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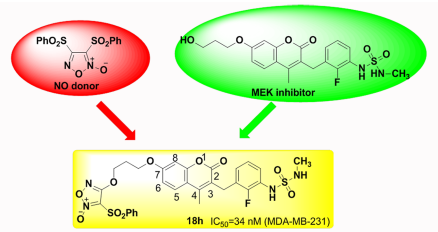


18h shows multitarget antitumor effects and better cell proliferation inhibition effect than **RO5126766** in MDA-MB-231 cells

Hybrids of MEK inhibitor and NO donor as multitarget antitumor drugs

Chao Wang, [a,1] Dandan Xi, [b,1] Han Wang, [b] Yan Niu, [b] Lei Liang, [b] Fengrong Xu, [b] Yihong Peng, [c] and Ping Xu*[b]

[a] National Pharmaceutical Teaching Laboratory Center, School of Pharmaceutical Sciences, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China

[b] Department of Medicinal Chemistry, School of Pharmaceutical Sciences, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China

[c] Department of Microbiology, School of Basic Medical Sciences, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China

*Corresponding author:

Email: pingxu@bjmu.edu.cn

[1]These authors contributed equally to this work.

Abstract

A series of hybrids of MEK inhibitor and nitric oxide donor have been designed and synthesized. Compound **18h [**4-(3-((3-(2-fluoro-3-((N-methylsulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy) propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide] was proven to be more potent than the clinical compound **RO5126766** in MDA-MB-231 cells. Compound **18h** can significantly reduce the levels of pMEK and pERK, induce cell apoptosis in MDA-MB-231 cells, and release NO in cells efficiently, suggesting that these hybrids, while displaying the properties of both MEK inhibitors and NO donors have a mechanism of action different from that of MEK inhibitors and NO donors. Thus, we are able to report a series of multitarget hybrids with better antitumor potency than a known MEK inhibitor and NO donor.

Keywords: Hybrid; MEK; NO donor; multitarget; antitumor.

Introduction

The RAS/RAF/MEK/ERK system is a key signaling pathway involved in the regulation of cell proliferation, differentiation and apoptosis. Aberrant activation of this pathway contributes to various cancers and other diseases. The mitogen-activated protein kinase (MEK) is one of the key kinases in this pathway and the extracellular signal-regulated kinase (ERK) appears to be the main substrate of MEK [1]. Allosteric MEK inhibitors have demonstrated *bona fide* antitumor efficacy and low toxicity in clinical trials. Two MEK inhibitors, **trametinib** (**1**) and **cobimetinib** (**2**) have received FDA approval for the treatment of metastatic melanoma [2]. The preliminary work showed that substituted 3-benzylcoumarins such as 3-benzyl-4-methyl-2-oxo-2H-chromen-7-yl dimethylcarbamate (**4**) and 3-benzyl-1,3-benzoxazine-2,4-dione analogues are potent allosteric MEK inhibitors [3-6]. The clinical compound **RO5126766** (**3**), which is in Phase I clinical trial, is a result of further optimization of the substituted 3-benzyl coumarins [2]. MEK inhibitors have the following characteristics: (1) they bind to the allosteric binding site of the protein without competing against ATP or ERK, (2) they cause several conformational changes of MEK and lock the unphosphorylated MEK in a catalytically inactive state, and (3) there is no homologous sequence between the inhibitor binding site of MEK and other protein kinases [7]. These properties made MEK a highly selective and very promising target for drug discovery.

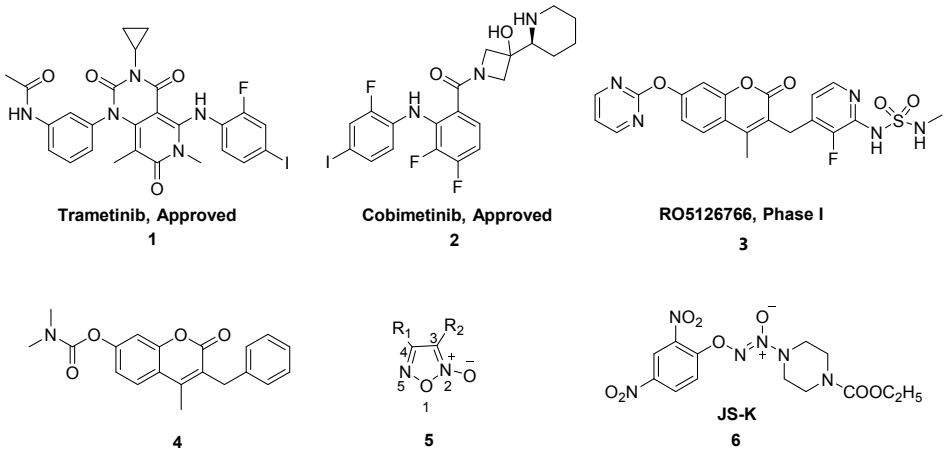


Fig. 1 The structures of MEK inhibitors and NO donors

In recent years, combination therapy has gained momentum over monotherapies in oncology due to a decreased likelihood of drug resistance. The combination of MEK inhibitors with other drugs such as BRAF inhibitors or PI3K/mTOR inhibitors has provided a new treatment choice for various cancers [8]. It has been reported that the combination of an MEK inhibitor and a nitric oxide (NO) donor can synergistically inhibit proliferation and invasion of cancer cells [9]. Hybrids of MEK inhibitor and NO donor already showed potent antitumor effects in many cancer cell lines[10-11]. Nitric oxide is a signaling molecule in many physiological and pathological processes. It has been reported that high levels of NO show very good anti-proliferation activity in various tumor cells [12]. There are several classes of NO donors, including organic nitrates, diazeniumdiolates, metal-NO complexes, furoxans, *S*-nitrosothiols and sydnonimines [12]. Of these, furoxans (**5**) are an important class of NO donors and can release NO when thiol-containing molecules or proteins attack either C3 or C4 of furoxans [13].

Both MEK inhibitors and NO donors have anti-proliferative activities in cells and we have designed and synthesized hybrids of a MEK inhibitory motif containing the coumarin core and a NO donor motif possessing phenylsulfonyl-substituted furoxan as multitarget antitumor drugs (Fig. 2) which would be expected to have synergistic antitumor effects and to be more potent than the individual known drugs (**1**, **2** or **3**). Their anti-proliferative effects have been evaluated in several cancer cells and their effects on the RAS/RAF/MEK/ERK pathway. Their pro-apoptotic effects and NO-releasing effects were also evaluated in an effort to gain a better understanding of the mechanism of action of these hybrid compounds.

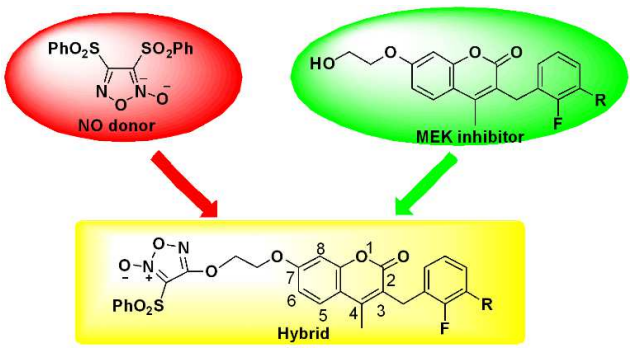


Fig. 2 Strategy for the discovery of hybrids containing a MEK inhibitor and a NO donor.

2. Results and discussion

2.1 Docking

The crystal structure of human MEK1 kinase in a complex with **RO5126766** and MgAMP-PNP (3WIG) was used as the docking MEK protein. Fig. 3 shows the binding mode of 4-(2-((3-(2-fluoro-3-((N-methylsulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**18a**) and the MEK protein (PDB ID: 3WIG) using the docking program GOLD. A linker was added from position 7 of the coumarin, as this site is on the exterior of the protein and should have no detrimental effect on the ligand-protein binding.

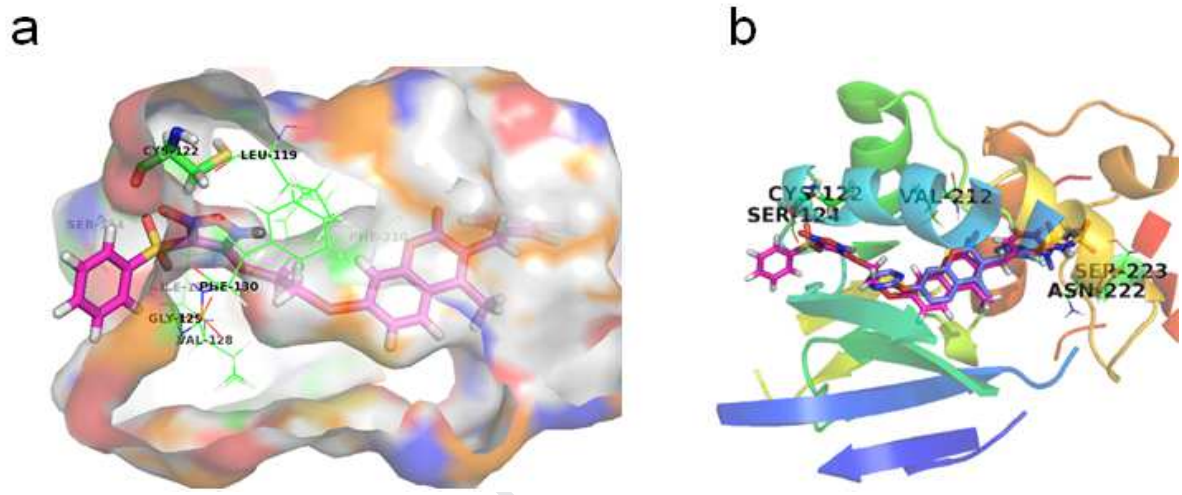


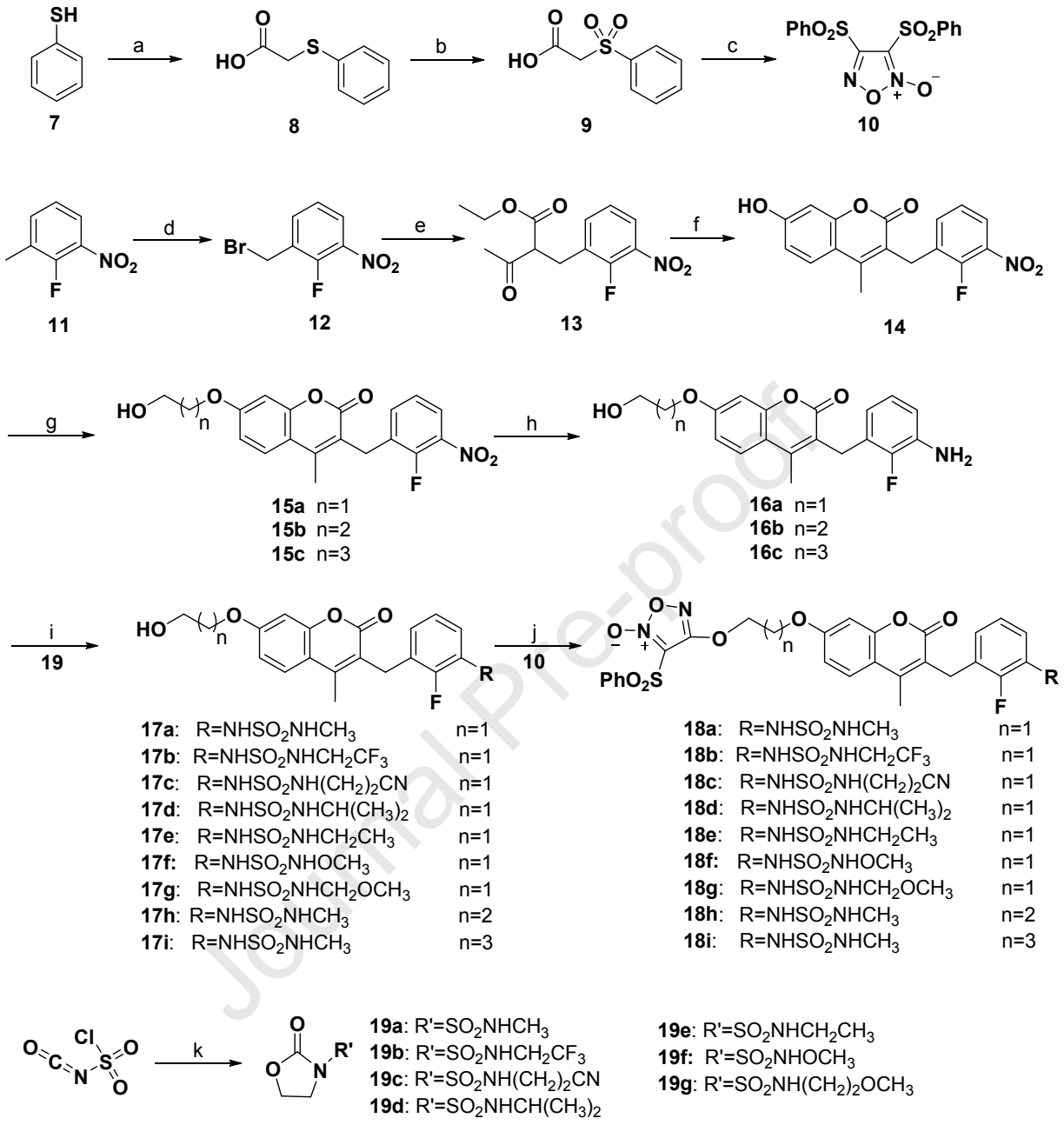
Fig. 3 (**a**) Binding mode of **18a** to the MEK protein (3WIG). (**b**) Superposition of the docked conformation of **18a** (pink) and **RO5126766** (purple) in the protein 3WIG crystal structure.

2.2 Chemistry

A series of hybrids of the MEK inhibitor and the NO donor with different substituents at the 3-benzyl group of the coumarin was synthesized using the synthetic route outlined in Scheme 1. The synthesis of the intermediate phenylsulfonyl-substituted furoxan (**10**) in three steps from benzenethiol (**7**) was reported previously[10, 14]. Briefly, benzenethiol (**7**) was reacted with chloroacetic acid to afford 2-(phenylthiol) acetic acid (**8**), which was then oxidized by 30% H_2O_2 to form compound **9** which was converted to **10** by treatment with fuming HNO_3. Compounds **16a**-**16c** were prepared according to the synthetic route showed below in Scheme 1. Commercially available 1-Methyl- 2-fluoro-3-nitrobenzene (**11**) was allowed to react with *N*-bromosuccinimide (NBS), to give 1-(bromomethyl)-2-fluoro-3-nitrobenzene (**12**). Compound **12** was reacted with ethyl acetoacetate affording the alkylation product ethyl 2-(2-fluoro-3-nitrobenzyl)-3-oxobutanoate (**13**). The coumarin (**14**) was synthesized by reacting compound **13** with resorcinol in a Pechmann reaction[15]. Compound **15**, which was obtained by reaction of coumarin **14** and halo alcohol (2-chloroethanol, 3-chloro-1-propanol or 4-chloro-1-butanol), was converted to **16a**-**16c** by reduction of the nitro group. Chlorosulfonylisocyanate was reacted with 2-bromoethanol followed by reaction of the in situ generated halogenosulfonyloxazolidinone with the appropriate primary

amine, in the presence of Et_3N to yield substituted oxazolidin-2-one **19a-19g**[15]. Compound **17a-17i**, which were made by reaction of **16a-16c** with **19a-19g** [16], was allowed to react with compound **10** affording the final product (**18a-18i**) using 8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a catalyst in CH_2Cl_2.

Scheme 1 Synthetic routes of target compounds.



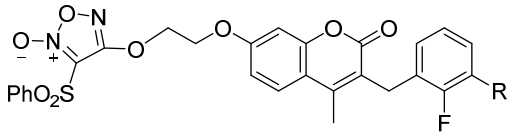
Reagents and conditions: (a) (i) NaOH (aq.), $ClCH_2COOH$, rt, 3 h; (ii) 6 mol/L HCl; (b) 30% H_2O_2, AcOH, rt, 3 h; (c) fuming HNO_3,100 ^oC, 4 h. (d) NBS, benzoyl peroxide, CCl_4, reflux, 13 h; (e) NaH (1.0 equiv), ethyl acetoacetate (1.0 equiv), THF, 0 ^oC, 12 h (2 steps); (f) resorcinol (1.0 equiv), conc. H_2SO_4, 0 ^oC, 12h; (g) 2-chloroethanol or 3-chloro-1-propanol or 4-chloro-1-butanol, K_2CO_3, acetone or DMF, reflux; (h) 10% Pd/C, H_2, MeOH, rt, 12 h. (i) pyridine or Et_3N, 12 h; (j) CH_2Cl_2, 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), -15 ^oC, 3 h; (k) 2-bromoethanol, amine, Et_3N, CH_2Cl_2.

2.3 Inhibition effects on cell proliferation.

We tested inhibition effects of compounds **17a-17g** on the cell growth in HCT116, A549, MDA-MB-231, A375 and HL60 cell lines (Table 1). The mutant KRAS was found in HCT116 and A549 cells, mutant KRAS and BRAF in MDA-MB-231 cells, mutant BRAF in A375 cells and NRAS

variants in HL60 cells [17-18]. In three cell lines, HCT116, A549 and MDA-MB-231, we found that the IC_{50} values of compounds **18a-18g** are better than the intermediates (**17a-17g**). In the A375 and HL60 cell lines the final products with a NO donor showed similar or reduced potency than the intermediates, suggesting that these NO-coupled MEK inhibitors have better effects in several but not all the cell lines. The HCT116, A549 and MDA-MB-231 cell lines are more sensitive than the A375 and HL60 cell lines to the compounds tested.

Table 1 Inhibition of cell viability by compounds in different cell lines (μM).[a]



	R	MDA-MB-231 ($KRAS^{G13D}$ /$BRAF^{G464V}$)	HCT116 ($KRAS^{G13D}$)	A549 ($KRAS^{G12S}$)	A375 ($BRAF^{V600E}$)	HL60 ($NRAS^{K61L}$)
18a	$NHSO_2NHCH_3$	0.093±0.05	0.67±0.31	1.10±0.57	0.33±0.05	0.53±0.27
18b	$NHSO_2NHCH_2CF_3$	0.13±0.09	0.61±0.16	2.51±1.31	0.5±0.09	0.27±0.11
18c	$NHSO_2NH(CH_2)_2CN$	0.27±0.16	0.76±0.28	2.61±1.31	0.66±0.21	0.64±0.19
18d	$NHSO_2NHCH(CH_3)_2$	0.86±0.59	1.23±0.82	2.99±0.70	0.97±0.22	0.55±0.53
18e	$NHSO_2CH_2CH_3$	0.18±0.11	0.72±0.18	2.57±1.24	0.54±0.18	0.29±0.10
18f	$NHSO_2NHOCH_3$	0.31±0.23	1.01±0.13	3.75±1.89	0.87±0.31	5.64±1.13
18g	$NHSO_2NHCH_2OCH_3$	0.38±0.24	0.71±0.23	2.27±0.97	0.54±0.15	0.31±0.25
Trametinib		0.018±0.011	0.019±0.012	0.46±0.27	0.0024±0.00076	0.003±0.0002
PD0325901		0.082±0.06	0.09±0.053	0.06±0.05	0.00077±0.00055	0.0013±0.0003
JS-K		2.90±1.65	0.50±0.18	0.69±0.08	1.99±0.37	0.53±0.02
RO5126766		0.17±0.17	0.053±0.017	1.24±0.06	0.017±0.009	0.006±0.001
17a	$NHSO_2NHCH_3$	2.19±1.33	3.47±2	>5	0.15±0.03	0.10±0.05
17b	$NHSO_2NHCH_2CF_3$	7.72±3.90	10.33±5.26	>5	0.93±0.14	0.78±0.23
17c	$NHSO_2NH(CH_2)_2CN$	5.49±2.55	15.40±3.79	>5	0.65±0.17	0.39±0.04
17d	$NHSO_2NHCH(CH_3)_2$	2.38±1.45	5.09±2.61	>5	0.15±0.08	0.14±0.06
17e	$NHSO_2CH_2CH_3$	2.48±1.33	5.79±2.08	>5	0.2±0.06	0.15±0.03
17f	$NHSO_2NHOCH_3$	ND	ND	ND	ND	ND
17g	$NHSO_2NHCH_2OCH_3$	2.99±1.45	6.50±1.85	>5	0.36±0.05	0.25±0

[a] Data are representative of 2-3 independent experiments. ND: not determined. MDA-MB-231: human breast cancer cell line. HCT116: human colorectal carcinoma cell line. A549: human non-small cell lung cancer cell line. A375: human malignant melanoma cell line. HL60: human promyelocytic leukemia cell line.

Among these 7 substituents at the meta-position of the 3-benzyl group, **18a**, which has the smallest R substituent, is the most potent and is as potent as **RO5126766** in A549 cells (1.1 μM vs. 1.24 μM) and more potent (0.093 μM) than the NO donor prodrug **JS-K** (**6**) (2.9 μM) and MEK inhibitor **RO5126766** (0.17 μM) in MDA-MB-231 cells. Compared to **18b-18g** with larger substituents, the potency of **18a** indicates that the protein pocket in R substituent area is not very big and small substituents are more favoured. Its intermediate (**17a**) is also more potent than the other MEK inhibitor intermediates **17b-17g**, suggesting that better inhibition effect of the MEK inhibitor motif is associated with better cell proliferation inhibition effect of the hybrid.

2.4 Compound **18a** reduces pMEK and pERK protein levels

We selected the most potent compound (**18a**) for western blot assays in the A549 cell line at the 2 h time point and compared it with the NO donor **JS-K** and the known MEK inhibitor **RO5126766**. The results showed that **18a** impacts the MAPK pathway immediately. At the 2 h time point, **18a** at concentrations of 10 μM and 1 μM, efficiently reduced the levels of pMEK and pERK proteins respectively, although somewhat less efficiently than **RO5126766** (Fig.4).

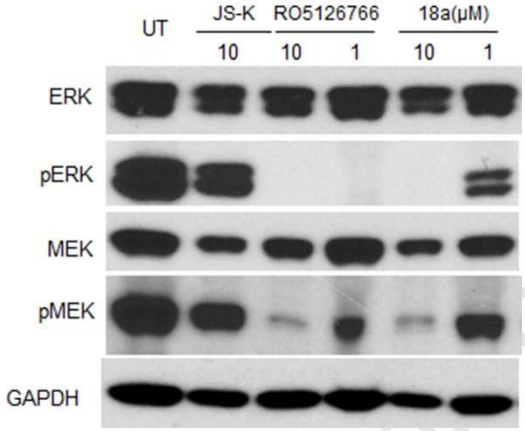


Fig. 4 Western blot results of **18a** in A549 cells at the 2 h time point with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as the loading control.

The effects of the best compound **18a** were further examined in MDA-MB-231 cells through the dose-dependent and the time-dependent assays. Fig. 5a shows that **18a** can efficiently reduce pMEK and pERK levels at 1, 3 and 10 μM after treatment for 24 h. Fig. 5b shows that the level of pERK and pMEK is obviously reduced by **18a** at a concentration of 10 μM at 2 h post-treatment. Almost complete depletion of these proteins was observed within 4 h.

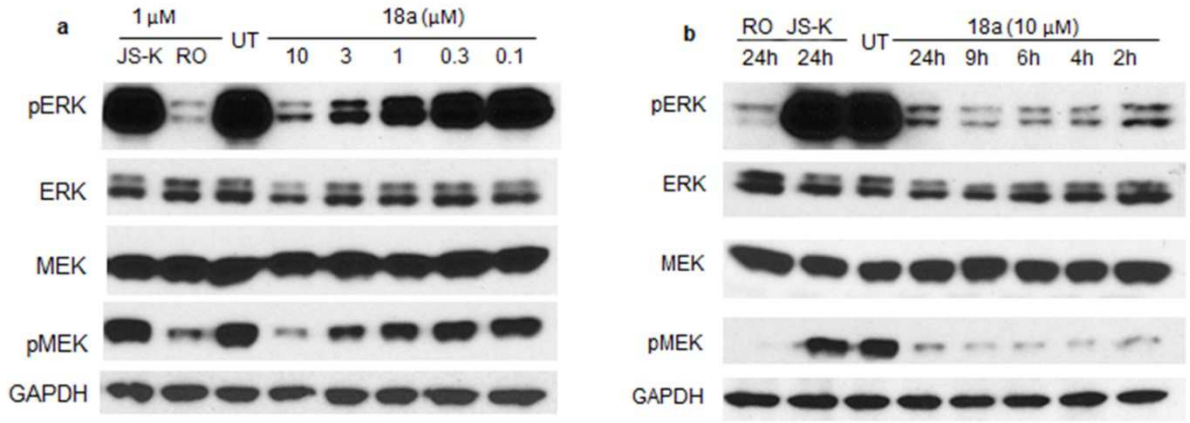


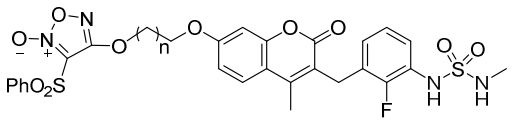
Fig. 5 (**a**)24 h dose-dependent western blot results of **18a** with 1 μM **JS-K** and **RO5126766** as negative and positive control, respectively. (**b**) time-dependent western blot results of **18a** with 10 μM **JS-K** and **RO5126766** in MDA-MB-231 cells.

2.5 Optimization of the linker

Focusing on the substituent group of **18a** we further optimized the linker connecting the NO donor moiety and the MEK inhibitor. Two compounds, **18h** and **18i**, with a longer linker (n=2-3) were made and compared with **18a** (n=1). The results in Table 2 show that **18h** with three carbons in the

linker has similar or better activity than **18a**, with IC_{50} values of 0.64 vs. 0.67 μM in HCT116, 1.35 vs. 1.10 μM in A549, and 0.034 vs. 0.093 μM in MDA-MB-231 cells. Compound **18i**, with the longest linker, displayed lower potency suggesting that longer linkers are not beneficial. It was concluded that a linker with 2-3 carbons is optimal for the activity.

Table 2 IC_{50} (μM) of compounds on cell viability against different cell lines.[a]



	n	MDA-MB-231	HCT116	A549	Vero	HL7702
RO5126766		0.17 ± 0.17	0.053 ± 0.017	1.24 ± 0.06	>50	17.27 ± 1.62
18a	1	0.093 ± 0.05	0.67 ± 0.31	1.10 ± 0.57	4.34 ± 0.32	2.78 ± 0.49
18h	2	0.034 ± 0.007	0.64 ± 0.30	1.35 ± 0.94	21.07 ± 1.32	5.62 ± 0.82
18i	3	0.20 ± 0.068	2.78 ± 1.08	3.49 ± 3.08	2.98 ± 0.14	1.93 ± 0.12
17a	1	2.19 ± 1.33	3.47 ± 2	>5	ND	ND
17h	2	1.90 ± 1.00	5.34 ± 3.10	>5	ND	ND
17i	3	3.20 ± 1.54	7.35 ± 2.15	>5	ND	ND
JS-K		2.90±1.65	0.50±0.18	0.69±0.08	1.16 ± 1.62	1.99 ± 0.22

[a]Data are representative of 2-3 independent experiments. ND: not determined. Vero: normal African green monkey kidney cell line. HL7702: human normal liver cell line.

We next determined the activity of **18a**, **18h-i** on normal cell lines including Vero cell line and HL7702 cell line. All the three compounds showed their activities at micromolar level. **18h** showed the lowest toxicity with IC_{50} values of 21.07 and 5.62 μM in these two cell lines, which possess lower toxicity than NO donor JS-K (1.99 μM). Therefore, **18h** may have a good therapeutic window in MDA-MB-231 breast cancer therapy in the future.

2.6 The reduction effect of **18h** on the levels of pERK and pMEK

We performed western blot assays in a dose-dependent and time-dependent manner of compound **18h**, the compound with the best cell growth inhibitory effect in MDA-MB-231 cells. Figure 6a shows that at the 2 h time point, **18h** at 1 μM and 10 μM concentrations can reduce the pMEK and pERK levels as efficiently as **RO5126766**. Figure 6b shows that a majority of pERK or pMEK is reduced by **18h** at 1 μM in 1 h or less.

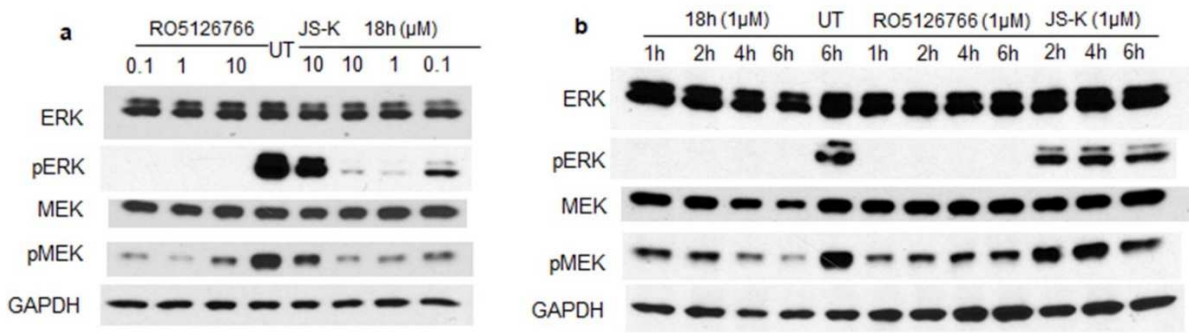


Fig. 6 (**a**)The effects of **RO5126766**, **JS-K** or **18h** on the levels of pERK and pMEK in MDA-MB-231 cells for 2 hours at different concentration and (**b**) at different time points.

2.7 Compound **18a** and **18h** induces apoptosis in MDA-MB-231 cells

Apoptosis assays in MDA-MB-231 cells showed that **18a** and **18h**, each at 10 μM and 1 μM can obviously induce cell apoptosis after treatment for 24 h. The NO donor **JS-K** at 10 μM can have similiar effects on cell apoptosis compared to **18a** and **18h** (Figure 7). In comparison, **RO5126766** has no obvious apoptotic effect on the cells, suggesting that hybrid and the MEK inhibitor may have different mechanisms of action.

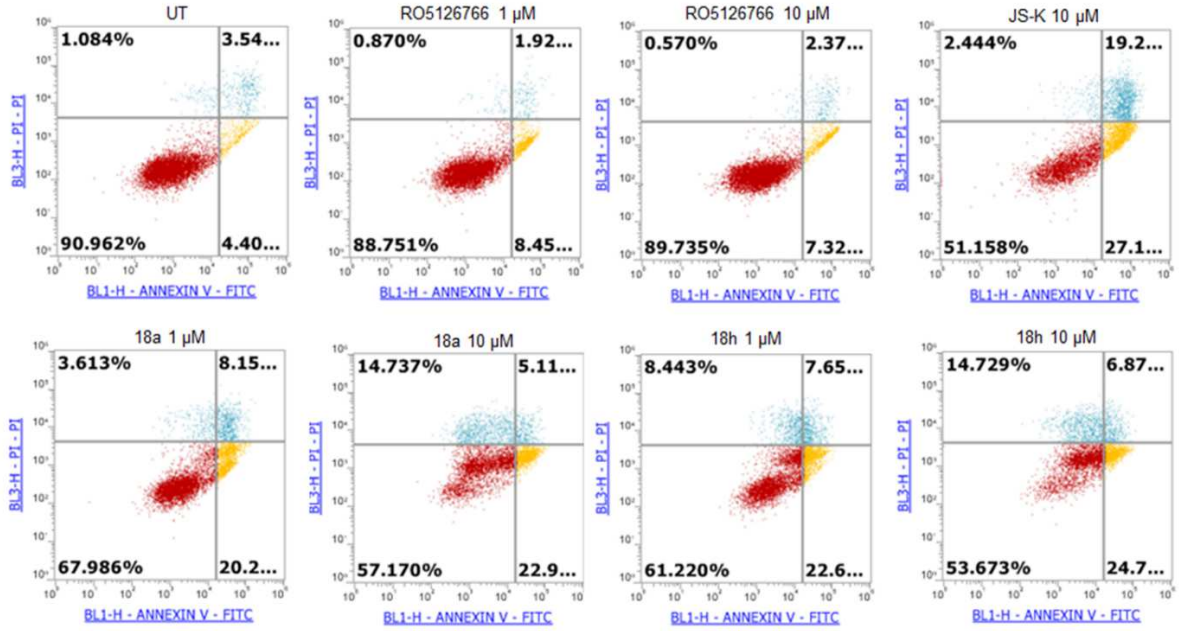


Fig. 7 18a and **18h** can induce cell apoptosis in MDA-MB-231 cells after 24 h treatment. MDA-MB-231 cells were treated with **DMSO**, **RO5126766**, **JS-K**, **18a or 18h** at the concentrations indicated for 24 h for flow cytometry analysis. Data are representative of 2 independent experiments. PI: propidium iodide.

2.8 NO release evaluation

NO release was evaluated in HCT116 cells with **RO5126766** and **JS-K** as negative and positive controls, respectively. The final compounds (**18a-i**) containing a NO donor can induce release of NO after 2 h treatment compared to the intermediates (**17a-i**). Compound **18h** can induce release of the highest amount of NO, even higher than **JS-K** (Fig.8a). Fig.8b shows the intracellular NO levels after 2 h treatment with **18a** or **18h** or the positive NO donor **JS-K** in HCT116 cells using the fluorescent indicator 4-Amino-5-methylamino-2', 7'-difluorofluorescein diacetate (DAF-FM-DA). These data suggest that our synthetic compounds (**18a-i**) can significantly induce NO release in cells.

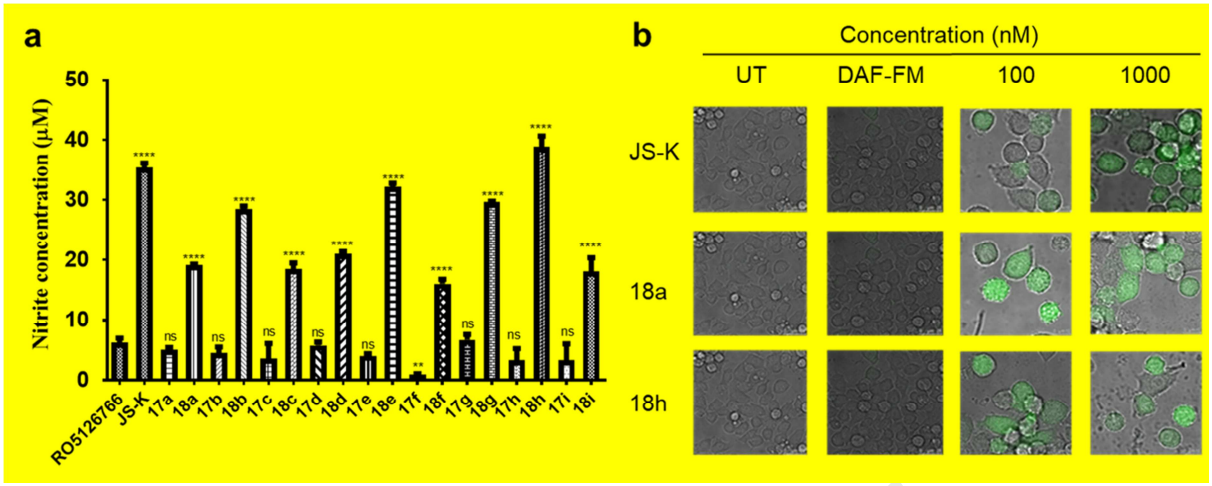


Fig. 8 Evaluation of NO release. (**a**) The NO-releasing effect in HCT116 cells after 2 h treatment with the compounds at 100 μM. The statistical significance was determined using 1way ANOVA. P value style GP:0.1234 (ns). 0.0332 (*). 0.0021 (**). 0.0002 (***). <0.0001 (****). (**b**) Intracellular NO levels in HCT116 cells changed 2 h treatment with the compounds as detected using the commercial fluorescent indicator DAF-FM DA. Experiments were repeated twice.

3. Conclusion

We have designed and synthesized a series of hybrids (**18a-i**) containing an MEK inhibitor motif and a NO donor motif. The structure-activity relationship indicated that small substituents at R position of the protein are more favoured. The linker connecting the NO donor moiety and the MEK inhibitor with 2-3 carbons is optimal for the activity. Better inhibition effect of the MEK inhibitor motif is associated with better cell proliferation inhibition effect of the hybrid. The release of NO can increase the anti-proliferation effect of MEK inhibitor.

Compound **18h** with 3 carbon linker induced five-fold more potent inhibition of cell proliferation than the MEK inhibitor **RO5126766** in MDA-MB-231 cells and showed low toxicity to normal cell lines tested, suggesting a broad therapeutic window. MDA-MB-231 cell line has been proved to be more sensitive to compound **18h** than other cell lines tested in this paper. Further research will be done to determine whether the compound is only specific to MDA-MB-231 cell line or also effective in other breast cancer cell lines.

Western blot analysis results in MDA-MB-231 and A549 cells demonstrated that compound **18h** efficiently reduces the levels of pMEK and pERK in a dose-dependent and time-dependent manner. Most of pERK and pMEK can be reduced by **18h** at 1 μM in 1 h, and **18h** is as efficient as the clinical candidate **RO5126766** in MDA-MB-231 cells while the NO donors do not affect this pathway. In contrast to **RO5126766**, **18a** and **18h** can also induce cell apoptosis in MDA-MB-231 cells. Qualitative and quantitative analysis of NO levels show that **18a** and **18h** can efficiently induce NO release in cells, suggesting that these hybrids have mechanism of action different from that in MEK inhibitors and NO donors since they produce the effects of both MEK inhibitors and NO donors. Thus, we are reporting a series of multitarget antitumor hybrids that have the

synergistic effects of both MEK inhibitors and NO donors. Further optimization of **18h** to improve its potency and PK/PD properties for in vivo studies is in progress.

4. Experimental section

4.1 Chemistry

4.1.1 General conditions

All reactions were carried out in oven-dried glassware, and monitored by thin layer chromatography (TLC). All reagents were reagent grade and purchased from commercial sources unless otherwise indicated. NMR spectra were recorded with a 400 MHz spectrometer for ^{1}H NMR, 100 MHz for ^{13}C NMR. Chemical shifts δ are given in ppm relative to the residual proton signals of the deuterated solvent DMSO in ^{1}H and ^{13}C NMR spectra. Multiplicities are reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q) or multiplet (m). High resolution mass spectra were recorded on an AB SCIEX FTMS ESI spectrometer. For column chromatography, silica gel (200-300 mesh) was used as the stationary phase.

4.1.2 Synthetic procedures

3-(2-Fluoro-3-nitrobenzyl)-7-hydroxy-4-methyl-2H-chromen-2-one (14).

The synthesis of the intermediate **14** from 2-fluoro-1-methyl-3-nitro-benzene has been reported previously[15].

General procedure for compounds 15a-15c.

The corresponding halo alcohol (2-chloroethanol, 3-chloro-1-propanol, 4-chloro-1-butanol) (2 mmol) and K_2CO_3 (3 mmol) were added to a stirred solution of **14** (1 mmol) in DMF (5 mL) at 70 °C . The mixture was refluxed for about 10 h and then poured into H_2O (50 mL). After filtration, the residue was washed with H_2O (3 × 10 mL) and dried with an infrared lamp, yielding **15a-15c** as a white solid.

3-(2-Fluoro-3-nitrobenzyl)-7-(2-hydroxyethoxy)-4-methyl-2H-chromen-2-one (15a)

The title compound was obtained from **14** and 2-chloro-1-ethanol as a white solid (60% yield, mp 163-165 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.49 (s, 3H, CH_3), 3.78 (dd, J = 9.9, 5.2 Hz, 2H, CH_2OH), 4.07 (s, 2H, CH_2), 4.14 (t, J = 4.8 Hz, 2H, OCH_2), 4.96 (t, J = 5.5 Hz, 1H, OH), 7.03 (dd, J = 4.6, 2.3 Hz, 2H, ArH), 7.35 (t, J = 8.0 Hz, 1H, ArH), 7.61 (t, J = 6.6 Hz, 1H, ArH), 7.80 (d, J = 9.6 Hz, 1H, ArH), 8.02 (t, J = 7.0 Hz, 1H, ArH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.62, 26.26, 59.83, 70.79, 101.47, 113.09, 113.83, 118.85, 124.55, 125.09 (d, $J_{C\text{-}F}$ = 4.6 Hz, 1C), 127.31, 129.65 (d, $J_{C\text{-}F}$ = 14.7 Hz, 1C), 136.31 (d, $J_{C\text{-}F}$ = 5.3 Hz, 1C), 137.76 (d, $J_{C\text{-}F}$ = 8.1 Hz, 1C), 150.25, 153.19 (d, $J_{C\text{-}F}$ = 259.4 Hz, 1C), 154.00, 161.28, 161.90; HRMS: exact mass calcd for $C_{19}H_{16}FNO_6$ $[M+H]^+$ 374.1040; found 374.1042.

3-(2-Fluoro-3-nitrobenzyl)-7-(3-hydroxypropoxy)-4-methyl-2H-chromen-2-one (15b)

The title compound was obtained from **14** and 3-chloro-1-propanol as a white solid (50% yield, mp 117-118 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 1.92 (t, J = 6.3 Hz, 2H, CH_2), 2.48 (s, 3H, CH_3), 3.59 (q, J = 6.0 Hz, 2H, CH_2OH), 4.07 (s, 2H, CH_2), 4.18 (t, J = 6.3 Hz, 2H, OCH_2), 4.64 (t, J = 5.1 Hz, 1H, OH), 7.01 (d, J = 3.6 Hz, 2H, ArH), 7.35 (t, J = 8.0 Hz, 1H, ArH), 7.61 (t, J = 6.9 Hz, 1H, ArH), 7.84 – 7.74 (m, 1H, ArH), 8.01 (t, J = 7.6 Hz, 1H, ArH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.60, 26.22 (d, $J_{C\text{-}F}$ = 3.6 Hz, 1C), 32.31, 57.57, 65.87, 101.40, 113.01, 113.78, 118.82, 124.53, 125.08 (d, $J_{C\text{-}F}$ = 4.6 Hz, 1C), 127.29, 129.64 (d, $J_{C\text{-}F}$ = 14.6 Hz, 1C), 136.30 (d, $J_{C\text{-}F}$ = 5.2 Hz, 1C), 137.75 (d, $J_{C\text{-}F}$ = 8.3 Hz, 1C), 150.22, 153.18 (d, $J_{C\text{-}F}$ = 259.5 Hz, 1C), 154.00, 161.27, 161.85; HRMS: exact mass calcd for $C_{20}H_{18}FNO_6$ $[M+H]^+$ 388.1196; found 388.1187.

3-(2-Fluoro-3-nitrobenzyl)-7-(4-hydroxybutoxy)-4-methyl-2H-chromen-2-one (15c)

The title compound was obtained from **8** and 4-chloro-1-butanol as a white solid (20% yield, mp 93-94 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 1.66 – 1.50 (m, 2H, CH_2), 1.88 – 1.73 (m, 2H, CH_2), 2.49 (s, 3H, CH_3), 3.49 (dd, *J* = 11.7, 6.3 Hz, 2H, CH_2O), 4.07 (s, 2H, CH_2), 4.12 (t, *J* = 6.5 Hz, 2H, OCH_2), 4.52 (t, *J* = 5.1 Hz, 1H, OH), 7.01 (d, *J* = 7.5 Hz, 2H, ArH), 7.35 (t, *J* = 8.0 Hz, 1H, ArH), 7.61 (t, *J* = 6.6 Hz, 1H, ArH), 7.80 (d, *J* = 9.5 Hz, 1H, ArH), 8.02 (t, *J* = 7.0 Hz, 1H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.40, 25.52, 26.41, 29.19, 62.45, 68.32, 101.09, 112.93, 113.72, 119.14, 124.01 (d, *J* = 4.8 Hz, 1C), 124.27, 125.96, 129.23 (d, *J* = 14.8 Hz, 1C), 136.57 (d, *J* = 5.4 Hz,1C), 149.10, 153.75 (t, *J* = 131.4 Hz, 1C), 154.02, 161.70, 161.97,; HRMS: exact mass calcd for $C_{21}H_{20}FNO_6$ $[M+H]^+$ 402.1353; found 402.1355.

3-(3-Amino-2-fluorobenzyl)-7-(2-hydroxyethoxy)-4-methyl-2H-chromen-2-one (16a-16c)

A catalytic amount of Pd/C (0.12 mmol) was added to a stirred solution of **15a-15c** (1.2 mmol) in MeOH (20 mL). The mixture was stirred under a hydrogen atmosphere (0.4 MPa) for 12 h at room temperature then the catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure. The fluoroaniline intermediates (**16a-16c)** were obtained without further purification.

General procedure for compounds 17a-17i.

Compound **19a-19g** (2.66 mmol) and Et_3N (3.99 mmol) were added to a solution of **16a-16c** (1.33 mmol) in anhydrous MeCN (15 mL) at room temperature. The reaction mixture was heated to 75 ^{o}C for 5 -12 h and then the mixture was cooled to room temperature and diluted with EtOAc. The mixture was washed with 1 mol/L aqueous HCl, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and evaporated. The residue was purified by silica gel chromatography (CH_2Cl_2/CH_3OH) to give compounds **17a-17i**.

3-(2-Fluoro-3-(N-methylsulfamoylamino)benzyl)-4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17a)

The title compound was obtained from **16** reacting with **19a** as a white solid (65% yield, mp 85-86 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.40 (s, 3H, CH_3), 2.53 (d, *J* = 5.0 Hz, 3H, $NHCH_3$), 3.75 (dd, *J* = 9.8, 5.1 Hz, 2H, CH_2OH), 3.94 (s, 2H, CH_2), 4.10 (t, *J* = 4.8 Hz, 2H, OCH_2), 4.94 (t, *J* = 5.5 Hz, 1H, OH), 6.85 (t, *J* = 6.8 Hz, 1H, ArH), 7.05 – 6.93 (m, 3H, ArH), 7.34 – 7.15 (m, 2H, ArH), 7.74 (d, *J* = 9.4 Hz, 1H, NH), 9.37 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.52, 25.99, 28.78, 59.83, 70.75, 101.44, 113.01, 113.85, 119.65, 122.69, 124.35 (d, J_{C-F} = 4.1 Hz, 1C), 125.23, 126.30 (d, J_{C-F} = 13.4 Hz, 1C), 126.70 (d, J_{C-F} = 14.4 Hz, 1C), 127.12, 149.65, 153.08 (d, J_{C-F} = 240.0 Hz, 1C), 153.90, 161.42, 161.77; HRMS: exact mass calcd for $C_{20}H_{21}FN_2O_6S$ $[M+H]^+$ 437.1183 ; found 437.1173.

3-(2-Fluoro-3-(N-(2,2,2-trifluoroethyl)sulfamoyl-amino)benzy)-l-4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17b)

The title compound was obtained from **16** reacting with **19b** as a white solid (52% yield, mp 101-102 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.40 (s, 3H, CH_3), 3.73 – 3.64 (m, 2H, CH_2CF_3), 3.75 (t, *J* = 4.8 Hz, 2H, CH_2OH), 3.94 (s, 2H, CH_2), 4.10 (t, *J* = 4.8 Hz, 2H, OCH_2), 6.91 (t, *J* = 6.7 Hz, 1H, ArH), 7.06 – 6.94 (m, 3H, ArH), 7.29 (dd, *J* = 7.7, 6.7 Hz, 1H, ArH), 7.81 – 7.68 (m, 1H, ArH), 8.35 (t, *J* = 6.8 Hz, 1H, NH), 9.60 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.52, 26.03, 43.78 (d, J_{C-F} = 34.3 Hz, 1C), 59.84

(2C), 70.77, 101.45, 113.03, 113.87, 119.63, 123.37 (d, J_{C-F} = 8.0 Hz, 1C), 124.44 (d, J_{C-F} = 4.2 Hz, 1C), 125.60 (d, J_{C-F} = 13.4 Hz, 1C), 126.11 (d, J_{C-F} = 15.4 Hz, 1C), 126.85 (d, J_{C-F} = 14.4 Hz, 1C), 127.14, 149.67, 153.54 (d, J_{C-F} = 244.78 Hz, 1C), 153.92, 161.43, 161.79; HRMS: exact mass calcd for $C_{21}H_{18}FNO_6$ $[M+H]^+$ 505.1056; found 505.1055.

3-(2-Fluoro-3-(N-(2-cyanoethyl)sulfamoylamino)benzyl)-4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17c)

The title compound was obtained from **16** and **19c** as a white solid (49% yield, mp 65-66 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.44 (s, 3H, CH_3), 2.70 (dd, *J* = 19.5, 12.9 Hz, 2H, CH_2CN), 3.28 – 3.13 (m, 2H, $NHCH_2$), 3.78 (d, *J* = 4.6 Hz, 1H), 3.97 (s, 2H, CH_2), 4.13 (t, *J* = 4.8 Hz, 1H), 4.42 (s, 2H, OCH_2), 5.78 (s, 1H, OH), 6.93 (d, *J* = 7.0 Hz, 1H, ArH), 7.09 – 6.97 (m, 3H, ArH), 7.31 (d, *J* = 7.6 Hz, 1H, ArH), 7.87 – 7.67 (m, 2H, ArH, NH), 9.53 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.55, 18.24, 55.37, 59.93, 66.46, 68.22, 70.83, 101.36, 112.98 (d, J_{C-F} = 9.5 Hz, 1C), 114.09 (d, J_{C-F} = 44.6 Hz, 1C) , 119.80 (d, J_{C-F} = 33.9 Hz, 1C), 123.26, 124.43, 125.77 (d, J_{C-F} = 8.5 Hz, 1C), 125.94, 126.86 (d, J_{C-F} = 5.6 Hz, 1C), 127.22 (d, J_{C-F} = 16.3 Hz, 1C), 149.64 (d, J_{C-F} = 6.1 Hz, 1C), 153.41 (d, J_{C-F} = 244.09 Hz, 1C), 160.89, 161.39 (d, J_{C-F} = 8.0 Hz, 1C), 161.78; HRMS: exact mass calcd for $C_{22}H_{22}FN_3O_6S$ $[M+H]^+$ 476.1292; found 476.1286.

3-(2-Fluoro-3-(N-isopropylsulfamoylamino)benzyl)-4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17d)

The title compound was obtained from **16** and **19d** as a white solid (48% yield, mp 178-179 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 0.99 (d, *J* = 6.3 Hz, 6H, CH_3CH_3), 2.39 (s, 3H, CH_3), 3.39 (dd, *J* = 12.9, 6.1 Hz, 1H, CH), 3.75 (t, *J* = 3.5 Hz, 2H, CH_2OH), 3.94 (s, 2H, CH_2), 4.10 (s, 2H, OCH_2), 4.93 (s, 1H, OH), 6.85 (d, *J* = 6.6 Hz, 1H, ArH), 7.00 (q, *J* = 7.6 Hz, 3H, ArH), 7.30 (d, *J* = 7.7 Hz, 2H, ArH), 7.74 (d, *J* = 8.9 Hz, 1H, NH), 9.33 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.53, 23.53, 25.96, 45.38, 59.84, 70.76, 101.43, 113.01, 113.87, 119.78, 121.63, 124.26, 124.76, 126.52, 126.70 (d, J_{C-F} = 7.1 Hz, 1C), 126.86, 127.10, 149.51, 152.44 (d, J_{C-F} = 243.3 Hz, 1C), 153.90, 161.40, 161.76; HRMS: exact mass calcd for $C_{22}H_{25}FN_2O_6S$ $[M+H]^+$ 465.1496 ; found 465.1485.

3-(2-Fluoro-3-(N-ethylsulfamoylamino)benzyl)4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17e)

The title compound was obtained from **16** and **19e** as a white solid (58% yield, mp 87-88 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 1.02 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.43 (s, 3H, CH_3), 3.05 – 2.84 (m, 2H, CH_2CH_3), 3.78 (dd, *J* = 9.9, 5.2 Hz, 2H, CH_2OH), 3.97 (s, 2H, CH_2), 4.13 (t, *J* = 4.8 Hz, 2H, OCH_2), 4.96 (t, *J* = 5.5 Hz, 1H, OH), 6.88 (t, *J* = 6.8 Hz, 1H, ArH), 7.11 – 6.94 (m, 3H, ArH), 7.33 (dd, *J* = 11.2, 5.8 Hz, 2H, ArH), 7.77 (d, *J* = 9.5 Hz, 1H, NH), 9.34 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.04, 15.53, 37.58, 59.84, 70.76, 101.44, 113.44 (d, J_{C-F} = 85.3 Hz, 1C), 119.73, 122.47, 124.31 (d, J_{C-F} = 4.1 Hz, 1C), 125.14, 126.41, 126.57 (d, J_{C-F} = 5.3 Hz, 1C), 126.74, 127.12, 149.59, 151.73, 153.90, 154.17, 161.42, 161.77; HRMS: exact mass calcd for $C_{21}H_{23}FN_2O_6S$ $[M+H]^+$ 451.1339; found 451.1332.

3-(2-Fluoro-3-(N-methoxylsulfamoylamino)benzyl)-4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17f)

The title compound was obtained from **16** and **19f** as a white solid (45% yield, mp > 300 °C). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 2.09 (s, 1H, NHO), 2.40 (s, 3H, CH_3), 3.62 (s, 3H, OCH_3), 3.74 (d, *J* = 4.6 Hz, 2H, CH_2OH), 3.93 (s, 2H, CH_2), 4.10 (t, *J* = 4.7 Hz, 2H, OCH_2), 4.94 (s, 1H, OH), 6.89 (s, 1H, ArH), 7.10 – 6.93 (m, 3H, ArH), 7.27 (t, *J* = 7.3 Hz, 1H, ArH), 7.76 (d, *J* = 8.8 Hz, 1H, ArH), 9.95 (s, 1H, NH); HRMS: exact mass calcd for $C_{20}H_{18}FNO_6$ $[M+H]^+$ 453.1132; found 453.1138.

3-(2-Fluoro-3-(N-methoxyethylsulfamoylamino)benzyl)-4-methyl-7-(2-hydroxyethoxy)-2H-chromen-2-one (17g)

The title compound was obtained from **16** and **19g** as a white solid (56% yield, mp 56-57 °C). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 2.43 (s, 3H, CH_3), 3.08 (d, *J* = 5.9 Hz, 2H, CH_2O), 3.19 (s, 3H, OCH_3), 3.35 (dd, *J* = 8.1, 3.9 Hz, 2H, $NHCH_2$), 3.78 (dd, *J* = 9.9, 5.1 Hz, 2H, OCH_2), 3.96 (s, 2H, CH_2), 4.13 (t, *J* = 4.8 Hz, 2H, OCH_2), 4.95 (t, *J* = 5.5 Hz, 1H, OH), 6.88 (t, *J* = 6.9 Hz, 1H, ArH), 7.08 – 6.96 (m, 3H, ArH), 7.34 (t, *J* = 7.4 Hz, 1H, ArH), 7.50 (t, *J* = 5.8 Hz, 1H, ArH), 7.77 (d, *J* = 9.6 Hz, 1H, NH), 9.38 (s, 1H, NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ 15.51, 26.02, 26.79, 42.19, 58.30, 59.83, 70.80, 101.42, 113.00, 113.85, 119.69, 122.35, 124.32 (d, J_{C-F} = 4.0 Hz, 1C), 125.15, 126.39 (d, J_{C-F} = 13.3 Hz, 1C), 126.64 (d, J_{C-F} = 14.2 Hz, 1C), 127.11, 149.58, 152.89 (d, J_{C-F} = 243.7 Hz, 1C), 153.89, 161.40, 161.76; HRMS: exact mass calcd for $C_{22}H_{25}FN_2O_7S$ $[M+H]^+$ 481.1445; found 481.1439.

3-(2-Fluoro-3-(N-methylsulfamoylamino)benzyl)-4-methyl-7-(3-hydroxyethoxy)-2H-chromen-2-one (17h)

The title compound was obtained from **16b** and **19a** as a white solid (60% yield, mp 59-60 °C). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ 1.97 – 1.87 (m, 2H, CH_2), 2.43 (s, 3H, CH_3), 2.56 (s, 3H, $NHCH_3$), 3.59 (q, *J* = 5.8 Hz, 2H, CH_2O), 3.96 (s, 2H, CH_2), 4.18 (t, *J* = 6.3 Hz, 2H, OCH_2), 4.61 (t, *J* = 4.9 Hz, 1H, OH), 6.88 (t, *J* = 7.1 Hz, 1H, ArH), 7.02 (dd, *J* = 17.1, 7.3 Hz, 3H, ArH), 7.22 (d, *J* = 5.0 Hz, 1H, ArH), 7.30 (t, *J* = 8.0 Hz, 1H, ArH), 7.78 (d, *J* = 9.6 Hz, 1H, NH), 9.36 (s, 1H, NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ 15.53, 26.83, 28.77, 32.32, 57.57, 65.85, 101.40, 112.96, 113.82, 119.63, 122.68, 124.35 (d, J_{C-F} = 4.2 Hz, 1C), 125.21 (d, J_{C-F} = 3.0 Hz, 1C), 126.30 (d, J_{C-F} = 13.1 Hz, 1C), 126.70 (d, J_{C-F} = 14.6 Hz, 1C), 127.15, 149.67, 153.07 (d, J_{C-F} = 245.5 Hz, 1C), 153.93, 161.43, 161.74; HRMS: exact mass calcd for $C_{21}H_{23}FN_2O_6S$ $[M+H]^+$ 451.1339; found 451.1336.

3-(2-Fluoro-3-(N-methylsulfamoylamino)benzyl)-4-methyl-7-(4-hydroxyethoxy)-2H-chromen-2-one (17i)

The title compound was obtained from **16c** and **19a** as a white solid (40% yield, mp 57-58 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.81 – 1.71 (m, 2H, CH_2), 1.97 – 1.83 (m, 2H, CH_2), 2.40 (s, 3H, CH_3), 2.75 (d, *J* = 5.2 Hz, 3H, $NHCH_3$), 3.64 (t, *J* = 7.9 Hz, 2H, CH_2O), 3.74 (t, *J* = 6.0 Hz, 2H, CH_2), 4.46 (t, *J* = 7.9 Hz, 2H, OCH_2), 4.71 (d, *J* = 5.0 Hz, 1H, OH), 6.75 (s, 1H, ArH), 6.81 (s, 1H, ArH), 6.87 (d, *J* = 8.6 Hz, 1H, ArH), 7.04 – 6.89 (m, 2H, ArH), 7.38 (t, *J* = 7.5 Hz, 1H, ArH), 7.53 (d, *J* = 8.9 Hz, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.28, 25.54, 29.21, 40.59, 62.43, 64.96, 68.33, 101.14, 112.79, 113.84, 119.57, 119.92, 124.48 (d, J_{C-F} = 4.3 Hz, 1C), 125.44 (d, J_{C-F} = 3.9 Hz, 1C),125.77, 126.40 (d, J_{C-F} = 14.3 Hz, 1C), 148.70, 151.47 (d, J_{C-F} = 242.5 Hz, 1C), 154.01, 160.29, 161.57, 162.07; HRMS: exact mass calcd for $C_{22}H_{25}FN_2O_6S$ $[M+H]^+$ 465.1496; found 465.1483.

General Procedure for the preparation of 18a-18i.

Compound **10** (1 mmol) was added to a stirred solution of compound **17a-17i** (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 H_2O (3 × 10 mL). The organic layer was dried with anhydrous Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by silica gel chromatography to give compounds **18a-18i**.

4-(2-((3-(2-Fluoro-3-((N-methylsulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18a)

The title compound was obtained from **10** and **17a** as a white solid (80% yield, mp 167-168 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.43 (s, 3H, CH_3), 3.96 (s, 2H, CH_2O), 4.51 (s, 2H, CH_2), 4.77 (s, 2H, OCH_2), 6.86 (d, *J* = 6.8 Hz, 1H, ArH), 7.14 – 6.96 (m, 3H, ArH), 7.33 – 7.14 (m, 2H, ArH), 7.67 (t, *J* = 7.7 Hz, 2H, ArH), 7.83 (dd, *J* = 14.8, 8.0 Hz, 2H, ArH), 7.98 (d, *J* = 7.5 Hz, 2H, ArH, NH), 9.38 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.58, 25.99, 28.78, 31.15, 66.57, 70.18, 101.74, 111.00, 113.10, 114.35, 120.03, 122.69, 124.36 (d, J_{C-F} = 4.4 Hz, 1C), 125.22, 126.33 (d, J_{C-F} = 13.5 Hz, 1C), 126.67 (d, J_{C-F} = 14.5 Hz, 1C), 127.32, 128.73, 130.41 (2C), 136.57, 137.63, 149.62, 151.87, 153.89, 154.31, 160.31 (d, J_{C-F} = 214.77 Hz, 1C), 161.00; HRMS: exact mass calcd for $C_{28}H_{25}FN_4O_{10}S_2$ $[M+Na]^+$ 683.0894; found 683.0874.

4-(2-((3-(2-Fluoro-3-((N-(2,2,2-trifluoroethyl)sulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18b)

The title compound was obtained from **10** and **17b** as a white solid (78% yield, mp 188-190 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.43 (s, 3H, CH_3), 3.71 (dd, *J* = 9.4, 6.9 Hz, 2H, CH_2CF_3), 3.96 (s, 2H, CH_2O), 4.57 – 4.43 (m, 2H, CH_2), 4.78 (dd, *J* = 4.0, 1.6 Hz, 2H, OCH_2), 6.91 (t, *J* = 6.7 Hz, 1H, ArH), 7.12 – 6.96 (m, 3H, ArH), 7.30 (t, *J* = 7.3 Hz, 1H, ArH), 7.66 (dd, *J* = 10.8, 5.0 Hz, 2H, ArH), 7.88 – 7.75 (m, 2H, ArH), 8.04 – 7.93 (m, 2H, ArH), 8.38 (t, *J* = 6.8 Hz, 1H, NH), 9.63 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.57, 26.02, 43.76 (d, J_{C-F} = 33.6 Hz, 1C), 66.56 (2C), 70.17, 101.72, 111.00, 113.09, 114.34, 119.98, 123.37 (d, J_{C-F} = 9.5 Hz, 1C), 124.45 (d, J_{C-F} = 4.2 Hz, 1C), 125.62 (d, J_{C-F} = 13.3 Hz, 1C), 126.00, 126.81 (d, J_{C-F} = 14.2 Hz, 1C), 127.30, 128.74, 130.40 (2C), 136.56, 137.62, 149.63, 153.53 (d, J_{C-F} = 244.12 Hz, 1C), 153.90, 159.24 (2C), 161.00, 161.39; HRMS: exact mass calcd for $C_{29}H_{24}F_4N_4O_{10}S_2$ $[M+Na]^+$ 751.0768; found 751.0742.

4-(2-((3-(3-((N-(2-Cyanoethyl)sulfamoyl)amino)-2-fluorobenzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18c)

The title compound was obtained from **10** and **17c** as a white solid (81% yield, mp 161-162 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.43 (s, 3H, CH_3), 2.66 (t, *J* = 6.5 Hz, 2H, CH_2CN), 3.16 (q, *J* = 6.4 Hz, 2H, $NHCH_2$), 3.96 (s, 2H, CH_2O), 4.56 – 4.43 (m, 2H, CH_2), 4.82 – 4.70 (m, 2H, OCH_2), 6.89 (t, *J* = 6.7 Hz, 1H, ArH), 7.13 – 6.95 (m, 3H, ArH), 7.29 (t, *J* = 7.2 Hz, 1H, ArH), 7.67 (dd, *J* = 8.3, 7.6 Hz, 2H, ArH), 7.88 – 7.73 (m, 2H, ArH), 7.98 (dd, *J* = 8.5, 1.1 Hz, 2H, ArH, NH), 9.53 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.50, 18.24, 25.89, 38.81, 66.54, 69.97, 101.69, 111.01, 113.10, 114.36, 119.33, 119.99, 123.26, 124.43, 125.72, 125.89 (d, J_{C-F} = 13.5 Hz, 1C), 126.78 (d, J_{C-F} = 14.5 Hz, 1C), 127.32, 128.74 (2C),

130.41 (2C), 136.57, 137.63, 149.63, 153.41 (d, J_{C-F} = 245.4 Hz, 1C), 153.90, 159.24, 161.00, 161.38; HRMS: exact mass calcd for $C_{30}H_{26}FN_5O_{10}S_2$ $[M+H]^+$ 722.1003; found 722.1002.

4-(2-((3-(2-Fluoro-3-((N-isopropylsulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18d)

The title compound was obtained from **10** and **17d** as a white solid (85% yield, mp 104-105 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 0.99 (d, *J* = 6.3 Hz, 6H, CH_3CH_3), 2.39 (s, 3H, CH_3), 2.66 (t, *J* = 6.5 Hz, 2H, CH_2CN), 3.16 (q, *J* = 6.4 Hz, 2H, $NHCH_2$), 3.96 (s, 2H, CH_2O), 4.56 – 4.43 (m, 2H, CH_2), 4.82 – 4.70 (m, 2H, OCH_2), 6.89 (t, *J* = 6.7 Hz, 1H, ArH), 7.13 – 6.95 (m, 3H, ArH), 7.29 (t, *J* = 7.2 Hz, 1H, ArH), 7.67 (dd, *J* = 8.3, 7.6 Hz, 2H, ArH), 7.88 – 7.73 (m, 2H, ArH), 7.98 (dd, *J* = 8.5, 1.1 Hz, 2H, ArH, NH), 9.53 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.51, 24.59 (2C), 24.09, 45.27, 66.35, 70.08, 101.71, 110.99, 113.07, 114.35 (2C), 120.15, 121.63, 124.26, 124.75, 126.54 (d, J_{C-F} = 14.5 Hz, 1C), 126.81 (d, J_{C-F} = 13.0 Hz, 1C), 128.73, 130.40, 136.56 (2C), 137.63 (2C), 149.46, 152.44 (d, J_{C-F} = 244.9 Hz, 1C), 153.66, 159.23, 160.97, 161.34; HRMS: exact mass calcd for $C_{30}H_{29}FN_4O_{10}S$ $[M+Na]^+$ 711.1207; found 711.1180.

4-(2-((3-(3-((N-Ethylsulfamoyl)amino)-2-fluorobenzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18e)

The title compound was prepared from **10** and **17e** as a white solid (82% yield, mp 95-96 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 0.99 (t, *J* = 7.2 Hz, 3H, CH_2CH_3), 2.42 (s, 3H, CH_3), 2.93 (dd, *J* = 7.2, 5.7 Hz, 2H, CH_2CH_3), 3.95 (s, 2H, CH_2OH), 4.61 – 4.38 (m, 2H, CH_2), 4.82 – 4.69 (m, 2H, OCH_2), 6.86 (t, *J* = 6.6 Hz, 1H, ArH), 7.04 (ddd, *J* = 15.7, 15.2, 5.2 Hz, 3H, ArH), 7.31 (dt, *J* = 12.2, 6.7 Hz, 2H, ArH), 7.75 – 7.55 (m, 2H, ArH), 7.86 – 7.75 (m, 2H, ArH), 7.98 (dd, *J* = 8.5, 1.1 Hz, 2H, ArH, NH), 9.35 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.58, 26.02, 43.51, 43.83, 66.55, 70.16, 101.69, 112.05 (d, J_{C-F} = 211.7 Hz, 1C), 114.33, 119.96, 123.35 (d, J_{C-F} = 11.1 Hz, 1C), 124.43, 125.60 (d, J_{C-F} = 13.3 Hz, 1C), 126.06 (d, J_{C-F} = 22.5 Hz, 1C), 126.8 (d, J_{C-F} = 14.4 Hz, 1C), 127.32, 128.74, 130.41 (2C), 136.58, 137.59, 149.66, 152.28, 153.88, 154.73, 159.23, 160.99, 161.39; HRMS: exact mass calcd for $C_{29}H_{27}FN_4O_{10}S_2[M+K]^+$ 713.0790; found 713.0405.

4-(2-((3-(2-Fluoro-3-((N-methoxysulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18f)

The title compound was obtained from **10** and **17f** as a white solid (78% yield, mp 127-128 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.45 (s, 3H, CH_3), 3.65 (s, 3H, OCH_3), 3.98 (s, 2H, CH_2OH), 4.59 – 4.46 (m, 2H, CH_2), 4.86 – 4.72 (m, 2H, OCH_2), 6.96 (d, *J* = 6.5 Hz, 1H, ArH), 7.08 (dt, *J* = 15.6, 5.5 Hz, 3H, ArH), 7 7.30 (t, *J* = 7.1 Hz, 1H, ArH), 7.69 (t, *J* = 7.9 Hz, 2H, ArH), 7.90 – 7.74 (m, 2H, ArH), 8.00 (dd, *J* = 8.4, 1.1 Hz, 2H, ArH), 9.97 (s, 1H, NH), 10.12 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.55, 25.86, 64.06, 66.47, 69.96, 101.73, 110.99, 113.10 (2C), 114.34, 119.93, 124.43 (d, J_{C-F} = 9.9 Hz, 1C), 125.11 (d, J_{C-F} = 13.4 Hz, 1C), 126.49, 126.81 (d, J_{C-F} = 14.5 Hz, 1C), 127.32, 128.73 (2C), 130.40 (2C), 136.56, 137.62, 149.67, 154.05 (d, J_{C-F} = 247.2 Hz, 1C), 153.89, 159.23, 161.00, 161.36; HRMS: exact mass calcd for $C_{28}H_{25}FN_4O_{11}S_2$ $[M+Na]^+$ 699.0843; found 699.0840.

4-(2-((3-(2-Fluoro-3-((N-(2-methoxyethyl)sulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18g)

The title compound was obtained from **10** and **17g** as a white solid (80% yield, mp 72-73 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.43 (s, 3H, CH_3), 3.05 (q, J = 6.0 Hz, 2H, CH_2O), 3.16 (s, 3H, OCH_3), 3.32 (dd, J = 10.4, 4.3 Hz, 2H, $NHCH_2$), 3.96 (s, 2H, CH_2OH), 4.56 – 4.38 (m, 2H, CH_2), 4.86 – 4.70 (m, 2H, OCH_2), 6.86 (t, J = 7.2 Hz, 1H, ArH), 7.14 – 6.94 (m, 3H, ArH), 7.31 (t, J = 7.2 Hz, 1H, ArH), 7.49 (t, J = 5.9 Hz, 1H, ArH), 7.75 – 7.58 (m, 2H, ArH), 7.84 (dt, J = 16.4, 5.0 Hz, 2H, ArH), 8.02 – 7.88 (m, 2H, ArH, NH), 9.38 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.55, 25.74, 42.26, 58.30, 66.35, 70.07, 70.81, 101.73, 111.00, 113.09, 114.36, 120.07, 122.36, 124.35, 125.13, 126.35, 126.50 (d, J_{C-F} = 4.7 Hz, 1C), 126.67, 127.31, 128.73, 130.41, 136.57, 137.63, 149.55, 151.68, 152.90 (d, J_{C-F} = 243.5 Hz, 1C), 153.89, 159.24, 160.99, 161.36; HRMS: exact mass calcd for $C_{30}H_{29}FN_4O_{11}S_2$ $[M+Na]^+$ 727.1156; found 727.1144.

4-(3-(3-(2-Fluoro-3-((N-methylsulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18h)

The title compound was obtained from **10** and **17h** as a white solid (80% yield, mp 81-82 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.33 – 2.18 (m, 2H, CH_2), 2.41 (s, 3H, CH_3), 3.95 (s, 2H, CH_2O), 4.22 (s, 2H, $ArCH_2$), 4.58 (t, J = 6.0 Hz, 2H, OCH_2), 6.86 (d, J = 6.6 Hz, 1H, ArH), 7.08 – 6.94 (m, 3H, ArH), 7.25 (dd, J = 21.1, 6.4 Hz, 2H, ArH), 7.70 (t, J = 7.9 Hz, 2H, ArH), 7.77 (d, J = 8.8 Hz, 1H, ArH), 7.86 (t, J = 7.5 Hz, 1H, ArH), 8.01 (dd, J = 8.4, 1.1 Hz, 2H, ArH, NH), 9.38 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.57, 25.97, 26.82, 28.16, 28.78, 64.99, 68.70, 101.51, 111.00, 112.94, 114.08, 119.83, 122.67, 124.37 (d, J_{C-F} = 3.6 Hz, 1C), 125.20 (d, J_{C-F} = 3.3 Hz, 1C), 126.33 (d, J_{C-F} = 13.3 Hz, 1C), 126.69 (d, J_{C-F} = 14.4 Hz, 1C), 127.25, 128.81, 130.45 (2C), 136.58, 137.64, 149.67, 151.85, 153.91, 159.31, 160.33 (d, J_{C-F} = 205.7 Hz, 1C), 161.41; HRMS: exact mass calcd for $C_{29}H_{27}FN_4O_{10}S_2$ $[M+Na]^+$ 697.1050; found 697.1068.

4-(4-((3-(2-Fluoro-3-((N-methylsulfamoyl)amino)benzyl)-4-methyl-2-oxo-2H-chromen-7-yl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (18i)

The title compound was obtained from **10** and **17i** as a white solid (85% yield, mp 75-76 °C). ^{1}H NMR (400 MHz, [D_6]DMSO): δ 2.04 – 1.77 (m, 4H, CH_2CH_2), 2.43 (s, 3H, CH_3), 3.97 (s, 2H, CH_2O), 4.21 (t, J = 6.1 Hz, 2H, $ArCH_2$), 4.51 (t, J = 5.9 Hz, 2H, OCH_2), 6.87 (t, J = 6.7 Hz, 1H, ArH), 7.02 (ddd, J = 8.8, 5.8, 2.5 Hz, 3H, ArH), 7.33 – 7.17 (m, 2H, ArH), 7.83 – 7.70 (m, 3H, ArH), 7.91 (t, J = 7.5 Hz, 1H, ArH), 8.03 (dd, J = 8.4, 1.1 Hz, 2H, ArH, NH), 9.38 (s, 1H, NH); ^{13}C NMR (100 MHz, [D_6]DMSO): δ 15.54, 25.16, 25.22, 25.89, 28.78, 68.20, 71.65, 101.45, 113.05, 113.91, 119.68, 122.68, 124.36 (d, J_{C-F} = 4.1 Hz, 1C), 125.18 (d, J_{C-F} = 3.1 Hz, 1C), 126.31 (d, J_{C-F} = 13.4 Hz, 1C), 126.70 (d, J_{C-F} = 14.4 Hz, 1C), 127.15, 128.75, 129.61 , 130.47 (2C), 136.59, 137.65, 149.68, 153.08 (d, J_{C-F} = 245.4 Hz, 1C), 153.95, 159.33, 160.42, 161.56; HRMS: exact mass calcd for $C_{30}H_{29}FN_4O_{10}S_2[M+Na]^+$ 711.1201; found 711.1154.

General Procedure for the Preparation of 19a-19g.

The synthesis of the intermediate **19a-19g** was reported previously[17]**.**

4.2 Biology

4.2.1 General

Medium. HCT116 cells were cultured in McCoy's 5A medium; A549, MDA-MB-231, HL60 and HL7702 cells were cultured in RPMI-1640 medium. A375 and Vero cells were grown in DMEM medium. All of the cells were supplemented with 10% fetal bovine serum and cultured at 37 °C in a humidified 5% CO_2 incubator.

Antibodies. Rabbit mAbs for MEK (#9126), pMEK (#9154P), ERK (#9102L), pERK (#9101S) and GAPDH (#3683S HRP conjugate) were obtained from Cell Signaling Technology. The secondary antibody conjugated with HRP (A16172) was from Invitrogen.

Reagents. CellTiter Glo assay reagent was purchased from Promega. Annexin V/Dead cell apoptosis kit (#1825833) was from Invitrogen. RIPA lysis buffer (#89900), protease and phosphatase inhibitor cocktail (#78442) were obtained from Thermo Scientific.

4.2.2 Anti-proliferation assay

The activity was determined using CellTiter-Glo assay. Cells were plated in 384 well plates, compounds were added and cells were incubated at 37 °C for 96 h in a humidified incubator containing 5% CO_2. CellTiter-Glo Reagent was added directly to the cells and OD values were read in a microplate reader. The IC_{50} values were calculated using GraphPad Prism.

4.2.3 Western blot assay

Cells were lysed using RIPA lysis buffer supplemented with protease and phosphatase inhibitors on ice for 20 min, and the supernatant was collected after centrifugation. The protein concentrations were determined using Bio-Rad protein assay kit. Equal amounts of protein were loaded into the wells of 4-20% SDS-PAGE gels (Invitrogen) and electrophoresed. Then the proteins were transferred from the gels to PVDF membranes. After blocking in 5% milk for 1 h at room temperature, membranes were incubated with indicated primary antibodies at 4 °C overnight, washed with TBST and incubated with HRP conjugated secondary antibody for 1 h at room temperature. Membranes were washed three times with TBST. The images were obtained using Clarity™ Western ECL Substrate from Bio-Rad.

4.2.4 Apoptosis analysis

Apoptosis analysis was performed using an Annexin V/Dead cell apoptosis kit according to the manufacturer's instruction.

4.2.5 NO release evaluation

Total NO production was estimated by measurement of the accumulation of nitrite and nitrate using the Griess reagent in the Total Nitric Oxide Assay Kit (Beyotime, China). Nitrate was measured after enzymatic conversion to nitrite by nitrate reductase. Briefly, the lysis buffer of every sample was added in duplicate wells in a 96-well plate at room temperature. The mixture was incubated with 5 μl of nicotinamide adenine dinucleotide phosphate (NADPH), 10 μl of flavin adenine dinucleotide (FAD) and 5 μl of nitrate reductase for 30 min at 37 °C. Then, 10 μl of lactate dehydrogenase (LDH) buffer and 10 μl of LDH were added in the above mixture for another 30 min at 37 °C. Finally, 50 μl of Griess reagent I and 50 μl of Griess reagent II were added into the

wells before incubation for 10 min. Optical density at 540 nm was measured with a FlexStation 3 multi-plate reader. Concentrations were calculated using a standard curve (80, 60, 40, 20, 10, 5 and 2 μM sodium nitrite).

4.2.6 Intracellular NO measurement

A NO-specific fluorescent probe, DAF-FM DA, purchased from Beyotime Institute of Biotechnology (China), was diluted to a final concentration of 5 μM with kit diluents. To incubate the probe and cells sufficiently, HCT116 cells (1×10^7) were pretreated with 5 μM DAF-FM DA at 37 °C for 20 min. The cells were washed 3 times with PBS to remove the remaining DAF-FM DA that did not enter into the cells. Compounds were incubated at 37 °C for 2 h before NO determination using a laser confocal scanning microscope (Olympus, Japan) with excitation at 495 nm and emission at 515 nm.

Conflicts of interest

There are no conflicts to declare.

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Highlights

A series of hybrids of MEK inhibitor and NO donor were designed and synthesized.

Compound **18h** shows better cell proliferation inhibition effects than **RO5126766** in MDA-MB-231 cells.

Compound **18h** significantly reduces levels of pMEK and pERK in MDA-MB-231 cells.

Compound **18h** can induce apoptosis in the MDA-MB-231 cell line.

Compound **18h** can release NO in cells.

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: