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Synthesis, cytotoxicity, and *in vivo* antitumor activity study of parthenolide semicarbazones and thiosemicarbazones

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Abstract:

Parthenolide is an important sesquiterpene lactone with potent anticancer activities. In order to further improve its biological activity, a series of parthenolide semicarbazone or thiosemicarbazone derivatives was synthesized and evaluated for their anticancer activity. Derivatives were tested *in vitro* against 5 human tumor cell lines, and many of these showed higher cytotoxicity than parthenolide. Five compounds were further studied for their antitumor activity in mice. The *in vivo* result indicated that compound **4d** showed both promising antitumor activity against mice colon tumor and small side effects on immune systems. The cell apoptosis and cell cycle distribution of compound **4d** were also studied. Molecular docking studies revealed multiple interactions between **4d** and NF-*κ*B. Our findings demonstrate the potential of semicarbazones as a promising type of compounds with anticancer activity.

Keywords:

Anticancer

Parthenolide

Semicarbazone

In vivo

1. Introduction

The lethality of cancer has encouraged numerous efforts to develop new anticancer drugs. Parthenolide (PTL, **1a**), a sesquiterpene lactone isolated from feverfew, has shown biological activity in many illnesses, including inflammatory diseases, kidney stones, infant colic, etc.[1] In recent years, parthenolide attracted particular attention due to its inhibiting activity on acute myeloid leukemia (AML) and glioblastoma (GBM). It is proposed that the antitumor activity of PTL resulted from its ability to inhibit NF-κB and induce redox stress.[2] Moreover, PTL was identified as the first small molecule to selectively eliminate cancer stem cells.[1, 3] Structure-activity relationship studies revealed that the α-methylene-γ-lactone structure and the epoxide group of PTL, which could react with biological nucleophiles of proteins, are the most important moieties for its biological activities.[4, 5]

Despite of its exceptional efficacy in eradicating cancer stem cells, it is reported that PTL were still not as effective in killing bulk tumor cells as in killing cancer stem cells.[1] Therefore, modifying the structure of PTL to enhance its interaction with target molecules while retaining the crucial α,β-unsaturated carbonyl structure could be an effective strategy to design PTL derivatives and reveal structure-activity relationships. Semicarbazones and thiosemicarbazones have been extensively studied for their broad bioactivities in antiviral, antibacterial, antimalarial, and anticancer applications.[6-8] Previous research showed that the oxygen/sulfur atom and nitrogen atoms of semicarbazone/thiosemicarbazone may interact with target kinases through the formation of hydrogen bonds, resulting in an inhibition of kinases.[9] In addition, structure-activity relationship studies on diaryl urea suggested that the aromatic ring attached to amide nitrogen could insert into pockets of some kinases and resulting in an inhibition effect on tumors.[10] Thiosemicarbazones were also widely studied as transition metal chelators with effective antitumor activity.[11] Therefore, inspired by these researches, the incorporation of semicarbazones or thiosemicarbazones scaffold into the PTL structure is expected to enhance the interaction between synthesized compounds and target molecules such as NF-κB, to increase their anticancer activity.

The goal of this research is to combine PTL structure with substituted semicarbazones or thiosemicarbazones to obtain PTL derivatives with higher anticancer activity and explore the structure-activity relationship between substituent groups of semicarbazone/thiosemicarbazone and the anticancer activity. The comparison between semicarbazones and thiosemicarbazones could also reveal valuable information about the importance of hydrogen bonds and metal chelation in anticancer activity. In order to attach semicarbazones or thiosemicarbazones to PTL, a two-step oxidation of PTL was conducted to yield an aldehyde (PTL-CHO, **1c**), which displayed slightly lower *in vitro* activity relative to PTL.[12] PTL-CHO was then condensed with semicarbazones or thiosemicarbazones to form a series of novel PTL derivatives, which were tested for their *in vitro* and *in vivo* anticancer activities.

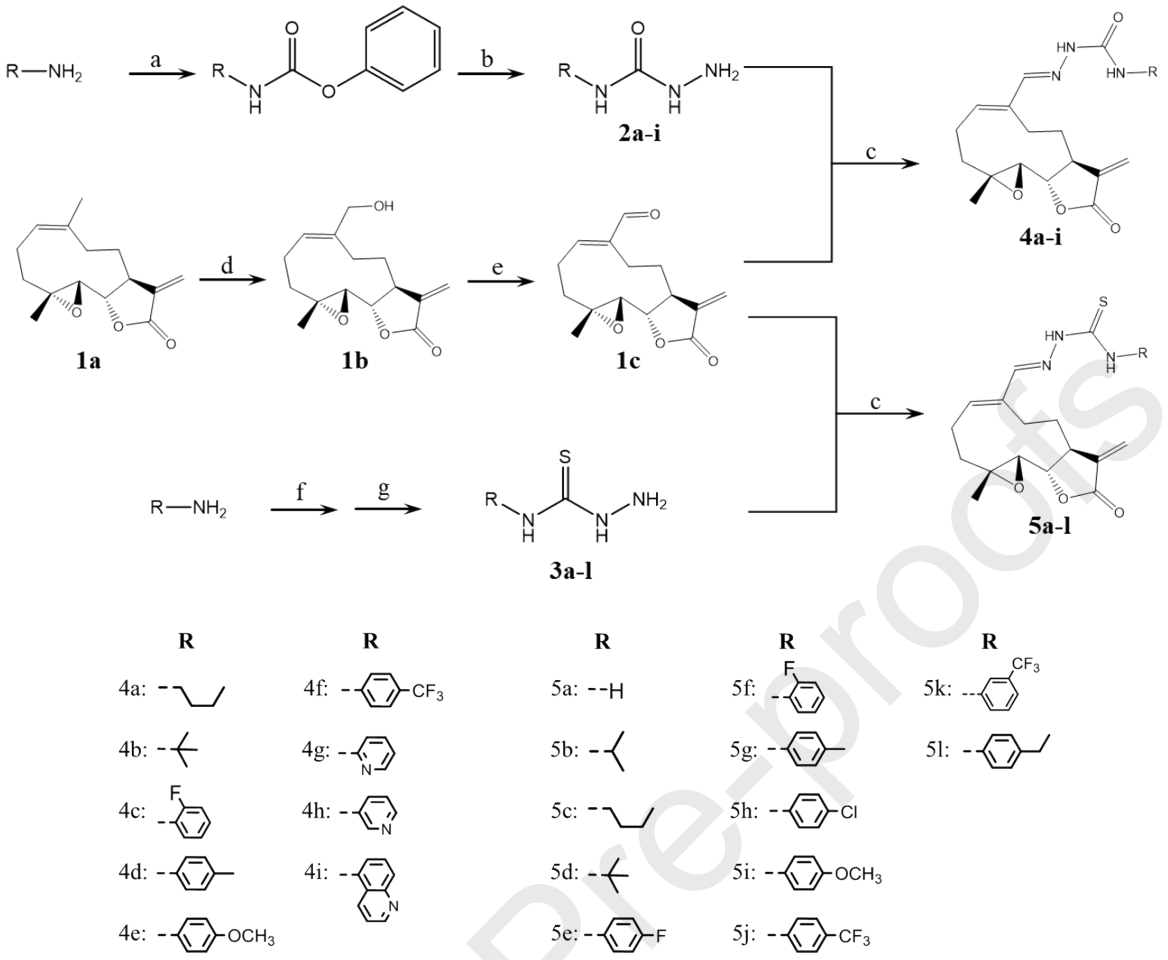
2. Results and discussion

2.1 Chemistry

The synthesis procedures of PTL semicarbazone or thiosemicarbazone derivatives are shown in Scheme 1. Initially, PTL (**1a**) was oxidized with SeO_2/*t*-BuOOH to give melampomagnolide B (**1b**), followed by the reaction with Dess-Martin periodinane to furnish the aldehyde PTL-CHO (**1c**).[12] Semicarbazones (**2a-i**) were synthesized by the condensation of amines with phenyl chloroformate to yield the carbamate, followed by the reaction with hydrazine hydrate.[13, 14] Substituted thiosemicarbazones (**3a-l**) were either purchased from chemical companies or synthesized by reacting functionalized amines with carbon disulphide, followed by the reaction with hydrazine hydrate.[15] The synthesized semicarbazones/thiosemicarbazones were refluxed with PTL-CHO (**1c**) catalyzed by acetic acid to yield the target compounds (**4a-i, 5a-l**).[15] The chemical structures of all compounds (**4a-i, 5a-l**) were confirmed by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectroscopy. Although both *E* and *Z* isomeric forms are possible for the imine double bond (-CH=N-), acidic synthesis environment as in this study generally yields *E* isomers.[8, 13] This is corroborated by the single peak observed in ^{1}H NMR of synthesized compounds for the hydrogen attached to carbon of imine, which is usually located at around δ 7.8 ppm for most compounds.[9] [15]

clog*P* values of synthesized derivatives were calculated by the ALOGPS 2.1 software from the Virtual Computational Chemistry Laboratory (http://www.vcclab.org).[16, 17] It could be seen from the result in Table 1 that synthesized PTL derivatives were generally more lipophilic compared to PTL or PTLCHO, despite the introduction of hydrogen bond donors/receptors as the =N-NH-CO-NH- or =N-NH-CS-NH- structure. Also, thiosemicarbazones were more lipophilic than semicarbazones, perhaps due to the lower polarity of the sulfur atom compared to that of the oxygen atom.

