Bioorganic & Medicinal Chemistry 24 (2016) 3218–3230

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Development of hydroxy-based sphingosine kinase inhibitors and anti-inflammation in dextran sodium sulfate induced colitis in mice

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ARTICLE INFO

Article history:
Received 11 April 2016
Revised 20 May 2016
Accepted 21 May 2016
Available online 24 May 2016

Keywords:
SphK
DSS
Inflammation
Colitis

ABSTRACT

Sphingosine kinase (SphK)-catalyzed production of sphingosine-1-phosphate (S1P) regulates cell growth, survival and proliferation as well as inflammatory status in animals. In recent study we reported the *N*′-(3-(benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide scaffold as a potent SphK inhibitor. As a continuation of these efforts, 51 derivatives were synthesized and evaluated by SphK1/2 inhibitory activities for structure–activity relationship (SAR) study. Among them, **33** was identified as the most potent SphK inhibitor. Potency of **33** was also observed to efficiently decrease SphK1/2 expression in human colorectal cancer cells (HCT116) and significantly inhibit dextran sodium sulfate (DSS)-induced colitis as well as the decreased expression of interleukin (IL)-6 and cyclooxygenase-2 (COX-2) in mouse models. Collectively, **33** was validated as an effective SphK inhibitor, which can be served as anti-inflammatory agent to probably treat inflammatory bowel diseases in human.

1. Introduction

The conserved lipid kinase, sphingosine kinase (SphK), catalyzes the transfer of phosphate from ATP to the 1-OH in sphingosine (Sph) to form sphingosine 1-phosphate (S1P), which plays an important role in regulating cell growth, survival, proliferation, neovascularization, and migration.[1–3] There are two known isoforms of SphK exist in mammals, SphK1 and SphK2. The amino acid sequence of these two kinases is 80 percent similar and 45 percent overall identical.[4] SphK has been involved with a diverse range of disease including sickle cell disease,[5,6] cancer,[7,8] atherosclerosis,[9,10] asthma,[11,12] and inflammation,[13,14] among others.

SphK1's role in colitis is widely studied, where correlation between elevated expression and severity of disease has been reported. Deletion of SphK1 reduces colitis severity.[15] However, SphK2 knockout induces increased severe colitis. It is thought that the two enzymes have some compensatory mechanism. SphK2 knockout up-regulating SphK1 expression is one main reason.[16] Studies in mice indicate a redundant role of SphK1 and SphK2 because the individual knockout remains viable, but double knockouts are embryonically lethal.[4,17] In addition, there are also differences in their subcellular localization and structure elucidation: SphK1 is mainly localized in cytosol while SphK2 is mainly in nuclei;[18] a high resolution X-ray crystal structure of human SphK1 was announced but the structure of SphK2 was not resolved.[19,20]

At present, there are several classes of SphK inhibitors reported: (1) SphK-nonselective inhibitors, such as *N*,*N*-dimethylsphingosine (DMS),[21] SKI-II[22] and ABC294735;[21] (2) SphK1-selective inhibitors, such as PF-543,[19] Compound **56** (derived from VPC94075),[18,23] RB005[24,25] and SLP7111228;[17] (3) SphK2-selective inhibitors, such as ABC294640,[26] SG-12[27] and SLP120701[17] (Fig. 1). Some inhibitors are reported to exert anti-inflammatory activities in animal models.[28–30] For example, ABC747080, a derivative of known SKI-II, was reported to have influence on the expression of S1P levels and on inflammation progression including colonic levels of tumor necrosis factor (TNF) α, interleukin (IL)-1β, IL-6 in dextran sodium sulfate (DSS)-induced colitis in mice.[4,31] In this model, decreased severity of DSS-induced colitis was observed when SphK was inhibited. Actually, this model is often used to evaluate potency against colitis and elucidate mechanisms involved in colitis, being considered as one of the most widely used models for understand-

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http://dx.doi.org/10.1016/j.bmc.2016.05.047
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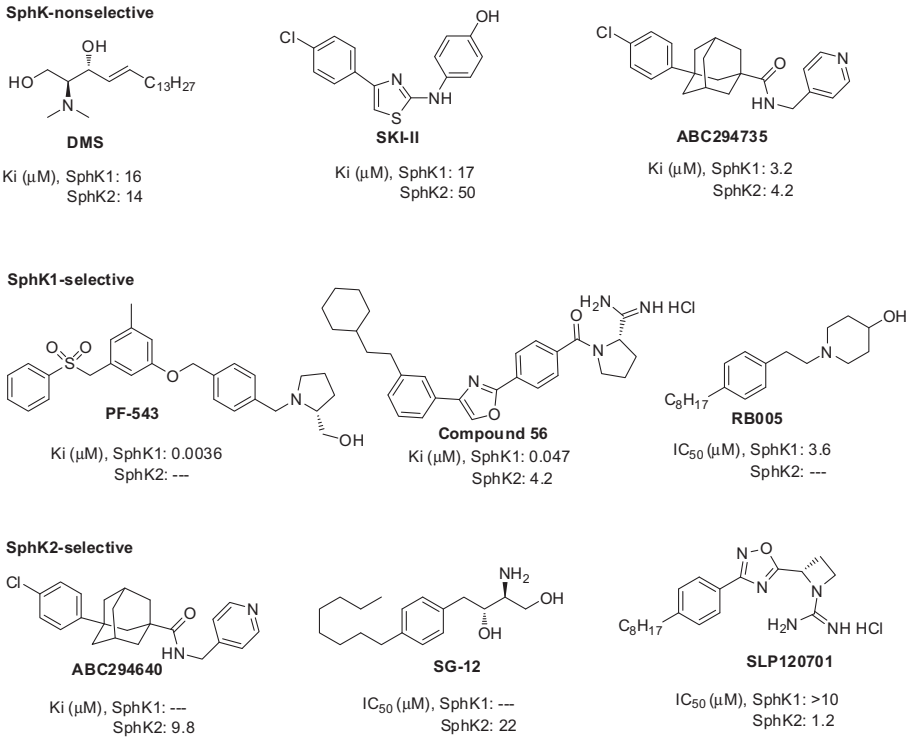


Figure 1. Representative structures and potencies of SphK inhibitors.

ing inflammatory bowel disease in human.[32,33] Herein, targeting the SphK pathway can treat colitis progression with concomitant decrease in inflammatory cytokines. Considering the importance of SphK inhibition in the therapy of colitis, novel SphK inhibitors are urgently needed to develop and attract the attention of medicinal chemists throughout the world.

In this study, we report a series of 51 compounds derived from *N*′-(3-(benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide scaffold. Among these compounds, **1** was first identified via structure-based hierarchical screening in recent study.[34] It showed potent activity against SphK in vitro. Thus **1** was selected as a hit for further optimization. Other 50 derivatives were designed, synthesized, and evaluated their ability to decrease SphK1 and SphK2 levels. Structure–activity relationship (SAR) studies indicated a more promising compound **33** for further analysis. It showed potent activity against SphK in cellular level and suppressed colitis in DSS mouse models. As far as we know, these compounds are first confirmed as SphK inhibitors by us, exhibiting favorable suppressive activity and serving as an ideal lead compound for further development of anti-inflammatory agents.

2. Results and discussion

2.1. Chemical synthesis

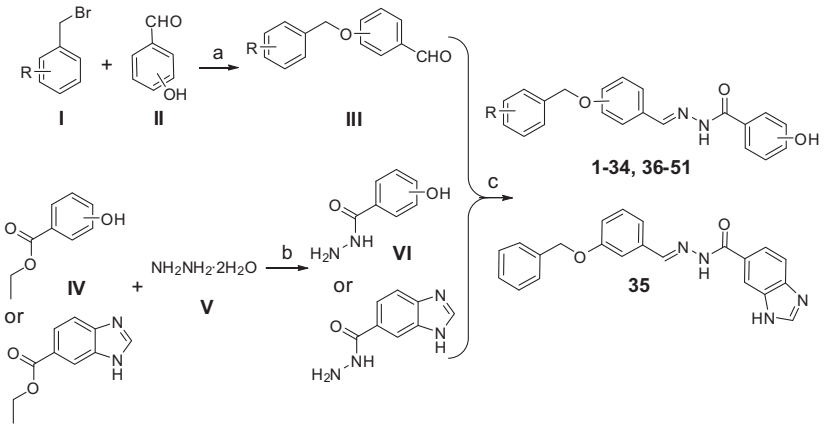
The synthetic route we employed to synthesize a class of aromatic compounds **1**–**51** was shown in Scheme 1. Accordingly, 3- or 4-(substituted benzyloxy)benzaldehyde (**III**) was synthesized via substitution reaction of 3- or 4-hydroxybenzaldehyde (**II**) with substituted benzyl bromide (**I**).[35,36] Treatment of substituted carboxylate (**IV**) with hydrazine monohydrate (**V**) in refluxing ethanol afforded the common intermediate or substituted carbohydrazide (**VI**).[37] Subsequent condensation was accomplished with **III**, which produced the corresponding desired target compounds **1**–**51** at reflux in excellent yield.[38,39]

2.2. Inhibitory activity on SphK1 and SphK2 and preliminary SAR

In an effort to discover new SphK inhibitors, we performed an enzyme-based screening for each compound to evaluate their ability to decrease SphK1 and SphK2 activity in 384-well plates.[40–42] Compound **33** showed the best inhibitory effect on SphK1 and SphK2 activity followed by **37** and **20**. In comparison, the positive control DMS displayed modest activity (Table 1).

To determine the effect of different alkyl or halogen substituent on ring A toward SphK1 activity, a set of compounds (**1**–**21**) around the phenyl ring were afforded to assess the extent of SphK1 inhibition. First, several examples incorporating either –F or –Cl atom were prepared. The great lack of inhibitory activity displayed by –F or –Cl substitution against SphK1 indicated that the halogen group was not tolerated except 2-Cl substituent (**11**). We also assessed the role of electron-drawing groups in the lipophilic tail of compounds. Changing the $-CF_3$ to –CN substitution at *ortho*- or *para*-position, the inhibitory activity against SphK1 was generally eliminated. The result confirmed that $-CF_3$ substituent played a more important role in increasing activity. However, it is worth mentioning that the $-CF_3$ substituents (**6**) at *meta*-position was inactive and –CN substituents (**16**) showed little inhibition. At this position, the introduction of electron-drawing groups exerted a negative influence on inhibition.

To further evaluate the effect of the electron-donating group on the ring A against SphK1 activity, we designed and synthesized some derivatives with $-CH_3$, $-C(CH_3)_3$ or $-OCH_3$ substitution. The compound with 3-OCH_3 substitution (**20**) increased SphK1 inhibition remarkably, suggesting that the 3-OCH_3 substituent maintained an efficient binding with SphK1 and had a positive effect on activity. When 4-OCH_3 group (**21**) was substituted, the activity was abolished completely, indicating that the substitution position was responsible for mediating the SphK1 activity. At *para*-position, when a $-C(CH_3)_3$ substituent (**14**) was further shortened to a $-CH_3$



Scheme 1. Synthetic route of 1–51. (Reagents and conditions: (a) K_2CO_3, DMF, 80 °C, 3 h; (b) EtOH, reflux, 36 h; (c) EtOH, reflux, 6 h.)

one (**4**), a slightly potency loss was observed indicating that a larger bulk of a substituent was beneficial.

