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# Hybrids of Phenylsulfonylfuroxan and Coumarin as Potent Antitumor Agents

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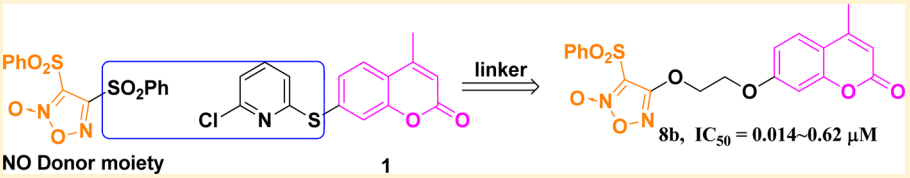
Supporting Information




**ABSTRACT:** Sixteen furoxan-based nitric oxide (NO) releasing coumarin derivatives (**6a−c**, **8a−g**, **10a**, **13a,b**, **15**, and **17a,b**) were designed, synthesized, and evaluated against the A549, HeLa, A2780, A2780/CDDP, and HUVEC cell lines. Most derivatives displayed potent antiproliferation activities. Among them, **8b** exhibited the strongest antiproliferation activity on the four sensitive cell lines mentioned above and three drug resistant tumor cell lines A2780/CDDP, MDA-MB-231/Gem, and SKOV3/CDDP with $IC_{50}$ values from 14 to 53 nM and from 62 to 140 nM, respectively. Furthermore, **8b** inhibited the growth of A2780 in vivo and displayed lower toxicity on nontumorigenesis T29, showing good selectivity against malignant cells in vitro. Preliminary pharmacological studies showed that **8b** induces apoptosis, arrests the cell cycle at the G2/M phase in the A2780 cell line, and disrupts the phosphorylation of MEK1 and ERK1. Overall, the NO-releasing capacity and the inhibition of ERK/MAPK pathway signaling may explain the potent antineoplastic activity of these compounds.

## INTRODUCTION

The Ras/Raf/MEK/ERK pathway is one of the most important signal transduction pathways in human cancer, and plays an important role in cell proliferation and cytokine production.[1−3] Oncogenic mutations to Ras or Raf contribute to the constitutive activation of this pathway and have been observed in lung, colon, pancreas, kidney, and ovary primary human tumor samples.[4] MEK is a pivotal node in the ERK/MAPK pathway, and extracellular regulated kinase (ERK) is the only known downstream target of MEK. All these characters make MEK a promising molecular target for tumor therapy.[5] Trametinib[6] is the first MEK inhibitor and was approved for the treatment of melanoma by FDA in 2013. Selumetinib[7] and MEK162[8] are diarylamine derivatives and MEK inhibitors; they have been at phase II and phase III clinical trials, respectively (Figure 1).

Recently, many coumarin derivatives were widely reported to have antitumor activity through binding to different targets and diverse pharmacological mechanisms. For example, 3,8-dibromo-7-hydroxycoumarin[9] (DBC, Figure 2) inhibited CK2 function. The 7,8-dihydroxy-4-methylcoumarin[10] (DHMC, Figure 2) induced apoptosis of human lung adenocarcinoma cells by a ROS-independent mitochondrial pathway through partial inhibition of ERK/MAPK signaling. Some other coumarin derivatives, such as 3-(benzo[*d*]thiazol-2-yl)-7-hydroxycoumarin[11] and 3-benzyl-6-chloro-7-(dimethylaminoformyloxy)coumarin[12] (G8935, Figure 2), showed antiproliferation of cancer cells via MEK1 targeting.

In our prior research,[13] a series of S- and O-substituted 7-mercaptocoumarin analogs were designed, synthesized, and evaluated in vitro against four human tumor cell lines (KB (nasopharyngeal), KB-vin (vincristine-resistant subline), A549 (lung), and DU145 (prostate)). The 7-(6-chloropyridin-2-ylthio)-4-methycoumarin[13] (**1**, Figure 2), which increased cellular apoptosis in a concentration-dependent manner and induced A549 cell cycle arrest at the G2/M phase, exhibited potent cytotoxic activity. The result prompted us to further modify the structures for the desirable compounds with the possible pharmacological mechanism(s) such as MEK1 target.

Additionally, nitric oxide (NO) plays pivotal roles in diverse physiological and pathophysiological processes.[14] Generally, high levels of NO generated from NO donors can induce apoptosis, inhibit metastasis of tumor cells, and sensitize tumor cells to chemotherapy, radiation, and immunotherapy in vitro and in vivo.[15−17] Furoxans are an important class of NO donors that can produce high levels of NO in vitro and inhibit the growth of tumors in vivo.[18] The mechanisms of NO release by

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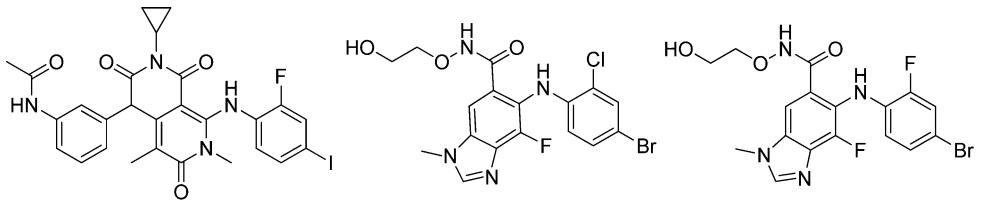


**Figure 1.** Approved or in clinical trial MEK1 inhibitors.

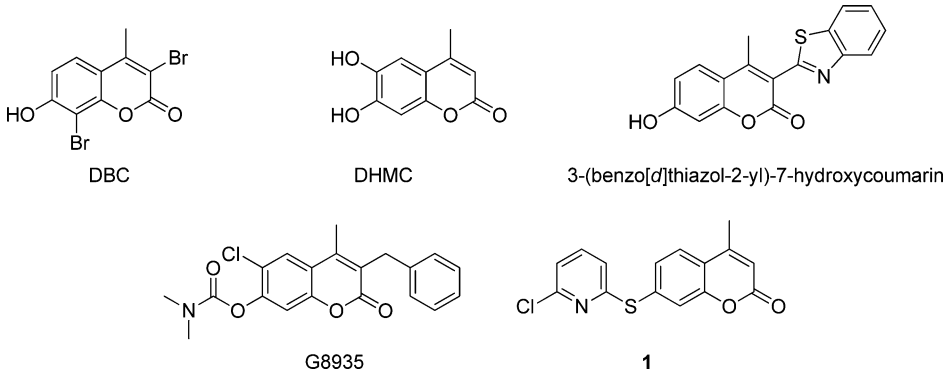


**Figure 2.** Some bioactive coumarin derivatives.

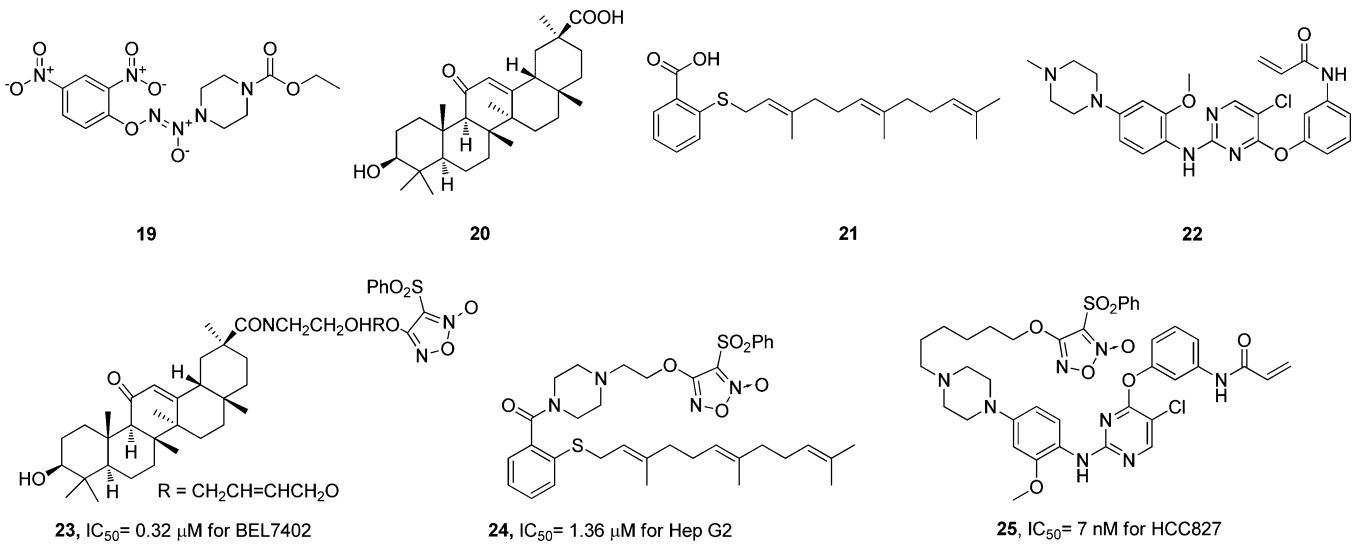


**Figure 3.** Compounds **20**–**22**, their corresponding furoxan hybrids **23**–**25**, and compound **19**.

furoxans were complex. Generally, it is hypothesized that thiolate attacked the position 3 or 4 followed by ring opening to give a nitroso derivative. The broken derivative then released the nitroxyl anion $NO^-$, which was further oxidized to NO.[19−21] The NO release can be modulated by varying the nature of the substituents.[22] Zhang and Tian's group reported hybrids from furoxan based NO donor and different active compounds like **20** (glycyrrhetinic acid, GA),[23] **21** (farnesylthiosalicylic acid, FTS),[24] and **22** (*N*-(3-((5-chloro-2-((2-methoxy-4-(4-methylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)oxy)phenyl)acrylamide, WZ4002).[25] Hybrids like **23** (GA/furoxan hybrid),[23] **24** (FTS/furoxan hybrid),[24] and **25** (WZ4002/furoxan hybrid)[25] displayed potent antitumor activity both in vivo and in vitro (Figure 3). Besides, it has also been reported that combined treatment of NO donor and MEK inhibitor synergistically inhibits proliferation and invasion of cancer cells and can be an effective and promising strategy for cancer treatment.[26]

To enhance the pharmacological activity of compound **1**, the furoxan based NO donor was introduced at 7-position of the coumarin core to design and synthesize 16 coumarin/furoxan hybrids. At the same time, their antitumor activities were screened in vitro and in vivo. Furthermore, MEK1 inhibitory activities, apoptosis inductive activities, and NO-releasing ability were also biologically evaluated to uncover the mechanisms of action underlying them.

## RESULTS AND DISCUSSION

**Chemistry.** The synthesis of target compounds is illustrated in Scheme 1. 3,4-Diphenylsulfonyl-1,2,5-oxadiazole 2-oxide (**4**) was synthesized in a three-step sequence according to literature procedures.[27] Commercial available reagents **5a**–**e** and **14** were


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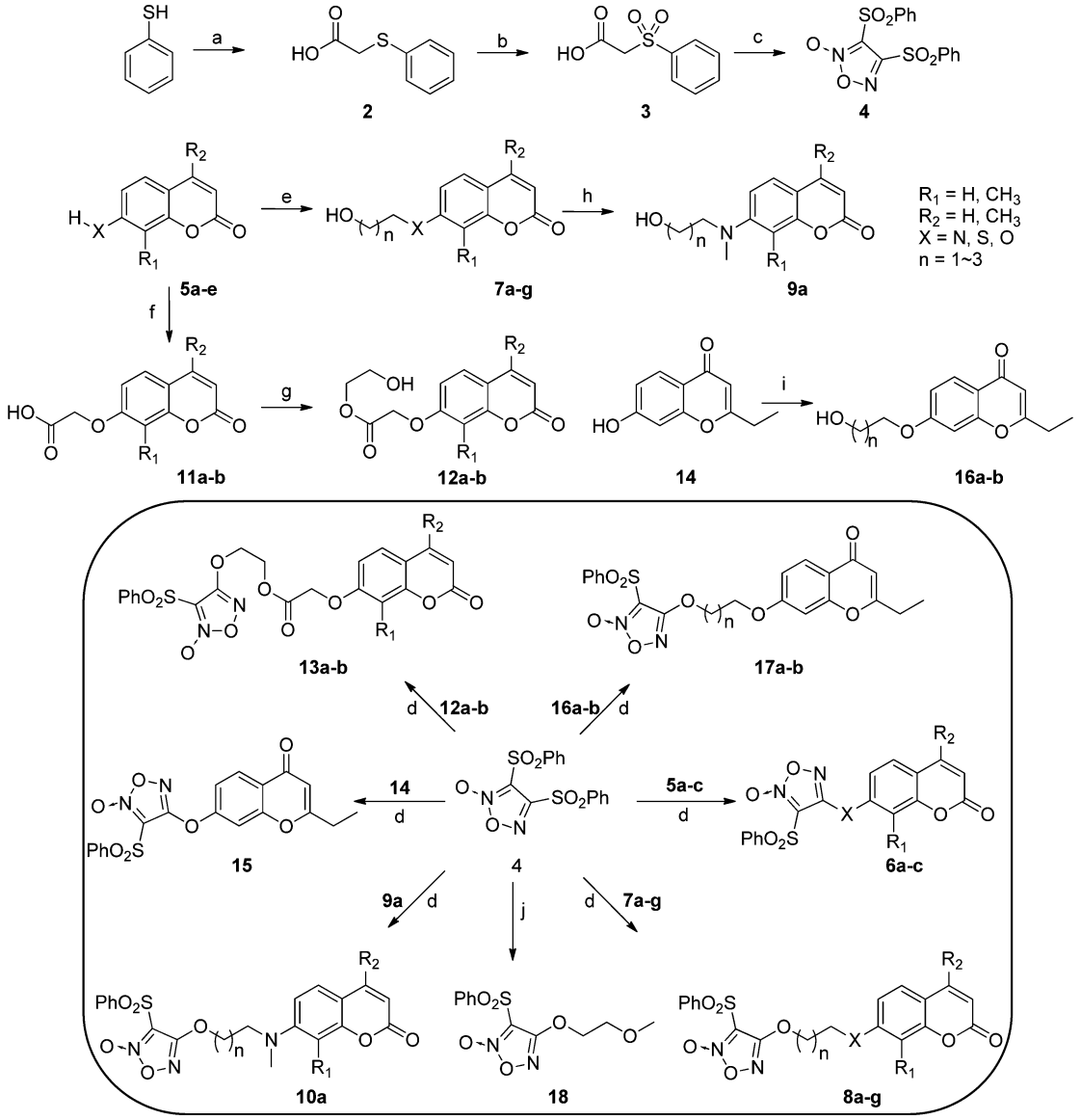
**Scheme 1. Synthetic Routes of Target Compounds**[a]




**5a**: $R_1$=H, $R_2$=H X=O
**5b**: $R_1$=H, $R_2$=$CH_3$ X=O
**5c**: $R_1$=$CH_3$, $R_2$=$CH_3$ X=O
**5d**: $R_1$=H, $R_2$=$CH_3$ X=S
**5e**: $R_1$=H, $R_2$=$CH_3$ X=NH
**6a**: $R_1$=H, $R_2$=H X=O
**6b**: $R_1$=H, $R_2$=$CH_3$ X=O
**6c**: $R_1$=$CH_3$, $R_2$=$CH_3$ X=O
**7a**: $R_1$=H, $R_2$=H, X=O, n=1
**7b**: $R_1$=H, $R_2$=$CH_3$, X=O, n=1
**7c**: $R_1$=H, $R_2$=$CH_3$, X=S, n=1
**7d**: $R_1$=H, $R_2$=$CH_3$, X=O, n=2
**7e**: $R_1$=H, $R_2$=$CH_3$, X=O, n=3
**7f**: $R_1$=$CH_3$, $R_2$=$CH_3$, X=O, n=1
**7g**: $R_1$=H, $R_2$=$CH_3$, X=NH, n=1
**8a**: $R_1$=H, $R_2$=H, X=O, n=1
**8b**: $R_1$=H, $R_2$=$CH_3$, X=O, n=1
**8c**: $R_1$=H, $R_2$=$CH_3$, X=S, n=1
**8d**: $R_1$=H, $R_2$=$CH_3$, X=O, n=2
**8e**: $R_1$=H, $R_2$=$CH_3$, X=O, n=3
**8f**: $R_1$=$CH_3$, $R_2$=$CH_3$, X=O, n=1
**8g**: $R_1$=H, $R_2$=$CH_3$, X=NH, n=1
**9a**: R1=H, $R_2$=$CH_3$, n=1
**10a**: $R_1$=H, $R_2$=$CH_3$, n=1
**11a**: $R_1$=H, $R_2$=H, X=O
**11b**: $R_1$=H, $R_2$=$CH_3$, X=O
**12a**: $R_1$=H, $R_2$=H, X=O
**12b**: $R_1$=H, $R_2$=$CH_3$, X=O
**13a**: $R_1$=H, $R_2$=H, X=O
**13b**: $R_1$=H, $R_2$=$CH_3$, X=O
**16a**: n=1
**16b**: n=2
**17a**: n=1
**17b**: n=2

[a]Reagents and conditions: (a) $ClCH_2COOH$, NaOH (aq), 140 °C, 2 h; (b) 30% $H_2O_2$, AcOH, room temperature, 3.5 h; (c) fuming $HNO_3$, 90 °C, 4 h; (d) $CH_2Cl_2$, DBU, −15 °C, 3 h, 95%; (e) corresponding halo alcohol, $K_2CO_3$, acetone or DMF, reflux; (f) $ClCH_2COOH$, $K_2CO_3$, DMF, reflux; (g) DMAP, EDC, 0 °C to room temperature; (h) $CH_3I$, $K_2CO_3$, DMF, reflux; (i) corresponding halo alcohol, $K_2CO_3$, acetone or DMF, reflux; (j) 2-methoxyethanol, $CH_2Cl_2$, DBU, room temperature, 3 h, 46%.

treated with various halo alcohols to provide the intermediates **7a−g** and **16a,b**. Methylation of compound **7g** with $CH_3I$ gave **9a**. Compounds **5a,b** were reacted with 2-chloroactic acid to form **11a,b**, which then condensed with ethanediol in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC) and 4-(dimethylamino)pyridine (DMAP) to gain intermediates **12a,b**. Finally, condensation of **4** with various hydroxyl linkers coupling compounds **5a−c**, **7a−g**, **9a**, **12a,b**, **14**, **16a,b**, and 2-methoxyethanol provided the 16 target compounds **6a−c**, **8a−g**, **10a**, **13a,b**, **15**, **17a,b**, and the NO donor moiety compound **18** using 8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a catalyst in $CH_2Cl_2$.

**Activities of Antiproliferation in Vitro.** Sixteen newly synthesized coumarin/furoxan hybrids (**6a−c**, **8a−g**, **10a**,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**13a,b**, **15**, and **17a,b**) were screened for antiproliferation in A549, HeLa, A2780, and A2780/CDDP with **1**, **4**, and a NO releasing prodrug **19** (JS-K, Figure 3)[28] as references. Because it is a first-line drug in the clinic for patients with ovarian cancer, cisplatin was also chosen as the positive control. As shown in Table 1, most target compounds showed considerable anticancer activities and had much more potency than that of the NO donor moiety **4**, hit **1**, cisplatin, and **19**. Among them, compounds **8a−f** and **17b** exhibited activities in the nanomolar range. In particular, 4-(2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**8b**) was the most potent compound. It not only inhibited the proliferation of A549, HeLa, and A2780 with $IC_{50}$ values at 24, 53, and 14 nM, respectively, but also had a very strong cytotoxicity for drug resistance in A2780/CDDP with $IC_{50}$ value of 62 nM. This result encouraged us to further measure its antiproliferative activity on the other two drug resistant cancer cell lines MDA-MB-231/gem and SKOV3/CDDP as well as their sensitive parent cell lines MDA-MB-231 and SKOV3.

**Table 1. Antiproliferation Activities for 6a−c, 8a−g, 10a, 13a,b, and 17a,b**

| | $IC_{50}$[a] (μM) | | | | |
|---|---|---|---|---|---|
| compd | A549[b] | HeLa[c] | A2780[d] | A2780/CDDP[e] | HUVEC[f] |
| cisplatin | 25.23 | 2.07 | 1.06 | >50 | ND[g] |
| **1** | 3.25 | 1.48 | 1.35 | 1.00 | 5.89 |
| **4** | 2.35 | 1.45 | 0.88 | 0.89 | 4.33 |
| **6a** | 8.86 | 4.55 | 0.11 | 3.44 | 6.75 |
| **6b** | 5.55 | >20 | 2.99 | 3.15 | 5.43 |
| **6c** | 11.68 | >20 | 9.35 | >20 | >20 |
| **8a** | 0.26 | 0.11 | 0.17 | 0.20 | 0.37 |
| **8b** | 0.024 | 0.053 | 0.014 | 0.062 | 0.034 |
| **8c** | 0.12 | 0.024 | 0.036 | 0.14 | 0.22 |
| **8d** | 0.28 | 0.26 | 0.35 | 0.36 | 0.68 |
| **8e** | 0.65 | 0.32 | 0.66 | 0.99 | 0.25 |
| **8f** | 1.00 | 0.25 | 0.046 | 0.33 | 0.097 |
| **8g** | 1.44 | 1.99 | 2.45 | 5.45 | 3.04 |
| **10a** | 1.02 | 0.51 | 0.95 | 2.38 | 0.89 |
| **13a** | >20 | >20 | >20 | >20 | >20 |
| **13b** | 6.92 | 4.31 | 3.65 | 7.36 | >20 |
| **15** | 3.29 | >20 | 0.13 | 2.99 | 3.35 |
| **17a** | 5.45 | 0.83 | 3.33 | 2.88 | 1.03 |
| **17b** | 0.40 | 0.19 | 0.29 | 0.89 | 0.35 |
| **19** | 1.26 | 3.56 | 0.63 | 1.05 | 5.39 |

[a]The antiproliferation activities of individual compound to tumor cells were determined by the MTT assay. The data are the mean of triplicate determinations. [b]A549 is a human lung cancer cell line. [c]HeLa is a human cervical carcinoma cell line. [d]A2780 is a human ovarian cancer cell line. [e]A2780/CDDP is a cisplatin-resistant human ovarian cancer cell line. [f]HUVEC are human umbilical vein endothelial cells. [g]Not determined.

As shown in Table 2, compound **8b** had an activity against both of these sensitive and drug resistant cancer cell lines with 0.15, 0.14, 0.083, and 0.14 μM $IC_{50}$ values, respectively. The results indicated that **8b** might be a broad-spectrum inhibitor for both sensitive and drug resistant cancer cell lines. Moreover, angiogenesis is necessary for tumor growth and metastasis, which made it a promising target for cancer treatment. Human umbilical vein endothelial cells (HUVECs) are a normal cell useful for assessing antiangiogenic potential. The HUVEC line was also screened in company with the previous four cancer cell lines. Eight compounds showed potent activity with $IC_{50}$ values less than 1 μM. The **8f** and **17a** demonstrated activity against HUVEC with $EC_{50}$ of 0.097 and 1.03 μM, respectively. Again, compound **8b** exhibited the highest potency with an $IC_{50}$ value of 0.034 μM. The results implied that these new compounds might also be active on angiogenesis targets like vascular endothelial growth factor receptor (VEGFR) and stimulated us to explore their pharmacological mechanism in the future.

Considering that **19** exerted selective antitumor activity,[28,29] the selectivity of **8b** on tumor and nontumorigenic cells also was studied via the inhibition of compound **8b** testing on the proliferation of nontumorigenic T29 cells (immortalized but nontumorigenic ovarian epithelial cells) in vitro. As Table 3 and Figure 4 illustrate, the $IC_{50}$ value of compound **8b** on T29 cells ($IC_{50}$ > 2 μM) was at least 140-fold more than that of compound **8b** on A2780 cells ($IC_{50}$ = 14 nM), indicating that **8b** has selective inhibition on the proliferation of A2780 cell in vitro. Notably, antiproliferation activity of **8b** ($IC_{50}$ = 14 nM) against A2780 cells was much more potent than that of the coumarin core moiety 7-hydroxy-4-methylcoumarin ($IC_{50}$ > 2 μM) and the NO donor moiety compound **18** ($IC_{50}$ > 2 μM),

**Table 3. Antiproliferation Activities for 8b, 7-Hydroxy-4-methylcoumarin, and 18**

| | $IC_{50}$[a] | |
|---|---|---|
| compd | T29[b] | A2780[c] |
| **8b** | >2 μM | 14 nM[d] |
| 7-hydroxy-4-methylcoumarin | >2 μM | >2 μM |
| **18** | >2 μM | >2 μM |

[a]The antiproliferation activities of individual compound to tumor cells were determined by the MTT assay. The data are the mean from at least three independent experiments. [b]T29 cells are immortalized but nontumorigenic ovarian epithelial cells. [c]A2780 is ovarian cancer cell line. [d]Data presented here were the first batch that was agreed with the second batch.

**Table 2. Antiproliferation Activities for 8b against Drug Resistant Cell Lines**

| | $IC_{50}$[a] (μM) | | | |
|---|---|---|---|---|
| compd | MDA-MB-231[b] | MDA-MB-231/Gem[c] | SKOV3[d] | SKOV3/CDDP[e] |
| **8b** | 0.15 | 0.14 | 0.083 | 0.14 |
| cisplatin | | | 5.95 | >100 |
| gemcitabine | 0.025 | >10 | | |

[a]The antiproliferation activities of individual compound to tumor cells were determined by the MTT assay. The data are the mean from at least three independent experiments. [b]MDA-MB-231 is a human breast cancer cell. [c]MDA-MB-231/Gem is a gemcitabine resistant breast cancer cell. [d]SKOV3 is a human ovarian carcinoma cell line. [e]SKOV3/CDDP is a cisplatin resistant human ovarian cancer cell line.


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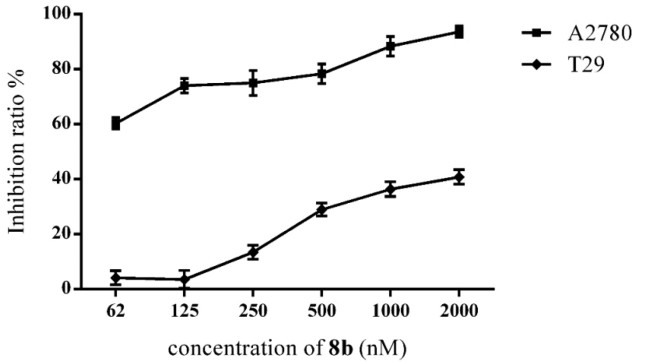


**Figure 4.** Antiproliferation activities of compound **8b** against A2780 tumor cells and nontumor T29 cells. T29 cells are immortalized but nontumorigenic ovarian epithelial cells.

which suggested that the combination of the NO donor moiety with coumarin core might lead to a synergetic antitumor effect.

**Structure–Activity Relationships (SARs).** According to the activities mentioned above, the preliminary SAR of target compounds can be inferred. First, the bioisosteric modification at the 7-position with O, S, and N showed that oxygen atom substitution was more favorable for antiproliferation activity. Second, the replacement of the coumarin scaffold with 4*H*-chromen-4-one core decreased the antiproliferation activity. Most importantly, for both the coumarin scaffold and its analog 4*H*-chromen-4-one scaffold, the length of the spacers connecting the NO donors was crucial to preserve the strong anticancer activity. For example, the coumarin/furoxan derivative **8b** with a C2 spacer was much more potent than those with no (**6b**) or longer spacers (**8d**, **8e**), respectively. Similarly, the 4*H*-chromen-4-one/furoxan derivative, however, with a C3 spacer (**17b**) would be better than those with C2 (**17a**) or no linkers (**15**).

**Compound 8b Inhibited Angiogenesis in Vitro.** To further evaluate the antiangiogenesis activity of compound **8b**, we performed capillary-like tube formation assay in vitro. Endothelial cells could form capillary-like structures in Matrigel, which are useful indicators of angiogenesis potential. Compound **19** was employed as a positive control. As shown in Figure 5, HUVEC cells formed a complete network structure overnight with serum stimulation. After compound **8b** treatment, the tubule formation was significantly suppressed even in a nonantiproliferative concentration (10 nM). At the concentration of 100 nM, **8b** completely disrupted the formation of tube structures.

**NO Releasing Assessment.** With the ability to produce high levels of NO in vitro,[18,30] furoxans are an important class of NO donors. As shown in Figure 6A, the intracellular NO production capability of these furoxan/coumarin hybrids (**6a–c**, **8a–g**, **10a**, **13a,b**, **15**, and **17a,b**) and furoxan moiety **4**, **18** was determined and presented as that of nitrite in the cell lysates using a Griess assay, with **19** and compound **1** as positive and negative controls, respectively. As expected, compound **1** without furoxan group was hardly detected with nitrite in A2780 cells, whereas the compounds bearing the furoxan moiety could produce various levels of nitrite intracellularly with the releasing percentage range from 21 to 97 $\mu$M, in which the most active compound **8b** released the highest concentration of nitrite (97 $\mu$M) and higher than the positive control **19** (48 $\mu$M). Most importantly, compound **8b** released more NO in A2780 tumor cancer cells than in T29

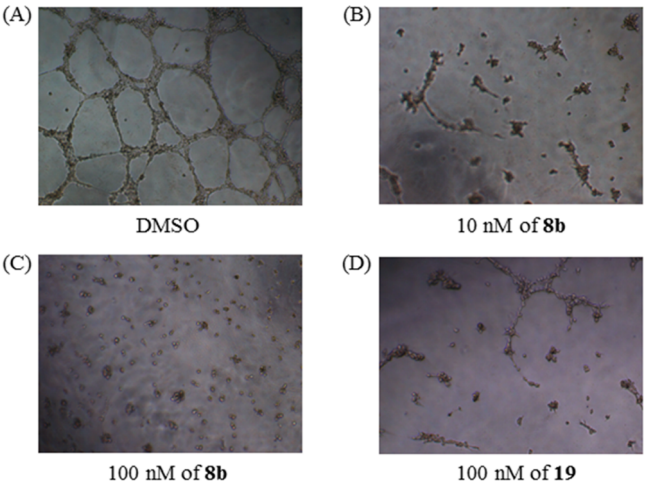


**Figure 5.** Capillary-like tube formation assay. (A) Tube formation for the vehicle control. HUVECs formed robust tube structures when they were only treated with vehicle (DMSO). (B) Tubule formation was significantly suppressed with 10 nM **8b** treatment. (C) 100 nM **8b** completely inhibits the formation of tube structures. (D) Compound **19** was used as a positive control.

cells, and its activity was diminished by pretreatment with a NO scavenger in a dose-dependent manner (Figure 6B and Figure 6C). The results above agreed with the study that **8b** might have selective inhibition on the proliferation of A2780 cell in vitro. This indicated that the potent antiproliferation activities and selectivity may be partly attributed to the release of nitric oxide.

Next we determined the intracellular NO level produced by compound **8b** and the reference compound **19** using fluorescent indicator DAF-FM DA. As shown in Figure 6D, when compared with the DMSO treated group, exposure of A2780 cells to **8b** for 1 h led to an increase in DAF-FM fluorescence in a dose-dependent manner. And the fluorescence intensity induced by **8b** was higher than that induced by **19**, which may indicate the better NO-releasing activity of **8b** compared with that of **19**.

**Compound 8b Inhibited Colony Formation and Blocked the Cell Cycle.**[31] We tested the colony formation ability of cells after treatment with compound **8b**. As shown in Figure 7 (parts A and B), the exposure of A2780 and MDA-MB-231/Gem cell lines to nanomolar concentrations of compound **8b** resulted in a significant inhibition of colony formation. The **8b** completely abolished growth of A2780 cell line at doses above 20 nM. Moreover, because chemical antitumor agents can inhibit cell proliferation through induction of cell cycle arrest, DNA-based cell cycle analysis was performed by flow cytometry to understand how the cell growth was inhibited by compound **8b**. As illustrated in Figure 7C, the treatment of **8b** caused blockage of the cell cycle in the G2/M phase. Compared with the control cells treated with DMSO, when A2780 cells were treated with increasing concentrations of **8b** (20, 40, and 80 nM), the mean percentage of cells in the G2/M phase increased from 10.5% to 64.9%, and the percentages of cells in S and G0/G1 phase decreased concomitantly (Figure 7D).

**Compound 8b Induced Cellular Apoptosis.** Considering that NO induces cellular apoptosis,[32] our coumarin/furoxan hybrids with the ability to release NO might also have a similar mechanism of inducing apoptosis against tumor cells. To


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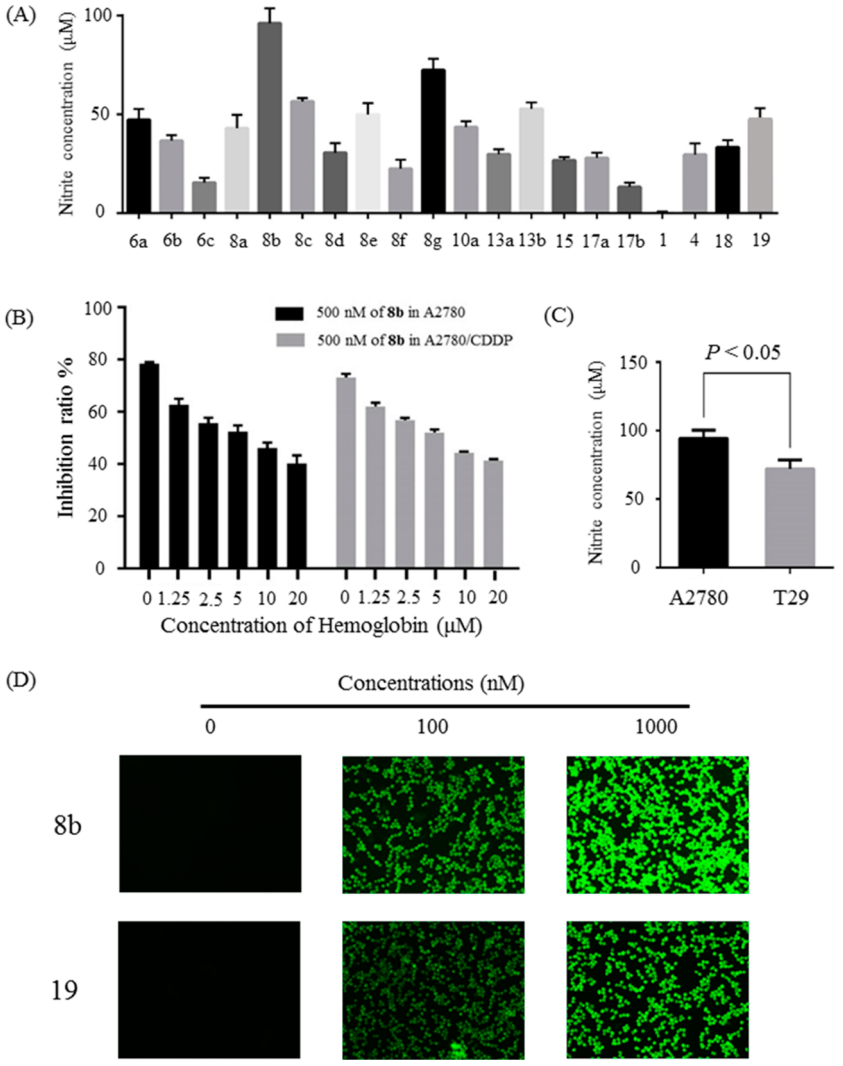


**Figure 6.** (A) Variable levels of NO produced by some coumarin/furoxan hybrids in A2780 cells. Results are indicated as the mean ± SD (standard error) of three independent experiments. (B) Effects of hemoglobin on the antiproliferative effect of **8b**. A2780 and A2780/CDDP cells were pretreated with the indicated concentrations of hemoglobin (0, 1.25, 2.5, 5, 10, or 20 $\mu$M) for 1 h and treated with 500 nM **8b** for 24 h. The results are expressed as the percentage of cell growth inhibition relative to control cells. Data are the mean value ± SD obtained from three determinations. (C) **8b** releases a higher level of NO in A2780 cells than in T29 cells, $P < 0.05$. Results are indicated as the mean ± SD (standard error) of three independent experiments. (D) Intracellular NO level produced by compound **8b** or the reference compound **19** using fluorescent indicator DAF-FM DA.

determine the number and stage of apoptotic cells, the annexin-V/PI double staining assay was applied to quantitate **8b** treated A2780 cells using flow cytometry. As depicted in Figure 8A and Figure 8B, the total proportion of annexin V+/PI− (the right lower quadrant, representing early apoptotic) and annexin V +/PI+ (the right upper quadrant representing late apoptotic and necrotic) cells increased from 2.5% to 29.5% after they were exposed to 20, 40, and 80 nM **8b** for 24 h.

We next investigated the signaling pathway involved in compound **8b** induced apoptosis. Western blot analysis showed that **8b** significantly up-regulated the expression of proapoptotic protein Bax but down-regulated the expression of the antiapoptotic proteins Bcl-2 in a dose dependent manner (Figure 8C). Meanwhile, **8b** could also up-regulate the expression and the phosphorylation levels of p53, an important tumor suppressor. Moreover, compared with control cells, exposure to **8b** significantly induced the cleavage of poly ADP-ribose polymerase 1 (PARP-1), a marker of cells undergoing apoptosis.[33] These results suggested that **8b** triggers cellular apoptosis through regulation of apoptosis-associated proteins.

Compound **8b** induced the expression and the phosphorylation levels of p53, which subsequently promoted Bax and reduced Bcl-2 expression. The increase of Bax/Bcl-2 ratio may be responsible for the concomitant execution phase of apoptosis which included disruption of mitochondrial membrane potential and finally cleavage and inactivation of key cellular proteins such as PARP-1.

**Effects of Compound 8b on MEK/ERK Signaling and Kinase Inhibitory Activity.** Our newly synthesized coumarin/furoxan hybrids also shared the coumarin scaffold. It promoted us to study the effect of these compounds on the ERK/MAPK pathway signaling, and **8b** was selected to explore the effect on the protein expression of MEK1, P-MEK1, ERK1, and P-ERK1. As described in Figure 9, the phosphorylation of MEK1 (P-MEK1) and ERK1 (P-ERK1) was decreased after treatment with **8b**. The result means that **8b** not only could prevent the inactive MEK1 being phosphorylated by upstream


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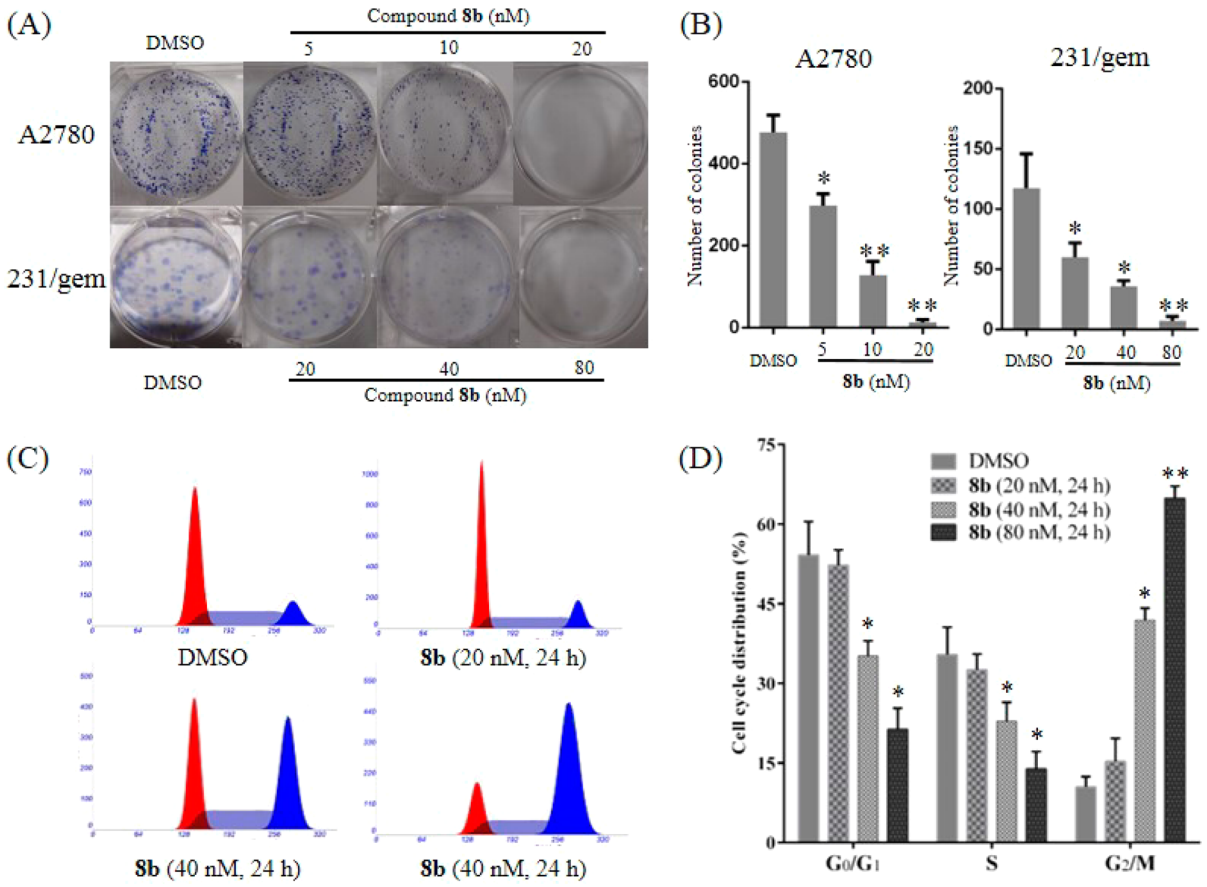


**Figure 7.** Compound **8b** inhibited colony formation and blocked cell cycle. (A) Treatment of ovarian cancer cell line A2780 and breast cancer drug resistant subline MDA-MB-231/gem with the compound **8b** resulted in a does-dependent inhibition of colony formation. (B) The profiles show the number of colonies. One colony was defined to be an aggregate of >50 cells. Results represent the mean ± SD from three independent experiments: (*) $p < 0.05$, (**) $p < 0.01$ compared to control. (C) **8b** dose-dependent induced cell cycle arrest in G2/M phase of A2780 cells. (D) The profiles showed the proportions (%) in each phase of A2780 cells treated with **8b** and diluent (DMSO). The experiments were repeated three times, and a representative experiment is shown: (*) $p < 0.05$, (**) $p < 0.01$ compared to control.

kinase B-RAF triggering but also inhibited the active MEK1 activating the downstream protein ERK1.

On the basis of the Western blot assay, we subsequently examined the inhibitory effects of **1**, **6a−c**, **8a−g**, **10a**, **13a,b**, **15**, and **17a,b** on MEK1. As depicted in Table 4, most of these NO release compounds had weak inhibitory ratios of less than 50% except compound **8c** with 53.4% inhibitory effect for MEK1 at a concentration of 5 $\mu$M. Merging the MEK inhibitory activity with the antitumor activity showed an interesting result that the antitumor action of compounds (**6a−c**, **8g**, **13a,b**, and **15**) with very poor MEK inhibitory activity (inhibitory ratio of <15%) generally was not very potent ($IC_{50}$ < 1 $\mu$M) even if they could release high levels of NO. On the contrary, compounds **8a−f** exhibited stronger antiproliferation activity that might be attributed to their higher MEK inhibitory activity together with high NO release capability. This phenomenon was in accordance with the report that NO donor combined with MEK inhibitor exerted synergistically anticancer effect.[26]

**In Vivo Antitumor Activity of Compound 8b.** To evaluate the safety of compound **8b**, groups of female KM mice were injected intraperitoneally with a single dose of **8b** at 500, 300, 275, 250, 225, 200 mg/kg or vehicle control. As illustrated in Table 5, treatment with **8b** less than 200 mg/kg caused no abnormality throughout the observation period similar to control. The $LD_{50}$ value calculated was 263.6 mg/kg.

The in vivo antitumor activity of **8b** was further evaluated in a human ovarian cancer xenograft mouse model established by subcutaneous inoculation of the A2780 cells in the female BALB/C nude mice. After establishment of solid tumor, the mice were randomized and treated with 15 or 30 mg/kg **8b** or vehicle once every 3 days, respectively. As shown in Figure 10A, treatment of 15 mg/kg **8b** significantly inhibited the tumor growth and produced a 55.8% reduction by the end of the observation period (15th day) versus vehicle only. Treatment with 30 mg/kg **8b** enhanced the inhibitory effect on the tumor growth with an inhibition rate of 65.5%. When the observation time ended, the mice were sacrificed and the tumors were excised and weighed. The results (Figure 10B) again showed a statistically significant reduction in tumor weight in both doses of **8b**-treated mice. Moreover, there was no significant weight loss in animals treated with **8b** compared with those in the vehicle control group (Figure 10C).

## CONCLUSIONS

In summary, a series of hybrids from phenylsulfonylfuroxan and coumarin derivatives (**6a−c**, **8a−g**, **10a**, **13a,b**, **14**, and **17a,b**) were synthesized and biologically evaluated. Most of them could produce high levels of NO and show strong antiproliferation activities for A549, HeLa, A2780, and A2780/CDDP cell lines. The antiproliferation of these hybrids


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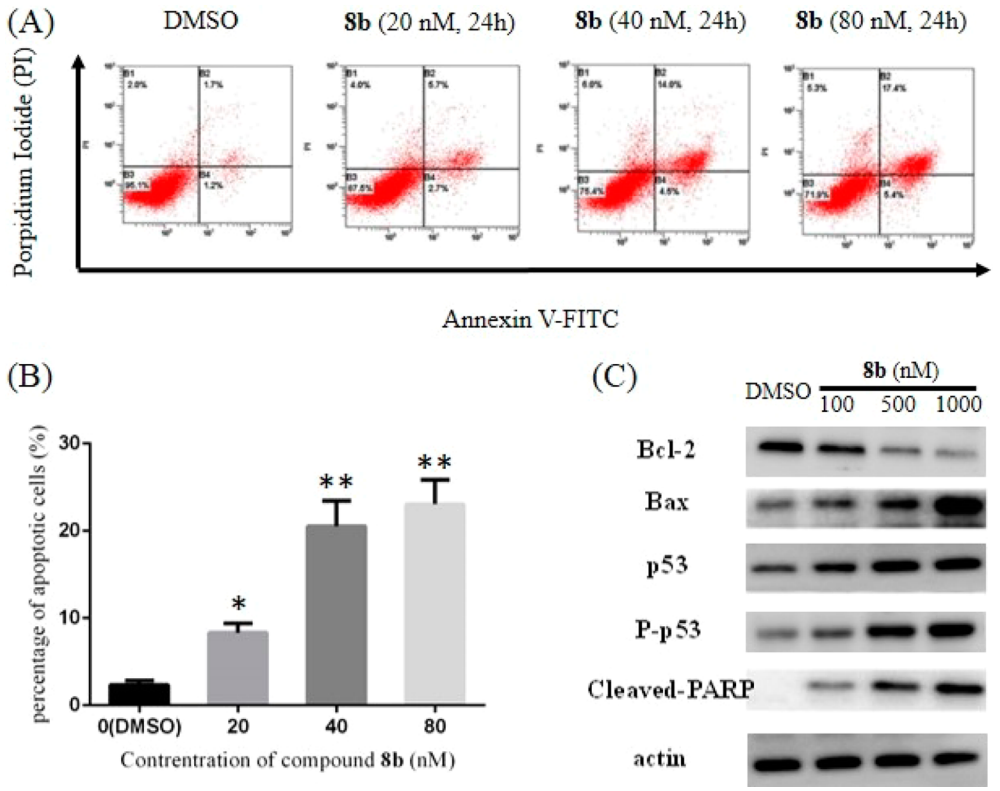


**Figure 8.** Apoptosis induced by compound **8b**. (A) Apoptotic cells were detected with annexin V/PI double staining after incubation with various concentrations of compound **8b** or diluent (DMSO) for 24 h. (B) The profiles showed the apoptotic proportion (%) of A2780 cells. The experiments were performed three times: (*) $p < 0.05$, (**) $p < 0.01$ compared to control. (C) Western blotting analysis showed that **8b** significantly up-regulated the expression of proapoptotic protein Bax but down-regulated the expression of the antiapoptotic proteins Bcl-2 in a dose dependent manner. Meanwhile, **8b** could also up-regulate the expression and the phosphorylation levels of p53 and induced the cleavage of PARP.

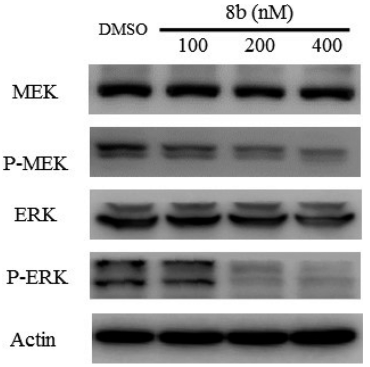


**Figure 9.** Effects of **8b** on the ERK/MAPK signaling pathway.

against HUVEC combined with the result that **8b** disrupted the formation of tube structures indicated that these compounds might have antiangiogenesis effect and pushed us to explore active targets such as VEGFR in the next study. By far, the most potent compound **8b** displayed a wide-spectrum anticancer activity against eight cancer cell lines including the five sensitive cell lines (A549, HeLa, A2780, MDA-MB-231, and SKOV3) and three drug resistant tumor cell lines (A2780/CDDP, MDA-MB-231/Gem, and SKOV3/CDDP) with nearly comparative $IC_{50}$ values. Interestingly, **8b** had a distinguishing antiproliferative selectivity between nontumorigenic T29 cell and A2780 tumor cell line. In addition, compound **8b** also inhibited human xenograft growth in the A2780 ovarian tumor-bearing nude mice in vivo. In our investigation of pharmacological mechanism, most of compounds were weakly active against MEK1, but the introduction of the NO group resulted in considerable antiproliferation activity against several cancer cell lines. In particular, **8b** showed cellular apoptosis, cell cycle arrest at the G2/M phase, and inhibition of MEK1 and ERK1 phosphorylation in A2780 cells. The preliminary SAR showed that the two to three C length of linker between the NO moiety and coumarin core was crucial to increase the anticancer activity. A detailed SAR study is ongoing. In conclusion, the NO-releasing capacity and the inhibition of ERK/MAPK pathway signaling, which exerts synergistic multitarget actions, may explain the potent antineoplastic activity of these compounds.

**Table 4. In Vitro MEK1 Inhibitory Activities of Target Compounds[a]**

| | compd[b] | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **6a** | **6b** | **6c** | **8a** | **8b** | **8c** | **8d** | **8e** | **8f** | **8g** | **10a** | **13a** | **13b** | **15** | **17a** | **17b** |
| MEK1 (%) | 1.6 | 8.4 | 10 | 11.3 | 22.7 | 35.2 | 53.4 | 31.6 | 43 | 48.1 | 5.7 | 47.6 | 16.4 | 14.4 | 10.9 | 29.8 | 20.4 |

[a]The data are the mean from two independent experiments. [b]The concentrations of tested compounds were 5 $\mu$M.


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Table 5. Acute Toxicity of 8b in Mice[a]

| dose (mg/kg) | no. of mice | no. of dead mice | | | | | | | total death | survivial (%) on day 14 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 h | 4 h | 1 day | 2 days | 3 days | 4 days | 5−14 days | | |
| 500 | 10 | 0 | 1 | 3 | 2 | 0 | 1 | 2 | 9 | 10 |
| 300 | 10 | 0 | 1 | 2 | 2 | 1 | 0 | 1 | 7 | 30 |
| 275 | 10 | 0 | 0 | 2 | 2 | 1 | 0 | 1 | 6 | 40 |
| 250 | 10 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 4 | 60 |
| 225 | 10 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 90 |
| 200 | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| vehicle | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |

[a]$LD_{50}$ = 263.6 mg/kg.

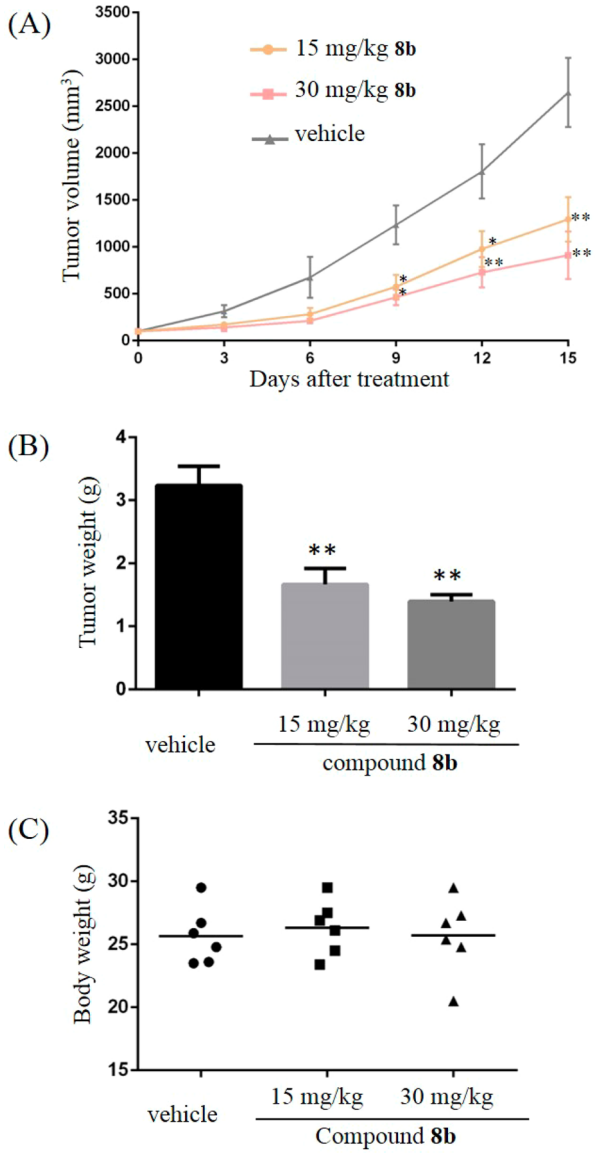


**Figure 10.** Inhibition of human xenograft growth in vivo by compound **8b**. (A) A2780 human ovarian tumor-bearing nude mice were administered 15 or 30 mg/kg **8b** or the vehicle, as controls ($n$ = 6). Injections were given intraperitoneally once every 3 days. The figures show the average measured tumor volumes (A), the weight of the excised tumor at the end of the observation period (B), and the body weights of the mice recorded at the end of the observation period (C). Data are expressed as the mean ± SD ($n$ = 6): (*) $p < 0.05$ and (**) $p < 0.01$ vs control.

## EXPERIMENTAL SECTION

**Chemistry.** Melting points were measured on a SGW X-4 microscopy melting point apparatus without correction. $^1$H and $^{13}$C NMR spectral data were recorded with a Varian 400 MHz spectrometer at 303 K using TMS as an internal standard. Mass spectra were recorded on Agilent Technologies 1260 infinity LC/MS instrument, and HRMS spectra were recorded on an Agilent Technologies LC/MSD TOF instrument. Analytical and preparative TLC was performed on silica gel HSGF/UV 254. The chromatograms were conducted on silica gel (100−200 mesh) and visualized under UV light at 254 and 365 nm. Tested compounds were analyzed by reverse-phase HPLC (ODS-$C_{18}$ column (5 $\mu$m, 4.6 mm × 150 mm or 3.5 $\mu$m, 4.6 mm × 150 mm) using a Waters 1525 binary HPLC pump system equipped with a Waters 2489 UV/visible detector. The mobile phase was 70% $CH_3CN$/30% $H_2O$ (v/v) and with a total flow rate of 1 mL/min. The purity was determined by monitoring at 254 nm. The purities of the synthesized compounds were confirmed to be ≥95% by this HPLC analysis.

**3,4-Diphenylsulfonyl-1,2,5-oxadiazole 2-Oxide (4).** The synthesis of the intermediate **4** from benzenethiol in a three-step sequence was reported previously in ref 27.

**2-Ethyl-7-hydroxy-4*H*-chromen-4-one (14).** The synthesis of the intermediate **14** from resorcinol was reported previously in ref 34.

**General Procedure for the Preparation of 7a−g.** To a stirred solution of **5a−e** (1 mmol) in DMF (5 mL) at room temperature were added corresponding halo alcohol (2 mmol) and $K_2CO_3$ (3 mmol). The mixture was refluxed for about 2−10 h and then poured into water (50 mL). After filtration, the residue was washed with water (3 × 10 mL) and dried with infrared lamp, yielding a yellow or white solid **7a−g** (40−96%).

**7-(2-Hydroxyethoxy)-2*H*-chromen-2-one (7a).** The title compound was obtained starting from **5a** and 2-chloroethanol (white solid, 88% yield, mp 85−86 °C). Analytical data for **7a**: ESI-MS $m/z$ 207.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.64 (d, 1H, −CH═CH−, $J$ = 9.5 Hz), 7.38 (d, 1H, ArH, $J$ = 8.6 Hz), 6.87 (dd, 1H, ArH, $J$ = 8.6, 2.3 Hz), 6.82 (d, 1H, ArH, $J$ = 2.0 Hz), 6.26 (d, 1H, −CH═CH−, $J$ = 9.5 Hz), 4.19−4.11 (m, 2H, $-OCH_2-$), 4.01 (m, 2H, $-CH_2O-$), 2.15 (s, 1H,OH).

**7-(2-Hydroxyethoxy)-4-methyl-2*H*-chromen-2-one (7b).** The title compound was obtained starting from **5b** and 2-chloroethanol (white solid, 90% yield, mp 145−147 °C). Analytical data for **7b**: ESI-MS $m/z$ 221.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.50 (d, 1H, ArH, $J$ = 8.8 Hz), 6.89 (d, 1H, ArH, $J$ = 8.8 Hz), 6.82 (s, 1H, ArH), 6.14 (s, 1H, −CH═), 4.14 (d, 2H, $-CH_2O-$, $J$ = 3.0 Hz), 4.02 (s, 2H, $-CH_2O-$), 2.40 (s, 3H, $-CH_3$), 2.20 (s, 1H, OH).

**7-(2-Hydroxyethylthio)-4-methyl-2*H*-chromen-2-one (7c).** The title compound was obtained starting from **5d** and 2-chloroethanol (yellow solid, 89% yield, mp 117−118 °C). Analytical data for **7c**: ESI-MS $m/z$ 237.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.48 (d, 1H, ArH, $J$ = 8.2 Hz), 7.26−7.18 (m, 2H, ArH), 6.23 (d, 1H, ═CH−, $J$ = 1.1 Hz), 3.86 (q, 2H, $-OCH_2-$, $J$ = 5.6 Hz), 3.22 (t, 2H, $-CH_2S-$, $J$ = 6.0 Hz), 2.41 (d, 3H, $-CH_3$, $J$ = 1.1 Hz), 2.07 (s, 1H, OH).

**7-(3-Hydroxypropoxy)-4-methyl-2*H*-chromen-2-one (7d).** The title compound was obtained starting from **5b** and 3-


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chloropropan-1-ol (white solid, 96% yield, mp 77−80 °C). Analytical data for **7d**: ESI-MS $m/z$ 235.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.48 (dd, 1H, ArH, $J$ = 8.7, 2.6 Hz), 6.90−6.79 (m, 2H, ArH), 6.12 (d, 1H, =CH−, $J$ = 1.0 Hz), 4.17 (t, $-CH_2O-$, $J$ = 6.0 Hz), 3.87 (d, 2H, $-CH_2-$, $J$ = 2.7 Hz), 2.39 (d, 3H, $-CH_3$, $J$ = 1.1 Hz), 2.08 (p, $-CH_2-$, $J$ = 6.0 Hz).

**7-(4-Hydroxybutoxy)-4-methyl-2*H*-chromen-2-one (7e).** The title compound was obtained starting from **5b** and 4-chlorobutan-1-ol (white solid, 72% yield, mp 127−130 °C). Analytical data for **7e**: ESI-MS $m/z$ 249.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.49 (dd, 1H, ArH, $J$ = 8.8, 3.0 Hz), 6.90−6.72 (m, 2H, ArH), 6.13 (s, 1H, −CH=), 4.53 (t, 2H, $-OCH_2-$, $J$ = 6.12 Hz), 4.13 (t, 2H, $-CH_2O-$, $J$ = 5.92 Hz), 2.39 (d, $J$ = 1.2 Hz, 3H), 2.08 (m, 4H, $-CH_2CH_2-$).

**7-(2-Hydroxyethoxy)-4,8-dimethyl-2*H*-chromen-2-one (7f).** The title compound was obtained starting from **5c** and 2-chloroethanol (white solid, 84% yield, mp 116−118 °C). Analytical data for **7f**: ESI-MS $m/z$ 235.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.42 (d, 1H, ArH, $J$ = 8.8 Hz), 7.26 (s, 1H, ArH), 6.85 (d, 1H, ArH, $J$ = 8.8 Hz), 6.15 (d, 1H, −CH=, $J$ = 1.0 Hz), 4.23−4.15 (m, 2H, $-CH_2O$), 4.08−3.99 (m, 2H, $-CH_2O$), 2.40 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 1.98 (t, 1H, OH, $J$ = 6.3 Hz).

**7-(2-Hydroxyethylamino)-4-methyl-2*H*-chromen-2-one (7g).** The title compound was obtained starting from **5e** and 2-bromoethanol (white solid, 40% yield, mp 145−147 °C). Analytical data for **7g**: ESI-MS $m/z$ 220.1 $[M + H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.40 (d, 1H, ArH, $J$ = 8.7 Hz), 6.61 (dd, 1H, ArH, $J$ = 8.7, 1.7 Hz), 6.41 (d, 1H, ArH, $J$ = 1.7 Hz), 5.89 (s, 1H, −CH=), 3.54 (t, 2H, $-OCH_2-$, $J$ = 5.8 Hz), 3.19−3.10 (m, 2H, $-CH_2N-$), 2.28 (s, 3H,-$CH_3$).

**General Procedure for the Preparation of 16a,b.** The procedure was identical to that used in the preparation of **7a−g** (75−93%).

**7-(2-Hydroxyethoxy)-2-ethyl-4*H*-chromen-4-one (16a).** The title compound was obtained starting from **14** and 2-bromoethanol (white crystal, 93% yield, mp 97−98 °C). Analytical data for **16a**: ESI-MS $m/z$ 235.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.07 (d, 1H, ArH, $J$ = 8.9 Hz), 6.95 (dd, 1H, ArH, $J$ = 8.9, 2.4 Hz), 6.84 (d, 1H, ArH, $J$ = 2.4 Hz), 6.12 (s, 1H, −CH=), 4.21−4.13 (m, 2H, $-CH_2-$), 4.07−3.98 (m, 2H, $-CH_2-$), 2.68−2.58 (m, 2H, $-CH_2-$), 2.26 (t, 1H, −OH, $J$ = 6.2 Hz), 1.30 (t, 3H, $CH_3$, $J$ = 7.5 Hz).

**7-(3-Hydroxypropoxy)-2-ethyl-4*H*-chromen-4-one (16b).** The title compound was obtained starting from **14** and 3-chloropropan-1-ol (white solid, 75% yield, mp 71−73 °C). Analytical data for **16b**: ESI-MS $m/z$ 249.1 $[M + H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.86 (d, 1H, ArH, $J$ = 8.8 Hz), 7.07 (s, 1H, ArH), 6.99 (d, 1H, ArH, $J$ = 8.9 Hz), 6.11 (s, 1H, −COCH=), 4.59 (t, 1H, OH, $J$ = 4.7 Hz), 4.15 (t, 2H, $-OCH_2-$, $J$ = 6.0 Hz), 3.54 (q, 2H, $-CH_2-$, $J$ = 5.4 Hz), 2.62 (q, 2H, $-CH_2O-$, $J$ = 7.4 Hz), 2.05−1.67 (m, 2H, $-CH_2CH_3$), 1.20 (dd, 3H, $-CH_2CH_3$, $J$ = 8.1, 6.9 Hz).

**General Synthetic Procedure for 9a.** A mixture of **7g** (100 mg, 0.46 mmol) and $CH_3I$ (133 mg, 0.92 mmol) in DMF (3 mL) was refluxed for 2 h. Then the reaction mixture was poured into ice−water (30 mL) and filtrated. The filtrate was washed with water (3 × 5 mL) and dried under infrared lamp to give 7-(*N*-(2-hydroxyethyl)-*N*-methylamino)-4-methyl-2*H*-chromen-2-one (**9a**), which was used in the next reaction without further purification. ESI-MS $m/z$ 234.1 $[M + H]^+$ (95 mg, 88%).

**General Procedure for the Preparation of 11a,b.** At room temperature, 2-chloroacetic acid (2 mmol) and $K_2CO_3$ (3 mmol) were added into a stirred solution of **5a−e** (1 mmol) in DMF (5 mL). After reflux for 1 h, water (20 mL) was added to dissolve the mixture and further acidified by 2 N aq HCl to reprecipit the solid. After filtration, the residue was washed with water (3 × 10 mL) and dried with infrared lamp to gain white solid **11a,b** (69−85%).

**2-(2-Oxo-2*H*-chromen-7-yloxy)acetic Acid (11a).** The title compound was obtained starting from **5a** and 2-chloroacetic acid (white solid, 69% yield, mp 198−200 °C). Analytical data for **11a**: ESI-MS $m/z$ 221.0 $[M + H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.01−7.88 (m, 2H, ArH), 7.62 (d, 1H, −CH=CH−, $J$ = 9.3 Hz), 6.93 (m, 1H, ArH), 6.28 (d, 1H, −CH=CH−, $J$ = 9.5 Hz), 4.81 (s, 2H, $-COCH_2O-$).

**2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)acetic Acid (11b).** The title compound was obtained starting from **5b** and 2-chloroacetic acid (white solid, 85% yield, mp 201−203 °C). Analytical data for **11b**: ESI-MS $m/z$ 235.1 $[M + H]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.67 (d, 1H, ArH, $J$ = 9.0 Hz), 7.01−6.91 (m, 2H, ArH), 6.21 (s, 1H, −CH=), 4.81 (s, 2H, $-COCH_2O-$), 2.38 (s, 3H, $-CH_3$).

**General Synthetic Procedure for 12a,b.** A mixture of **11a,b** (2 mmol), EDC (2.1 mmol), and DMAP (0.6 mmol) in $CH_2Cl_2$ (5 mL) was stirred at 0 °C for 0.5 h. After addition of glycol (4 mL) to the solution, the mixture was further stirred at room temperature for 24 h. The solution was washed with water (3 × 20 mL). After removal of the solvent in vacuo, the crude product was purified by column chromatography (eluent, $CH_2Cl_2$/MeOH 50:1) to give **12a,b** (40−51%).

**2-Hydroxyethyl 2-(2-Oxo-2*H*-chromen-7-yloxy)acetate (12a).** The title compound was obtained starting from **11a** and glycol (white solid, 40% yield, mp 85−87 °C). Analytical data for **12a**: ESI-MS $m/z$ 265.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.66 (d, 1H, −CH=CH−, $J$ = 9.5 Hz), 7.42 (d, 1H, ArH, $J$ = 8.6 Hz, 1H), 6.91 (dd, 1H, ArH, $J$ = 8.6, 2.5 Hz), 6.81 (d, 1H, ArH, $J$ = 2.4 Hz), 6.29 (d, 1H, −CH=CH−, $J$ = 9.5 Hz), 4.77 (s, 2H, $-COCH_2O-$), 4.38 (dd, 2H, $-CH_2O-$, $J$ = 5.4, 3.9 Hz), 3.90 (dd, 2H, $-CH_2O-$, $J$ = 5.4, 3.9 Hz).

**2-Hydroxyethyl 2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-acetate (12b).** The title compound was obtained starting from **11b** and glycol (white solid, 51% yield, mp 111−112 °C). Analytical data for **12b**: ESI-MS $m/z$ 279.1 $[M + H]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.52 (d, 1H, ArH, $J$ = 8.8 Hz), 6.92 (dd, 1H, ArH, $J$ = 8.8, 2.6 Hz), 6.79 (d, 1H, ArH, $J$ = 2.5 Hz), 6.16 (d, 1H, −CH=, $J$ = 1.1 Hz), 4.75 (s, 2H, $-COCH_2O-$), 4.37 (t, 2H, $-OCH_2-$, $J$ = 4.6 Hz), 3.89 (t, 2H, $-CH_2O-$, $J$ = 4.6 Hz), 2.40 (d, 3H, $-CH_3$, $J$ = 1.1 Hz).

**General Procedure for the Target Compounds 6a−c, 8a−g, 10a, 13a,b, 15, and 17a,b as Well as the NO Donor Moiety Compound 18.** Compound **4** (1 mmol) was added to a stirred solution of compound alcohol selected from the group **5a−c**, **7a−g**, **9a**, **12a,b**, **14**, **16a,b**, or 2-methoxyethanol (1.2 mmol) in the presence of 8-diazabicyclo[5.4.0]undec-7-ene (DBU) (3 mmol) in $CH_2Cl_2$. The reaction mixture was stirred at room temperature for 2−5 h and then washed with water (3 × 10 mL). The organic layer was dried with anhydrous $Na_2SO_4$, and the solvent was removed in vacuo to give an oil. The oil was added methanol to precipitate. After filtration, the filter residue was purified by recrystallizing using ethyl acetate or ethanol to give the respective title products listed below.

**4-(2*H*-Chromen-2-one-7-yloxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (6a).** The title compound was obtained starting from **5a** and **4** (white solid, 84% yield, mp 177−179 °C). Analytical data for **6a**: ESI-MS $m/z$ 387.0 $[M + H]^+$, 409.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.16 (d, 1H, −HC=CH−, $J$ = 9.56 Hz), 7.28 (dd, 1H, ArH, $J$ = 8.54, 2.40 Hz), 7.32 (d, 1H, ArH, $J$ = 2.12 Hz), 7.58 (d, 1H, ArH, $J$ = 8.48 Hz), 7.67 (t, 2H, ArH, $J$ = 7.56 Hz), 7.72 (d, 1H, −CH=CH−, $J$ = 9.60 Hz), 7.81 (t, 1H, ArH, $J$ = 7.56 Hz) 8.09 (d, 2H, ArH, $J$ = 7.44 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 159.79, 157.37, 154.80, 154.57, 147.47, 137.55, 136.02, 129.84, 129.29, 128.64, 117.23, 116.71, 116.09, 110.59, 108.46; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{17}H_{10}N_2O_7S$ 387.0287, obsd 387.0292, ppm error 1.3.

**4-(4-Methyl-2*H*-chromen-2-one-7-yloxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (6b).** The title compound was obtained starting from **5b** and **4** (white solid, 90% yield, mp 201−203 °C). Analytical data for **6b**: ESI-MS $m/z$ 401.0 $[M + H]^+$, 423.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.46 (s, 3H, $-CH_3C=$), 6.32 (s, 1H, $-CH_3C=CHCOO$), 7.31 (m, 2H, ArH), 7.68 (m, 3H, ArH), 7.81 (t, 1H, ArH, $J$ = 6.4 Hz), 8.10 (d, 2H, ArH, $J$ = 7.48 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 159.98, 157.44, 154.44, 154.30, 151.59, 137.55, 136.01, 129.84, 128.64, 126.16, 118.48, 115.80, 115.05, 110.60, 108.50, 18.77. ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{18}H_{12}N_2O_7S$ 401.0443, obsd 401.0446, ppm error 0.7.

**4-(4,8-Dimethyl-2*H*-chromen-2-one-7-yloxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (6c).** The title compound was


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obtained starting from **5c** and **4** (white solid, 80% yield, mp 243−245 °C). Analytical data for **6c**: ESI-MS $m/z$ 415.0 $[M + H]^+$, 437.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.39 (s, 3H, $=CCH_3$), 2.46 (s, 3H, $ArCH_3$), 6.33 (s, 1H, $-CH_3C=CH-COO-$), 7.27 (m, 1H, ArH), 7.54 (d, 1H, ArH, $J$ = 8.76 Hz), 7.68 (t, 2H, ArH, $J$ = 7.68 Hz), 7.83 (t, 3H, ArH, $J$ = 7.48 Hz), 8.14 (d, 2H, ArH, $J$ = 7.40 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 160.22, 157.88, 152.78, 152.63, 152.01,137.73, 135.99, 129.88, 128.57,122.80, 118.76, 118.51, 115.84, 114.86, 110.48, 18.89, 9.12; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{19}H_{14}N_2O_7S$ 415.0600, obsd 415.0600, ppm error 0.

**4-(2-(2-Oxo-2*H*-chromen-7-yloxy)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8a).** The title compound was obtained starting from **7a** and **4** (white solid, 84% yield, mp 139−141 °C). Analytical data for **8a**: ESI-MS $m/z$ 431.0 $[M + H]^+$, 453.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.02 (d, 2H, ArH, $J$ = 7.4 Hz), 7.73 (t, 1H, ArH, $J$ = 7.5 Hz), 7.67 (d, 1H, $-CH=CH-$, $J$ = 9.5 Hz), 7.56 (t, 2H, ArH, $J$ = 7.9 Hz), 7.44 (d, 1H, ArH, $J$ = 8.6 Hz), 6.90 (dd, 1H, ArH, $J$ = 8.6, 2.3 Hz), 6.85 (d, 1H, ArH, $J$ = 2.1 Hz), 6.30 (d, 1H, $-CH=CH-$, $J$ = 9.5 Hz), 4.86−4.73 (m, 2H, $-CH_2O-$), 4.47−4.37 (m, 2H, $-CH_2O-$). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 161.32, 158.76, 158.79, 152.68, 152.61, 137.91, 135.68, 129.62, 128.36, 122.64, 114.69, 114.46, 112.37, 110.35, 107.74, 69.30, 66.03, 18.77, 8.29; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{19}H_{14}N_2O_8S$ 431.0549, obsd 431.0557, ppm error 1.8.

**4-(2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8b).** The title compound was obtained starting from **7b** and **4** (white solid, 93% yield, mp 162−165 °C). Analytical data for **8b**: ESI-MS $m/z$ 445.0 $[M + H]^+$, 467.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.43 (s, 3H,$=CCH_3$), 4.45 (t, 2H, $-CH_2O-$, $J$ = 4.12 Hz), 4.81 (t, 2H, $-OCH_2-$, $J$ = 4.20 Hz), 6.18 (s, 1H, $-CH_3C=CH-COO-$), 6.85 (d, 1H, ArH, $J$ = 2.32 Hz), 6.92 (dd, 1H, ArH, $J$ = 8.80, 2.36 Hz), 7.57 (m, 3H, ArH), 7.73 (t, 1H, ArH, $J$ = 7.57 Hz), 8.03 (d, 2H, ArH, $J$ = 7.72 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 161.05, 160.97, 158.65, 155.09, 152.42, 137.89, 135.68, 129.63, 128.51, 125.87, 114.27, 112.46, 112.20, 110.33, 101.81, 69.03, 65.63, 18.71; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{20}H_{16}N_2O_8S$ 445.0706, obsd 445.0712, ppm error 1.3.

**4-(2-(4-Methyl-2-oxo-2*H*-chromen-7-ylthio)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8c).** The title compound was obtained starting from **7c** and **4** (white solid, 83% yield, mp 162−165 °C). Analytical data for **8c**: ESI-MS $m/z$ 461.0 $[M + H]^+$, 483.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.42 (s, 3H, $=CCH_3$), 3.49 (t, 2H, $-CH_2S-$, $J$ = 6.76 Hz), 4.63 (t, 2H, $-OCH_2-$, $J$ = 6.76 Hz), 6.25 (s, 1H, $-CH_3C=CH-COO-$), 7.28 (m, 2H, ArH), 7.53 (d, 1H, ArH, $J$ = 8.8 Hz), 7.63 (t, 2H, ArH, $J$ = 7.68 Hz), 7.77 (t, 1H, ArH, $J$ = 7.44 Hz), 8.05 (d, 2H, ArH, $J$ = 8.24 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 160.33, 158.46, 153.77, 152.00, 140.25, 137.79, 135.72, 129.68, 128.54, 125.12, 123.81, 118.13, 115.50, 114.50, 110.31, 68.91, 30.74, 18.71; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{20}H_{16}N_2O_7S_2$ 461.0477, obsd 461.0489, ppm error 2.6.

**4-(3-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)propanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8d).** The title compound was obtained starting from **7d** and **4** (white solid, 79% yield, mp 116−118 °C). Analytical data for **8d**: ESI-MS $m/z$ 459.0 $[M + H]^+$, 480.9 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.40 (m, 5H, $=CCH_3$ and $-CH_2-$), 4.23 (t, 2H, $-CH_2O-$, $J$ = 5.84 Hz), 4.65 (t, 2H, $-OCH_2-$, $J$ = 6.00 Hz), 6.16 (s, 1H, $-CH_3C=CH-COO-$), 6.84 (d, 1H, ArH, $J$ = 1.84 Hz), 6.89 (dd, 1H, ArH, $J$ = 8.76, 2.28 Hz),7.52 (d, 1H, ArH, $J$ = 8.80 Hz),7.58 (t, 2H, ArH, $J$ = 7.60 Hz), 7.74 (t, 1H, ArH, $J$ = 7.40 Hz), 8.02 (d, 2H, ArH, $J$ = 8.32 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$161.46, 161.17, 158.78, 155.16, 152.49, 137.86, 135.67, 129.63, 128.44, 125.67, 113.84, 112.39, 112.15, 110.41, 109.72, 101.46, 67.78, 63.99, 28.24, 18.68; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{21}H_{18}N_2O_8S$ 459.0862, obsd 459.0864, ppm error 0.4.

**4-(4-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)butanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8e).** The title compound was obtained starting from **7e** and **4** (white solid, 70% yield, mp 153−155 °C). Analytical data for **8e**: ESI-MS $m/z$ 473.3 $[M + H]^+$, 495.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.08 (m, 4H, $-CH_2CH_2-$), 2.40 (s, 3H, $=CCH_3$), 4.13 (t, 2H, $-CH_2O-$, $J$ = 5.92 Hz), 4.53 (t, 2H, $-OCH_2-$, $J$ = 6.12 Hz), 6.14 (s, 1H, $-CH_3C=CH-COO-$), 6.82 (d, 1H, ArH, $J$ = 2.20 Hz), 6.87 (dd, 1H, ArH, $J$ = 8.76, 2.36 Hz),7.51 (d, 1H, ArH, $J$ = 8.80 Hz), 7.61 (t, 3H, ArH, $J$ = 8.00 Hz), 7.76 (t, 1H, ArH, $J$ = 7.32 Hz), 8.05 (d, 2H, ArH, $J$ = 8.16 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 161.73,161.27, 158.89, 155.19, 152.55, 137.91, 135.62, 129.62, 128.48, 125.59, 113.65, 112.44, 111.98, 110.43, 101.39, 71.08, 67.64, 25.41, 25.34, 18.68; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{22}H_{20}N_2O_8S$ 473.1019, obsd 473.1021, ppm error 0.4.

**4-(2-(4,8-Dimethyl-2-oxo-2*H*-chromen-7-yloxy)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8f).** The title compound was obtained starting from **7f** and **4** (yellow solid, 84% yield, mp 181−185 °C). Analytical data for **8f**: ESI-MS $m/z$ 459.1 $[M + H]^+$, 481.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.98 (d, 2H, ArH, $J$ = 7.6 Hz), 7.69 (t, 1H, ArH, $J$ = 7.5 Hz), 7.50 (t, 2H, ArH, $J$ = 7.9 Hz), 7.44 (d, 1H, ArH, $J$ = 8.8 Hz), 6.89 (d, 1H, ArH, $J$ = 8.8 Hz), 6.16 (s, 1H, $-CH=$), 4.84-.79 (m, 2H, $-OCH_2-$), 4.51−4.44 (m, 2H, $-OCH_2-$), 2.40 (s, 3H, $-CH_3$), 2.26 (s, 3H, $-CH_3$). $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 161.32, 158.76, 158.69, 152.68, 152.61, 137.91, 135.68, 129.62, 128.36, 122.64, 114.69, 114.46, 112.37, 107.74, 69.30, 66.03, 18.77, 8.29. ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{21}H_{18}N_2O_8S$ 459.0862, obsd 459.0864, ppm error 0.4.

**4-(2-(4-Methyl-2-oxo-2*H*-chromen-7-azyl)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (8g).** The title compound was obtained starting from **7g** and **4** (yellow solid, 80% yield, mp 193−195 °C). Analytical data for **8g**: ESI-MS $m/z$ 444.0 $[M + H]^+$, 466.0 $[M + Na]^+$; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.31 (s, 3H, $=CCH_3$), 3.60 (m, 2H, $-CH_2NH-$), 4.53 (t, 2H, $-OCH_2-$, $J$ = 5.04 Hz), 5.93 (s, 1H, $-CH_3C=CH-COO-$), 6.54 (d, 1H, ArH, $J$ = 1.52 Hz), 6.67 (d, 1H, ArH, $J$ = 8.72 Hz), 6.87 (t, 1H, ArH, $J$ = 5.64 Hz), 7.47 (d, 1H, ArH, $J$ = 8.76 Hz), 7.66 (t, 2H, ArH, $J$ = 7.72 Hz), 7.85 (t, 1H, ArH, $J$ = 7.36 Hz), 7.96 (d, 2H, ArH, $J$ = 7.88 Hz); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 160.62, 158.93, 155.59, 153.66, 151.93, 137.08, 136.06, 129.88, 128.30, 126.04, 110.54, 110.31, 109.12, 107.80, 96.53, 69.68, 40.93, 18.00; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{20}H_{17}N_3O_7S$ 444.0865, obsd 444.0867, ppm error 0.4.

**4-(2-(Methyl(4-methyl-2-oxo-2*H*-chromen-7-yl)amino)-ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (10a).** The title compound was obtained starting from **9a** and **4** (yellow solid, 40% yield, mp 181−185 °C). Analytical data for **10a**: ESI-MS $m/z$ 458.0 $[M + H]^+$, 480.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 2.36 (s, 3H, $=CCH_3$), 3.19 (s, 3H, $-NCH_3$), 3.95 (t, 2H, $-CH_2N-$, $J$ = 5.32 Hz), 4.63 (t, 2H, $-OCH_2-$, $J$ = 5.28 Hz), 6.02 (s, 1H, $-CH_3C=CH-COO-$), 6.59 (d, 1H, ArH, $J$ = 2.52 Hz), 6.76 (dd, 1H, ArH, $J$ = 8.92, 2.56 Hz), 7.46 (d, 1H, ArH, $J$ = 8.92 Hz), 7.57 (t, 2H, ArH, $J$ = 7.88 Hz), 7.74 (t, 1H, ArH, $J$ = 7.48 Hz), 7.95 (d, 2H, ArH, $J$ = 7.40 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 206.05, 161.81, 158.72, 155.64, 152.79, 151.27, 137.71, 135.72, 129.64, 128.41, 125.72, 110.32, 109.90, 108.92, 98.48, 68.51, 50.64, 39.34, 18.51; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{21}H_{19}N_3O_7S$ 458.1022, obsd 458.1033, ppm error 2.4.

**4-(2-(2-(2-Oxo-2*H*-chromen-7-yloxy)acetyl)oxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (13a).** The title compound was obtained starting from **12a** and **4** (white solid, 50% yield, mp 174−177 °C). Analytical data for **13a**: ESI-MS $m/z$ 488.9 $[M + H]^+$, 510.9 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.68 (s, 4H, $-OCH_2CH_2O-$), 4.80 (s, 2H, $-COCH_2O-$), 6.28 (d, 1H, $-HC=CH-$, $J$ = 9.44 Hz), 6.83 (s, 1H, ArH), 6.90 (d, 1H, ArH, $J$ = 8.64 Hz),7.41 (d, 1H, ArH, $J$ = 8.52 Hz),7.63 (m, 3H, ArH and $-HC=CH-$), 7.76 (t, 1H, ArH, $J$ = 7.28 Hz), 8.07 (d, 2H, ArH, $J$ = 7.60 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 167.85,160.82, 160.52, 158.49, 155.56, 143.14, 137.74, 135.70, 129.65, 129.08, 128.61, 113.88, 113.45, 112.49, 101.91, 68.41, 65.11, 61.91, 29.67; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{21}H_{16}N_2O_{10}S$ 489.0604, obsd 489.0613, ppm error 1.8.

**4-(2-(2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)acetyl)-oxyethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (13b).** The title compound was obtained starting from **12b** and **4** (white solid, 65% yield, mp 113−115 °C). Analytical data for **13b**: ESI-MS $m/z$ 502.9 $[M + H]^+$, 525.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$)


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$\delta$ 2.39 (s, 3H, =$CCH_3$), 4.67 (s, 4H, $-OCH_2CH_2O-$), 4.80 (s, 2H, $-COCH_2O-$), 6.16 (s, 1H, $-CH_3C$=CH$-COO-$), 6.82 (d, 1H, ArH, $J$ = 2.32 Hz), 6.92 (dd, 1H, ArH, $J$ = 8.88, 2.08 Hz), 7.53 (d, 1H, ArH, $J$ = 8.80 Hz), 7.62 (t, 3H, ArH, $J$ = 7.69 Hz), 7.76 (t, 1H, ArH, $J$ = 7.44 Hz), 8.06 (d, 2H, ArH, $J$ = 8.16 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 167.91, 160.94, 160.35, 158.50, 154.96, 152.35, 137.74, 135.70, 129.66, 128.60, 125.91, 114.55, 112.58, 112.15, 110.37, 101.90, 68.44, 65.09, 61.93, 18.67; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{22}H_{18}N_2O_{10}S$ 503.0760, obsd 503.0762, ppm error 0.4.

**4-(2-Ethyl-4*H*-chromen-4-one-7-yloxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (15).** The title compound was obtained starting from **14** and **4** (white solid, 89% yield, mp 159−161 °C). Analytical data for **15**: ESI-MS $m/z$ 415.0 $[M + H]^+$, 437.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.32 (t, 3H, $CH_2CH_3$, $J$ = 7.56 Hz), 2.47 (q, 2H, $-CH_2CH_3$, $J$ = 7.56 Hz), 6.21 (s, 1H, $-OC$=CH$-$), 7.32 (dd, 1H, ArH, $J$ = 8.80, 2.36 Hz), 7.46 (d, 1H, ArH, $J$ = 2.32 Hz), 7.66 (t, 2H, ArH, $J$ = 8.28 Hz), 7.81 (t, 1H, ArH, $J$ = 7.56 Hz), 8.08 (dd, 2H, ArH, $J$ = 1.28, 8.48 Hz), 8.27 (d, 1H, ArH, $J$ = 8.80 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 177.22, 171.33, 157.12, 156.97, 155.89, 137.55, 135.99, 129.83, 128.60, 128.01, 121.93, 116.62, 110.61, 109.20, 108.60, 27.41, 10.85; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{19}H_{14}N_2O_7S$ 415.0600, obsd 415.0604, ppm error 0.9.

**4-(2-(2-Ethyl-4*H*-chromen-4-one-7-yloxy)ethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (17a).** The title compound was obtained starting from **16a** and **4** (white solid, 81% yield, mp 167−169 °C). Analytical data for **17a**: ESI-MS $m/z$ 458.9 $[M + H]^+$, 480.9 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.31 (t, 3H, $-CH_2CH_3$, $J$ = 7.52 Hz), 2.65 (q, 2H, $-CH_2CH_3$, $J$ = 7.56 Hz), 4.49 (t, 2H, $-CH_2O-$, $J$ = 4.44 Hz), 4.82 (t, 2H, $-OCH_2-$, $J$ = 4.28 Hz), 6.14 (s, 1H, $-C$=CH$-$), 6.90 (d, 1H, ArH, $J$ = 2.36 Hz), 6.97 (dd, 1H, ArH, $J$ = 8.82, 2.24 Hz), 7.53 (t, 2H, ArH, J = 7.76 Hz), 7.71 (t, 1H, ArH, $J$ = 7.52 Hz), 8.00 (d, 2H, ArH, $J$ = 7.56 Hz), 8.14 (d, 1H, ArH, $J$ = 8.84 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 177.80, 170.52, 162.22, 158.63, 158.01, 137.87, 135.66, 129.62, 128.46, 127.31, 118.14, 114.12, 110.31, 108.81, 101.09, 69.04, 65.60, 27.37, 10.91; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{21}H_{18}N_2O_8S$ 459.0862, obsd 459.0859, ppm error 0.6.

**4-(3-(2-Ethyl-4*H*-chromen-4-one-7-yloxy)propanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (17b).** The title compound was obtained starting from **16b** and **4** (yellow solid, 74% yield, mp 153−155 °C). Analytical data for **17b**: ESI-MS $m/z$ 473.0 $[M + H]^+$, 495.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.28 (t, 3H, $-CH_2CH_3$, $J$ = 7.52 Hz), 2.40 (m, 2H, $-CCH_2C-$), 2.62 (q, 2H, $-CH_2CH_3$, $J$ = 7.60 Hz), 4.25 (t, 2H, $-CH_2O-$, $J$ = 5.84 Hz), 4.64 (t, 2H, $-OCH_2-$, $J$ = 6.00 Hz), 6.11 (s, 1H, $-C$=CH$-$), 6.86 (d, 1H, ArH, $J$ = 2.32 Hz), 6.93 (dd, 1H, ArH, $J$ = 8.86, 2.32 Hz), 7.51 (t, 2H, ArH, $J$ = 8.16 Hz), 7.69 (t, 1H, ArH, $J$ = 7.56 Hz), 7.96 (d, 2H, ArH, $J$ = 7.32 Hz), 8.09 (d, 1H, ArH, $J$ = 8.84 Hz); $^{13}$C NMR (101 MHz, $CDCl_3$) $\delta$ 177.87, 170.41, 162.73, 158.77, 158.10, 137.81, 135.64, 129.60, 128.41, 127.11, 117.77, 114.20, 110.40, 108.74, 100.79, 67.66, 63.96, 28.23, 27.35, 10.92; ESI-HRMS ($m/z$) $[M + H]^+$ calcd for $C_{22}H_{20}N_2O_8S$ 473.1019, obsd 473.1011, ppm error 1.7.

**4-(2-Methoxyethanoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-Oxide (18).** The title compound was obtained starting from 2-methoxyethanol and **4** (white solid, 46% yield, mp 104−106 °C). ESI-MS $m/z$ 301.0 $[M + H]^+$, 323.0 $[M + Na]^+$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.08 (d, 2H, ArH, $J$ = 7.8 Hz), 7.75 (d, 1H, ArH, $J$ = 7.4 Hz), 7.62 (t, 2H, ArH, $J$ = 7.8 Hz), 4.61−4.53 (m, 2H, $-CH_2-$, 2H), 3.85−3.77 (m, 2H, $-CH_2-$), 3.46 (s, 3H, $-CH_3$); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 159.26, 138.33, 135.89, 129.91, 128.91, 110.77, 70.76, 70.00, 59.59.

**In Vitro Antiproliferative Assay.** The in vitro antiproliferation of the chemical compounds was measured by the MTT reagent, as described in the literature.[35] Briefly, $5 \times 10^3$ cells in 100 $\mu$L of medium per well were plated in 96-well plates. After incubated for 24 h, the cells were treated with different concentration of tested compound or DMSO (as negative control) for 48 h. Then the medium with compound or DMSO was replaced with 200 $\mu$L of fresh medium containing 10% MTT (5 mg/mL in PBS) in each well and incubated at 37 °C for 4 h. Last, the MTT-containing medium was discarded and 150 $\mu$L of DMSO per well was added to dissolve the formazan crystals newly formed. Absorbance of each well was determined by a microplate reader (Synergy H4, Bio-Tek) at a 570 nm wavelength. The inhibition rates of proliferation were calculated with the following equation:

$$\text{inhibition ratio (\%)} = \frac{OD_{DMSO} - OD_{compd}}{OD_{DMSO} - OD_{blank}} \times 100$$

The concentrations of the compounds that inhibited cell growth by 50% ($IC_{50}$) were calculated using GraphPad Prism, version 6.0. For the NO scavenge experiment, cells were pretreated with the indicated concentrations of hemoglobin (Hb) (0, 1.25, 2.5, 5, 10, or 20 $\mu$M) for 1 h and treated with 500 nM **8b** for 24 h. Then the viability of the cells was determined by MTT reagent as described above.

**Capillary-like Tube Formation Assay.** Capillary-like tube formation assay was performed by following the procedure published previously.[36] Briefly, 70 $\mu$L per well Matrigel (Corning, NY) was added to 96-well plates and incubated at 37 °C for 30 min to allow gelation to occur. HUVECs were added to the top of the gel at a density of 30 000 cells/well in the presence of tested compound or vehicle control (DMSO). Cells were incubated at 37 °C with 5% $CO_2$ overnight, and pictures were captured with a CCD Sensicam camera mounted on a Olympus inverted microscope.

**Nitrite Measurement in Vitro.** The levels of NO released by tested compounds in the cells are presented as that of nitrite and were determined by the Griess reagent (Beyotime, China), according to the literature with some modifications.[25] Briefly, cells ($1 \times 10^7$ per 10 cm dish) were treated with a 100 $\mu$M concentration of each compound for 150 min. Subsequently, the cells were harvested and lysed with 100 $\mu$L of RIPA lysis buffer (Beyotime, China) for 30 min on ice. The cell lysates were mixed with Griess for 30 min in a dark place, followed by measurement by a microplate reader (Synergy H4, Bio-Tek) at 540 nm wavelength. The cells treated with diluent were used to determine the background levels of nitrite production, while sodium nitrite at different concentrations was measured to generate a standard curve.

**Measurement of Intracellular NO.** Intracellular NO was measured with 3-amino,4-aminomethyl-2′,7′-difluorescein, diacetate (DAF-FM DA, purchased from beyotime,China), as previously described.[37] Briefly, A2780 cells were pretreated with 5 $\mu$M DAF-FM DA at 37 °C for 20 min. And then the cells were washed with PBS three times and incubated with tested compound or vehicle control (DMSO) for 2 h. Finally, the cells were observed under a fluorescence microscope (Olympus, Japan) and pictures were captured with a CCD Sensicam camera.

**Colony Formation Assay.**[38] $1 \times 10^3$ cells per well were seeded in six-well plates at a single cell density. At 48 h later, the cells were treated with DMSO or different concentrations of compound **8b** for 48 h. Then the medium was replaced with fresh medium to allow cell growth for at least 1 week. The cells were fixed with methyl alcohol for 15 min and stained by gentian violet staining for 30 min. The colonies consisting of more than 50 cells were counted.

**Cell Cycle Analysis.** Cell cycle status was detected by flow cytometry according to a previously published method[39] and were analyzed by Multicycle AV (for Windows, version 320) software. Briefly, cells were first treated with DMSO or different concentrations of compound **8b** for 24 h and then harvested, washed twice with 1× PBS, and resuspended in 200 $\mu$L of 1× PBS. The cells were fixed in 4 mL of ice-cold 75% ethanol at −20 °C overnight and stained with 500 $\mu$L of propidium iodide (50 $\mu$g/mL, Sigma) containing 0.1% RNase (1 mg/mL, Sigma) for 15 min in dark conditions at room temperature. The cells were then analyzed by flow cytometry (Cytomics FC 500 MPL, Beckman Coulter). The results were indicated as mean values from three independent determinations.

**Cell Apoptosis Analysis.** Cell apoptosis was detected by flow cytometry according to a previously published method.[40] Briefly, cells were incubated with DMSO or different concentrations of compound **8b** for 24 h. The cells were harvested, washed twice with cold 1× PBS, and resuspended in 200 $\mu$L of binding buffer at a density of $1 \times 10^5$ cells/mL. The cells were then stained with 5 $\mu$L of annexin-V and PI


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for 15 min in dark conditions at room temperature and subjected to analysis by flow cytometry (Cytomics FC 500 MPL, Beckman Coulter). The early apoptosis was evaluated based on the percentage of cells with annexin V+/PI−, while the late apoptosis was that of annexin V+/PI+. The results were indicated as mean values from three independent determinations.

**Western Blot Analysis.** A2780 cells were treated with DMSO or different concentrations of compound **8b** for 24 h. Cells were harvested, washed with cold 1× PBS, and lysed with RIPA lysis buffer (Beyotime, China) for 30 min on ice, then centrifuged at 12000*g* for 15 min at 4 °C. The total protein concentration was determined by BCA protein assay kit (Beyotime, China). Equal amounts (30 $\mu$g per load) of protein samples were subjected to SDS−PAGE electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore) which were then blocked in 10% nonfat milk (BD Biosciences) and reacted with primary antibodies. The antibodies against Bcl-2, Bax, p53, MEK 1/2, phosphor-MEK1/2(Ser217/221), ERK1/2, phosphor-ERK1/2(Thr202/Tyr204) were purchased from Santa Cruz Biotechnology, and phosphor-p53, cleaved-PARP, and $\beta$-actin were purchased from Cell Signaling Technology. The secondary antibodies conjugated with horseradish peroxidase (HRP) were from Cell Signaling Technology. The protein bands were developed by the chemiluminescent reagents (Millipore).

**Acute Toxicity.** Female KM mice were purchased from Department of Laboratory Animal Science, Fudan University (Shanghai, China), and housed individually in a specific pathogen free facility. Groups of mice ($n$ = 10 per group) were injected intraperitoneally once with different concentrations of **8b** (500, 300, 275, 250, 225, 200 mg/kg) or vehicle control, respectively. The mouse death was monitored daily and recorded up to 14 days after injection. The animal experimental protocols were approved by the Animal Ethics Committee of School of Pharmacy, Fudan University.

**In Vivo Tumor Growth Inhibition Assessment.** Female BALB/c nude mice at 4−5 weeks of age were purchased from Shanghai Slac Laboratory Animal Co. Ltd. (Shanghai, China) and housed individually in a specific pathogen free facility. The mice were inoculated subcutaneously with A2780 cells ($2 \times 10^6$ suspended in 0.2 mL of PBS for each mouse). After reaching an average tumor volume of 100−150 $mm^3$, the animals were randomized into groups and treated intraperitoneally with 15 or 30 mg/kg **8b** (compound dissolved in 0.2 mL of olive oil) or vehicle control (0.2 mL olive oil). Administration of vehicle or compound and monitoring tumor progression were done once every 3 days. The tumor volumes were estimated by measuring the two dimensions of the tumors using a digital caliper and calculated by the formula $V = L \times W^2 \times 0.52$, with $V$ being volume, $L$ being length, and $W$ being width of the tumor nodules. At the end of the experiment, the mice were weighed and sacrificed, and their tumors were dissected out and weighed. The animal experimental protocols were approved by the Animal Ethics Committee of School of Pharmacy, Fudan University.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

The effect of hemoglobin on the proliferation of cells, method for MEK1 kinase inhibitory activity, high resolution mass spectral data for target compounds, HPLC assessment of compound purity, and $^1$H NMR and $^{13}$C NMR spectra of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Author Contributions

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

NO, nitric oxide; MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated protein kinase; MAPK, mitogen-activated protein kinase; VEGFR, vascular endothelial growth factor receptor; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-(dimethylamino)-pyridine; SAR, structure−activity relationship; DBU, 8-diazabicyclo[5.4.0]undec-7-ene; FDA, Food and Drug Administration; JS-K, 4-(2,4-dinitrophenoxy)-NNO-azoxy]piperazine-1-carboxylic acid ethyl ester

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