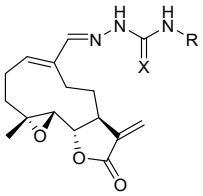
Scheme 1. General synthesis of PTL semicarbazone/thiosemicarbazone derivatives (**4a-i**, **5a-l**). Reagents and conditions: (a) phenyl chloroformate, pyridine, 50 °C, 4 h; (b) hydrazine hydrate, dimethoxyethane, 80 °C, 12 h; (c) ethanol, acetic acid, 50 °C, 4 h; (d) SeO_2/*t*-BuOOH, CH_2Cl_2, 45 °C, 4 h; (e) Dess-Martin periodinane, CH_2Cl_2, $NaHCO_3$, room temperature, 2 h; (f) CS_2, DMF, NaOH, room temperature, 4 h; (g) hydrazine hydrate, 65 °C, 2 h.

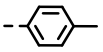
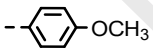
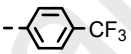


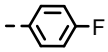
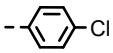
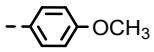
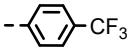
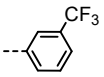
2.2 *In vitro* cytotoxic studies

The cytotoxic activities of target compounds (**4a-i, 5a-l**) were evaluated in HCT116 (human colorectal carcinoma), U87-MG (human glioblastoma), HepG2 (human liver carcinoma), BGC823 (human gastric cancer), and PC9 (human lung cancer) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Both Taxol (paclitaxel) and PTL (**1a**) were used as positive control, while PTL was also a well-known NF-*κ*B inhibitor.[2, 18, 19] PTL-CHO (**1c**) were also included for comparison. The IC_{50} values were reported in Table 1. PTL was more cytotoxic than PTL-CHO, which is consistent with a previous study.[20] All synthesized derivatives displayed moderate to prominent anticancer activities against 5 cancer cell lines. All semicarbazones except **4b** displayed stronger *in vitro* inhibitory activities against 5 cell lines compared to PTL, with most of IC_{50} values lower than PTL.

Table 1. *In vitro* antitumor activity of target compounds **4a-4i**, **5a-5l** against 5 human cancer cell lines.



Compound	X	R	clog*P* [a]	IC_{50} (μmol/L)				
				HCT116	U87-MG	HepG2	BGC823	PC9
4a	O		2.95	2.72	4.77	5.88	14.40	8.67
4b	O		3.02	14.47	14.02	7.43	15.60	6.44
4c	O		3.65	3.48	4.58	2.77	12.83	5.02
4d	O		3.61	5.35	2.81	2.83	2.02	3.90
4e	O		3.50	4.24	3.16	2.86	2.90	13.60
4f	O		4.08	4.16	2.49	1.74	1.44	4.66
4g	O		2.96	8.09	3.05	3.23	1.08	4.18
4h	O		2.75	6.91	2.72	6.54	3.77	16.74
4i	O		3.71	3.31	1.47	3.66	1.77	3.12
5a	S		2.58	16.09	2.27	8.99	16.48	11.47
5b	S		3.42	19.11	5.44	17.44	34.17	34.20
5c	S		3.91	11.98	17.29	6.15	19.69	22.36

Compound	X	R	clog*P* [a]	IC_{50} (μmol/L)				
5d	S		3.84	15.21	18.74	18.31	25.63	35.08
5e	S		4.23	14.01	5.99	3.33	15.35	15.55
5f	S		4.26	7.77	3.24	14.86	15.26	5.10
5g	S		4.50	4.48	2.43	4.75	13.52	4.27
5h	S		4.76	17.92	14.74	17.25	18.70	18.05
5i	S		4.40	15.60	4.38	3.77	15.17	7.31
5j	S		4.96	17.92	17.16	17.15	24.21	9.22
5k	S		4.97	18.78	9.75	16.82	34.36	7.73
5l	S		4.86	8.64	14.55	7.33	9.30	12.40
1a (PTL)	-		3.03	3.13	5.75	5.71	15.77	6.61
1c (PTL-CHO)	-		2.01	14.46	7.37	4.77	16.39	10.69
Taxol	-			0.000206	0.000380	0.000762	0.000077	0.000056

[a] clog*P*: calculated by the ALOGPS 2.1 software from Virtual Computational Chemistry Laboratory (VCCLAB).

In general, semicarbazone derivatives were more cytotoxic than thiosemicarbazones, as can be seen in the comparison between **4a** and **5c**, **4d** and **5g**, etc. This is indicative of the importance of the oxygen atom in the carbonyl group. Thiosemicarbazones are an important type of anticancer agents, which could inhibit the DNA synthesis by chelating with metal ions.[7] However, the inferior activity of PTL thiosemicarbazones compared to PTL as can be seen in

Table 1 suggested that the ability to chelate did not contributed to the cytotoxicity in this study. In addition, the lower electronegativity of the thiosemicarbazone sulfur atom than the semicarbazone oxygen atom may also hamper the ability to form hydrogen bonds and weaken the interaction between synthesized compounds and target proteins.

One noticeable trend for both semicarbazone and thiosemicarbazone derivatives is that the alkyl-substituted compounds were generally less potent than aryl-substituted ones, as can be seen for **4a**, **4b**, **5b**, **5c** and **5d**. Previous docking study on diaryl urea indicated that an aromatic ring attached to the urea moiety may insert into the hydrophobic pockets of various kinases and inhibit proliferation-related kinases.[10] Due to the structure similarity, this mechanism could also be a possible explanation for the higher potency of aryl-substituted compounds, implying that an aromatic ring attached to the urea moiety may be beneficial for increasing cytotoxicity. Among aryl-substituted derivatives, for both semicarbazones and thiosemicarbazones, the *p*-methylphenyl-substituted compound (**4d** and **5g**) stood out in terms of their cytotoxicity against all 5 cell lines.

2.3 *In vivo* anticancer activity studies

Based on the promising results of *in vitro* assay, five synthesized compounds, including **4d**, **4f**, **4g**, **4i**, and **5g**, were selected for further evaluation of *in vivo* antitumor activity in mice bearing murine colon adenocarcinoma cell MC38. The mice were divided into one control group and six experimental groups (6 mice per group) treated with 5-fluorouracil (5-FU), **4d**, **4f**, **4g**, **4i**, and **5g**. As shown in Table 2 and Figure 1, 5-FU showed the highest tumor inhibition rate among all tested compounds, while its toxicity was also severe according to the decreased body weight, spleen index, and thymus index compared to the control group. Five synthesized compounds showed distinct *in vivo* antitumor activities, with compound **4d** and **4f** displayed the highest inhibition activity against MC38. However, **4f** showed considerable toxicity during the treatment, with the mice body weight much lower than the control or other groups. **4i**, which showed prominent activity in MTT assay, showed the lowest tumor inhibition rate among all compounds as well as pronounced toxicity. Interestingly, although previous studies have suggested that the low water solubility of PTL inhibited its activity,[21] no direct correlation between clog*P* values and tumor inhibition rates was observed in this study. For example, compound **4h** with 2-pyridyl substituent showed the lowest clog*P* value among all semicarbazone derivatives, while its *in vivo* antitumor activity was moderate compared to other compounds.

