) at room temperature for 2 h. Finally, the blots were washed in TBST/TBS. The antibody-reactive were revealed by enhanced chemiluminescence (ECL) and exposed on Kodak radiographic film.

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Highlights

- The pteridin-7*(8H)*-one derivatives showed potent inhibition against the cancer cells.
- Compound **12** exerted the most potent and broad-spectrum antiproliferative activity.
- Compound **12** induced the apoptosis and G2/M arrest of MKN-45 cells.
- A novel bicyclic 8,9-dihydro-7*H*-purine-8-carboxylate scaffold was constructed.