In a second step, we paid attention to the substitution position of ring B in the linker. Substituted benzyloxy groups attached to the *para*-position on ring B gave rise to representative analogues (**22**–**34**). Their inhibitory activity toward SphK1 was evaluated as to facilitate exploration of SAR. Among these monosubstituted derivatives, the slightly changed activity between *para*- and *meta*-position except 3-CF_3 substituted benzyloxy group (**6**, **24**) implied that the substitution position may be not the key factor in affecting the potency of SphK1 inhibition.

To exam the role of –OH groups on ring C, an imidazole group (bioisosterism, **35**), 3,5-OH, 3,4-OH, 3-OH or 4-OH (**35**–**51**) was introduced to get access to a last set of compounds. As it turned out, most of the compounds were inactive except 3,4-OH substituents (**37**, **41**, **45** and **49**). The result suggested that the polar head of 3,4-OH was essential for mediating SphK1 inhibitory activity but the 5-OH group was dispensable. For SphK2 inhibition, most of these compounds showed similar activity to SphK1. In a word, these compounds can be considered as SphK inhibitors. A brief description of SAR was presented in Figure 2. On the basis of these data, the derivative **33** can serve as an ideal lead compound for further optimization and evaluation.

2.3. Potency of compound 33 to inhibit the expression of SphK1 and SphK2

Through enzyme-based activity studies, **33** was identified as the most potent SphK inhibitor. In order to exam the inhibitory effect for this compound in cellular level, human colorectal cancer cells (HCT116) were used to evaluate the expression of the target enzymes. Treatment with **33** decreased the expression of SphK1 and SphK2 in a time dependent manner by measuring protein levels in HCT116 (Fig. 3). At 24 h, SphK levels were significantly lower compared to the Con panel ($p < 0.01$).[43,44]

2.4. Effect of administration with identified compound 33 on development of DSS mouse models

As described above, it has been showed that pharmacological SphK inhibitors can inhibit DSS-induced colitis. To confirm **33** was able to inhibit colon inflammation, mice were measured for their body weight, colon length and histological score. 5-aminosalicylic acid (5-ASA) was used as the positive control.[45,46] In Figure 4A, no obvious change of body weight was observed in the DSS group and 3 other compound administration groups until the 7th day. After day 8, a significant body weight decrease was observed in the DSS group. Body weight in compound administration groups was increased by approximately 12% average compared to the DSS group at the end of the study, showing apparent preventive effect on DSS treated weight loss (Table 2). In addition, DSS treatment induced a reduction in colon length in 4 groups on day 10. However, colon length in compound treated groups was significantly longer than in the DSS group (Fig. 4B).

2.5. Pathology findings of DSS mice treated with 33

To evaluate the colitis, we observed the cellular infiltration of the intestinal wall, mucosal lesion and crypt damage on the day of dissection.[47–49] Histological signs of colitis indicated cellular infiltrations of predominantly mononuclear macrophages and neutrophils, mucosal and submucosal lesion, degeneration and necrosis of crypt cells were severe in the DSS group when compared with the DSS + 5-ASA or **33** treated mice. Consequently, mean histologic score in mice treated with 5-ASA or **33** was significantly lower than in the DSS group. In a word, the inflammation level of **33** at a dose of 10 mg/kg treated mice was the lowest among the 4 DSS treated groups, which revealed that **33** effectively attenuated the colitis (Fig. 5A and HE).

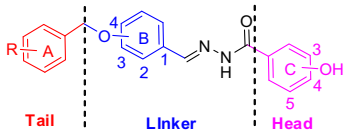
2.6. Suppression of 33 on the overexpression of IL-6 and COX-2 in DSS induced colitis in mice

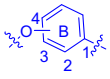
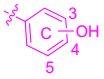
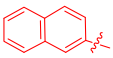
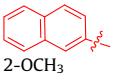
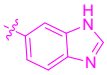
Some of the inflammatory mediators such as interleukin-6 (IL-6) and cyclooxygenase-2 (COX-2) were characterized recently. The importance of pro-inflammatory cytokines IL-6 in colon tissue has been demonstrated in animal models of colitis. It showed that inhibiting IL-6 efficiently ameliorated the colitis.[50,51] COX-2 was also overexpressed in DSS induced colitis.[52,53] Therefore, we evaluated anti-inflammatory activity by IL-6 and COX-2 immunohistochemistry[54,55] in mice. Overexpression of IL-6 and COX-2 in the DSS group was observed. After treated with 5-ASA or **33**, the evidently decreased expression was observed, indicating that **33** inhibiting this factor in the inflammatory environment was able to suppress the colitis effectively (Fig. 5B, C, IL-6 and COX-2).

3. Conclusion

We have identified a series of SphK inhibitors derived from *N*′-(3-(benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide scaffold via structure-based hierarchical screening in recent study. According to this finding, we made a structural optimization and SAR Study. The result indicated that electrostatic state and substitution position in lipophilic ring was responsible for mediating the

Table 1
SphK1 and SphK2 inhibitory activity



Compd ID				IC_{50} (μM)[a]	
				SphK1	SphK2
1	H	3-O–	3,4,5-OH	40.21 ± 3.05	26.33 ± 2.55
2	2-CH_3	3-O–	3,4,5-OH	63.24 ± 5.61	81.17 ± 4.29
3	3-CH_3	3-O–	3,4,5-OH	52.10 ± 3.28	78.69 ± 6.04
4	4-CH_3	3-O–	3,4,5-OH	45.79 ± 4.15	65.38 ± 3.72
5	2-CF_3	3-O–	3,4,5-OH	19.57 ± 2.43	16.32 ± 2.31
6	3-CF_3	3-O–	3,4,5-OH	>100	>100
7	4-CF_3	3-O–	3,4,5-OH	16.26 ± 1.25	22.15 ± 1.96
8	2-F	3-O–	3,4,5-OH	>100	98.90 ± 5.21
9	3-F	3-O–	3,4,5-OH	>100	>100
10	4-F	3-O–	3,4,5-OH	>100	>100
11	2-Cl	3-O–	3,4,5-OH	36.80 ± 4.83	58.79 ± 6.52
12	3-Cl	3-O–	3,4,5-OH	>100	67.37 ± 5.97
13	4-Cl	3-O–	3,4,5-OH	>100	61.20 ± 4.28
14	4-$C(CH_3)_3$	3-O–	3,4,5-OH	35.22 ± 3.24	62.17 ± 5.16
15	2-CN	3-O–	3,4,5-OH	45.38 ± 2.31	68.55 ± 3.71
16	3-CN	3-O–	3,4,5-OH	85.26 ± 4.64	>100
17	4-CN	3-O–	3,4,5-OH	69.44 ± 3.85	35.11 ± 4.58
18		3-O–	3,4,5-OH	31.30 ± 2.74	40.58 ± 5.16
19	2-OCH_3	3-O–	3,4,5-OH	20.52 ± 2.61	19.53 ± 1.74
20	3-OCH_3	3-O–	3,4,5-OH	8.57 ± 2.50	8.70 ± 0.14
21	4-OCH_3	3-O–	3,4,5-OH	>100	>100
22	H	4-O–	3,4,5-OH	22.02 ± 2.71	28.51 ± 1.88
23	2-CF_3	4-O–	3,4,5-OH	15.01 ± 2.12	15.08 ± 1.16
24	3-CF_3	4-O–	3,4,5-OH	54.72 ± 0.98	23.83 ± 1.42
25	4-CF_3	4-O–	3,4,5-OH	37.40 ± 2.81	15.64 ± 1.06
26	2-CN	4-O–	3,4,5-OH	>100	44.54 ± 3.70
27	3-CN	4-O–	3,4,5-OH	45.76 ± 5.82	22.59 ± 4.53
28	4-CN	4-O–	3,4,5-OH	27.74 ± 1.09	17.79 ± 0.87
29	4-CH_3	4-O–	3,4,5-OH	32.73 ± 1.85	44.50 ± 4.91
30	4-$C(CH_3)_3$	4-O–	3,4,5-OH	40.55 ± 7.06	43.28 ± 5.79
31		4-O–	3,4,5-OH	20.61 ± 3.38	30.23 ± 2.14
32	2-OCH_3	4-O–	3,4,5-OH	18.44 ± 4.25	26.62 ± 3.29
33	3-OCH_3	4-O–	3,4,5-OH	4.36 ± 1.08	2.17 ± 0.77
34	4-OCH_3	4-O–	3,4,5-OH	>100	>100
35	H	3-O–		>100	>100
36	3-OCH_3	3-O–	3,5-OH	>100	>100
37	3-OCH_3	3-O–	3,4-OH	5.00 ± 0.65	4.60 ± 0.67
38	3-OCH_3	3-O–	3-OH	>100	>100
39	3-OCH_3	3-O–	4-OH	>100	>100
40	3-OCH_3	4-O–	3,5-OH	>100	>100
41	3-OCH_3	4-O–	3,4-OH	25.32 ± 3.11	10.15 ± 1.07
42	3-OCH_3	4-O–	3-OH	>100	>100
43	3-OCH_3	4-O–	4-OH	>100	>100
44	2-CF_3	3-O–	3,5-OH	>100	>100
45	2-CF_3	3-O–	3,4-OH	15.53 ± 0.97	13.84 ± 1.21
46	2-CF_3	3-O–	3-OH	>100	>100
47	2-CF_3	3-O–	4-OH	>100	>100
48	2-CF_3	4-O–	3,5-OH	>100	>100
49	2-CF_3	4-O–	3,4-OH	16.48 ± 2.08	11.40 ± 0.70
50	2-CF_3	4-O–	3-OH	>100	>100
51	2-CF_3	4-O–	4-OH	>100	>100
DMS				50.56 ± 3.12	26.29 ± 4.66

[a] Values shown are mean ± SD (n = 3, 1 h of treatment in triplicate). Each compound was exposed to human SphK, Sph and ATP measured their inhibitory ability.

activity. It was worth noting that there was no long lipophilic tail compared with a large portion of previous reported compounds.[56–58] It broke the limit of long-chain aliphatic hydrocarbons and was a notable exception. In the linker, the substitution position was not the key factor for mediating the inhibitory activity. In an effort to discover a surrogate for polar head, 3,4,5-OH was replaced with imidazole according to the biosostere theory as well as 3,5-OH, 3,4-OH, 3-OH, 4-OH. The result suggested that the 3,4-

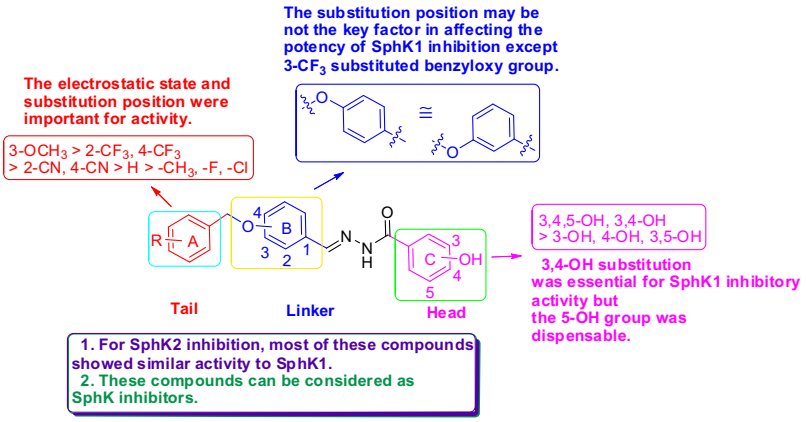


Figure 2. SAR of compounds **1**–**51**.

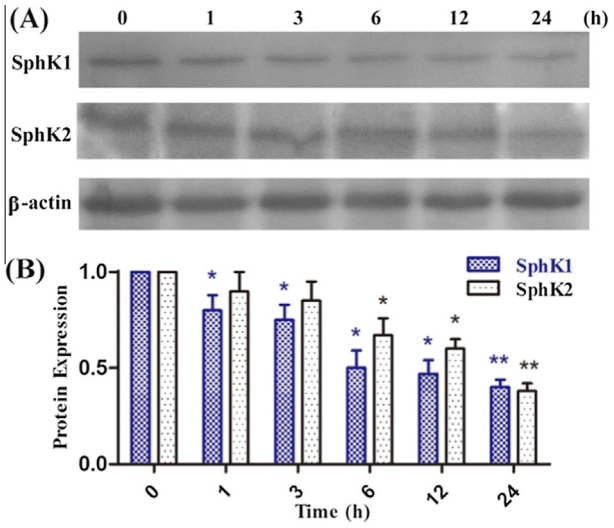


Figure 3. **33** inhibited SphK1 and SphK2 activity in vitro. (A) HCT116 cells were treated with vehicle or **33** (30 μM) for different time points and analyzed by western blot for total SphK1, SphK2 and β-actin. (B) Quantification of expression of SphK1 and SphK2. β-Actin was used for normalization. $^{*}p < 0.05$, $^{**}p < 0.01$, statistically significant difference from the vehicle group.

OH group was essential for potent SphK inhibitory activity but the 5-OH was dispensable. In the future plan, amidine, guanidine, animo alcohol or other groups will be introduced to measure the inhibitory activity to probe the polar head.

According to SAR, a potent compound **33** was discovered as the most active SphK inhibitor. The examination of protein expression was used to prove that **33** was a potent SphK inhibitor in cellular level. In DSS treated mouse models, **33** diminished loss in body weight and reduction in colon length. Furthermore, **33** significantly inhibited colitis as well as decreased expression of pro-inflammatory mediators IL-6 and COX-2. It was believed that SphK inhibitors with micromolar level were effective in vivo in suppressing inflammation response.[18] However, in further plan, there is still a need for optimizing this structure that could be active at submicromolar or nanomolar level. In summary, we demonstrated the efficacy of **33** not only in vitro but also in vivo. Targeting SphK not only gave insight into the treatment of colitis in mice, but also a possibly novel therapeutic strategy to treat inflammatory bowel diseases in human.

4. Experimental procedures

4.1. Synthetic chemistry

All of reagents were purchased from commercial sources. Organic solutions were concentrated in a rotary evaporator (Büchi)

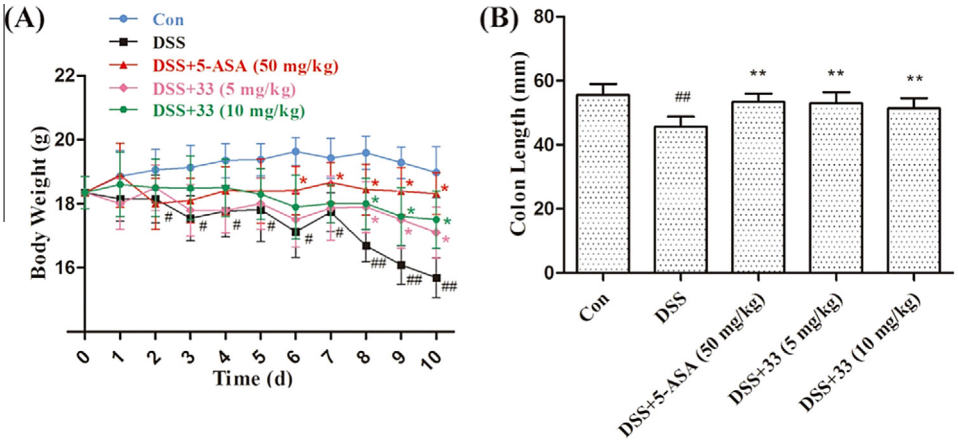


Figure 4. Changes in (A) body weight and (B) colon length at the end of the study in DSS-induced colitis in C57BL/6 mice. The following groups of mice were used: (1) Con: water + vehicle; (2) DSS: 2.5% DSS + vehicle; (3) DSS + 5-ASA (50 mg/kg): 2.5% DSS + 5-ASA (50 mg/kg, *ig*); (4) DSS + **33** (5 mg/kg): 2.5% DSS + **33** (5 mg/kg, *ip*); (5) DSS + **33** (10 mg/kg): 2.5% DSS + **33** (10 mg/kg, *ip*). Data are shown as mean ± SD ($n = 10$). $^{*}p < 0.05$, $^{**}p < 0.01$, statistically significant difference from the DSS group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with the Con group.

Table 2
Overview of DSS models[a]

Group	Body weight (g) day 10	Colon length (mm)
Con	18.98 ± 0.84	55.64 ± 2.31
DSS	15.69 ± 0.65	45.68 ± 1.34[##]
DSS + 5-ASA (50 mg/kg)	18.32 ± 0.58	53.40 ± 2.53[**]
DSS + **33** (5 mg/kg)	17.10 ± 0.82	53.04 ± 2.14[**]
DSS + **33** (10 mg/kg)	17.50 ± 0.77	51.41 ± 2.67[*]

[a] Data are shown as mean ± SD (n = 10).
[*] p <0.05,
[**] p <0.01, statistically significant difference from the DSS group.
[##] P <0.01, compared with the Con group.

below 50 °C under reduced pressure. Reactions were monitored by thin layer chromatography silica gel slabs on 0.2 ± 0.03 mm and visualized under UV light at 254 nm. Melting points were determined with a Yanaco MP-J3 microscope melting point apparatus. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-400 instrument using deuterated solvents with tetramethylsilane (TMS) as internal standard. High resolution mass spectra (HRMS) and ESI-mass were recorded on a Thermo Exactive Orbitrap plus spectrometer. The purity (⩾95%) of the compounds is verified by the HPLC study performed on YMC-Pack ODS-A (4.6 × 150 mm, 5.0 μm) column using a mixture of solvent methanol/water or acetonitrile/water with 0.5% TFA at the flow rate of 1 mL/min and peak detection at 210 nm under UV. 51 compounds were synthesized as follows:

To a solution of **II** (8.2 mmol) in DMF (15 mL), K_2CO_3 (1.35 g, 9.8 mmol) and **I** (9.0 mmol) was added. The resulting mixture was stirred at 80 °C for 3 h, then it was cooled to room temperature and poured into EtOAc (40 mL). The organic layer was washed with H_2O (15 mL) and brine (15 mL), then dried over Na_2SO_4 and concentrated in vacuo to remove solvents. The crude was purified by flash column chromatography (eluent: 10–15% EtOAc/petroleum ether) to afford **III** as a white solid. Yield 75.5–86.8%.

To a solution of **IV** (15.12 mmol) in ethanol (35 mL), Hydrazine monohydrate (8.6 ml, 120.9 mmol) was added. The resulting mixture was heated under reflux for 36 h. After being cooled to room temperature, the mixture was filtered. The filter cake was washed with EtOAc (45 mL) to afford **VI** as a white solid. Yield 70.1–93.3%.

To a solution of **VI** (4.89 mmol) in ethanol (60 mL), **I** (4.08 mmol) was added. The resulting mixture was heated under reflux for 6 h. After being cooled to room temperature, the mixture was filtered. The filtrate was evaporated in vacuo and the crude was purified by flash column chromatography (eluent: 5–10% MeOH/DCM) to afford **1**–**51**. Yield 56.7–92.0%.

33 (50 mg-scale) was synthesized for *ig* administration of DSS treated mice.

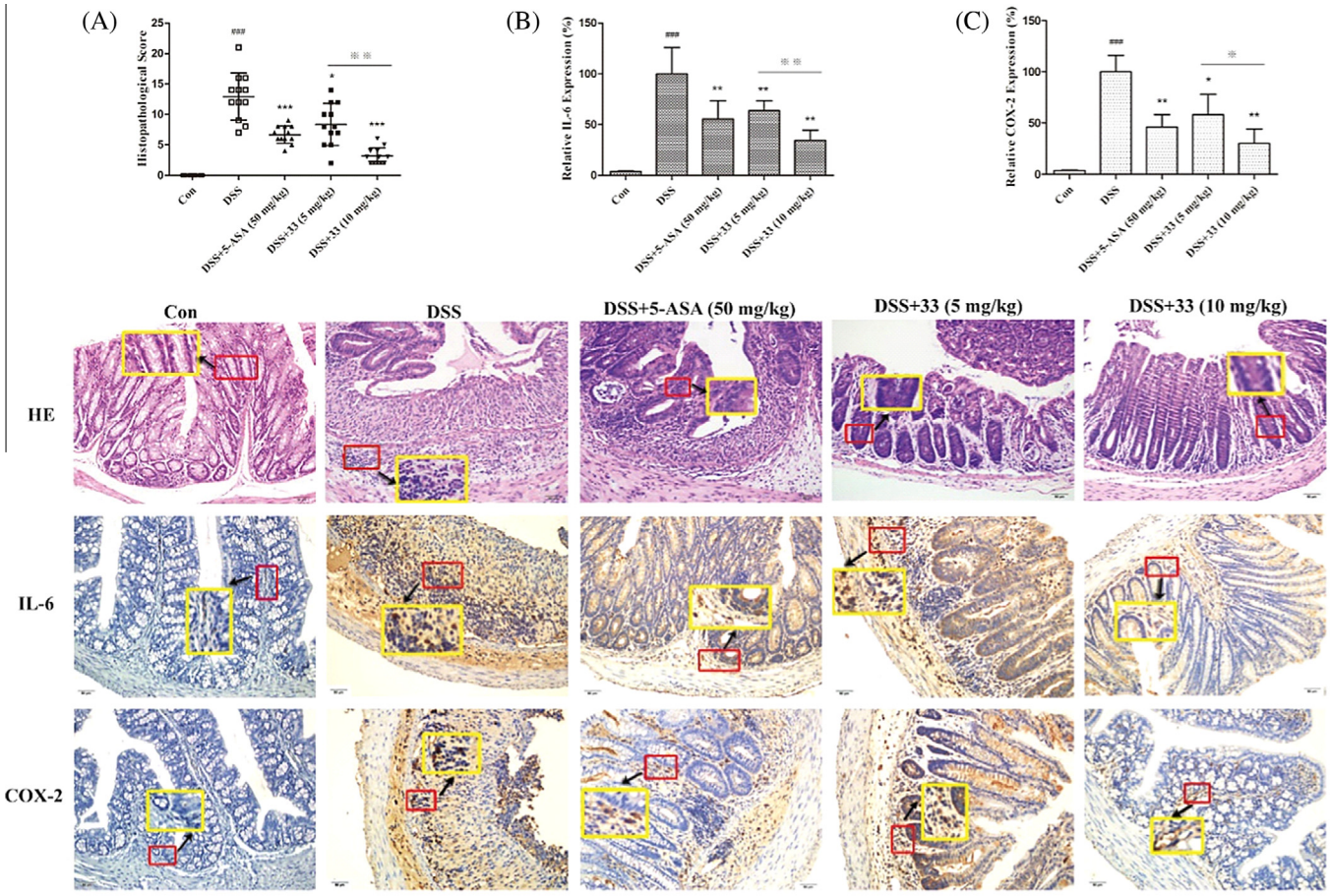


Figure 5. Histological evaluation of the DSS-induced colitis and **33** inhibited the expression of IL-6 and COX-2. (A) Histological score on day 10. Values represent the individual scores of each mouse (n = 10). (B and C) Quantification of IL-6 and COX-2 staining. Data are shown as mean ± SD value for each group. Asterisk represents significant difference from the DSS group ($^{*}p$ < 0.05, $^{**}p$ < 0.01, $^{***}p$ < 0.001). $^{\#\#\#}P$ < 0.001, compared with the Con group. p < 0.05, p < 0.01, significant difference between the DSS + **33** (5 mg/kg) group and the DSS + **33** (10 mg/kg) one. (HE) Representative pictures of colon from groups in C57BL/6 mice. (IL-6 and COX-2) Representative pictures of IL-6 and COX-2 immunohistochemistry staining in indicated treatment. Bars inserted indicate magnification (50 μm).

4.1.1. *N′*-(3-(Benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (1)

Yield 35.0%; White solid; mp 213–215 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.04 (s, 3H), 8.36 (s, 1H), 7.46 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.3 Hz, 2H), 7.36–7.30 (m, 2H), 7.25 (d, J = 7.7 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 6.92 (s, 2H), 5.14 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.62, 159.10, 146.87, 146.00, 137.44, 137.39, 136.56, 130.40, 128.90, 128.32, 128.15, 123.75, 120.40, 117.06, 112.73, 107.63, 69.70; HRMS calcd. For $C_{21}H_{19}N_2O_5$ $[M+H]^+$ 379.1288, found 379.1282; HPLC (40% acetonitrile and 0.5% TFA in water): t_R = 6.500 min, 98.66%.

4.1.2. 3,4,5-Trihydroxy-*N′*-(3-((2-methylbenzyl)oxy)benzylidene)benzohydrazide (2)

Yield 37.8%; White solid; mp 206–208 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.53 (s, 1H), 9.13 (s, 2H), 8.82 (s, 1H), 8.37 (s, 1H), 7.42 (d, J = 7.2 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 4.4 Hz, 1H), 7.23 (m, 4H), 7.07 (m, 1H), 6.91 (s, 2H), 5.12 (s, 2H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.23, 158.82, 146.49, 145.61, 137.08, 136.73, 136.15, 134.85, 130.19, 130.00, 128.68, 128.17, 125.83, 123.32, 120.02, 116.59, 112.22, 107.20, 68.05, 18.53; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1445, found 393.1442; HPLC (63% methanol and 0.5% TFA in water): t_R = 6.956 min, 99.25%.

4.1.3. 3,4,5-Trihydroxy-*N′*-(3-((3-methylbenzyl)oxy)benzylidene)benzohydrazide (3)

Yield 36.0%; White solid; mp 156–158 °C; ^{1}HNMR (400 MHz, DMSO) δ 11.53 (s, 1H), 9.03 (s, 3H), 8.36 (s, 1H), 7.34 (m, 2H), 7.26 (m, 4H), 7.13 (d, J = 6.7 Hz, 1H), 7.05 (d, J = 8.1 Hz, 1H), 6.91 (s, 2H), 5.09 (s, 2H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 162.69, 159.15, 158.98, 146.86, 146.02, 139.05, 138.08, 137.50, 137.32, 136.57, 132.31, 130.41, 128.96, 128.81, 128.72, 128.06, 125.25, 123.73, 120.38, 117.03, 112.72, 110.34, 107.62, 70.53, 21.47; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1445, found 393.1441; HPLC (63% methanol and 0.5% TFA in water): t_R = 7.554 min, 99.67%.

4.1.4. 3,4,5-Trihydroxy-*N′*-(3-((4-methylbenzyl)oxy)benzylidene)benzohydrazide (4)

Yield 39.6%; White solid; mp 213–215 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 9.13 (s, 2H), 8.81 (s, 1H), 8.35 (s, 1H), 7.35 (m, 3H), 7.29 (s, 1H), 7.24 (d, J = 7.4 Hz, 1H), 7.19 (d, J = 7.7 Hz, 2H), 7.04 (d, J = 7.9 Hz, 1H), 6.90 (s, 2H), 5.09 (s, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.16, 160.03, 158.68, 146.42, 145.57, 137.10, 137.04 136.09, 133.90, 129.93, 129.00, 127.79, 127.69, 123.27, 119.87, 116.62, 112.32, 107.16, 69.16, 20.80; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1445, found 393.1441; HPLC (63% methanol and 0.5% TFA in water): t_R = 7.598 min, 98.57%.

4.1.5. 3,4,5-Trihydroxy-*N′*-(3-((2-(trifluoromethyl)benzyl)oxy)benzylidene)benzohydrazide (5)

Yield 43.3%; White solid; mp 206–207 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.11 (s, 3H), 8.37 (s, 1H), 7.79 (m, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.58 (t, J = 7.6 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.29 (m, 2H), 7.05 (d, J = 7.8 Hz, 1H), 6.90 (s, 2H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.20, 158.36, 146.30, 145.57, 137.05, 136.24, 134.80, 132.88, 130.57, 130.10, 128.83, 128.83 (q, J = 30.3 Hz), 126.20 (q, J = 5.4 Hz), 124.35 (q, J = 272.3 Hz), 123.28, 120.32, 116.41, 112.20, 107.17, 66.28; HRMS calcd. For $C_{22}H_{18}F_3N_2O_5$ $[M+H]^+$ 447.1162, found 447.1162; HPLC (63% methanol and 0.5% TFA in water): t_R = 8.722 min, 98.59%.

4.1.6. 3,4,5-Trihydroxy-*N′*-(3-((3-(trifluoromethyl)benzyl)oxy)benzylidene)benzohydrazide (6)

Yield 41.5%; White solid; mp 208–210 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.55 (s, 1H), 9.06 (s, 3H), 8.37 (s, 1H), 7.83 (s, 1H), 7.79 (d, J = 7.4 Hz, 1H), 7.70 (d, J = 7.6 Hz, 1H), 7.64 (m, 1H), 7.37 (m, 2H), 7.27 (d, J = 7.3 Hz, 1H), 7.08 (d, J = 8.0 Hz, 1H), 6.91 (s, 2H), 5.25 (s, 2H); ^{13}C NMR (125 MHz, DMSO) δ 163.60, 158.86, 146.75, 146.00, 138.93, 137.44, 136.62, 132.13, 130.46, 130.03, 129.61 (q, J = 31.5 Hz), 125.03 (q, J = 3.6 Hz), 124.65 (q, J = 270.8 Hz), 124.49(q, J = 3.9 Hz), 123.71, 120.64, 117.02, 112.64, 107.59, 68.78; HRMS calcd. For $C_{22}H_{18}F_3N_2O_5$ $[M+H]^+$ 447.1162, found 447.1159; HPLC (63% methanol and 0.5% TFA in water): t_R = 9.745 min, 99.72%.

4.1.7. 3,4,5-Trihydroxy-*N′*-(3-((4-(trifluoromethyl)benzyl)oxy)benzylidene)benzohydrazide (7)

Yield 46.4%; White solid; mp 129–131 °C; ^{1}HNMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.12 (s, 3H), 8.36 (s, 1H), 7.75 (d, J = 8.0 Hz, 2H), 7.69 (d, J = 8.0 Hz, 2H), 7.36 (m, 2H), 7.26 (d, J = 7.6 Hz, 1H), 7.07 (m, 1H), 6.91 (s, 2H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.65, 158.84, 146.78, 146.02, 142.35, 137.46, 136.65, 130.49, 128.76 (q, J = 31.6 Hz), 128.46, 125.81 (q, J = 3.7 Hz), 124.71 (q, J = 270.4 Hz), 123.75, 120.71, 117.03, 112.71, 107.64, 68.80; HRMS calcd. For $C_{22}H_{18}F_3N_2O_5$ $[M+H]^+$ 447.1162, found 447.1159; HPLC (63% methanol and 0.5% TFA in water): t_R = 10.849 min, 99.34%.

4.1.8. *N′*-(3-((2-Fluorobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (8)

Yield 38.6%; White solid; mp 228–230 °C; ^{1}HNMR (400 MHz, DMSO) δ 11.54 (s, 1H), 8.98 (s, 3H), 8.36 (s, 1H), 7.57 (t, J = 7.4 Hz, 1H), 7.37 (m, 3H), 7.24 (m, 3H), 7.07 (d, J = 8.0 Hz, 1H), 6.91 (s, 2H), 5.18 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 162.10, 159.66, 158.96, 146.78, 146.02, 137.47, 136.65, 131.18 (d, J = 3.0 Hz), 130.90 (d, J = 8.0 Hz) 130.47, 125.03 (d, J = 3.0 Hz), 124.19 (d, J = 14.0 Hz), 123.75, 120.52, 116.94, 11.88 (d, J = 21.0 Hz), 112.78, 107.63, 64.10 (d, J = 3.0 Hz); HRMS calcd. For $C_{21}H_{18}FN_2O_5$ $[M+H]^+$ 397.1194, found 397.1194; HPLC (60% methanol and 0.5% TFA in water): t_R = 6.592 min, 96.11%.

4.1.9. *N′*-(3-((3-Fluorobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (9)

Yield 35.3%; White solid; mp 211–213 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 8.94 (s, 3H), 8.36 (s, 1H), 7.51 (m, 2H), 7.34 (m, 2H), 7.22 (m, 3H), 7.05 (d, J = 7.9 Hz, 1H), 6.91 (s, 2H), 5.12 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.16, 162.99, 160.57, 158.56, 146.36, 145.57, 137.07, 136.14, 133.9 (d, J = 3.0 Hz), 129.98 (d, J = 9.0 Hz),129.96, 123.21, 120.02, 116.61, 115.37, 115.16, 112.24, 107.15, 68.54; HRMS calcd. For $C_{21}H_{18}FN_2O_5$ ($[M+H]^+$ 397.1194, found 397.1191; HPLC (60% methanol and 0.5% TFA in water): t_R = 6.997 min, 97.74%.

4.1.10. *N′*-(3-((4-Fluorobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (10)

Yield 36.8%; White solid; mp 202–204 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.03 (s, 3H), 8.36 (s, 1H), 7.57–7.47 (m, 2H), 7.35 (m, 2H), 7.23 (m, 3H), 7.05 (d, J = 8.1 Hz, 1H), 6.91 (s, 2H), 5.13 (s, 2H); ^{13}C NMR (125 MHz, DMSO) δ 163.21, 162.79, 160.85, 158.60, 146.42, 145.61, 137.06, 136.16, 133.21(d, J = 3.0 Hz), 130.03 (d, J = 8.1 Hz), 123.30, 120.07, 116.66, 115.31 (d, J = 21.3 Hz), 112.25, 107.19, 68.56; HRMS calcd. For $C_{21}H_{18}FN_2O_5$ $[M+H]^+$ 397.1194, found 397.1193; HPLC (60% methanol and 0.5% TFA in water): t_R = 6.992 min, 97.39%.

4.1.11. *N*′-(3-((2-Chlorobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (11)

Yield 35.2%; White solid; mp 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.53 (s, 1H), 9.13 (s, 2H), 8.81 (s, 1H), 8.37 (s, 1H), 7.62 (t, J = 4.8 Hz, 1H), 7.51 (t, J = 5.2 Hz, 1H), 7.43–7.36 (m, 3H), 7.34 (d, J = 9.6 Hz, 1H), 7.27 (d, J = 7.7 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 6.90 (s, 2H), 5.19 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.61, 158.94, 146.78, 146.01, 137.44, 136.65, 134.65, 133.10, 130.63, 130.50, 130.38, 129.87, 127.86, 123.75, 120.68, 116.98, 112.65, 107.64, 67.38; HRMS calcd. For $C_{21}H_{18}N_2O_5Cl$ [M+H]$^+$ 413.0899, found 413.0886; HPLC (63% methanol and 0.5% TFA in water): t_R = 7.883 min, 99.69%.

4.1.12. *N*′-(3-((3-Chlorobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (12)

Yield 36.4%; White solid; mp 140–142 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.02 (s, 3H), 8.36 (s, 1H), 7.53 (s, 1H), 7.42 (m, 2H), 7.40–7.29 (m, 3H), 7.26 (d, J = 7.3 Hz, 1H), 7.06 (m, 1H), 6.91 (s, 2H), 5.16 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.17, 158.43, 146.34, 145.57, 139.57, 137.07, 136.18, 133.12, 130.40, 130.00, 127.79, 127.31, 126.19, 123.24, 120.13, 116.60, 112.26, 107.16, 68.32; HRMS calcd. For $C_{21}H_{18}ClN_2O_5$ [M+H]$^+$ 413.0899, found 413.0898; HPLC (63% methanol and 0.5% TFA in water): t_R = 8.717 min, 97.80%.

4.1.13. *N*′-(3-((4-Chlorobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (13)

Yield 32.6%; White solid; mp 200–202 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.10 (s, 2H), 8.36 (s, 1H), 7.49 (d, J = 8.5 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.34 (m, 2H), 7.25 (d, J = 7.4 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.91 (s, 2H), 5.14 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.26, 158.52, 146.41, 145.59, 137.02, 136.19, 136.07, 132.46, 130.02, 129.55, 128.49, 123.34, 120.13, 116.65, 112.30, 107.23, 68.44; HRMS calcd. For $C_{21}H_{18}ClN_2O_5$ [M+H]$^+$ 413.0899, found 413.0898; HPLC (63% methanol and 0.5% TFA in water): t_R = 8.912 min, 99.88%.

4.1.14. *N*′-(3-((4-(*tert*-Butyl)benzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (14)

Yield 35.6%; White solid; mp 126–128 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.53 (s, 1H), 8.98 (s, 3H), 8.36 (s, 1H), 7.44–7.28 (m, 6H), 7.25 (s, 1H), 7.04 (d, J = 7.1 Hz, 1H), 6.91 (s, 2H), 5.09 (s, 2H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, DMSO) δ 163.27, 158.77, 150.39, 146.47, 145.61, 137.06, 136.16, 133.97, 130.00, 127.66, 125.24, 123.34, 119.94, 116.64, 112.23, 107.21, 69.10, 34.35, 31.18; HRMS calcd. For $C_{25}H_{26}N_2O_5$ [M+H]$^+$ 435.1915, found 435.1902; HPLC (70% methanol and 0.5% TFA in water): t_R = 10.454 min, 99.20%.

4.1.15. *N*′-(3-((2-Cyanobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (15)

Yield 37.7%; White solid; mp 239–240 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.07 (s, 3H), 8.37 (s, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.75 (d, J = 4.3 Hz, 2H), 7.62–7.51 (m, 1H), 7.45–7.24 (m, 3H), 7.09 (d, J = 8.1 Hz, 1H), 6.90 (s, 2H), 5.28 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.62, 158.84, 146.72, 146.01, 140.36, 137.45, 136.68, 133.92, 133.79, 130.52, 130.19, 129.62, 123.75, 120.85, 117.70, 116.97, 112.78, 107.64, 68.12; HRMS calcd. For $C_{22}H_{18}N_3O_5$ [M+H]$^+$ 404.1241, found 404.1228; HPLC (55% methanol and 0.5% TFA in water): t_R = 5.328 min, 99.77%.

4.1.16. *N*′-(3-((3-Cyanobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (16)

Yield 39.1%; White solid; mp 168–170 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.49 (s, 1H), 8.95 (s, 3H), 8.37 (s, 1H), 7.93 (s, 1H), 7.81 (t, J = 8.7 Hz, 2H), 7.62 (t, J = 7.8 Hz, 1H), 7.37 (m, 2H), 7.28 (d, J = 7.5 Hz, 1H), 7.09 (m, 1H), 6.93 (s, 2H), 5.22 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ163.27, 158.39, 145.60, 138.77, 137.03, 136.23, 132.48, 131.73, 131.08, 130.06, 129.80, 123.34, 120.29, 118.74, 116.66, 112.27, 111.49, 107.24, 68.12; HRMS calcd. For $C_{25}H_{26}N_2O_5$ [M+H]$^+$ 404.1236, found 404.1241; HPLC (55% methanol and 0.5% TFA in water): t_R = 11.906 min, 95.60%.

4.1.17. *N*′-(3-((4-Cyanobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (17)

Yield 43.9%; White solid; mp 243–245 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.53 (s, 1H), 9.13 (s, 2H), 8.81 (s, 1H), 8.36 (s, 1H), 7.86 (d, J = 7.8 Hz, 2H), 7.66 (d, J = 7.9 Hz, 2H), 7.42–7.21 (m, 3H), 7.06 (d, J = 8.1 Hz, 1H), 6.90 (s, 2H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.61, 158.75, 146.74, 146.00, 143.26, 137.44, 136.65, 132.89, 130.48, 128.53, 123.74, 120.76, 119.23, 117.04, 112.64, 110.95, 107.63, 68.75; HRMS calcd. For $C_{22}H_{18}N_3O_5$ [M+H]$^+$ 404.1241, found 404.1229; HPLC (55% methanol and 0.5% TFA in water): t_R = 6.476 min, 99.98%.

4.1.18. 3,4,5-Trihydroxy-*N*′-(3-(naphthalen-1-ylmethoxy) benzylidene)benzohydrazide (18)

Yield 41.1%; White solid; mp 128–130 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.55 (s, 1H), 9.15 (s, 2H), 8.83 (s, 1H), 8.39 (s, 1H), 8.11 (d, J = 7.7 Hz, 1H), 7.96 (m, 2H), 7.70 (d, J = 6.7 Hz, 1H), 7.56 (m, 3H), 7.40 (m, 2H), 7.30 (s, 1H), 7.15 (d, J = 7.4 Hz, 1H), 6.92 (s, 2H), 5.60 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.19, 158.76, 146.44, 145.57, 137.00, 136.18, 133.32, 132.40, 131.20, 130.00, 128.78, 128.51, 126.83, 126.52, 126.02, 125.41, 123.97, 123.33, 119.95, 116.59, 112.57, 107.20, 67.94; HRMS calcd. For $C_{25}H_{21}N_2O_6$ [M+H]$^+$ 429.1445, found 429.1445; HPLC (63% methanol and 0.5% TFA in water): t_R = 10.578 min, 99.33%.

4.1.19. 3,4,5-Trihydroxy-*N*′-(3-((2-methoxybenzyl)oxy) benzylidene)benzohydrazide (19)

Yield 39.8%; White solid; mp 163–165 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 9.13 (s, 2H), 8.81 (s, 1H), 8.36 (s, 1H), 7.41 (d, J = 7.5 Hz, 1H), 7.36 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 7.1 Hz, 2H), 7.23 (d, J = 7.6 Hz, 1H), 7.09–7.00 (m, 1H), 6.96 (t, J = 7.4 Hz, 1H), 6.90 (s, 2H), 5.10 (s, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.61, 159.22, 157.34, 146.91, 146.01, 137.44, 136.55, 130.41, 129.85, 129.46, 124.95, 123.76, 120.77, 120.49, 117.16, 112.18, 111.35, 107.63, 64.95, 55.95; HRMS calcd. For $C_{22}H_{21}N_2O_6$ [M+H]$^+$ 409.1394, found 409.1383; HPLC (60% methanol and 0.5% TFA in water): t_R = 6.988 min, 97.30%.

4.1.20. 3,4,5-Trihydroxy-*N*′-(3-((3-methoxybenzyl)oxy) benzylidene)benzohydrazide (20)

Yield 36.7%; White solid; mp 181–183 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 9.04 (s, 3H), 8.35 (s, 1H), 7.39–7.21 (m, 4H), 7.09–6.97 (m, 3H), 6.96–6.85 (m, 3H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.61, 159.79, 159.06, 146.84, 146.01, 138.98, 137.48, 136.56, 130.40, 130.02, 123.73, 120.42, 120.15, 117.07, 113.77, 113.54, 112.70, 107.63, 69.55, 55.52; HRMS calcd. For $C_{22}H_{21}N_2O_6$ [M+H]$^+$ 409.1394, found 409.1382; HPLC (60% methanol and 0.5% TFA in water): t_R = 6.301 min, 98.67%.

4.1.21. 3,4,5-Trihydroxy-*N*′-(3-((4-methoxybenzyl)oxy) benzylidene)benzohydrazide (21)

Yield 36.1%; White solid; mp 127–129 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 9.12 (s, 3H), 8.35 (s, 1H), 7.42–7.31 (m, 3H), 7.29 (s, 1H), 7.24 (d, J = 7.5 Hz, 1H), 7.03 (m, 1H), 6.96–6.89 (m, 4H), 5.05 (s, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.37, 159.02, 158.71, 146.42, 145.55, 136.97, 136.07, 129.91, 129.51, 128.79, 123.31, 119.80, 116.64, 113.83, 112.32, 107.17, 69.04, 55.10; HRMS calcd. For $C_{22}H_{21}N_2O_6$ [M+H]$^+$ 409.1394, found

409.1381; HPLC (60% methanol and 0.5% TFA in water): t_R = 5.915 min, 96.92%.

4.1.22. *N*′-(4-(Benzyloxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (22)

Yield 34.2%; White solid; mp 214–215 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.38 (s, 1H), 8.94 (s, 3H), 8.33 (s, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.36–7.28 (m, 1H), 7.07 (d, J = 8.1 Hz, 2H), 6.89 (s, 2H), 5.14 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.47, 160.15, 146.90, 145.99, 137.28, 137.25, 128.92, 128.38, 128.23, 127.85, 123.98, 115.59, 107.56, 69.80; HRMS calcd. For $C_{21}H_{19}N_2O_5$ $[M+H]^+$ 379.1288, found 379.1277; HPLC (55% methanol and 0.5% TFA in water): t_R = 8.813 min, 99.42%.

4.1.23. 3,4,5-Trihydroxy-*N*′-(4-((2-(trifluoromethyl)benzyl)oxy)benzylidene)benzohydrazide (23)

Yield 32.5%; White solid; mp 190–192 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.12 (s, 2H), 8.78 (s, 1H), 8.34 (s, 1H), 7.85–7.68 (m, 3H), 7.68–7.54 (m, 3H), 7.08 (d, J = 8.3 Hz, 2H), 6.90 (s, 2H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.47, 159.79, 146.79, 145.99, 137.29, 135.09, 133.34, 131.07, 129.33, 129.01, 128.25, 127.40 (q, J = 30.7 Hz), 126.66 (q, J = 5.5 Hz), 124.77 (q, J = 275.0 Hz), 123.95, 115.50, 107.55, 66.80; HRMS calcd. For $C_{22}H_{18}O_5N_2F_3$ $[M+H]^+$ 447.1162, found 447.1147; HPLC (60% methanol and 0.5% TFA in water): t_R = 11.426 min, 99.82%.

4.1.24. 3,4,5-Trihydroxy-*N*′-(4-((3-(trifluoromethyl)benzyl)oxy)benzylidene)benzohydrazide (24)

Yield 32.0%; White solid; mp 188–190 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.11 (s, 2H), 8.80 (s, 1H), 8.34 (s, 1H), 7.82 (s, 1H), 7.77 (d, J = 7.6 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 7.8 Hz, 3H), 7.10 (d, J = 8.3 Hz, 2H), 6.89 (s, 2H), 5.26 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.46, 159.87, 146.79, 145.99, 138.82, 137.28, 132.20, 130.12, 129.65 (q, J = 31.8 Hz), 128.96, 128.11, 125.09 (q, J = 4.0 Hz), 124.64 (q, J = 273.1 Hz),124.57 (q, J = 3.8 Hz), 123.96, 115.61, 107.55, 68.89; HRMS calcd. For $C_{22}H_{18}O_5N_2F_3$ $[M+H]^+$ 447.1162, found 447.1148; HPLC (45% acetonitrile and 0.5% TFA in water): t_R = 7.386 min, 99.86%.

4.1.25. 3,4,5-Trihydroxy-*N*′-(4-((4-(trifluoromethyl)benzyl)oxy)benzylidene)benzohydrazide (25)

Yield 38.8%; White solid; mp >250 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.76 (d, J = 7.9 Hz, 2H), 7.67 (d, J = 8.2 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H), 7.09 (d, J = 8.3 Hz, 2H), 6.89 (s, 2H), 5.27 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 163.47, 159.82, 146.79, 145.99, 142.19, 137.29, 128.97, 128.82 (q, J = 31.8 Hz), 128.52, 128.12, 125.83 (q, J = 3.8 Hz), 124.64 (q, J = 273.1 Hz), 123.95, 115.62, 107.55, 68.87; HRMS calcd. For $C_{22}H_{18}O_5N_2F_3$ $[M+H]^+$ 447.1162, found 447.1148; HPLC (60% methanol and 0.5% TFA in water): t_R = 12.108 min, 99.97%.

4.1.26. *N*′-(4-((2-Cyanobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (26)

Yield 33.3%; White solid; mp >250 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.41 (s, 1H), 9.12 (s, 2H), 8.78 (s, 1H), 8.35 (s, 1H), 7.91 (s, 1H), 7.74 (s, 2H), 7.65 (s, 2H), 7.58 (s, 1H), 7.12 (s, 2H), 6.90 (s, 2H), 5.29 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.48, 159.80, 146.76, 145.99, 140.20, 137.29, 133.95, 133.81, 130.21, 129.67, 128.98, 128.36, 123.95, 117.66, 115.60, 111.83, 107.56, 68.13; HRMS calcd. For $C_{22}H_{18}O_5N_3$ $[M+H]^+$ 404.1241, found 404.1227; HPLC (50% methanol and 0.5% TFA in water): t_R = 8.778 min, 95.94%.

4.1.27. *N*′-(4-((3-Cyanobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (27)

Yield 38.1%; White solid; mp >250 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.93 (s, 1H), 7.80 (s, 2H), 7.62 (s, 3H), 7.09 (s, 2H), 6.89 (s, 2H), 5.21 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.47, 159.78, 146.80, 145.99, 139.02, 137.29, 132.96, 132.19, 131.58, 130.24, 128.96, 128.15, 123.95, 119.13, 115.62, 111.92, 107.55, 68.60; HRMS calcd. For $C_{22}H_{18}O_5N_3$ $[M+H]^+$ 404.1241, found 404.1227; HPLC (50% methanol and 0.5% TFA in water): t_R = 9.628 min, 95.30%.

4.1.28. *N*′-(4-((4-Cyanobenzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (28)

Yield 37.5%; White solid; mp >250 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.40 (s, 1H), 9.12 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.86 (s, 2H), 7.64 (s, 4H), 7.08 (s, 2H), 6.89 (s, 2H), 5.26 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.48, 159.73, 146.79, 145.99, 143.10, 137.29, 132.91, 128.98, 128.59, 128.17, 123.94, 119.21, 115.62, 111.00, 107.55, 68.82; HRMS calcd. For $C_{22}H_{18}O_5N_3$ $[M+H]^+$ 404.1241, found 404.1226; HPLC (50% methanol and 0.5% TFA in water): t_R = 9.605 min, 95.65%.

4.1.29. 3,4,5-Trihydroxy-*N*′-(4-((4-methylbenzyl)oxy)benzylidene)benzohydrazide (29)

Yield 32.7%; White solid; mp 243–245 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.38 (s, 1H), 9.11 (s, 2H), 8.77 (s, 1H), 8.32 (s, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 7.6 Hz, 2H), 7.19 (d, J = 7.6 Hz, 2H), 7.05 (d, J = 8.3 Hz, 2H), 6.89 (s, 2H), 5.09 (s, 2H), 2.29 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.44, 160.19, 146.91, 145.99, 137.63, 137.27, 134.20, 129.46, 128.91, 128.33, 127.78, 123.98, 115.60, 107.54, 69.71, 21.25; HRMS calcd. For $C_{22}H_{21}O_5N_2$ $[M+H]^+$ 393.1445, found 393.1431; HPLC (60% methanol and 0.5% TFA in water): t_R = 8.514 min, 98.09%.

4.1.30. *N*′-(4-((4-(*tert*-Butyl)benzyl)oxy)benzylidene)-3,4,5-trihydroxybenzohydrazide (30)

Yield 38.4%; White solid; mp 232–234 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.39 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 6.3 Hz, 4H), 7.06 (d, J = 8.2 Hz, 2H), 6.89 (s, 2H), 5.09 (s, 2H), 1.26 (s, 9H); ^{13}C NMR (100 MHz, DMSO) δ 163.45, 160.22, 150.86, 146.91, 145.98, 137.27, 134.23, 128.93, 128.14, 127.77, 125.66, 123.98, 115.55, 107.54, 69.61, 34.77, 31.59; HRMS calcd. For $C_{25}H_{27}O_5N_2$ $[M+H]^+$ 435.1914, found 435.1899; HPLC (70% methanol and 0.5% TFA in water): t_R = 9.100 min, 98.31%.

4.1.31. 3,4,5-Trihydroxy-*N*′-(4-(naphthalen-2-ylmethoxy)benzylidene)benzohydrazide (31)

Yield 39.4%; White solid; mp 246–248 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.39 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.99 (s, 1H), 7.97–7.89 (m, 3H), 7.63 (d, J = 8.3 Hz, 2H), 7.58 (d, J = 8.5 Hz, 1H), 7.56–7.46 (m, 2H), 7.13 (d, J = 8.3 Hz, 2H), 6.89 (s, 2H), 5.32 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.46, 160.17, 146.88, 145.99, 137.28, 134.89, 133.23, 133.03, 132.29, 128.96, 128.57, 128.27, 128.08, 127.92, 126.82, 126.64, 126.18, 123.98, 115.68, 107.56, 69.93; HRMS calcd. For $C_{25}H_{21}O_5N_2$ $[M+H]^+$ 429.1445, found 429.1431; HPLC (60% methanol and 0.5% TFA in water): t_R = 13.448 min, 99.89%.

4.1.32. 3,4,5-Trihydroxy-*N*′-(4-((2-methoxybenzyl)oxy)benzylidene)benzohydrazide (32)

Yield 35.4%; White solid; mp 238–240 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.38 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.61 (s, 2H), 7.36 (d, J = 24.5 Hz, 2H), 7.05 (s, 3H), 6.95 (s, 1H), 6.89 (s, 2H), 5.09 (s, 2H), 3.82 (s, 4H); ^{13}C NMR (100 MHz, DMSO)

δ 163.45, 160.32, 157.43, 146.91, 145.99, 137.26, 129.99, 129.70, 128.95, 127.75, 124.78, 123.99, 120.77, 115.43, 111.39, 107.54, 65.19, 55.92; HRMS calcd. For $C_{22}H_{21}O_6N_2$ $[M+H]^+$ 409.1394, found 409.1374; HPLC (60% methanol and 0.5% TFA in water): t_R = 6.318 min, 95.82%.

4.1.33. 3,4,5-Trihydroxy-*N*′-(4-((3-methoxybenzyl)oxy) benzylidene)benzohydrazide (33)

Yield 33.6%; White solid; mp 202–204 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.39 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.61 (d, *J* = 8.3 Hz, 2H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 7.02 (s, 2H), 6.89 (s, 3H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.44, 160.11, 159.80, 146.88, 145.98, 138.83, 137.27, 130.05, 128.92, 127.86, 123.97, 120.24, 115.60, 113.80, 113.66, 107.54, 69.65, 55.52; HRMS calcd. For $C_{22}H_{21}O_6N_2$ $[M+H]^+$ 409.1394, found 409.1379; HPLC (58% methanol and 0.5% TFA in water): t_R = 6.446 min, 99.60%.

4.1.34. 3,4,5-Trihydroxy-*N*′-(4-((4-methoxybenzyl)oxy) benzylidene)benzohydrazide (34)

Yield 38.7%; White solid; mp196–198 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.38 (s, 1H), 9.11 (s, 2H), 8.78 (s, 1H), 8.33 (s, 1H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.05 (d, *J* = 8.3 Hz, 2H), 6.94 (d, *J* = 8.3 Hz, 2H), 6.89 (s, 2H), 5.05 (s, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.45, 160.23, 159.52, 146.94, 145.99, 137.27, 130.08, 129.08, 128.90, 127.72, 123.99, 115.59, 114.29, 107.55, 69.59, 55.56; HRMS calcd. For $C_{22}H_{21}O_6N_2$ $[M+H]^+$ 409.1394, found 409.1378; HPLC (55% methanol and 0.5% TFA in water): t_R = 7.797 min, 99.99%.

4.1.35. *N*′-(3-(Benzyloxy)benzylidene)-1H-benzo[d]imidazole-5-carbohydrazide (35)

Yield 31.6%; White solid; mp 222–224 °C; ^{1}H NMR (400 MHz, DMSO) δ 12.74 (s, 1H), 11.86 (s, 1H), 8.44 (s, 1H), 8.36 (s, 1H), 8.23 (s, 1H), 7.80 (d, *J* = 7.9 Hz, 1H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.47 (m, 2H), 7.36 (m, 5H), 7.08 (m, 1H), 5.15 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.56, 158.68, 147.00, 144.18, 136.94, 136.00, 129.99, 128.45, 127.87, 127.71, 126.93, 121.84, 120.01, 116.72, 112.42, 69.26; HRMS calcd. For $C_{22}H_{19}N_4O_2$ $[M+H]^+$ 371.1503, found 371.1496; HPLC (35% acetonitrile and 0.5% TFA in water): t_R = 7.777 min, 99.77%.

4.1.36. 3,5-Dihydroxy-*N*′-(3-((3-methoxybenzyl)oxy) benzylidene)benzohydrazide (36)

Yield 62.3%; White solid; mp 103–105 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.72 (s, 1H), 9.59 (s, 2H), 8.40 (s, 1H), 7.35 (s, 1H), 7.27 (d, *J* = 7.9 Hz, 2H), 7.04 (q, *J* = 6.9, 5.5 Hz, 3H), 6.87 (d, *J* = 8.2 Hz, 1H), 6.77 (s, 2H), 6.45 (s, 1H), 5.10 (s, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 164.09, 160.02, 159.29, 159.11, 148.06, 139.17, 136.57, 136.12, 130.64, 130.24, 120.83, 120.37, 117.53, 113.98, 113.76, 113.01, 109.99, 106.50, 69.79, 55.70; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1372, found 393.1450; HPLC (57% methanol and 0.5% TFA in water): t_R = 11.483 min, 99.75%.

4.1.37. 3,4-Dihydroxy-*N*′-(3-((3-methoxybenzyl)oxy) benzylidene)benzohydrazide (37)

Yield 62.2%; White solid; mp 170–172 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.56 (s, 1H), 9.60 (s, 1H), 9.21 (s, 1H), 8.37 (s, 1H), 7.40–7.33 (m, 2H), 7.33–7.20 (m, 4H), 7.09–6.99 (m, 3H), 6.88 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.66, 160.03, 159.30, 149.76, 147.24, 145.75, 139.19, 136.74, 130.63, 130.25, 124.99, 120.70, 120.38, 117.34, 116.14, 115.68, 113.99, 113.78, 112.95, 69.79, 55.73; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1372, found 393.1436; HPLC (57% methanol and 0.5% TFA in water): t_R = 12.434 min, 99.23%.

4.1.38. 3-Hydroxy-*N*′-(3-((3-methoxybenzyl)oxy)benzylidene) benzohydrazide (38)

Yield 66.1%; White solid; mp 183–185 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H), 9.74 (s, 1H), 8.40 (s, 1H), 7.43–7.23 (m, 7H), 7.08 (s, 1H), 7.03 (d, *J* = 4.8 Hz, 2H), 6.96 (d, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.3 Hz, 1H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.87, 160.03, 159.31, 158.11, 148.18, 139.17, 136.53, 135.49, 130.66, 130.24, 130.20, 120.81, 120.38, 119.40, 118.80, 117.55, 115.23, 113.99, 113.78, 113.09, 69.81, 55.73; HRMS calcd. For $C_{22}H_{21}N_2O_4$ $[M+H]^+$ 377.1423, found 377.1491; HPLC (65% methanol and 0.5% TFA in water): t_R = 7.558 min, 99.86%.

4.1.39. 4-Hydroxy-*N*′-(3-((3-methoxybenzyl)oxy)benzylidene) benzohydrazide (39)

Yield 45.7%; White solid; mp 174–176 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.62 (s, 1H), 10.09 (s, 1H), 8.38 (s, 1H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.40–7.20 (m, 4H), 7.06 (d, *J* = 8.2 Hz, 1H), 7.02 (d, *J* = 4.9 Hz, 2H), 6.89 (s, 1H), 6.84 (d, *J* = 8.2 Hz, 2H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.23, 161.16, 159.80, 159.07, 147.12, 138.96, 136.46, 130.42, 130.16, 130.02, 124.30, 120.45, 120.16, 117.12, 115.47, 113.76, 113.56, 112.78, 69.56, 55.51; HRMS calcd. For $C_{22}H_{21}O_4N_2$ $[M+H]^+$ 377.1496, found 377.1481; HPLC (63% methanol and 0.5% TFA in water): t_R = 8.086 min, 99.24%.

4.1.40. 3,5-Dihydroxy-*N*′-(4-((3-methoxybenzyl)oxy) benzylidene)benzohydrazide (40)

Yield 59.6%; White solid; mp 214–216 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H), 9.74 (s, 1H), 8.40 (s, 1H), 7.43–7.19 (m, 7H), 7.07 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 4.6 Hz, 2H), 6.96 (d, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.3 Hz, 1H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.85, 160.49, 160.03, 159.06, 147.99, 139.03, 136.32, 130.26, 129.29, 127.90, 120.46, 115.84, 114.02, 113.88, 106.41, 106.20, 69.89, 55.73; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1372, found 393.1459; HPLC (62% methanol and 0.5% TFA in water): t_R = 9.417 min, 98.41%.

4.1.41. 3,4-Dihydroxy-*N*′-(4-((3-methoxybenzyl)oxy) benzylidene)benzohydrazide (41)

Yield 61.5%; White solid; mp 198–200 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.42 (s, 1H), 9.57 (s, 1H), 9.19 (s, 1H), 8.34 (s, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.33 (s, 1H), 7.29 (d, *J* = 8.1 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 4.8 Hz, 2H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.79 (d, *J* = 8.2 Hz, 1H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.42, 160.36, 160.02, 149.57, 147.21, 145.70, 139.04, 130.26, 129.16, 128.04, 125.18, 120.45, 120.15, 116.04, 115.83, 115.63, 114.01, 113.87, 69.88, 55.73; HRMS calcd. For $C_{22}H_{21}N_2O_5$ $[M+H]^+$ 393.1372, found 393.1445; HPLC (57% methanol and 0.5% TFA in water): t_R = 10.171 min, 99.17%.

4.1.42. 3-Hydroxy-*N*′-(4-((3-methoxybenzyl)oxy)benzylidene) benzohydrazide (42)

Yield 67.2%; White solid; mp 174–176 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.61 (s, 1H), 9.71 (s, 1H), 8.36 (s, 1H), 7.64 (d, *J* = 8.3 Hz, 2H), 7.31 (d, *J* = 7.8 Hz, 3H), 7.27 (d, *J* = 8.6 Hz, 3H), 7.08 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 4.7 Hz, 2H), 6.95 (d, *J* = 6.6 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 5.12 (s, 2H), 3.75 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.69, 160.53, 160.03, 158.09, 148.18, 139.01, 135.67, 130.26, 130.16, 129.34, 127.85, 120.45, 119.26, 118.74, 115.85, 115.19, 114.01, 113.88, 69.89, 55.72; HRMS calcd. For $C_{22}H_{21}N_2O_4$ $[M+H]^+$ 377.1423, found 377.1497; HPLC (55% methanol and 0.5% TFA in water): t_R = 14.280 min, 98.29%.

4.1.43. 4-Hydroxy-*N*′-(4-((3-methoxybenzyl)oxy)benzylidene) benzohydrazide (43)

Yield 50.2%; White solid; mp 188–190 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.49 (s, 1H), 10.07 (s, 1H), 8.35 (s, 1H), 7.78 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H), 7.29 (t, J = 8.0 Hz, 1H), 7.07 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 4.9 Hz, 2H), 6.89 (s, 1H), 6.84 (d, J = 8.2 Hz, 2H), 5.12 (s, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO) δ 163.04, 161.04, 160.17, 159.80, 147.13, 138.82, 130.05, 128.98, 127.77, 124.48, 120.24, 115.62, 115.44, 113.80, 113.66, 69.65, 55.52; HRMS calcd. For $C_{22}H_{21}O_4N_2$ $[M+H]^+$ 377.1496, found 377.1478; HPLC (43% acetonitrile and 0.5% TFA in water): t_R = 8.276 min, 99.87%.

4.1.44. 3,5-Dihydroxy-*N*′-(3-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (44)

Yield 61.5%; White solid; mp 239–241 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.68 (s, 1H), 9.55 (s, 2H), 8.39 (s, 1H), 7.83–7.76 (m, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.35–7.25 (m, 2H), 7.07 (d, J = 8.2 Hz, 1H), 6.72 (s, 2H), 6.41 (s, 1H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 164.08, 159.11, 159.04, 147.90, 136.71, 136.11, 135.44, 133.48, 131.19, 130.77, 129.44, 127.62 (d, J = 30.4 Hz), 126.83 (q, J = 5.6 Hz), 125.00 (d, J = 274.1 Hz), 121.14, 117.30, 112.95, 106.49, 106.37, 66.96; HRMS calcd. For $C_{22}H_{18}F_3N_2O_4$ $[M+H]^+$ 431.1140, found 431.1191; HPLC (62% methanol and 0.5% TFA in water): t_R = 12.727 min, 96.04%.

4.1.45. 3,4-Dihydroxy-*N*′-(3-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (45)

Yield 62.1%; White solid; mp 193–195 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.57 (s, 1H), 9.60 (s, 1H), 9.21 (s, 1H), 8.38 (s, 1H), 7.79 (t, J = 6.3 Hz, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.43–7.21 (m, 5H), 7.06 (d, J = 8.5 Hz, 1H), 6.80 (d, J = 8.2 Hz, 1H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.63, 159.03, 149.76, 147.09, 145.74, 136.86, 135.45, 133.49, 131.19, 130.76, 129.45, 127.60 (d, J = 30.3 Hz), 126.83 (q, J = 5.5 Hz), 125.00 (d, J = 272.2 Hz), 124.97, 121.01, 120.30, 117.11, 116.12, 115.67, 112.89, 67.13; HRMS calcd. For $C_{22}H_{18}F_3N_2O_4$ $[M+H]^+$ 431.1140, found 431.1218; HPLC (62% methanol and 0.5% TFA in water): t_R = 13.959 min, 96.97%.

4.1.46. 3-Hydroxy-*N*′-(3-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (46)

Yield 64.6%; White solid; mp 175–177 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H), 9.74 (s, 1H), 8.41 (s, 1H), 7.79 (t, J = 5.9 Hz, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.39 (t, J = 7.8 Hz, 1H), 7.37–7.25 (m, 5H), 7.08 (d, J = 8.1 Hz, 1H), 6.96 (d, J = 6.9 Hz, 1H), 5.28 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.90, 159.05, 158.12, 148.07, 136.67, 135.47, 133.50, 131.19, 130.79, 130.21, 129.46, 129.09, 127.61 (q, J = 30.3 Hz), 126.84 (d, J = 6.0 Hz), 125.01 (d, J = 273.8 Hz), 121.16, 119.42, 118.80, 117.35, 115.23, 113.01, 66.96; HRMS calcd. For $C_{22}H_{18}F_3N_2O_3$ $[M+H]^+$ 415.1191, found 415.1258; HPLC (65% methanol and 0.5% TFA in water): t_R = 13.108 min, 96.54%.

4.1.47. 4-Hydroxy-*N*′-(3-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (47)

Yield 55.67%; White solid; mp 175–177 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.63 (s, 1H), 10.10 (s, 1H), 8.39 (s, 1H), 7.79 (d, J = 8.3 Hz, 4H), 7.72 (t, J = 7.6 Hz, 1H), 7.58 (t, J = 7.7 Hz, 1H), 7.38 (t, J = 7.9 Hz, 1H), 7.31 (d, J = 11.0 Hz, 2H), 7.06 (d, J = 8.1 Hz, 1H), 6.84 (d, J = 8.2 Hz, 2H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.50, 161.41, 159.04, 147.24, 136.81, 135.45, 133.49, 131.16, 130.76, 130.38, 129.44, 127.59 (q, J = 30.2 Hz), 126.82 (q, J = 5.5 Hz), 125.00 (q, J = 272.2 Hz), 124.51, 121.03, 117.13, 115.70, 112.91, 66.93; HRMS calcd. For $C_{22}H_{18}F_3N_2O_3$ $[M+H]^+$ 415.1191, found 415.1263; HPLC (65% methanol and 0.5% TFA in water): t_R = 12.879 min, 97.38%.

4.1.48. 3,5-Dihydroxy-*N*′-(4-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (48)

Yield 64.0%; White solid; mp 218–220 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.54 (s, 1H), 9.53 (s, 2H), 8.35 (s, 1H), 7.83–7.75 (m, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.59 (t, J = 7.6 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 6.71 (s, 2H), 6.39 (s, 1H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.90, 160.17, 159.07, 147.91, 136.30, 135.28, 133.50, 131.24, 129.50, 129.38, 128.29, 127.63 (d, J = 30.7 Hz), 126.85 (q, J = 5.5 Hz), 124.97 (d, J = 274.2 Hz), 115.73, 106.43, 106.24, 67.02; HRMS calcd. For $C_{22}H_{18}F_3N_2O_4$ $[M+H]^+$ 431.1140, found 431.1203; HPLC (62% methanol and 0.5% TFA in water): t_R = 11.616 min, 99.98%.

4.1.49. 3,4-Dihydroxy-*N*′-(4-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (49)

Yield 63.4%; White solid; mp 223–225 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.45 (s, 1H), 9.58 (s, 1H), 9.20 (s, 1H), 8.35 (s, 1H), 7.83–7.75 (m, 2H), 7.72 (t, J = 7.6 Hz, 1H), 7.65 (d, J = 8.2 Hz, 2H), 7.58 (t, J = 7.7 Hz, 1H), 7.34 (s, 1H), 7.27 (d, J = 8.2 Hz, 1H), 7.08 (d, J = 8.3 Hz, 2H), 6.80 (d, J = 8.2 Hz, 1H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 166.85, 163.48, 160.05, 149.61, 147.12, 145.71, 135.31, 133.52, 131.25, 129.52, 129.26, 128.44, 127.62 (d, J = 30.3 Hz), 126.88, 125.17, 124.99 (d, J = 272.2 Hz), 120.17, 116.06, 115.72. 67.05; HRMS calcd. For $C_{22}H_{18}F_3N_2O_4$ $[M+H]^+$ 431.1140, found 431.1214; HPLC (62% methanol and 0.5% TFA in water): t_R = 12.624 min, 97.35%.

4.1.50. 3-Hydroxy-*N*′-(4-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (50)

Yield 65.6%; White solid; mp 200–202 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.63 (s, 1H), 9.71 (s, 1H), 8.37 (s, 1H), 7.84–7.76 (m, 2H), 7.75–7.69 (m, 1H), 7.67 (d, J = 8.2 Hz, 2H), 7.59 (t, J = 7.6 Hz, 1H), 7.30 (s, 1H), 7.28 (d, J = 11.0 Hz, 2H), 7.09 (d, J = 8.3 Hz, 2H), 6.95 (s, 1H), 5.28 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.72, 160.21, 158.09, 148.09, 135.64, 135.27, 133.51, 131.24, 130.16, 129.51, 129.42, 128.23, 127.63 (q, J = 30.3 Hz), 126.85 (q, J = 5.5 Hz), 124.98 (d, J = 273.8 Hz), 119.27, 118.73, 115.74, 115.19, 67.03; HRMS calcd. For $C_{22}H_{18}F_3N_2O_3$ $[M+H]^+$ 415.1191, found 415.1245; HPLC (65% methanol and 0.5% TFA in water): t_R = 11.832 min, 99.86%.

4.1.51. 4-Hydroxy-*N*′-(4-((2-(trifluoromethyl)benzyl)oxy) benzylidene)benzohydrazide (51)

Yield 40.7%; White solid; mp 191–193 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.50 (s, 1H), 10.07 (s, 1H), 8.36 (s, 1H), 7.79 (t, J = 7.9 Hz, 4H), 7.72 (t, J = 7.6 Hz, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.59 (t, J = 7.7 Hz, 1H), 7.08 (d, J = 8.3 Hz, 2H), 6.84 (d, J = 8.2 Hz, 2H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 163.05, 161.04, 159.85, 147.03, 135.08, 133.33, 131.06, 130.05, 129.32, 129.06, 128.15, 127.41 (q, J = 30.5 Hz), 126.65 (q, J = 5.6 Hz), 124.77 (q, J = 274.7 Hz), 124.46, 115.51, 115.44, 66.80; HRMS calcd. For $C_{22}H_{18}O_3N_2F_3$ $[M+H]^+$ 415.1264, found 415.1249; HPLC (65% methanol and 0.5% TFA in water): t_R = 10.552 min, 99.26%.

^{1}H and ^{13}CNMR spectral data of representative compounds for characterization were presented in the supporting information.

4.2. Biology

4.2.1. Enzyme-based screening

Human recombinant SphK1 (PV5214, 0.37 mg/mL), SphK2 (PV5216, 0.39 mg/mL), Sph (PV5372, 400 nmol), ATP (10 mM) were supplied from Life technologies (NY, USA). ADP Quest™ Assay Kit were provided by DiscoveRx (Fremont, CA, USA). A typical reac-

tion contained 5 μL compound solution, 5 μL sph, 5 μL ATP and 5 μL SphK. The mixture was incubated at 28 °C for 1 h with a 10 min preincubation of SphK and compounds. All reactions were proceeded by the addition of ADP Quest™ reagent A and B, then incubated at room temperature for 30 min. For fluorescence intensity measurement, the PerkinElmer envision enspire multimode reader (Waltham, USA) was used at 530 nm excitation and 590 nm emission.

4.2.2. Cell culture conditions

HCT116 (China Infrastructure of Cell Line Resources, Beijing, China) were maintained in IMDM medium (GiBco, Invitrogen Corp., USA) with 10% fetal bovine serum (FBS) (GiBco, Invitrogen Corp., USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

4.2.3. Western blot

Anti-SphK2 (sc-22704, goat, polyclonal, 1:500) were from Santa Cruz Biotechnology (CA, USA). Anti-SphK1 (#3297, rabbit, polyclonal, 1:1000) and β-actin (#4970S, rabbit, monoclonal, 1:1000) were purchased from cell signaling technology (Boston, USA). HCT116 (5×10^6 cells/well) were washed once with cold PBS and driven down with 1 mL of trypsin. Cells were centrifuged at 1000 rpm and resuspended in NP-40 lysis buffer (Beyotime, NT, China) and 1 mM PMSF for 1 h on ice. Then cells were centrifuged again at 12000 rpm for 15 min at 4 °C. The protein concentration was determined by the BCA assay at 562 nm. Samples were stored at −80 °C. The extracts were separated by SDS–PAGE (12% gel) and transferred to a PVDF membrane (Perkin-Elmer, Northwalk, CT, USA) followed by blocking with 1% BSA, detecting via a primary antibody and an anti-rabbit HRP-labeled secondary antibody using the UVP EC3 Imaging System (UVP, USA). The intensity data was analyzed and quantified to the comparable value according to the protocol of VisionWorks LS software (The imaging system itself contains this software).

4.2.4. DSS mouse models

Animal studies were conducted according to protocols approved by Institutional Animal Care and Use Committee of Peking Union Medical College and Chinese Academy of Medical Sciences. All animals were appropriately used on a scientifically valid and ethical manner. DSS (molecular weight at 36–50 kDa) (MP Biomedicals, Morgan Irvine, CA) was dissolved at 2.5% with distilled water. 5-ASA was purchased from Energy Chemical (SH, China). C57BL/6 female mice (6–8 weeks old) were divided into five groups in random: Con group, DSS group, DSS + 5-ASA group (50 mg/kg, *ig*), DSS + **33** group (5 mg/kg, *ip*) and DSS + **33** group (10 mg/kg, *ip*). Colitis was induced by 2.5% w/v DSS in drinking water for 5 days followed by another 5 days with normal water. Mice were weighted every day, and administration of 5-ASA or **33** lasted until the end of the experiment. After 10 days, mice were sacrificed. The colon was fixed in 10% buffered formalin (pH 7.4) for at least 24 h for further histopathological assessment and immunohistochemical study.

4.2.5. Histopathological evaluation

The colon was spread onto a plastic sheet, fixed with 10% neutral-buffered formalin for 48 h, and embedded in paraffin block. Sections of paraffin-embedded tissues were subjected to hematoxylin & eosin (H & E, KeyGEN, Nanjing, China) staining for measuring the severity of inflammation. Inflammation score was performed in a blind fashion by a pathologist, with a combined score for the severity of inflammation (score, 0–3), extent of inflammation (score, 0–3), crypt damage (score, 0–4) and percent involvement (score, 0–4). The grade of inflammation in the colon was classified as follows: (1) for the severity of inflammation, no inflammation was assigned a value of 0, sligt inflammation was assigned a value of 1, moderate inflammation was assigned a value of 2, and severe inflammation was assigned a value of 3; (2) for extent of inflammation, normal colonic mucosa was scored as 0, mucosal lesion was scored as 1; mucosal and submucosal lesion was scored as 2; transmural lesion was scored as 3; (3) for crypt damage, no crypt damage was assigned as 0, 1/3 of crypt damage was assigned as 1, 2/3 of crypt damage was assigned as 2, complete loss of crypts and normal surface epithelium were assigned as 3, complete loss of crypt and surface epithelium were assigned as 4; (4) for percent involvement, the proportion of involvement being 0% was scored as 0, 1–25% was scored as 1, 26–50% was scored as 2, 51–75% was scored as 3, 76–100% was scored as 4. The score of percent involvement (0–4) was multiplied by the sum of former three parameters (0–10) to yield the final score.

4.2.6. Immunohistochemistry

Tissues were prepared from formalin fixed, paraffin-embedded colon tissue. Stains against IL-6 (ab6672, rabbit, polyclonal, 1:500) and COX-2 (sc-1746, goat, polyclonal, 1:200) were performed according to the kit protocol (KeyGEN, NanJing, China). Briefly, the slides were deparaffinized. Antigen unmasking was carried out by incubation in 100 °C water bath in 10 mM sodium citrate buffer with 0.1% Tween 20 for 20 min. Slides were incubated with primary antibodies in PBS containing 5% BSA and 10% goat serum. HRP Polymer were added and incubated at room temperature for 30 min. And the sections were stained with DAB substrate and counterstained with hematoxylin after 40 min. Image Pro Plus 6.0 software (Media Cybernetics, Silver Spring, USA) was used for automatic counting of labeled inflammatory cytokines to quantify the experimental data.

4.2.7. Statistical analysis

All results are shown as mean ± SD. Each study was of 3 separate experiments and performed in parallel experiments for triplicate unless otherwise indicated. *P*-Values less than 0.05 ($p < 0.05$) were considered statistically significant. They were undertaken using the GraphPad Prism software (GraphPad Software Inc., Avenida, CA).

Acknowledgments

This work was financially supported by National Natural Science Foundation of China (NSFC No. 81473096), Peking Union Medical College Youth Fund (No.2013CHX12, 2015CX10).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.05.047.

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