Spleen and thymus are important immune organs, which are also the major sites for NF-*κ*B to function.[22] Mice treated with 5-FU displayed much lower thymus indices than the control group ($P < 0.001$), demonstrating the suppression effect of 5-FU on immune system as reported

previously.[23] In contrast, all 5 PTL derivatives showed higher spleen indices and much higher thymus indices than 5-FU, suggesting that synthesized compounds induced beneficial influences on immune systems compared to 5-FU during treatments in tumor-bearing mice. In addition, synthesized compounds exerted less side effects than 5-FU as the body weights of **4d**, **4g**, and **4i** treated mice showed increased body weights compared to the control group (Table 2).

Table 2. *In vivo* antitumor effects in MC38-bearing mice (mean ± SD).

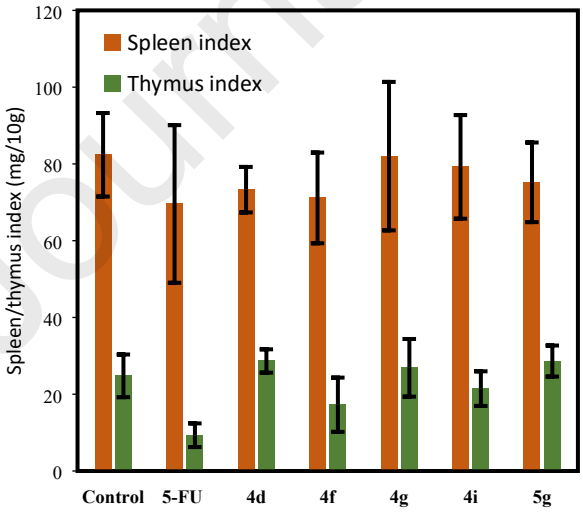
Groups	*N*	Dose (mg/kg)	Mice body weight (g)		Tumor weight (g)	Inhibition rate (%)
			Begin	End		
Control	6	-	14.5 ± 0.5	16.6 ± 1	2.67 ± 0.59	-
5-FU	6	40.0	14.2 ± 0.6	16.0 ± 1.5	1.76 ± 0.32**	34.27
4d	6	40.0	14.0 ± 0.9	17.4 ± 0.9	1.97 ± 0.36*	26.22
4f	6	40.0	14.5 ± 0.6	14.5 ± 2.5	1.96 ± 0.24*	26.78
4g	6	40.0	14.2 ± 0.7	17.8 ± 1.5	2.03 ± 0.76	24.09
4i	6	40.0	14.0 ± 0.5	18.0 ± 1.4	2.29 ± 0.49	14.23
5g	6	40.0	14.0 ± 0.6	16.1 ± 1.1	2.11 ± 0.33	20.85

N, number of mice in each group.

* $P < 0.05$, compared with model group.

** $P < 0.01$, compared with model group.

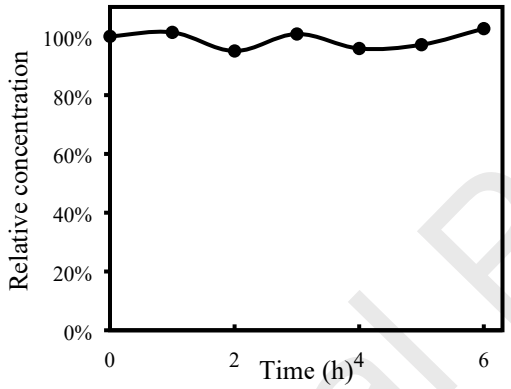
Figure 1. Evaluation of spleen indices and thymus indices of tumor-bearing mice.



Previous studies have reported that PTL alone was not able to significantly inhibit tumor growth.[1, 24, 25] According to the *in vivo* experiment in the present study, PTL semicarbazone **4d** showed promising antitumor activity, with slightly lower tumor inhibition rate but less overall side effects compared to 5-FU. Therefore, it is deduced that an increased dose of **4d** might be more effective against tumor *in vivo*, and this will be studied in future research.

Considering the possibility of semicarbazone decomposition, the chemical stability of the most active compound (**4d**) over 6 h was studied in phosphate buffer solution of pH = 7.4 at 37 °C, to simulate its stability in serum. The relative change of **4d** concentration versus time is shown in Figure 2. It could be seen in the figure that **4d** is relatively stable, with no obvious decrease in concentration for 6 h. This is consistent with a previous study, reporting that semicarbazone scaffold is stable in both buffer solutions and rat plasma.[13]

Figure 2. Chemical stability of compound **4d** in phosphate buffer solution (pH = 7.4) at 37 °C in 6 h.



2.4 Cell apoptosis and cell cycle studies

Based on the *in vitro* and *in vivo* result, PTL semicarbazone derivative **4d** displayed the best biological activity among synthesized compounds. In order to provide further insights on the antitumor activity of **4d**, effects of **4d** on cell apoptosis and cell cycle of human glioblastoma U87-MG were studied by flow cytometry. It could be seen in Figure 3 that **4d** induced concentration-dependent apoptosis in U87-MG tumor cell, demonstrating the antitumor activity of **4d**, in agreement with the MTT assay in this study. Also, it is observed that the cell apoptosis induced by **4d** is mainly the early stage of apoptosis (Q2), while the percentage of late apoptosis and necrosis cells (Q3) is generally unchanged.

The effect of compound **4d** on U87-MG cell cycle was also studied and the result is shown in Figure 4. The cell population in G0/G1 phase of the cell cycle was slightly decreased in **4d**-treated cells compared to the control group, suggesting a cancer cell cycle arrest in G0/G1 phase.

Although NF-κB has been reported previously to induce G0/G1 phase arrest,[26, 27] the role of NF-κB on cell cycle distribution is still controversial and has yet reached an agreement.[28] Therefore, further experiments will be necessary to reveal the actual influence of **4d** on cell cycle.

Figure 3. Compound **4d** induced apoptosis of U87-MG cells incubated at various concentrations (0, 4, and 16 μM).

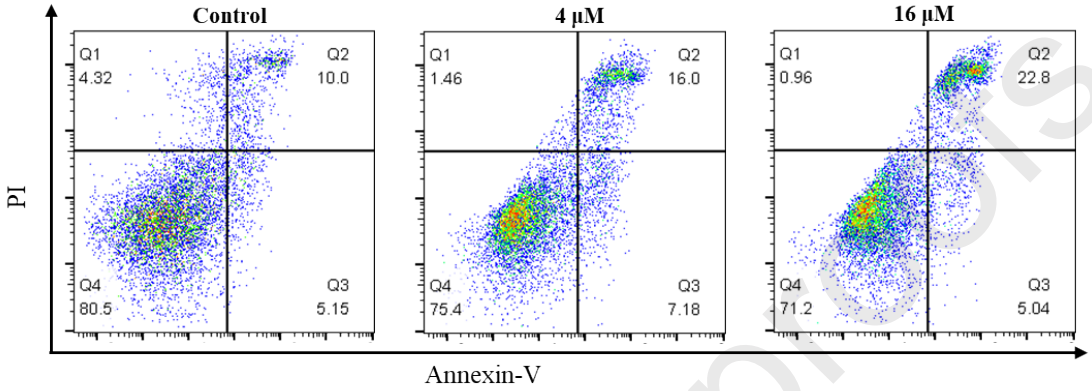
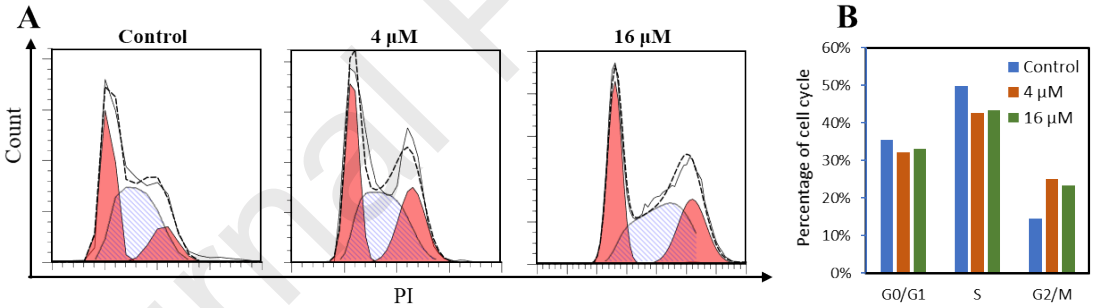


Figure 4. The effect of compound **4d** on cell cycle in U87-MG cancer cells. (A) U87-MG cells were incubated at various concentrations of **4d** (0, 4, and 16 μM). (B) Histograms display the percentage of cell cycle distribution.

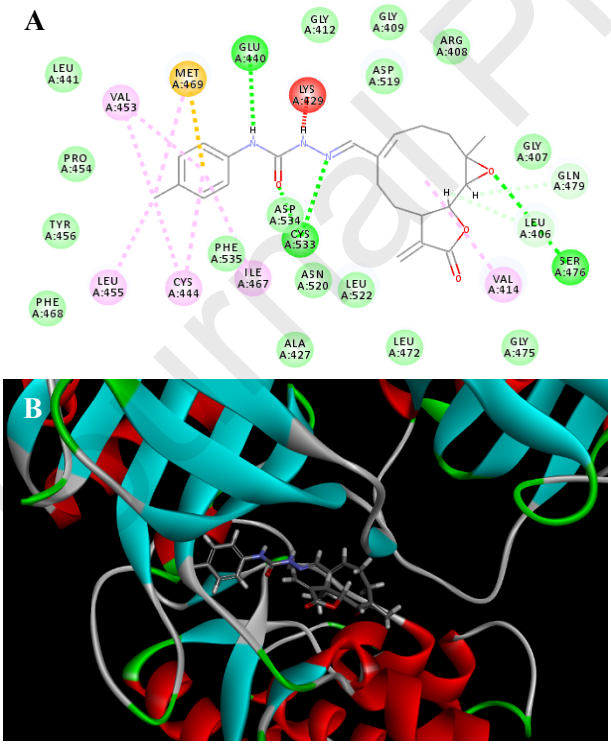


2.5 Molecular docking studies:

In literature, PTL has been widely regarded as a strong NF-κB inhibitor.[1] In order to reveal the binding modes of compound **4d** with NF-κB, molecular docking simulations were conducted using Discovery Studio (Accelrys Inc., USA). Compound **4d** was docked into the active site of NF-κB. As shown in Figure 5, multiple interactions were observed between compound **4d** and the NF-κB residues. For the PTL moiety of compound **4d**, a hydrogen bond was formed between residue SER476 and the epoxide group, consistent with other studies proposing that the epoxide group is crucial for the anticancer activity of PTL.[29] Three H-bond interactions

were noticed for the semicarbazone structure: one between CYS533 and the imine nitrogen atom, one between CYS533 and the carbonyl oxygen atom, and one between GLU440 and the amide hydrogen atom. Previous research proposed that the -NH-CO-NH- structure could bind various enzymes and receptors through hydrogen bond interaction, with the amide hydrogen being the proton donors and the carbonyl oxygen as the proton receptor.[10] The *p*-methylphenyl group of compound **4d** also interacts with various residues of NF-*κ*B. Three π-alkyl interactions were formed between the benzene ring and residue ILE467, CYS444, and VAL453. The methyl group is also interacting with residue LEU455, VAL453, and CYS444. In addition, this compound would insert into the pocket formed by multiple protein residues via van der Waals interaction, which further stabilized the ligand-receptor conformation. Previous research suggested the formation of hydrogen bond between amide hydrogen of the urea group and the carbamoyl moieties of I*κ*B kinase, an important kinase complex in the NF-*κ*B pathway, resulting in a significant increase in anticancer activity.[30] In this study, direct interactions between the synthesized semicarbazone **4d** and NF-*κ*B were also discovered and could account for the anticancer activity of **4d** by inhibiting NF-*κ*B activity.

Figure 5. Docking models of representative semicarbazone **4d**. (A) 2D molecular docking model of compound **4d** and surrounding residues of NF-*κ*B. (B) 3D molecular docking model of compound **4d** with NF-*κ*B.



3. Conclusion

In this study, 21 novel parthenolide semicarbazone or thiosemicarbazone derivatives were synthesized. Most semicarbazones exhibited higher cytotoxicity against human cancer cell lines than parthenolide. Preliminary analysis indicated that aryl-substituted semicarbazones were generally more cytotoxic. Five synthesized compounds, including 4 semicarbazones and 1 thiosemicarbazone were further tested in MC38-bearing mice. The *in vivo* results showed that **4d** possessed relatively high tumor inhibitory effect, as well as nearly unchanged spleen and thymus indices compared to the control group. Cell apoptosis experiments indicated a concentration-dependent apoptosis induced by **4d** in U87-MG cells, with a G0/G1 phase arrest observed in cell cycle analysis. Molecular studies demonstrated the formation of various interactions between NF-κB and semicarbazone moiety of **4d**. This indicated that PTL semicarbazones showed promising antitumor activity and are worth further investigation in anticancer experiments.

4. Experimental section

4.1 Chemistry

Reagents, solvents and starting compounds were purchased from Innochem, Alladin, Adamas, Alfa, or other chemical companies. Thin-layer chromatography (TLC) with Qingdao Haiyang GF254 were used to monitor reaction. Column chromatograph were performed with silica gel (100-200 mesh) from Qingdao Haiyang. The chromatograms were viewed under ultraviolet light (254-265 nm). NMR analysis on Bruker AV 400 (Bruker Company, USA) were performed to determine the structure of target compounds. $CDCl_3$, or DMSO-d_6 were used as solvents and tetramethylsilane was used as reference. ^{1}H NMR were recorded at 400 or 500 MHz, and ^{13}C NMR were recorded at 100 or 126 MHz. Chemical shifts (δ) were reported in ppm and coupling constants (J) were reported in Hz. High-resolution mass spectroscopy of target compounds was conducted on Agilent Technologies 6224 TOF LC/MS equipped with an ESI source.

4.1.1 General method for semicarbazones preparation

Typically, 10 mmol of substituted amines were dissolved in 10 mL of pyridine and allowed to stir. 11 mmol of phenyl chloroformate were added dropwise into the pyridine solution. The reaction was proceeded at 50 °C for 4 hours. After the total conversion of amines monitored by TLC, 20 mL of dichloromethane were added, and the mixture was then washed with water (20 mL × 3). The organic phase was concentrated in a rotary evaporator at 50 °C to afford crude carbamates. These carbamates could be directed used in the next step without further purification.

All crude carbamates from the previous step were dissolved in 20 mL dimethoxyethane, followed by the addition of 2.5 mL of 80% hydrazine hydrate. The reaction was stirred and refluxed at 80 °C for 12 h. Afterwards, the mixture was cooled and concentrated to give unpurified semicarbazones **2a**-**2i** in the form of oily liquid. This liquid was either recrystallized by 80% ethanol in water or purified by silica gel chromatography using a mixture of petroleum ether and ethyl acetate (2:1 ~ 1:2) as the eluent, to yield semicarbazones (**2a** - **2i**).

4.1.2 General method for thiosemicarbazones preparation

Thiosemicarbazone **3a**, **3j** and **3k** were purchased from chemical companies. For other compounds, the following synthesis protocol was used. To 15 mL of *N*, *N*-dimethylformamide (DMF), 10 mmol of substituted amines, 10 mmol of carbon disulphide, and 11 mmol of sodium hydroxide were added. The reaction was stirred at room temperature for 4 h. 2.5 mL of 80% of hydrazine hydrate was then added and the temperature was increased to 65 °C. The reaction was continued with stirring for 2 hours. After the reaction is completed checked by TLC, 30 mL water was added, and the mixture was then cooled in the fridge overnight. In some cases, a solid separated out which could be filtrated to yield the crude thiosemicarbazones. Otherwise, the mixture was extracted by CH_2Cl_2 (30 mL × 3). The organic phases were combined and purified by column chromatography. The solids obtained by filtration or column chromatography were then recrystallized by 90% ethanol in water to yield thiosemicarbazones **3b**-**3i,** and **3l**.

4.1.3 General method for PTL-CHO preparation

The synthesis method was adapted from a previous research.[20] 1 mmol of parthenolide (**1a**) in 15 mL of CH_2Cl_2 were oxidized with 0.4 mmol of SeO_2 and 10 mmol of 70% *t*-BuOOH at 45 °C for 4 h. After the reaction was completed checked by TLC, the mixture was filtered through kieselguhr. The liquid was concentrated under reduced pressure and purified by column chromatography to give melampomagnolide B (**1b**) in 50% yield and PTL-CHO (1c) in yield of 24%. 1 mmol of **1b** could be further oxidized by 1.5 mmol of Dess-Martin periodinane in 10 mL CH_2Cl_2 and 10 mmol $NaHCO_3$ at room temperature for 2 h. To the mixture saturated $Na_2S_2O_3$ (10 mL) aqueous solution, 10 mL of water, and 20 mL of CH_2Cl_2 were added and stirred. After the liquid phases turned clear, the organic phase was washed with brine, dried over anhydrous Na_2SO_4 overnight, and then concentrated to afford the residue. The residue was then purified by column chromatography using a mixture of petroleum ether and ethyl acetate (2:1 ~ 1:2) as the eluent, to give PTL-CHO (**1c**) in the yield of 87%.

4.1.4 General synthesis method for PTL semicarbazone/thiosemicarbazone derivatives

To 5 mL of ethanol, 0.25 mmol of PTL-CHO (**1c**), 0.3 mmol of semicarbazone (**2a-2i**) or thiosemicarbazone (**3a-3l**), and 0.1 mL of acetic acid were added. The reaction mixture was stirred at 50 °C for 4 h, monitored by TLC. After the complete disappearance of PTL-CHO, the liquid was cooled in the fridge overnight. In some cases, solids were separated out and direct filtration will yield target compounds. Otherwise, 10 mL of saturated sodium bicarbonate solution were added to the liquid, and the mixture was extracted by 30 mL of CH_2Cl_2 3 times. The organic phases were combined, concentrated, and the solid residue was purified by column chromatography to yield the target compounds.

4.1.4.1: *(E)-N-butyl-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carboxamide (**4a**)*. 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.82 (s, 1H), 7.40(s, 1H), 6.20 (d, J = 3.5 Hz, 1H), 5.93 (t, J = 8.3 Hz, 1H), 5.85 (t, J = 6.0 Hz, 1H), 5.38 (d, J = 3.2 Hz, 1H), 3.82 (t, J = 9.4 Hz, 1H), 3.42-3.24 (m, 2H), 2.91-2.82 (m, 1H), 2.80 (d, J = 9.6 Hz, 1H), 2.69-2.63 (m, 2H), 2.58-2.39 (m, 2H), 2.38-2.29 (m, 1H), 2.27-2.20 (m, 1H), 1.71-1.63 (m, 1H), 1.59-1.50 (m, 5H), 1.45-1.34 (m, 2H), 1.18 (t, J = 13.2 Hz, 1H), 0.96 (t, J = 7.43 Hz, 3H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 169.27, 156.08, 144.42, 138.73, 138.50, 137.12, 119.70, 81.77, 63.05, 59.66, 41.62, 39.48, 36.79, 32.52, 25.87, 24.66, 23.19, 20.07, 18.00, 13.82; HRMS (ESI) calcd for $C_{20}H_{29}N_3O_4$ [M + H$^+$] 376.2236; found 376.2236.

4.1.4.2: *(E)-N-(tert-butyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carboxamide (**4b**)*. 16% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.39 (s, 1H), 7.34 (s, 1H), 6.23 (d, J = 3.4 Hz, 1H), 5.90 (t, J = 8.4 Hz, 1H), 5.80 (s, 1H), 5.42 (d, J = 3.0 Hz, 1H), 3.82 (t, J = 9.4 Hz, 1H), 2.91-2.76 (m, 2H), 2.71-2.60 (m, 2H), 2.60-2.30 (m, 3H), 2.29-2.19 (m, 1H), 1.72-1.61 (m, 1H), 1.56 (s, 3H), 1.42 (s, 9H), 1.18 (t, J = 13.3 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 169.26, 154.83, 143.61, 138.84, 138.33, 137.11, 119.53, 81.82, 63.04, 59.64, 50.46, 41.60, 36.82, 29.71, 29.45, 29.32, 25.80, 24.66, 23.24, 18.01; HRMS (ESI) calcd for $C_{20}H_{29}N_3O_4$ [M + H$^+$] 376.2236; found 376.2246.

4.1.4.3: *(E)-N-(2-fluorophenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carboxamide (**4c**)*. 31% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.00(s, 1H), 8.29-8.25 (m, 2H), 7.50 (s, 1H), 7.18-7.11 (m, 2H), 7.07-7.01 (m, 1H), 6.16 (d, J = 3.5 Hz, 1H), 6.02 (t, J = 8.5 Hz, 1H), 5.49 (d, J = 3.2 Hz, 1H), 3.83 (t, J = 9.5 Hz, 1H), 3.05-2.95 (m, 1H), 2.82 (d, J = 9.5 Hz, 1H), 2.77-2.56 (m, 3H), 2.53-2.44 (m, 1H), 2.43-2.34 (m, 1H), 2.29-2.23 (m, 1H), 1.79-1.69 (m, 1H), 1.58 (s, 3H), 1.21 (t, J = 13.2 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 169.57, 153.54, 152.94, 151.62, 145.87, 140.39, 138.53, 137.29, 126.61,

125.04, 123.67, 120.76, 115.10, 81.97, 63.22, 59.85, 41.99, 36.95, 25.66, 24.99, 23.44, 18.20; HRMS (ESI) calcd for $C_{22}H_{24}FN_3O_4$ [M + H^+] 414.1829; found 414.1839.

4.1.4.4: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(p-tolyl)hydrazine-1-carboxamide* ***(4d)***. 63% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.83 (s, 1H), 7.77 (s, 1H), 7.45 (s, 1H), 7.37 (s, 1H), 7.35 (s, 1H), 7.17 (s, 1H), 7.14 (s, 1H), 6.15 (d, J = 3.5 Hz, 1H), 5.98 (t, J = 8.8 Hz, 1H), 5.37 (d, J = 3.2 Hz, 1H), 3.83 (t, J = 9.5 Hz, 1H), 2.96-2.87 (m, 1H), 2.81 (d, J = 9.5 Hz, 1H), 2.74-2.65 (m, 2H), 2.64-2.51 (m, 1H), 2.51-2.42 (m, 1H), 2.41-2.30 (m, 4H), 2.29-2.23 (m, 1H), 1.77-1.67 (m, 1H), 1.59-1.54 (s, 3H), 1.20 (m, J = 13.3 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 169.25, 153.35, 145.17, 139.54, 138.42, 136.92, 134.86, 133.57, 129.69, 120.07, 119.87, 81.72, 63.03, 59.64, 41.61, 36.73, 25.88, 24.74, 23.23, 20.84, 18.00; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_4$ [M + H^+] 410.2084; found 410.2080.

4.1.4.5: *(E)-N-(4-methoxyphenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carboxamide* ***(4e)***. 38% yield; ^{1}H NMR (500MHz, $CDCl_3$) δ: 8.98 (s, 1H), 7.69 (s, 1H), 7.46 (s, 1H), 7.39-7.35 (m, 2H), 6.92-6.88 (m, 2H), 6.17 (d, J = 3.5 Hz, 1H), 5.97 (t, J = 8.5 Hz, 1H), 5.38 (d, J = 3.2 Hz, 1H), 3.85-3.80 (m, 4H), 2.97-2.86 (m, 1H), 2.81 (d, J = 9.5Hz, 1H), 2.75-2.65 (m, 2H), 2.63-2.42 (m, 2H), 2.40-2.32 (m, 1H), 2.28-2.23 (m, 1H), 1.67-1.61 (m, 1H), 1.57 (s, 3H), 1.20 (t, J = 13.5 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 169.23, 156.36, 153.66, 145.16, 139.44, 138.47, 136.94, 130.34, 122.13, 119.99, 114.35, 81.71, 63.03, 59.63, 55.53, 41.63, 36.74, 25.88, 24.73, 23.22, 18.00; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_5$ [M + H^+] 426.2024; found 426.2029.

4.1.4.6: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(4-(trifluoromethyl)phenyl)hydrazine-1-carboxamide* ***(4f)***. 34% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.00 (s, 1H), 8.05 (s, 1H), 7.67-7.58 (m, 4H), 7.51 (s, 1H), 6.15 (d, J = 3.5 Hz, 1H), 6.04 (t, J = 8.4 Hz, 1H), 5.34 (d, J = 3.1 Hz, 1H), 3.84 (t, J = 9.4 Hz, 1H), 2.95-2.85 (m, 1H), 2.80 (d, J = 9.5 Hz, 1H), 2.76-2.57 (m, 3H), 2.55-2.35 (m, 2H), 2.30-2.24 (m, 1H), 1.80-1.70 (m, 1H), 1.58 (s, 3H), 1.21 (t, J = 13.3 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 169.15, 166.85, 152.85, 145.99, 140.79, 140.32, 138.46, 136.74, 126.45, 126.42, 119.93, 118.79, 81.66, 62.98, 59.65, 41.62, 36.66, 25.79, 24.78, 23.20, 17.97; HRMS (ESI) calcd for $C_{23}H_{24}F_3N_3O_4$ [M + H^+] 464.1801; found 464.1797.

4.1.4.7: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(pyridin-2-yl)hydrazine-1-carboxamide* ***(4g)***. 33% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.88-8.38 (m, 2H), 8.06 (s, 1H), 8.28 (d, J = 4.5 Hz, 1H), 7.74-7.68 (m, 1H), 7.55-

7.48 (s, 1H), 7.04-7.00 (m, 1H), 6.14 (d, *J* = 3.5 Hz, 1H), 6.01 (t, *J* = 8.2 Hz, 1H), 5.68 (d, *J* = 3.1 Hz, 1H), 3.83 (t, *J* = 9.4 Hz, 1H), 3.10 (s, 1H),2.82-2.45 (m, 5H), 2.42-2.33 (m, 1H), 2.30-2.23 (m, 1H), 1.78-1.67 (m, 1H), 1.58 (s, 3H), 1.20 (t, *J* = 13.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) *δ*: 169.47, 152.92, 151.45, 147.63, 146.59, 140.12, 138.37, 138.16, 137.25, 120.68, 118.93, 112.76, 81.86, 63.05, 59.59, 41.89, 36.80, 25.86, 24.71, 23.21, 17.99; HRMS (ESI) calcd for $C_{21}H_{24}N_4O_4$ [M + H^+] 397.1881; found 397.1876.

4.1.4.8: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(pyridin-3-yl)hydrazine-1-carboxamide (**4h**)*. 45% yield; ^{1}H NMR (400MHz, $CDCl_3$) *δ*: 9.16 (s, 1H), 8.63 (d, *J* = 2.3 Hz, 1H), 8.36 (d, *J* = 4.6 Hz, 1H), 8.08 (d, *J* = 8.5 Hz, 1H), 7.92 (s, 1H), 7.52 (s, 1H), 7.32 (dd, *J* = 3.6, 4.7 Hz, 1H), 6.16 (d, *J* = 3.6 Hz, 1H), 6.04 (t, *J* = 8.3 Hz, 1H), 5.36 (d, *J* = 3.1 Hz, 1H), 3.84 (t, *J* = 9.4 Hz, 1H), 2.95-2.86 (m, 1H), 2.82 (d, *J* = 9.6 Hz, 1H), 2.78-2.44 (m, 4H), 2.42-2.33 (m, 1H), 2.30-2.23 (m, 1H), 1.78-1.71 (m, 1H), 1.58 (s, 3H), 1.21 (t, *J* = 13.7 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) *δ*: 169.22, 153.15, 146.13, 144.67, 140.77, 140.37, 138.51, 136.78, 134.64, 126.94, 123.92, 119.96, 81.68, 63.00, 59.67, 41.66, 36.67, 25.75, 24.79, 23.20, 17.99; HRMS (ESI) calcd for $C_{21}H_{24}N_4O_4$ [M + H^+] 397.1881; found 397.1877.

4.1.4.9: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(quinolin-5-yl)hydrazine-1-carboxamide (**4i**)*. 35% yield; ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.27 (s, 1H), 8.97 (d, *J* = 3.2 Hz, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.20 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.88 (d, *J* = 7.4 Hz, 1H), 7.76 (t, *J* = 7.9 Hz, 1H), 7.51-7.43 (m, 2H), 6.11 (d, *J* = 3.1Hz, 1H), 5.96 (t, *J* = 8.3 Hz, 1H), 5.31 (s, 1H), 3.82 (t, *J* = 9.4 Hz, 1H), 3.00-2.91 (m, 1H), 2.81-2.67 (m, 3H), 2.65-2.57 (m, 1H), 2.53-2.43 (m, 1H), 2.40-2.33 (m, 1H), 2.29-2.22 (m, 1H), 1.74-1.68 (m, 1H), 1.57 (s, 3H), 1.20 (t, *J* = 13.2 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) *δ*: 169.21, 154.33, 150.50, 148.68, 146.07, 140.01, 138.66, 136.80, 132.38, 129.87, 129.49, 127.27, 123.47, 121.76, 121.12, 119.83, 81.67, 62.95, 59.68, 41.69, 36.66, 25.73, 24.75, 23.21, 17.95; HRMS (ESI) calcd for $C_{25}H_{26}N_4O_4$ [M + H^+] 447.2040; found 447.2032.

4.1.4.10: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5a**)*. 43% yield; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.12 (s, 1H), 8.07 (s, 1H), 7.70 (s, 1H), 7.53 (s, 1H), 6.01 (s, 1H), 5.92 (t, *J* = 7.7 Hz, 1H), 5.52 (s, 1H), 4.04 (t, *J* = 9.2 Hz, 1H), 3.01-2.92 (m, 1H), 2.84 (d, *J* = 9.5 Hz, 1H), 2.65-2.57 (m, 1H), 2.55-2.39 (m, 3H), 2.29-2.21 (m, 1H), 2.16-2.09 (m, 1H), 1.60-1.45 (m, 4H), 1.09-1.00 (m, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) *δ*: 177.41, 169.19, 147.26, 140.05, 139.70, 137.18, 118.65, 81.55, 62.20, 59.52, 40.88, 36.19, 24.52, 24.14, 22.14, 17.26; HRMS calcd for $C_{16}H_{21}N_3O_3S$ [M + H^+] 336.1382; found 336.1371.

4.1.4.11: *(E)-N-isopropyl-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5b**)*. 32% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.27 (s, 1H), 7.49 (s, 1H), 6.92 (d, J = 8.51 Hz, 1H), 6.20 (d, J = 3.5 Hz, 1H), 6.06 (t, J = 8.50 Hz, 1H), 4.65-4.55 (m, 1H), 3.82 (t, J = 9.2 Hz, 1H), 2.86-2.75 (m, 2H), 2.70-2.47 (m, 4H), 2.44-2.32 (m, 1H), 2.30-2.22 (m, 1H), 1.73-1.60 (m, 2H), 1.58-1.55 (s, 3H), 1.24-1.20 (m, 6H), 1.21 (t, J = 13.0 Hz, 1H) ; ^{13}C NMR (126MHz, $CDCl_3$) δ: 176.18, 169.32, 146.28, 141.71, 138.89, 136.94, 119.83, 81.83, 63.12, 59.88, 46.49, 41.85, 36.83, 25.91, 25.11, 23.36, 23.07, 22.85, 18.21; HRMS (ESI) calcd for $C_{19}H_{27}N_3O_3S$ [M + H^+] 378.1851; found 378.1844.

4.1.4.12: *(E)-N-butyl-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5c**)*. 38% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.02 (s, 1H), 7.45 (s, 1H), 7.13 (t, J = 5.5 Hz, 1H), 6.21 (d, J = 3.6 Hz, 1H), 6.05 (t, J = 8.4 Hz, 1H), 5.34 (d, J = 3.2 Hz, 1H), 3.82 (m, 2H), 3.63 (m, 1H); 2.87-2.76 (m, 2H), 2.69-2.46 (m, 4H), 2.41-2.34 (m, 1H), 2.29-2.24 (m, 1H), 1.70-1.63 (m, 3H), 1.59-1.55 (m, 5H), 1.48-1.18 (m, 1H), 0.99 (t, J=7.2 Hz, 3H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 177.45, 169.34, 146.20, 141.53, 138.83, 136.99, 119.97, 81.80, 63.15, 59.89, 44.43, 41.84, 36.83, 31.68, 25.94, 25.12, 23.37, 20.35, 18.20, 14.04; HRMS (ESI) calcd for $C_{20}H_{29}N_3O_3S$ [M + H^+] 392.2008; found 392.2004.

4.1.4.13: *(E)-N-(tert-butyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5d**)*. 15% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 8.91 (s, 1H), 7.43 (s, 1H), 7.23 (s, 1H), 6.22 (d, J = 3.6 Hz, 1H), 6.04 (t, J = 8.6 Hz, 1H), 5.36 (d, J = 3.1 Hz, 1H), 3.82 (t, J = 9.3 Hz, 1H), 2.87-2.76 (m, 2H), 2.69-2.33 (m, 5H), 2.29-2.22 (m, 1H), 1.73-1.66 (m, 1H), 1.63-1.59 (m, 9H), 1.58-1.54 (m, 3H), 1.21 (t, J = 14.0 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 175.93, 169.29, 144.84, 141.30, 138.93, 137.02, 119.81, 81.84, 63.15, 59.85, 53.84, 41.80, 36.86, 29.29, 25.89, 25.11, 23.40, 18.20; HRMS (ESI) calcd for $C_{20}H_{29}N_3O_3S$ [M + H^+] 392.2008; found 392.2006.

4.1.4.14: *(E)-N-(4-fluorophenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5e**)*. 32% yield; ^{1}H NMR (500MHz, $CDCl_3$) δ: 9.47 (s, 1H), 8.74 (s, 1H), 7.57 (s, 1H), 7.52-7.47 (m, 2H), 7.12 (t, J = 7.8 Hz, 2H), 6.18 (s, 1H), 6.12 (t, J = 8.2 Hz, 1H), 5.33 (s, 1H), 3.83 (t, J = 9.4 Hz, 1H), 2.90-2.81 (m, 1H), 2.80 (d, J = 9.8 Hz, 1H), 2.73-2.56 (m, 3H), 2.54-2.45 (m, 1H), 2.43-2.34 (m, 1H), 2.30-2.24 (m, 1H), 1.75-1.55 (m, 4H), 1,27-1.19 (m, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 176.55, 169.08, 160.01 (d, J = 245.4 Hz), 146.81, 142.53, 138.54, 136.61, 133.46 (d, J = 3.4 Hz), 127.12 (d, J = 8.4 Hz), 119.85, 115.99 (d, J = 22.6 Hz), 81.56, 62.92, 59.62, 41.71, 36.58, 25.72, 24.99, 23.14, 17.98; HRMS (ESI) calcd for $C_{22}H_{24}FN_3O_3S$ [M + H^+] 430.1601; found 430.1597.

4.1.4.15: *(E)-N-(2-fluorophenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5f**)*. 23% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.40 (s, 1H), 9.20 (s, 1H), 8.64-8.57 (m, 1H), 7.57 (s, 1H), 7.22-7.15 (m, 3H), 6.18 (d, J = 3.4 Hz, 1H), 6.13 (t, J = 8.2 Hz, 1H), 5.46 (d, J = 3.1 Hz, 1H), 3.83 (t, J = 9.3 Hz, 1H), 3.02-2.91 (m, 1H), 2.81 (d, J = 9.5 Hz, 1H), 2.76-2.59 (m, 3H), 2.53-2.45 (m, 1H), 2.44-2.35 (m, 1H), 2.30-2.24 (m, 1H), 1.77-1.67 (m, 1H), 1.58 (s, 3H), 1.23 (t, J = 11.20 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 174.96, 169.32, 153.46 (d, J = 245.5 Hz), 146.84, 142.74, 138.21, 136.86, 126.37 (d, J = 8.4 Hz), 126.16 (d, J = 10.1 Hz), 124.59, 124.20 (d, J = 3.4 Hz), 120.41 (d, J = 3.6 Hz), 115.13 (d, J = 20.4 Hz), 81.62, 62.93, 59.74, 41.73, 36.57, 25.41, 25.02, 23.12, 17.98; HRMS (ESI) calcd for $C_{22}H_{24}FN_3O_3S$ [M + H$^+$] 430.1601; found 430.1586.

4.1.4.16: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(p-tolyl)hydrazine-1-carbothioamide (**5g**)*. 42% yield; ^{1}H NMR (500MHz, DMSO-d_6) δ: 11.46 (s, 1H), 9.59 (s, 1H), 7.79 (s, 1H), 7.34 (d, J = 8.2 Hz, 2H), 7.18 (d, J = 8.2 Hz, 2H), 5.98 (m, 2H), 5.40 (s, 1H), 4.05 (t, J = 9.2 Hz, 1H), 3.05-2.97 (m, 1H), 2.87 (d, J = 9.5 Hz, 1H), 2.70-2.62 (m, 2H), 2.53-2.41 (m, 2H), 2.33-2.24 (m, 4H), 2.17-2.11 (m, 1H), 1.62-1.54 (m, 1H), 1.49 (s, 3H), 1.07 (t, J = 13.4 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 176.57, 169.30, 153.84, 146.45, 142.40, 138.63, 136.93, 135.10, 129.86, 125.13, 120.23, 81.77, 63.18, 59.79, 41.91, 36.84, 25.97, 25.19, 23.40, 21.32, 18.20; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_3S$ [M + H$^+$] 426.1851; found 426.1839.

4.1.4.17: *(E)-N-(4-chlorophenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5h**)*. 27% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.53 (s, 1H), 8.80 (s, 1H), 7.59-7.52 (m, 3H), 7.41-7.36 (m, 2H), 6.16 (d, J = 3.3 Hz, 1H), 6.13 (t, J = 8.7 Hz, 1H), 5.31 (d, J = 3.1 Hz, 1H), 3.82 (t, J = 9.2 Hz, 1H), 2.90-2.77 (m, 2H), 2.74-2.58 (m, 3H), 2.52-2.35 (m, 2H), 2.30-2.24 (m, 1H), 1.76-1.66 (m, 1H), 1.57 (s, 3H), 1.23 (t, J = 13.7 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 175.96, 169.08, 146.84, 142.67, 138.48, 136.58, 136.08, 131.95, 129.11, 125.86, 119.91, 81.55, 62.91, 59.63, 41.69, 36.57, 25.71, 25.00, 23.15, 17.98; HRMS (ESI) calcd for $C_{22}H_{24}ClN_3O_3S$ [M + H$^+$] 446.1305; found 446.1291.

4.1.4.18: *(E)-N-(4-methoxyphenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide (**5i**)*. 27% yield; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 11.42 (s, 1H), 9.55 (s, 1H), 7.78 (s, 1H), 7.20 (d, J = 7.5 Hz, 2H), 6.94 (d, J = 7.0 Hz, 2H), 6.02-5.94 (m, 2H), 5.44 (s, 1H), 4.05 (d, J = 9.2 Hz, 1H), 3.77 (s, 3H), 3.08-2.98 (m, 1H), 2.86 (d, J = 9.3 Hz, 1H), 2.70-2.62 (m, 2H), 2.52-2.41 (m, 2H), 2.31-2.23 (m, 1H), 2.16-2.11 (m, 1H), 1.63-1.53 (m, 1H), 1.49 (s, 3H), 1.07 (t, J = 13.7 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ:

176.88, 169.09, 158.30, 146.28, 142.15, 138.48, 136.72, 130.30, 126.96, 119.96, 114.24, 81.57, 62.96, 59.58, 55.51, 41.72, 36.63, 25.76, 24.97, 23.17, 17.99; HRMS (ESI) calcd for $C_{23}H_{27}N_3O_4S$ [M + H^+] 442.1801; found 442.1794.

4.1.4.19: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(4-(trifluoromethyl)phenyl)hydrazine-1-carbothioamide* (***5j***). 36% yield; ^{1}H NMR (400MHz, DMSO-d_6) δ: 11.72 (s, 1H), 9.88 (s, 1H), 7.84-7.80 (m, 3H), 7.76-7.72 (s, 2H), 6.02 (t, J = 8.4 Hz, 1H), 5.86 (d, J = 3.9 Hz, 1H), 5.37 (d, J = 2.6 Hz, 1H), 4.06 (t, J = 8.5 Hz, 1H), 3.05-2.94 (m, 1H), 2.87 (d, J = 9.5 Hz, 1H), 2.73-2.64 (m, 2H), 2.54-2.42 (m, 2H), 2.34-2.25 (m, 1H), 2.18-2.12 (m, 1H), 1.65-1.55 (m, 1H), 1.50 (s, 3H), 1.08 (t, J = 12.5 Hz, 1H); ^{13}C NMR (126MHz, DMSO-d_6) δ: 175.51, 169.23, 148.49, 142.78, 141.34, 139.92, 137.16, 125.60, 125.28 (q, J = 30.8 Hz), 125.15 (q, J = 31.0 Hz), 123.12 (q, J = 271 Hz), 118.31, 81.60, 62.22, 59.63, 40.91, 36.20, 24.57, 24.28, 22.16, 17.33; HRMS (ESI) calcd for $C_{23}H_{24}F_3N_3O_3S$ [M + H^+] 480.1569; found 480.1555.

4.1.4.20: *(E)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)-N-(3-(trifluoromethyl)phenyl)hydrazine-1-carbothioamide* (***5k***). 18% yield; ^{1}H NMR (400MHz, $CDCl_3$) δ: 9.40 (s, 1H), 8.91 (s, 1H), 7.90-7.84 (m, 2H), 7.60-7.51 (m, 3H), 6.20-6.12 (m, 2H), 5.33 (d, J = 3.2 Hz, 1H), 3.83 (t, J = 9.1 Hz, 1H), 2.91-2.78 (m, 2H), 2.76-2.36 (m, 5H), 2.31-2.24 (m, 1H), 1.78-1.68 (m, 1H), 1.58 (s, 3H), 1.23 (t, J = 13.4 Hz, 1H); ^{13}C NMR (126MHz, $CDCl_3$) δ: 175.85, 169.08, 147.19, 142.94, 138.51, 138.10, 136.52, 131.49 (q, J = 33.0 Hz), 129.51, 127.77, 124.72 (q, J = 272 Hz), 123.02 (q, J = 4.0 Hz), 121.03 (q, J = 4.0 Hz), 119.88, 81.54, 62.89, 59.66, 41.72, 36.54, 25.69, 25.03, 23.14, 17.98; HRMS (ESI) calcd for $C_{23}H_{24}F_3N_3O_3S$ [M + H^+] 480.1569; found 480.1570.

4.1.4.21: *(E)-N-(4-ethylphenyl)-2-(((1aR,7aS,10aS,10bS,E)-1a-methyl-8-methylene-9-oxo-1a,2,3,6,7,7a,8,9,10a,10b-decahydrooxireno[2',3':9,10]cyclodeca[1,2-b]furan-5-yl)methylene)hydrazine-1-carbothioamide* (***5l***). 51% yield; ^{1}H NMR (500MHz, DMSO-d_6) δ: 11.47 (s, 1H), 9.59 (s, 1H), 7.79 (s, 1H), 7.36 (d, J = 7.9 Hz, 2H), 7.22 (d, J = 7.8 Hz, 2H), 6.02-5.94 (m, 2H), 5.75 (s, 2H), 5.41(s, 1H), 4.05 (t, J = 9.4 Hz, 1H), 3.06-2.96 (m, 1H), 2.87 (d, J = 9.6 Hz, 1H), 2.71-2.57 (m, 4H), 2.31-2.21 (m, 1H), 2.18-2.10 (m, 1H), 1.63-1.54 (m, 1H), 1.49 (s, 3H), 1.20 (t, J = 7.6 Hz, 3H), 1.07 (t, 13.1 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ: 169.24, 147.62, 140.91, 140.63, 139.96, 137.26, 136.63, 127.34, 126.00, 118.35, 81.63, 62.24, 59.63, 54.81, 40.92, 36.23, 27.62, 24.63, 24.24, 22.14, 17.33, 15.64; HRMS (ESI) calcd for $C_{24}H_{29}N_3O_3S$ [M + H^+] 440.2008; found 440.2011.

4.2 Cell proliferation assay

The growth inhibitory effect of compounds toward five cancer cell lines was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were plated at a density of 1500-2000 cells/well in 96-well plates and incubated for 24 h. Compounds were added to the wells with various concentrations. After 96 h, a total of 50 μL MTT stock solutions (2 mg/mL, BIOFROXX, Germany) were added to each well. The 96-well plates were subsequently incubated for a further 2 h at 37˚C. The medium was replaced with DMSO (200 μL/well, Biosharp, Inc., Hefei, China;). Following gentle agitation, an ELISA reader (WD-2102A, Beijing Liuyi Biotech Co., Ltd., China) was used to measure the absorbance at 570 nm. Three parallel samples were measured in each cell line. The absorbance values were normalized to the values obtained for vehicle-treated cells to determine the percentage of surviving cells. The median inhibitory concentration (IC_{50}) was assessed from the dose response curve.

4.3 *In vivo* antitumor experiments

The MC38 xenograft model was established in male C57BL/6 mice (6-8 weeks old). The animals were purchased from Beijing HFK Bioscience Co., Ltd (Beijing, China) and housed in controlled environment at 25 °C on a 12 h light/dark cycle. All animal protocols were conformed to the Guidelines for the Care and Use of Laboratory Animals approved by the Animal Care and Use Committee of Chinese Academy of Medical Sciences and Peking Union Medical College. C57BL/6 mice were subcutaneously implanted with 0.2 mL MC38 cell dilution (5×10^7 cells/mL) on the flank. 24 h after inoculation, mice were randomly divided into 7 groups (n = 6). One group received gavage (p.o.) of 25% PEG400 as a model control; one group received an intraperitoneal injection of 40.0 mg/kg 5-FU (once every three days) used as the positive control. 5 groups involved 40.0 mg/kg of **4d**, **4f, 4g**, **4i**, and **5g** (dissolved in 25% PEG400) were continuously administrated p.o. for 12 days, once a day. The weight of tumor, body, thymus and spleen at the end were measured.

4.4 Chemical stability studies:

The chemical stability assay was adapted from a previous research.[13] Compound **4d** was dissolved in DMSO to obtain a solution of 1.6 mM. 1 mL of this solution was diluted with pH 7.4 phosphate buffer solution to a final concentration of 0.08 mM. 1 mL of the 0.08 mM solution mixture were placed in water bath at 37 °C for different periods of time. At the end of each time period, the sample was mixed with 5 mL of acetonitrile and filtered (Jinteng, 0.45 μm pore size). Three parallel experiments were conducted for each time period. The liquid sample was analyzed by a high-performance liquid chromatography (Wufeng LC100) equipped with a Kromasil C18 column (4.6 mm * 250 mm, 5 μm) and an ultraviolet detector at the wavelength

of 220 nm. The mobile phase was 60% acetonitrile and 40% water at the flow rate of 1 mL/min. Each sample was analyzed 3 times, and average values were used for plotting.

4.5 Flow cytometric study of cell apoptosis and cell cycle

Cell apoptosis of **4d** was studied using AnnexinV-FITC/PI labeled Apoptosis Assay Kit (BestBio, Shanghai, China) according to the manufacturer's instructions. Briefly, U87-MG was cultured into 6-well plates and incubated for 48 h. Then, cells (10^6 cells/mL) were treated with 0 μM, 4 μM and 16 μM of **4d** in DMSO for 12 hours. After the cells were collected and washed twice with cold PBS and once with binding buffer, cells were stained by 5 μL of AnnexinV-FITC and 5 μL of PI. Apoptic cells were analyze by a flow cytometry ACEA Novocyte.

For cell cycle analysis, incubated U87-MG were treated with 0 μM, 4 μM and 16 μM of **4d** in DMSO for 12 hours. Cells were then harvested, washed with PBS, and then fixed with ice-cold 70% ethanol at -20 °C overnight. PI/RNase A (BestBio, Shanghai, China) solution was used to stain cells for 30 min in the dark. The samples were determined by a flow cytometry ACEA Novocyte.

4.6 Molecular docking studies:

Molecular docking was carried out using the Discovery Studio as implemented through the graphical user interface DS-CDOCKER protocol. The 3D structures of compound **4d** were constructed using Chem3D software (Chemical Structure Drawing Standard; Cambridge Soft corporation). The molecular docking in this study was performed by inserting compound **4d** into the binding pocket of NF-*κ*B (PDB code: 1NFK). Types of interactions of the docked protein with ligand-based pharmacophore model were analyzed after the end of molecular docking.

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Supplementary Information

Supplementary material is available on the publisher's website along with the published article.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: