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Research paper

Novel tertiary sulfonamide derivatives containing benzimidazole moiety as potent anti-gastric cancer agents: Design, synthesis and SAR studies

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ABSTRACT

With the expectation to find out new anti-gastric cancer agents with high efficacy and selectivity, a series of novel tertiary sulfonamide derivatives were synthesized and the anti-cancer activity was studied in three selected cancer cell lines (MGC-803, PC-3, MCF-7) in vitro. Some of the synthesized compounds could significantly inhibit the proliferation of these tested cancer cells and were more potent than the positive control (5-Fu). The structure-activity relationship of tertiary sulfonamide derivatives was explored in this report. Among the tested compounds, compound **13g** containing benzimidazole moiety showed the best anti-proliferation activities against MGC-803 cells ($IC_{50} = 1.02\ \mu M$), HGC-27 cells ($IC_{50} = 1.61\ \mu M$), SGC-7901 ($IC_{50} = 2.30\ \mu M$) cells as well as the good selectivity between the cancer and normal cells. Cellular mechanism studies elucidated compound **13g** inhibited the colony formation of gastric cancer cell lines. Meanwhile, compound **13g** arrested cell cycle at G2/M phase and induced cell apoptosis. Mechanistically, compound **13g** markedly decreased p-Akt and p-*c*-Raf expression, which revealed that compound **13g** targeted gastric cancer cell lines via interfering with AKT/mTOR and RAS/Raf/MEK/ERK pathways. All the findings suggest that compound **13g** might be a valuable lead compound for the anti-gastric cancer agents.

1. Introduction

Gastric cancer (GC), a very aggressive tumor, is the fifth most common malignancy with almost one million new cases diagnosed globally each year and remains one of the deadliest malignant tumors among Asians [1–3]. Although surgery procedures and chemotherapy have improved greatly in recent years, undesirable drug-induced toxicities and deleterious side effects owing to insufficient selectivity against healthy mammalian cells limit their therapeutic use [4,5]. Thus, it is highly desirable to develop newly targeted therapy for gastric cancer with minimal side-effects and new class of anti-cancer agents with the excellent selectivities between cancer and normal cells. Akt/mTOR and RAS/Raf/MEK/ERK pathways play an important role in cellular functions, and they are often overexpressed and uncontrolled in many human cancer cells such as gastric cancer cells, breast cancer cells, and prostate cancer cells [6–8]. Akt and mTOR are 2 pathways have cross-talks and compensations with MAPK pathway. Moreover, The Akt/mTOR and Ras/Raf/MEK/ERK pathways have been identified as promising therapeutic targets for cancer therapy including gastric cancer [9–11]. Therefore, targeting Akt/mTOR and Ras/Raf/MEK/ERK pathways is one of the effective ways to gastric tumors.

The sulfonamide derivatives are known for their numerous pharmacological activities in medicinal chemistry and modern

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drug discovery, such as anti-bacterial [12–14], anti-tumor [15–21], anti-HIV [22–24] and anti-inflammatory activities [25–27]. Recently, the tertiary sulfonamide fragment was frequently utilized to design novel anticancer agents to enhance the biological efficacy, such as tubulin polymerization inhibitors [15,16,19,28], carbonic anhydrase inhibitors [18,20,29], HIF-1 pathway inhibitors [30], cell cycle inhibitors [31] and MEK inhibitors [32,33]. Styryl-pyridine *N*-oxide sulfonamide **1** [28] demonstrated effective inhibition of tubulin polymerization and were found to be potent antimitotic agents. Compound **2** [15] showed excellent activity as tubulin polymerization inhibitor with an IC_{50} value of 1.1 μM and exhibited the antiproliferative activity with an IC_{50} value of 0.086 μM against HT29 cells. Tertiary sulfonamide derivatives of pyridyl-indole based heteroaryl chalcone **3** [20] exhibited the significant inhibition of hCA IX activity with an IC_{50} value of 0.13 μM. Compound **4** was observed strong selectivity toward tumor-associated hCA IX, without inhibiting the off target hCA II with an IC_{50} value of 4.2 nM against hCA IX [29]. Compound **5** inhibited HIF activity in an HRE-dependent cell-based assay with an IC_{50} value of approximate 590 nm and displayed promising antitumor activity in animal models [30]. 4-Methoxy-*N*-(3-chloro-7-indazolyl) benzenesulfonamide **6** [31] was identified as the most potent with an IC_{50} of 0.44 μM against murine L1210 cell line (Fig. 1). Based on the above findings mentioned, sulfonamide moiety was chosen as a core for designing tertiary sulfonamide derivatives with anti-gastric cancer activity.

Recently, our group has reported several series of novel tertiary amide derivatives that exhibited excellent antiproliferative activity against gastrointestinal track tumor cell lines [34–36]. In one of the studies mentioned above, tertiary amide derivative containing benzimidazole moiety (TRF-7, in Fig. 2) showed potent anti-gastric cancer cells (MGC-803) with an IC_{50} value of 1.23 μM but weak activities against human prostate cancer cell line (PC-3) and human esophageal cancer cell line (EC-109) [34]. In addition, benzimidazole and its derivatives occupy an important place in medicinal chemistry due to their synthetic utility and diversified biological activities [37]. Benzimidazole derivatives have been reported to have potent anti-proliferative effect against many types of cancer and benzimidazole moiety was important for anticancer activity [38–40]. Therefore, benzimidazole moiety was introduced into the designed hybrids to optimize for better efficacy and lower toxicity.

The molecular hybridization approach is a widely used strategy in drug discovery of rational design new molecular entities by incorporating two or more bioactive substructures through suitable

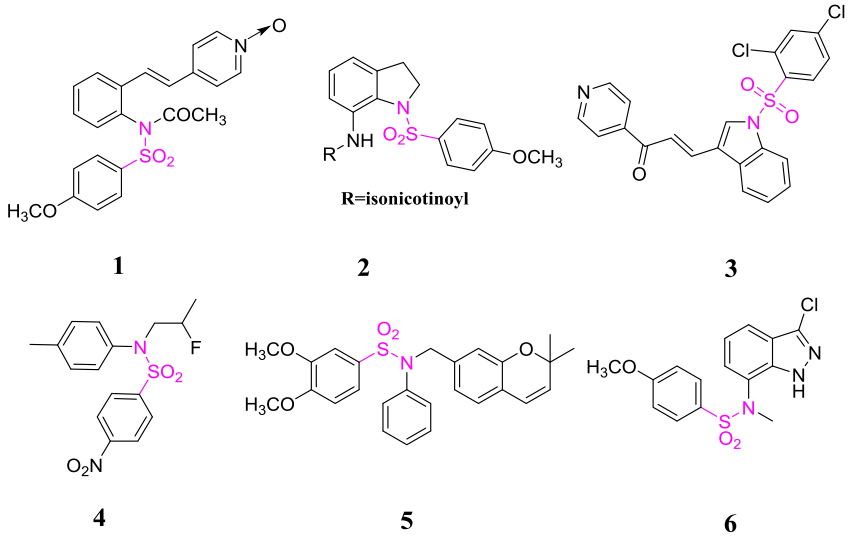


Fig. 1. Structures of tertiary sulfonamide derivatives with anticancer activity.

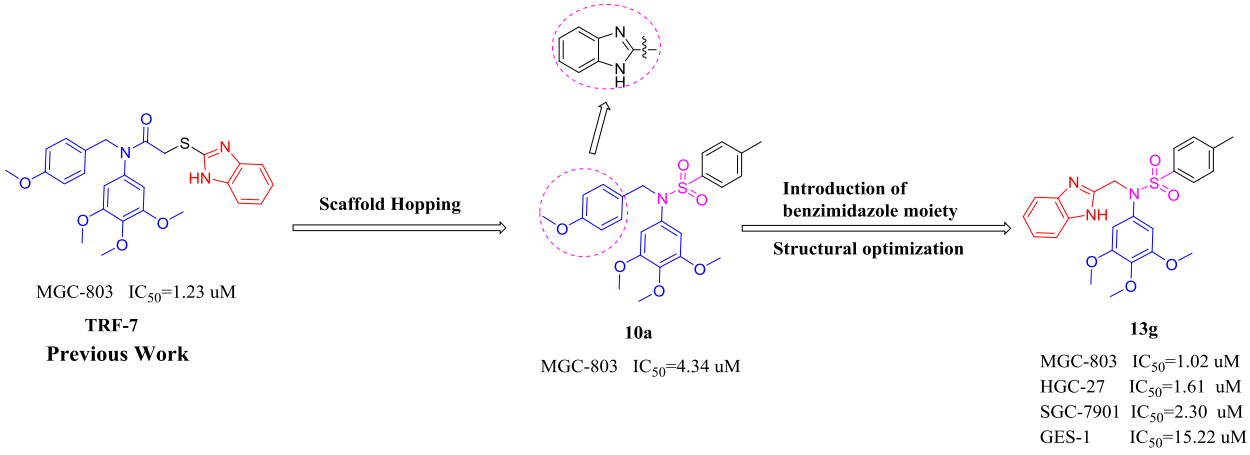
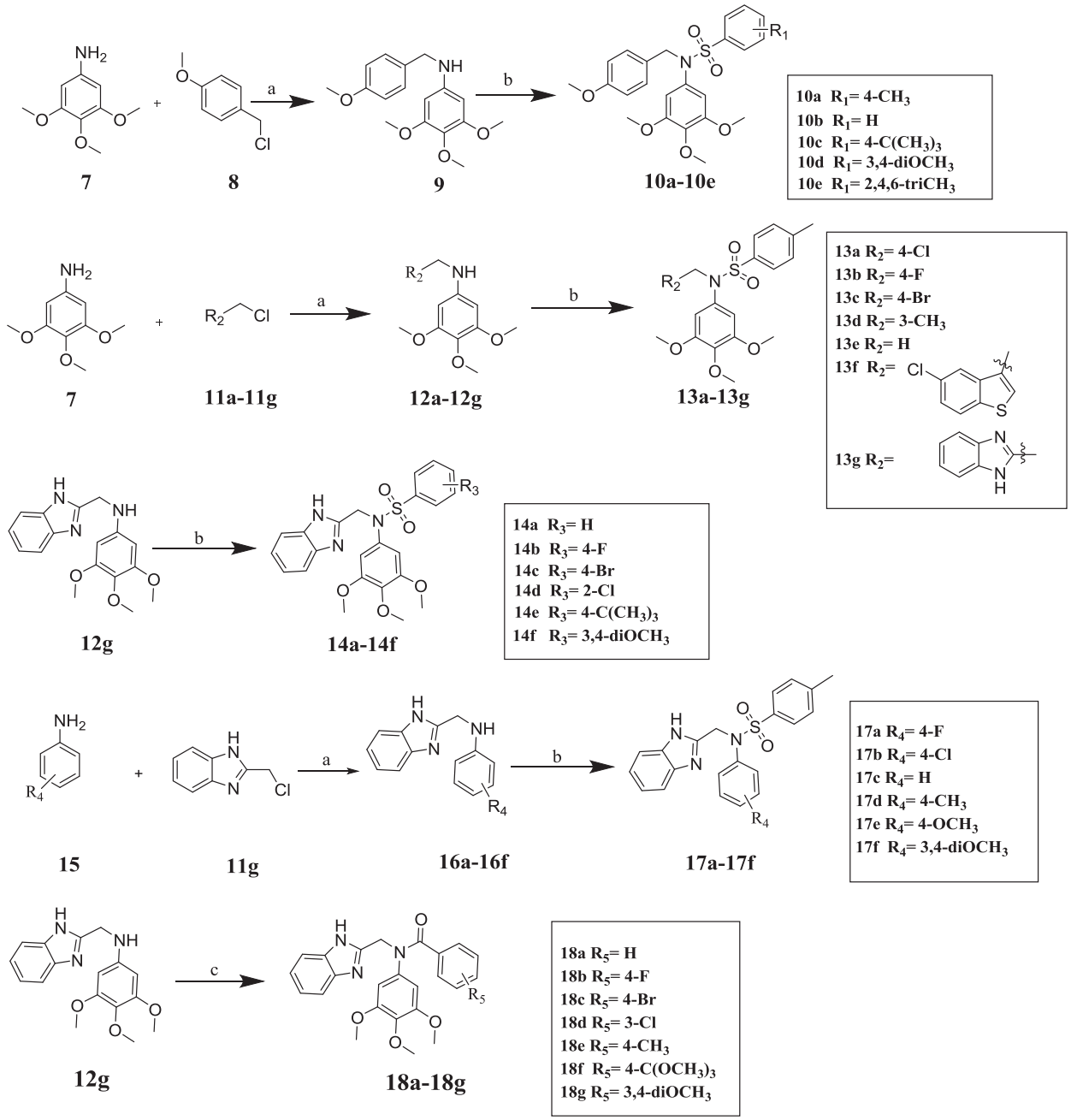


Fig. 2. Designed tertiary sulfonamide derivative **13g** in this work.

linkages. As the continuation of our studies on the development of anti-gastric cancer agents, we designed and synthesized a series of tertiary sulfonamide derivatives containing benzimidazole moiety by the scaffold hopping and molecular hybridization approaches. The structure-activity relationships of tertiary sulfonamide derivatives containing benzimidazole moiety were further discussed. Finally, the mechanistic study of compound **13g** containing benzimidazole moiety was investigated (Fig. 2).

2. Chemistry

The synthetic routes of target tertiary sulfonamide derivatives were shown in Scheme 1. First, 3,4,5-trimethoxyaniline **7** reacted with 4-methoxybenzyl chloride **8** in the presence of K_2CO_3 in acetone to give the secondary amine **9**. The intermediate compound **9** reacted with different benzenesulfonyl chloride derivatives in the presence of triethylamine in DMF to obtain the tertiary sulfonamide derivatives **10a**-**10e**. And, 3,4,5-trimethoxyaniline **7** reacted with benzyl chlorides **11a**-**11g** in the presence of K_2CO_3 in acetone to give the secondary amines **12a**-**12g**. The **12a**-**12g** reacted with 4-toluene sulfonyl chloride to obtain the tertiary sulfonamide derivatives **13a**-**13g**. The intermediate compound **12g** reacted with different benzene sulfonyl chloride derivatives or different acyl chloride derivatives to obtain the tertiary sulfonamide derivatives **14a**-**14f** and the tertiary amides **18a**-**18f,** respectively. The amines **15** reacted with **11g** obtain the tertiary sulfonamide derivatives **17a**-**17f**. Characterization of all the compounds was carried out by means of ^1H, ^{13}C NMR and HREI-mass



Scheme 1. Reagents and conditions: a) K_2CO_3, acetone, reflux, 6 h; b) benzenesulfonyl chloride derivatives, TEA, DMF, rt; c) acyl chloride derivatives, TEA, DMF, rt.

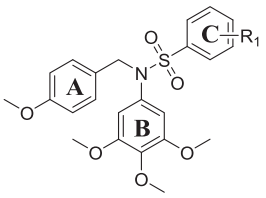
spectra. The detailed physical and analytical data are given in the experimental part.

3. Results and discussion

3.1. Anti-proliferative activity and SAR discussion

The anticancer activity of synthesized compounds was evaluated against human gastric cancer MGC-803 cell line, PC-3 human prostate cancer cell line and MCF-7 human breast adenocarcinoma cell line using MTT assay and the well-known anticancer drug 5-fluorouracil as reference drug. The preliminary results are listed in Table 1, Table 2, Table 3, Table 4 and Table 5. The anticancer activity of compound **13g** was also evaluated against human gastric cancer cell lines (MGC-803 and SGC-7901), human cell line derived from the metastatic lymph node of gastric cancer (HGC-27) and gastric epithelial cell line (GES-1) as shown in Table 6. Based on the obtained data, structure-activity relationship was analyzed and summarized in Fig. 3.

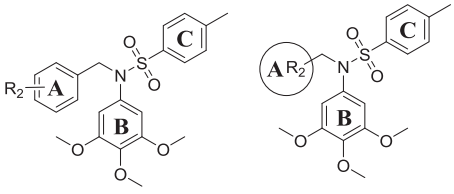
Table 1
In vitro anti-proliferative activity of tertiary sulfonamide derivatives **10a**-**10e** against human cancer cell lines (MGC-803, PC-3, and MCF-7 cells).

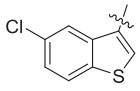
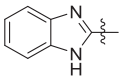


Compound	R_1	IC_{50} (μM)[a]		
		MGC-803	PC-3	MCF-7
10a	4-CH_3	4.34 ± 0.67	8.22 ± 1.08	10.14 ± 1.12
10b	H	>50	>50	>50
10c	4-$C(CH_3)_3$	10.31 ± 1.93	28.56 ± 2.04	32.72 ± 3.27
10d	3,4-diOCH_3	8.31 ± 2.67	20.14 ± 2.28	22.71 ± 1.42
10e	2,4,6-triCH_3	6.44 ± 1.58	14.82 ± 2.04	18.65 ± 1.58
5-Fu	–	6.82 ± 1.17	18.42 ± 1.73	17.11 ± 2.94

[a] Antiproliferative activity was assayed by exposure for 48 h.

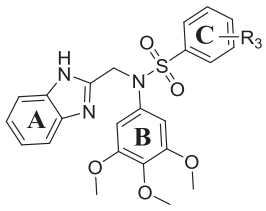
Table 2
In vitro anti-proliferative activity of tertiary sulfonamide derivatives **13a**-**13g** against human cancer cell lines (MGC-803, PC-3, and MCF-7 cells).



Compound	R_2	IC_{50} (μM)[a]		
		MGC-803	PC-3	MCF-7
13a	4-Cl	1.72 ± 0.26	14.59 ± 1.12	18.64 ± 1.08
13b	4-F	3.75 ± 0.48	20.71 ± 2.01	19.20 ± 1.61
13c	4-Br	1.61 ± 0.36	4.68 ± 0.68	10.89 ± 1.47
13d	3-CH_3	4.34 ± 2.19	8.58 ± 1.24	14.28 ± 1.82
13e	H	3.57 ± 0.61	7.81 ± 0.72	10.22 ± 1.06
13f		3.45 ± 0.29	15.25 ± 1.02	8.95 ± 0.74
13g		1.02 ± 0.03	3.34 ± 0.09	5.40 ± 0.51
5-Fu	–	6.82 ± 1.17	18.42 ± 1.73	17.11 ± 2.94

[a] Antiproliferative activity was assayed by exposure for 48 h.

Table 3
In vitro anti-proliferative activity of tertiary sulfonamide derivatives **14a**-**14f** against human cancer cell lines (MGC-803, PC-3, and MCF-7 cells).



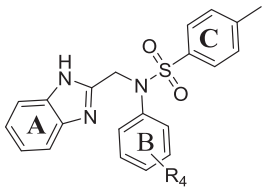
Compound	R_3	IC_{50} (μM)[a]		
		MGC-803	PC-3	MCF-7
14a	H	12.38 ± 0.99	25.49 ± 2.73	27.29 ± 2.34
14b	4-F	5.68 ± 0.86	7.99 ± 1.24	9.38 ± 1.08
14c	4-Br	2.19 ± 0.07	6.89 ± 0.46	7.74 ± 0.39
14d	2-Cl	19.12 ± 2.19	34.52 ± 1.94	38.12 ± 2.19
14e	4-$C(CH_3)_3$	24.10 ± 0.14	29.81 ± 1.93	33.64 ± 1.18
14f	3,4-diOCH_3	22.51 ± 2.14	30.37 ± 1.34	47.73 ± 1.96
5-Fu	–	6.82 ± 1.17	18.42 ± 1.73	17.11 ± 2.94

[a] Antiproliferative activity was assayed by exposure for 48 h.

Compounds **10a**-**10e** were initially evaluated for their anti-proliferative activities against MGC-803, PC-3, and MCF-7 cancer cell lines to research the effect of tertiary sulfonamide derivatives with **5-Fu** as the positive control. As shown in Table 1, the majority of the synthesized compounds exhibited moderate to potent activity against the tested cancer cell lines. The potency of the compounds varies with respect to substitutions on the simple phenyl ring (C ring). The benzene sulfonyl group of C ring displayed weak cytotoxicity(**10b**). To our delight, the compound **10a** with 4-methylbenzene sulfonyl group of C ring showed the most potent activity against MGC-803, PC-3, and MCF-7 cells with IC_{50} values of 4.74 μM, 8.22 μM and 10.14 μM, respectively.

As illustrated above with Table 1, the 4-methylbenzene sulfonyl group of C ring(**10a**) showed the most potent activity against MGC-803 cells, PC-3 cells and MCF-7 cells. To further investigate the influence of A ring in activity, compounds **13a**-**13g** containing different aryl substituents on A ring with the 4-methyl benzene sulfonyl group of C ring and a 3,4,5-trimethoxy group of B ring at the N-1 position were synthesized and the anti-proliferative activities were listed in Table 2. The results indicated that most of the synthesized compounds displayed high anti-proliferative activities against selected cancer cell lines, especially for MGC-803 cells. The potency of the compounds varies with respect to substitutions on the A ring. Compounds **13a**-**13c**, with electron-withdrawing groups on the A ring, showed improved inhibitory activity against MGC-803 cells compared to both its electron-donating groups on the A ring (compounds **10a** and **13d**) and unsubstituted phenyl group on the A ring (compound **13e**). Compared with compound **13e**, the electron-donating group substituted compound **10a** and compound **13d** showed a slightly lower activity. What's more, compounds **13f**-**13g**, with heterocyclic groups on the A ring showed improved inhibitory activity against MGC-803 cells with IC_{50} values of 1.02 ± 0.03 μM and 3.45 ± 0.29 μM, respectively. And, compound **13g** showed the most potent activities against MGC-803, PC-3 and MCF-7 cells with IC_{50} values of 1.02 ± 0.03 μM, 3.34 ± 0.09 μM, and

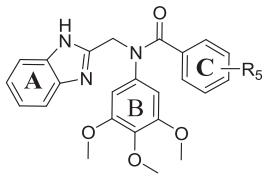
Table 4
In vitro anti-proliferative activity of tertiary sulfonamide derivatives **17a**-**17f** against human cancer cell line (MGC-803, PC-3, and MCF-7 cells).



Compound		R_5	IC_{50} (μM)[a]		
			MGC-803	PC-3	MCF-7
17a		4-F	34.62 ± 1.41	44.96 ± 1.84	>50
17b		4-Cl	24.77 ± 2.28	42.19 ± 2.41	38.29 ± 2.04
17c		H	45.18 ± 1.39	>50	49.67 ± 1.04
17d		4-CH_3	41.25 ± 2.64	48.91 ± 2.08	>50
17e		4-OCH_3	>50	>50	>50
17f		3,4-diOCH_3	>50	>50	>50
5-Fu	–		6.82 ± 1.17	18.42 ± 1.73	17.11 ± 2.94

[a] Antiproliferative activity was assayed by exposure for 48 h.

Table 5
In vitro anti-proliferative activity of tertiary amides **18a**-**18g** against human cancer cell lines (MGC-803, PC-3, and MCF-7 cells).



Compound	R_6	IC_{50} (μM)[a]		
		MGC-803	PC-3	MCF-7
18a	H	>50	>50	>50
18b	4-F	23.55 ± 1.17	21.86 ± 1.89	45.23 ± 5.78
18c	4-Br	14.55 ± 2.21	19.93 ± 1.63	>50
18d	3-Cl	>50	>50	34.86 ± 2.62
18e	4-CH_3	30.47 ± 2.48	42.04 ± 1.00	49.25 ± 1.42
18f	4-C$(CH_3)_3$	>50	46.04 ± 1.00	48.81 ± 1.32
18g	3,4-diOCH_3	22.38 ± 1.08	34.86 ± 2.62	>50
5-Fu	–	6.82 ± 1.17	18.42 ± 1.73	17.11 ± 2.94

[a] Antiproliferative activity was assayed by exposure for 48 h.

Table 6
In vitro anti-proliferative activity of **13g** against gastric cancer cells (MGC-803, HGC-27 and SGC-7901) and non-cancer cell lines (GES-1).

Compd.	IC_{50} (μM)[a]				Fold selectivity		
	MGC-803	HGC-27	SGC-7901	GES-1	A	B	C
13g	1.02	1.61	2.30	15.22	14.9	9.4	6.6
5-Fu	6.82	4.28	5.54	10.21	1.49	2.4	1.84

A = IC_{50} (GES-1)/IC_{50} (MGC-803).
B = IC_{50} (GES-1)/IC_{50} (HGC-27).
C = IC_{50} (GES-1)/IC_{50} (SGC-7901).

5.40 ± 0.51 μM respectively. This observation suggests that the electron deficiency or richness of the A ring system may affect the antiproliferative activity to a certain extent and benzimidazole or benzothiophene motifs enhanced anticancer activity.

With the identification of benzimidazole region established based on the SAR analysis, the focus shifted to the optimization of C ring. As shown in Table 3, The potency of the compounds varies with respect to substitutions on the C ring. MGC-803 cells were also more sensitive to the compounds **14a-14f** than PC-3 and MCF-7 cells. Compound **13g** with the methyl group on the C ring showed the most potent activity against MGC-803 cells with an IC_{50} value of 1.02 ± 0.03 μM, which was higher than compound **14a** with hydrogen atom and **14b, 14c, 14d** with halogen atoms on the C ring. Compound **14c** exhibited slightly lower activity compared to **13g** against MGC-803 cells with an IC_{50} value of 2.19 μM. The relationships between the halogen substituents and the antiproliferative activities were 4-Br>4-F>2-Cl. In addition, compounds **14e** and **14f** were less potent activity than compound **10e**.

In this series of derivatives, we mainly explored the significance of the 3,4,5-trimethoxy group of phenyl ring (B ring) at the N-1 position. These inhibitory activity results indicated that the 3,4,5-trimethoxy group of B ring at the N-1 position was very crucial for the antitumor activity. When the 3,4,5-trimethoxy group was replaced by other groups (4-trimethoxy, 3,4-dimethoxy, 4-methyl, halogen substituents, and hydrogen atom), compounds **17a-17h** displayed very weak activity with almost IC_{50} values of >50 μM against three selected cancer cell lines. Based on the inhibitory activity results, the 3,4,5-trimethoxy group of B ring displayed an important effect for the antiproliferative activity. Other substituent groups may significantly impair anti-proliferative activity.

In order to complete the structure activity relationships, a series of tertiary amide derivatives were synthesized and evaluated for their antiproliferative activity. As shown in Table 5, some of these compounds retain the cytotoxic activity(**18a**-**18g**) but displayed weak activity against three selected cancer cell lines compared to tertiary sulfonamide derivatives (**13g** and **14a**-**14f**). All these modifications and the inhibitory results revealed that the sulfonyl groups were important for their inhibitory activity which can maintain or enhance the anti-proliferative activity against three tested human cancer cells.

Furthermore, MGC-803 cells were more sensitive to the compounds than PC-3 cells, and MCF-7 cells. What's more, as part of our group's research focus and continuation of our studies on the development of anti-gastric cancer agents, we chose two other common gastric cancer cell lines (HGC-27 and SGC-7901), so compound **13g** was examined for its activity against gastric cancer cells (MGC-803, HGC-27 and SGC-7901) and non-cancer cell lines (GES-1). As shown in Table 6, compounds **13g** exhibited high activity against MGC-803 cells, HGC-27 cells and SGC-7901 cells with

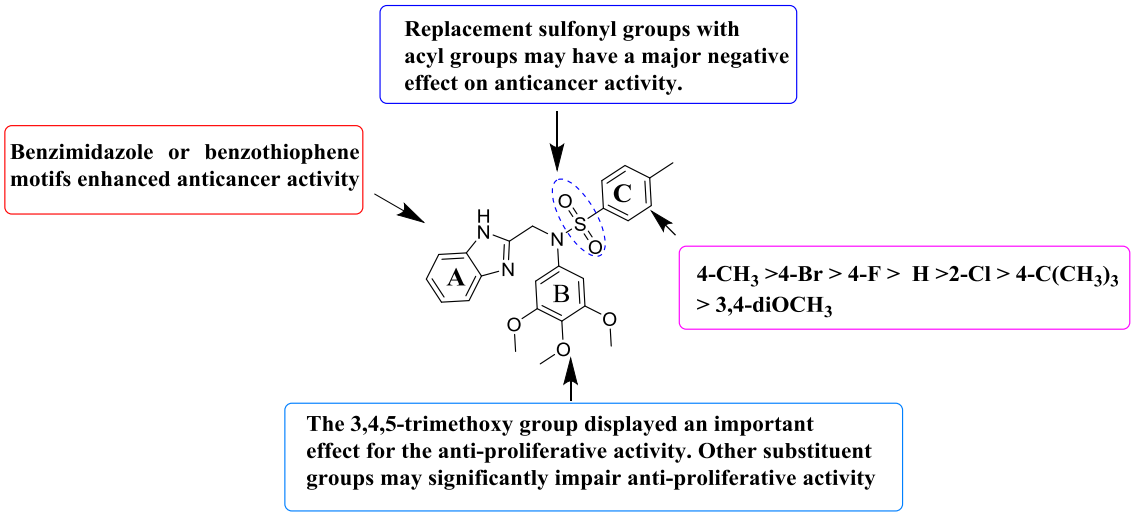


Fig. 3. Summary of the structure-activity relationships.

IC_{50} values of 1.02 μM, 1.61 μM and 2.30 μM, respectively. Compounds **13g** exhibited moderate activity against GES-1 cells with an IC_{50} value of 15.22 μM. Notably, compounds **13g** exhibited highest activity against three test human cancer cells. What's more, compounds **13g** also displayed the high selectivity between the gastric cancer cells and non-cancer cell lines (around 14.9-, 9.4- and 6.6-fold selectivity to MGC-803 cells, HGC-27 cells and SGC-7901 cells over GES-1 cells, respectively).

Based on above analysis of the structure-activity relationship, we can conclude that the sulfonyl groups are important for the activity. The electron deficiency or richness of the A ring system may affect the antiproliferative activity to a certain extent.

Benzimidazole or benzothiophene motifs may enhance selectivity to the tested cancer cell lines. The 3,4,5-trimethoxy group of B ring at the N-1 position displayed an important effect for the antiproliferative activity. The potency of the compounds varies with respect to substitutions of the C ring. The summary illustration for SAR studies of target derivatives was shown in Fig. 3.

In view of an effective growth inhibitory effect on both of the tested cancer cells, compound **13g** was chosen to further evaluate its possible anticancer mechanism of action against MGC-803, HGC-27 and SGC-7901 cells in all the subsequent experiments.

3.2. Compound 13 g selectively inhibited the cell viability of gastric cancer cells

Compound **13g** was selected to do the mechanism study based on the results of cell viability. It showed a highest activity against MGC-803 cells of compound **13g**, and also, compound **13g** inhibited two other gastric cancer cells with potent activities (HGC-27 and SGC-7901). We detected three gastric cancer cells in the manner way to determine whether the mechanisms by which compound **13g** inhibited three human gastric cell lines are the same. For this reason, two other gastric cancer cell lines HGC-27, SGC-7901 were selected to detect the activity of compound **13g** inhibiting gastric cancer cells. Normal gastric epithelial cell GES-1 was used as a control group to detect the selective inhibition against cancer cells. As shown in Fig. 4E, the IC_{50} values of three cancer cell lines were much lower than IC_{50} value of normal cell GES-1. The IC_{50} values were 1.02 μmol/L (MGC-803), 1.61 μmol/L (HGC-27), 2.30 μmol/L(SGC-7901), respectively, while the IC_{50} value of GES-1 was 15.22 μmol/L. As shown in Fig. 4, compound **13g** exhibited the proliferation inhibition against three gastric cancer cell lines(Fig. 4A for MGC-803, Fig. 4B for HGC-27, Fig. 4C for SGC-7901). After treatment for 48 h, compound **13g** limited three cancer cells growth lower than 2 folds. 3 gastric cancer cell lines and 1 normal cell line were next treated with different concentration of compound **13g**, compound **13g** inhibited cell viability of gastric cancer cells in a dose-dependent manner but had a low cytotoxicity against normal cell (Fig. 4D). These results indicated that compound **13g** selectively inhibited gastric cancer cells.

3.3. Compound 13 g inhibited the proliferation of gastric cancer cells

To detect the activity of compound **13g** influencing cell proliferation, colony formation assay was performed using three gastric cancer cells. The colony numbers were evidently reduced by low dose (0.5 μmol/L) treatment. In mid dose (1 μmol/L) and high dose (1.5 μmol/L), there was barely colony obtained (Fig. 5C). It was demonstrated that compound **13g** inhibited the proliferation of MGC-803, HGC-27 and SGC-7901 cells in a dose-dependent manner. Next, cell cycles of treated cells with different concentrations of compound **13g** or DMSO were analyzed. As shown in Fig. 5A and B, gastric cancer cells were significantly arrested in G2/M phase after 48 h treatment with compound **13g**. The G2/M related proteins were detected, CyclinB and CDK1 are two members of complex molecules which promote cell through G2 phase to M phase. After treatment with compound **13g** for 48 h, CDK1 and CyclinB was clearly decreased (Fig. 5D) which would result in down-regulation of CyclinB/CDK1 complex and lead to the G2 phase arrestment. As the cell cycle analysis cannot distinguish between G2 and M phases, the M phase arrest biomarker p-Histone3 was checked. As a result the up-regulation of p-Histone3 indicated the compound **13g** treatment also caused a M phase arrestment (Fig. 5D). To sum up, compound **13g** treatment lead to G2 and M phase arrestment resulting in proliferation inhibition against 3 gastric cancer cells.

3.4. Compound 13 g inhibited the Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways in gastric cancer cells

The Ras/Raf/MEK/ERK signaling pathway acts a critical role in cell survival and proliferation, this pathway promotes cell survival and proliferation through a cascade of RAS-Raf-MEK-ERK. The inhibition of Ras/Raf/MEK/ERK signaling pathway will lead to cell

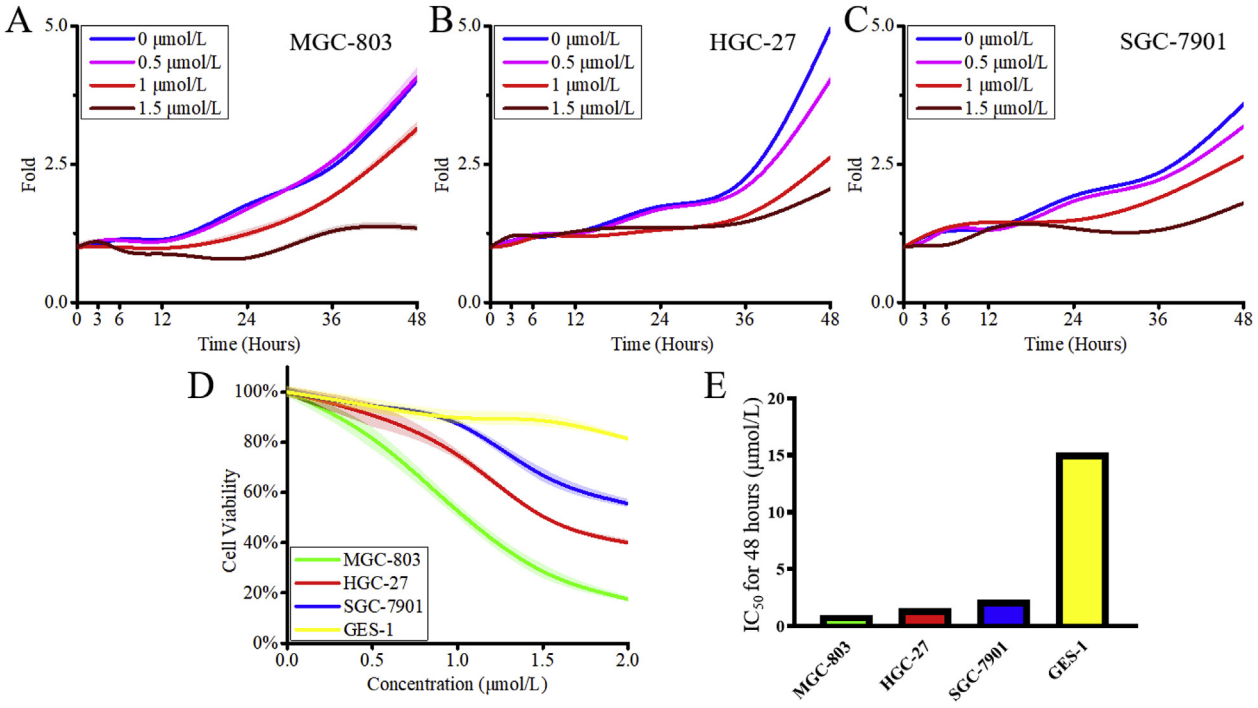


Fig. 4. (A) Growth Curve of MGC-803 cancer cells treated with different concentration of compound **13g**. (B) Growth Curve of HGC-27 cancer cells treated with different concentration of compound **13g**. (C) Growth Curve of SGC-7901 cancer cells treated with different concentration of compound **13g**. (D) Four gastric cells viabilities after treated with different concentration of compound **13g**. (E) IC_{50} values of compound **13g** against three gastric cell lines and normal cell line.

proliferation inhibition or cell apoptosis. Here as the Fig. 6A shown, compound **13g** did not show effect on RAS, but had an obvious influence on its down streams. The activation (phosphonate) of c-Raf, MEK1, ERK1/2 were evidently down-regulated in a dose-dependent manner. The transcription factors FoxO3a and c-Myc which regulated by Ras/Raf/MEK/ERK signaling pathway were also down-regulated (Fig. 6A). Akt and mTOR are 2 pathways have cross-talks and compensations with MAPK pathway, biomarkers of both pathway, p-Akt (473Ser) and p-mTOR (2448Ser), were both downregulated at same time which suggested compound **13g** influenced all these pathways (Fig. 6A). To detect the relationship of compound **13g** activity and Ras/Raf/MEK/ERK signaling pathway, combination treatment with another Ras/Raf/MEK/ERK signaling pathway inhibitor piperlongumine was performed [41,42]. As shown in Fig. 6B, the combination index (CI) was greater than 1, which exhibited the antagonism between compound **13g** and piperlongumine. The CI analysis verified that compound **13g** might inhibit Ras/Raf/MEK/ERK signaling pathway as piperlongumine did. These results indicated compound **13g** regulated 3 gastric cancer cells via the AKT/mTOR and Ras/Raf/MEK/ERK signaling pathway and down-regulated the level of FoxO3a and c-Myc.

3.5. Compound 13 g induced apoptosis of gastric cancer cells

Since the Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways were inhibited, the induction of cell apoptosis by compound **13g** was detected. After 48 h treatment, compound **13g** induced cell apoptosis in the dose-dependent manner (Fig. 7A and B). Meanwhile, compound **13g** induced cell nucleus' concentration and DNA fragment of 3 gastric cancer cells which were believed as a mark of cell apoptosis (Fig. 7C). Some apoptosis related proteins were then checked, the apoptosis executer Caspase-7 and its substrate PARP were cleaved. Pro-apoptosis protein Noxa was up-regulated, while the anti-apoptosis proteins Mcl-1, Bcl-2, c-IAP1 and XIAP were down-regulated (Fig. 7D). These data suggested that compound **13g** induced MGC-803, HGC-27 and SGC-7901 cells apoptosis.

4. Conclusions

In summary, a series of novel tertiary sulfonamide derivatives containing benzimidazole moiety were synthesized and evaluated for their antiproliferative activity. All the targeted compounds were first reported in this work except compounds **13a**, **13b** and **13e**. Among these compounds, in comparison of **5-FU**, compound **13g** showed better activity against the tested cells (MGC-803, PC-3 and MCF-7) with IC_{50} values of 1.02 μM, 3.34 μM and 5.40 μM, respectively. Moreover, compound **13g** not only had the high antiproliferative activity against MGC-803 cells, HGC-27 cells and SGC-7901 cells with IC_{50} values of 1.02 μM, 1.61 μM and 2.30 μM, respectively, but also it displayed the highly selectivity between the tested gastric cancer cell lines and normal cells (14.9-fold selectivity to the MGC-803 cancer cells over the GES-1 normal cells, 9.9-fold selectivity to the HGC-27 cancer cells over the GES-1 normal cells and 6.9-fold selectivity to the SGC-7901 cells over the GES-1 normal cells, respectively). Further results suggested that potent compound **13g** inhibited the colony formation in vitro, caused a cell cycle arrestment at G2/M phase and induced cell apoptosis in 3 gastric cancer cells. Importantly, Western blot analysis indicated that compound **13g** markedly decreased p-Akt and p-*c*-Raf expression, which revealed that compound **13g** targeted gastric cancer cell lines via interfering with Akt/mTOR and RAS/Raf/MEK/ERK pathways. All the findings suggested that tertiary sulfonamide derivatives containing benzimidazole moiety may offer significant potentiality for the discovery of a new class anti-gastric cancer agents with the ability to induce apoptosis and inhibit Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways.

Starting from the compound **10a**, the 4-methylbenzene sulfonyl group of C ring and benzimidazole group of A ring have been

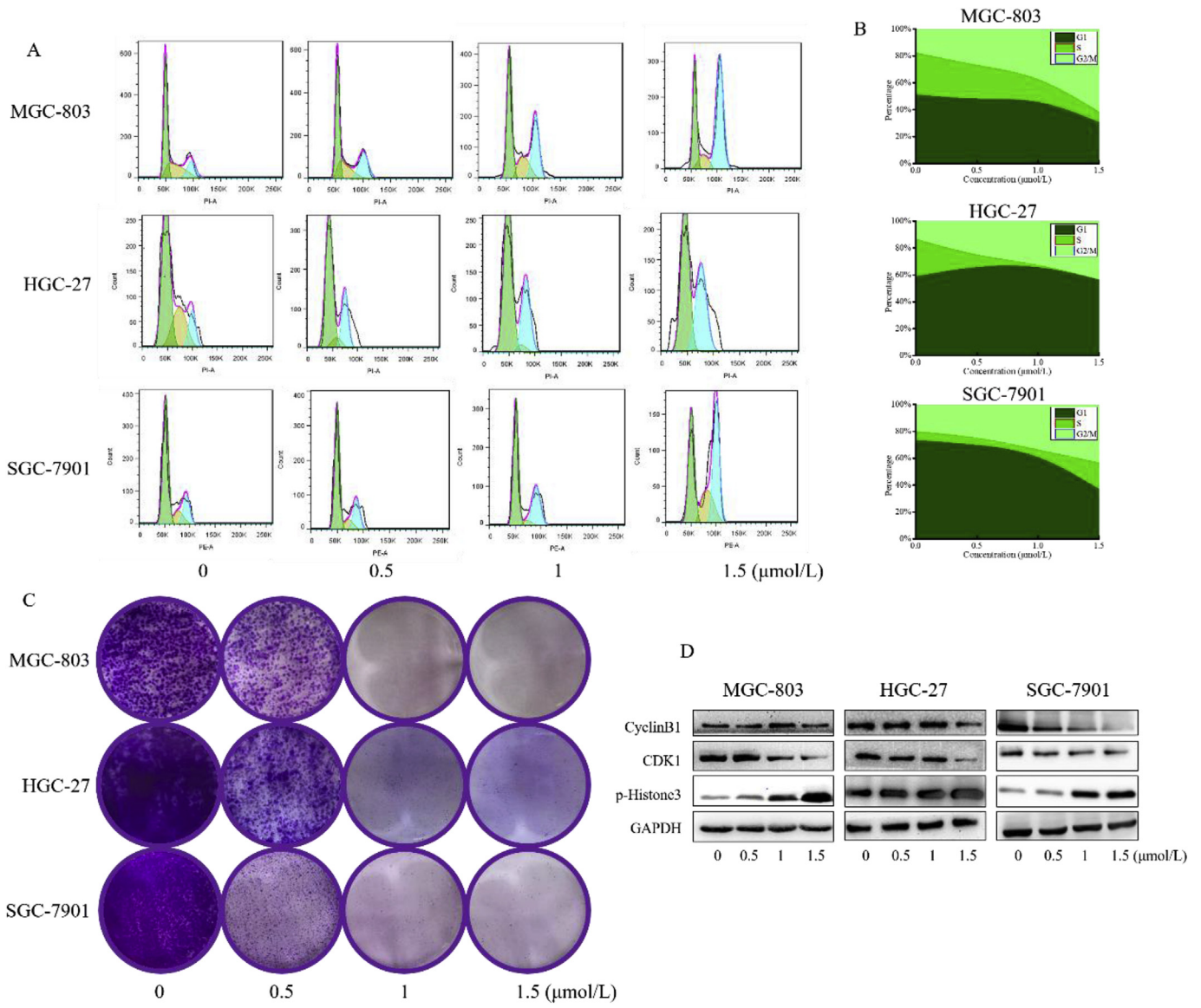


Fig. 5. (A) Effect of compound **13g** on cell cycle in three gastric cancer cells, three gastric cancer cells were treated with different concentration of compound **13g** for 48 h. (B) Quantitative analysis of the percentage of three gastric cancer cells cycle phase distribution. (C) Three gastric cancer cells were treated with different concentration of compound **13g** for 7 days and stained with Crystal Violet to determine cell colony formatting activity. (D) G2/M phase related proteins were detected using western blotting, three gastric cancer cells were treated with different concentration of compound **13g** for 48 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

optimized, which led to a gradually improved anti-gastric cancer cells activity. In addition, preliminary structure-activity relationship research revealed that the sulfonyl groups were important for the activity; incorporation of benzimidazole to the hybrids was the best optimal for bioactivity; 3,4,5-trimethoxy group of B ring at the N-1 position displayed a vital role for the antiproliferative activity. All of these findings suggested that the compound **13g** containing benzimidazole moiety may be a promising skeleton for the development of anti-gastric cancer agents with improved efficacy and fewer side effects.

5. Experimental section

5.1. General

All commercial materials were used without further purification. TLC analysis was carried out on silica gel plates GF254 (Qingdao Haiyang Chemical, China). Silica gel chromatography was carried out on 200−300 mesh gel. Anhydrous solvents and reagents were dried by routine protocols. Melting points were determined on an X-5 micromelting apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 400 MHz and 100 MHz spectrometer, respectively. High resolution mass spectra (HRMS) of all derivatives were recorded on a Waters Micromass Q-T of Micromass spectrometer by electrospray ionization (ESI).

5.2. General procedure for the synthesis of compound 9, 12a-12 g and 16a-16f

A solution of various aromatic amines (0.5 mmol, 1.0eq), benzyl chlorides derivatives (0.6 mmol, 1.2eq) and K_2CO_3 (0.75 mmol, 1.5eq) were added into DMF(5 ml). Then the mixture was stirred at room temperature for 5 h. Upon completion, ethyl acetate (20 ml) and H_2O (5 ml) were added. The aqueous layer was extracted with

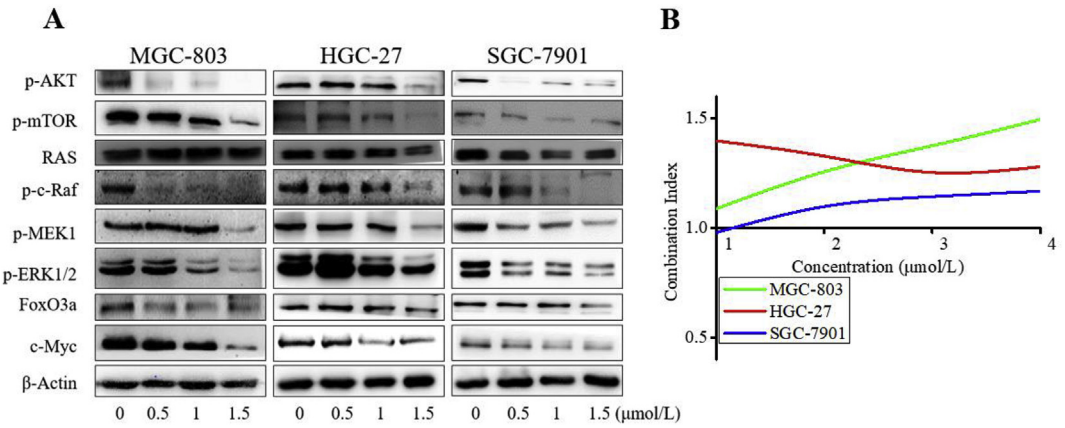


Fig. 6. The effect of compound **13g** on the Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways. (A) The Akt/mTOR and Ras/Raf/MEK/ERK signaling pathway related proteins were detected using western blotting, three gastric cancer cells were treated with different concentration of compound **13g** for 48 h. (B) Combination Index (CI) analysis of compound **13g** and piperlongumine, the concentration of **13g** is 1.5 μmol/L, the horizontal axis shows the concentration of piperlongumine.

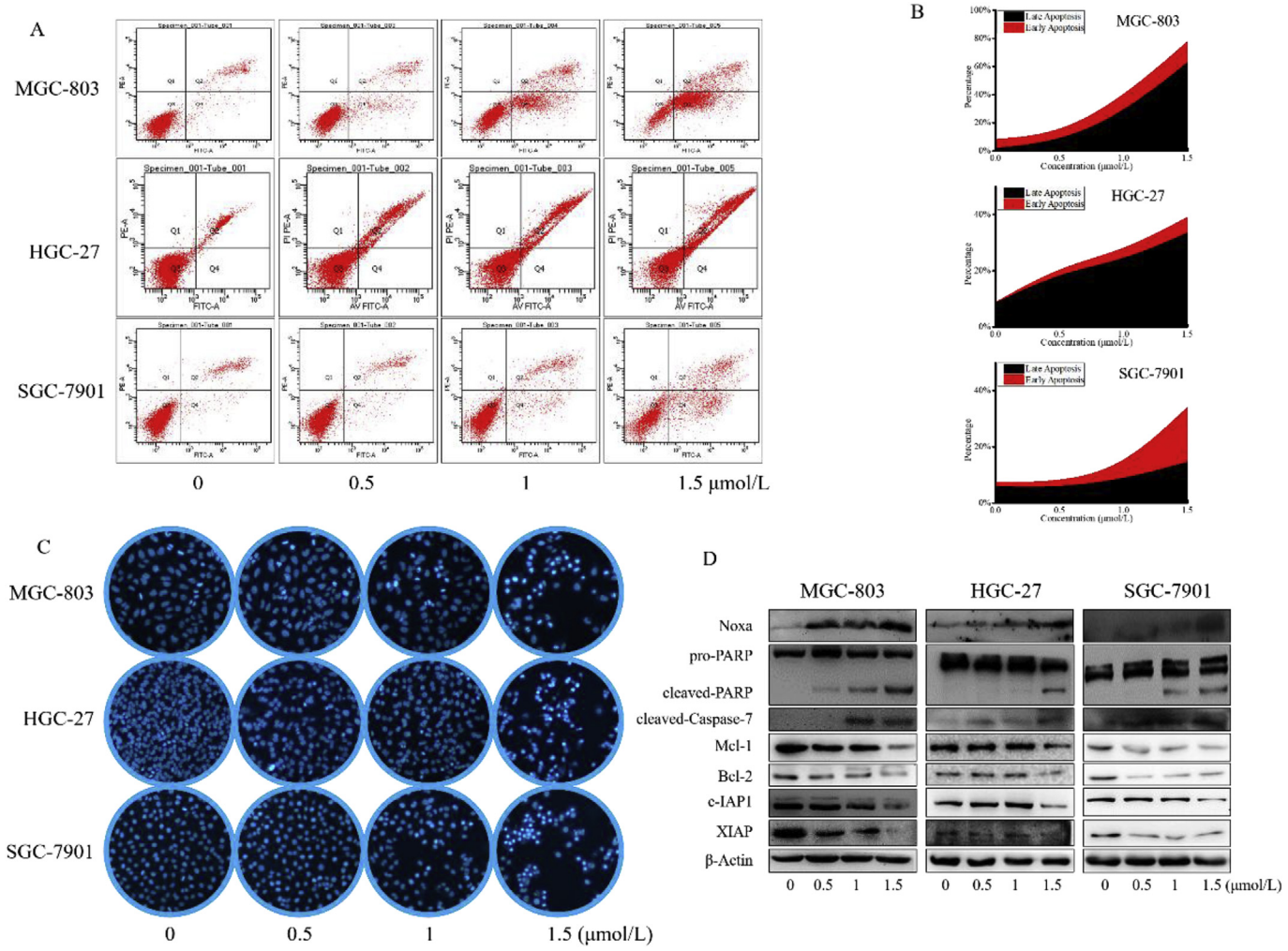


Fig. 7. (A) Cell apoptosis analysis were performed using flow cytometry, three gastric cancer cells were treated with different concentration of compound **13g** for 48 h. (B) Quantitative analysis of the apoptotic cells of three gastric cancer cells. (C) Images of 48 h treated gastric cells' nucleus. (D) Apoptosis related proteins were detected using western blotting, three gastric cancer cells were treated with different concentration of compound **13g** for 48 h.

ethyl acetate for several times, the combined organic layers were washed with H_2O for several times, and then washed with brine, dried over $MgSO_4$ and evaporated to give the products. The residue was purified with column chromatography (PE: EA = 2:1–3:1) to obtain compound **9**, **12a-12g,** and **16a-16f**.

5.3. General procedure for the synthesis of compound 10a-10e, 13a-13 g, 14a-14f, 17a-17f and 18a-18 g

A solution of compound **9**, **12a**-**12g** or **16a**-**16g** (1.0eq), substituted sulfonyl chloride (1.1eq) or substituted acyl chloride(1.1eq) and TEA(1.5eq) were added into DMF(5 ml. And then the mixture was stirred at room temperature for 6 h. Upon completion, ethyl acetate (20 ml) and H_2O (5 ml) were added. The aqueous layer was extracted with ethyl acetate for several times, the combined organic layers were washed with H_2O for several times, and then washed with brine, dried over MgSO4 and evaporated to give the products. The crude residue was purified by silica gel column chromatography eluted with a mixture of petroleum ether and ethyl acetate (2:1) to obtain target compound.

5.3.1. N-(4-methoxybenzyl)-4-methyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(10a)

White solid, yield, 64%, m.p. 156–157 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (d, J = 54.3 Hz, 4H), 7.17 (s, 2H), 6.83 (s, 2H), 6.22 (s, 2H), 4.65 (s, 2H), 3.69 (s, 3H), 3.58 (d, J = 15.1 Hz, 9H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.53, 152.32, 143.55, 136.88, 135.06, 134.29, 129.64, 129.56, 128.10, 127.58, 113.64, 106.47, 59.98, 55.84, 54.95, 53.19, 20.99; HRMS (ESI) m/z calcd for $C_{24}H_{27}NO_6S$ [M+Na]+480.1457, found 480.1456.

5.3.2. N-(4-methoxybenzyl)-N-(3,4,5-trimethoxyphenyl) benzene sulfonamide(10b)

White solid, yield, 58%, m.p. 161–162 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.82–7.75 (m, 3H), 7.70 (t, J = 7.5 Hz, 2H), 7.24 (d, J = 8.0 Hz, 2H), 6.89 (d, J = 7.8 Hz, 2H), 6.26 (s, 2H), 4.74 (s, 2H), 3.75 (s, 3H), 3.66 (s, 3H), 3.61 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.56, 152.34, 137.90, 136.93, 134.15, 133.15, 129.59, 129.25, 128.05, 127.50, 113.66, 106.49, 59.98, 55.82, 54.96, 53.36. HRMS (ESI) m/z calcd for $C_{23}H_{25}NO_6S$ [M+Na] $^+$ 466.1300, found 466.1300.

5.3.3. 4-(tert-butyl)-N-(4-methoxybenzyl)-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(10c)

White solid, yield, 49%, m.p. 138–139 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (q, J = 8.4 Hz, 4H), 7.24 (d, J = 8.2 Hz, 2H), 6.88 (d, J = 8.2 Hz, 2H), 6.20 (s, 2H), 4.73 (s, 2H), 3.75 (s, 3H), 3.65 (s, 3H), 3.59 (s, 6H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.54, 156.24, 152.30, 136.93, 134.99, 134.29, 129.57, 128.17, 127.44, 126.01, 113.64, 106.55, 59.98, 55.71, 54.96, 53.50, 34.90, 30.75. HRMS (ESI) m/z calcd for $C_{27}H_{33}NO_6S$ [M+Na] $^+$ 522.1926, found 522.1927.

5.3.4. 3,4-Dimethoxy-N-(4-methoxybenzyl)-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(10d)

White solid, yield, 67%, m.p. 126–127 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.29 (d, J = 8.4 Hz, 1H), 7.22–7.15 (m, 3H), 7.03 (s, 1H), 6.83 (d, J = 8.4 Hz, 2H), 6.26 (s, 2H), 4.65 (s, 2H), 3.86 (s, 3H), 3.75 (s, 3H), 3.69 (s, 3H), 3.60 (d, J = 9.4 Hz, 9H).^{13}C NMR (100 MHz, DMSO-$d6$) δ 158.51, 152.53, 152.30, 148.50, 136.92, 134.51, 129.58, 129.15, 128.22, 121.45, 113.63, 111.23, 110.18, 106.53, 59.97, 55.91, 55.87, 55.69, 54.93, 52.98. HRMS (ESI) m/z calcd for $C_{25}H_{29}NO_8S$ [M+Na] $^+$ 526.1512, found 526.1511.

5.3.5. N-(4-methoxybenzyl)-2,4,6-trimethyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(10e)

White solid, yield, 67%, m.p. 116–117 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.15 (d, J = 8.1 Hz, 2H), 7.04 (s, 2H), 6.83 (d, J = 8.1 Hz, 2H), 6.25 (s, 2H), 4.81 (s, 2H), 3.69 (s, 3H), 3.55 (d, J = 22.5 Hz, 9H), 3.36 (s, 3H), 2.42 (s, 6H), 2.26 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 158.57, 152.35, 142.62, 139.73, 136.90, 133.71, 132.40, 131.65, 129.85, 128.33, 113.65, 106.66, 60.03, 55.76, 54.94, 52.24, 22.42, 20.34. HRMS (ESI) m/z calcd for $C_{26}H_{31}NO_6S$ [M+H] $^+$ 486.1950, found 486.1950.

5.3.6. N-(4-chlorobenzyl)-4-methyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(13a)

White solid, yield, 69%, m.p. 162–163 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (d, J = 7.7 Hz, 2H), 7.44 (d, J = 7.9 Hz, 2H), 7.33 (s, 4H), 6.26 (s, 2H), 4.73 (s, 2H), 3.59 (d, J = 10.3 Hz, 9H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.40, 143.71, 137.01, 135.56, 134.80, 134.24, 131.97, 130.00, 129.68, 128.27, 127.61, 106.42, 59.98, 55.88, 53.02, 20.99. HRMS (ESI) m/z calcd for $C_{23}H_{24}ClNO_5S$ [M+Na] $^+$ 484.0961, found 484.0962.

5.3.7. N-(4-fluorobenzyl)-4-methyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(13b)

White solid, yield, 69%, m.p. 153–154 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 7.33 (dd, J = 14.3, 7.3 Hz, 1H), 7.20–7.01 (m, 3H), 6.28 (s, 2H), 4.75 (s, 2H), 3.59 (d, J = 9.5 Hz, 9H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.22, 160.80, 152.41, 143.74, 139.55, 139.48, 137.02, 134.76, 134.31, 130.28, 129.68, 127.63, 124.15, 124.13, 114.86, 114.29, 106.38, 59.98, 55.88, 53.18, 20.98. HRMS (ESI) m/z calcd for $C_{23}H_{24}FNO_5S$ [M+Na] $^+$ 468.1257, found 468.1255.

5.3.8. N-(4-bromobenzyl)-4-methyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(13c)

White solid, yield, 69%, m.p. 156–157 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.59 (d, J = 7.9 Hz, 2H), 7.46 (dd, J = 12.2, 8.4 Hz, 4H), 7.26 (d, J = 8.1 Hz, 2H), 6.26 (s, 2H), 4.71 (s, 2H), 3.59 (d, J = 10.0 Hz, 9H), 2.42 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 152.40, 143.71, 136.99, 135.99, 134.76, 134.24, 131.19, 130.34, 129.68, 127.62, 120.51, 106.39, 59.98, 55.87, 53.05, 20.99. HRMS(ESI) m/z calcd for $C_{23}H_{24}BrNO_5S$ [M+Na] $^+$ 528.0456, found 528.0458.

5.3.9. 4-Methyl-N-(3-methylbenzyl)-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide (13d)

White solid, yield, 51%, m.p. 121–122 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.60 (d, J = 7.9 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.17 (d, J = 7.7 Hz, 2H), 7.07 (d, J = 7.7 Hz, 2H), 6.24 (s, 2H), 4.68 (s, 2H), 3.58 (d, J = 13.7 Hz, 9H), 2.42 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.33, 143.57, 136.88, 136.50, 134.99, 134.35, 133.29, 129.64, 128.82, 128.14, 127.58, 106.41, 59.96, 55.83, 53.46, 20.98. HRMS (ESI) m/z calcd for $C_{24}H_{27}NO_5S$ [M+H] $^+$ 442.1688, found 442.1689.

5.3.10. N-benzyl-4-methyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(13e)

White solid, yield, 58%, m.p. 194–195 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.61 (d, J = 7.8 Hz, 2H), 7.45 (d, J = 7.8 Hz, 2H), 7.32–7.18 (m, 5H), 6.25 (s, 2H), 4.73 (s, 2H), 3.57 (d, J = 13.7 Hz, 10H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.36, 143.62, 136.95, 136.43, 135.02, 134.38, 129.66, 128.25, 128.15, 127.59, 127.36, 106.46, 59.97, 55.84, 53.80, 20.98. HRMS (ESI) m/z calcd for $C_{23}H_{25}NO_5S$ [M+Na] $^+$ 450.1351, found 450.13.

5.3.11. N-((5-chlorobenzo[b]thiophen-3-yl) methyl)-4-methyl-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide(13f)

White solid, yield, 58%, m.p. 140–141 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.98 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 7.3 Hz, 3H), 7.44 (dd, J = 29.0, 8.3 Hz, 3H), 6.21 (s, 2H), 4.98 (s, 2H), 3.59 (s, 3H), 3.52 (s, 6H), 2.44 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 152.30, 143.78, 139.08, 138.19, 137.08, 134.54, 134.08, 130.10, 129.71, 129.40, 129.22, 127.75, 124.56, 124.45, 121.46, 106.48, 59.97, 55.80, 48.09, 21.02. HRMS (ESI) m/z calcd for $C_{23}H_{25}NO_5S$ [M+Na] $^+$ 540.0682, found 540.0682.

5.3.12. N-((3a,7a-dihydro-1H-benzo[d]imidazol-2-yl) methyl)-4-methyl-N-(3,4,5- trimethoxyphenyl) benzenesulfonamide (13 g)

White solid, Yield, 73%, m.p. 204–205 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.46 (s, 1H), 7.62 (d, J = 8.3 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 7.14 (s, 2H), 6.48 (s, 2H), 4.99 (s, 2H), 3.58 (d, J = 9.5 Hz, 9H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 152.37,149.81, 143.91, 137.15, 134.70, 134.33, 129.64, 127.77, 121.68, 106.42, 59.94, 55.80, 49.48, 21.01. HRMS (ESI) m/z calcd for $C_{24}H_{25}N_3O_5S$ [M+H] $^+$ 468.1593, found 468.1590.

5.3.13. N-((3a,7a-dihydro-1H-benzo[d]imidazol-2-yl)methyl)-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide (14a)

White solid, Yield, 52%, m.p. 181–182 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.49 (s, 1H),7.79–7.71 (m, 3H),7.68–7.59 (m, 2H),7.50 (t, J = 8.4 Hz, 2H),7.14 (dt, J = 15.1, 6.6 Hz, 2H), 6.47 (s, 2H),5.03 (s, 2H),3.57 (d, J = 15.0 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 152.41, 149.73, 142.97,137.29,134.52,133.37, 129.21, 127.70, 122.10, 121.20, 118.51, 111.40,106.54,59.95,55.82,49.58. HRMS (ESI) m/z calcd for $C_{23}H_{23}N_3O_5S$ [M+H] $^+$ 454.1437, found, 454.1436.

5.3.14. N-((3a,7a-dihydro-1H-benzo[d]imidazol-2-yl) methyl)-4-fluoro-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide (14b)

Yellow solid, m.p. 192–193 °C; Yield, 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.48 (s, 1H), 7.89–7.72 (m, 2H), 7.59–7.38 (m, 4H), 7.14 (td, J = 14.7, 6.4 Hz, 2H), 6.53 (s, 2H), 5.02 (s, 2H), 3.59 (d, J = 5.2 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 166.01, 163.50,152.48,149.65, 142.96, 137.37, 134.48, 133.74, 133.71, 130.90, 130.81, 122.11, 121.20, 118.50, 116.46, 116.24,111.39,106.62,59.95, 55.89, 49.51. HRMS (ESI) m/z calcd for $C_{23}H_{22}FN_3O_5S$ [M+H] $^+$ 472.1342, found, 472.1341.

5.3.15. 4-Bromo-N-((3a,7a-dihydro-1H-benzo[d]imidazol-2-yl) methyl)-N-(3,4,5- trimethoxyphenyl) benzenesulfonamide (14c)

Yellow solid, m.p. 187–188 °C, Yield 70%. ^{1}HNMR (400 MHz, DMSO-d_6) δ12.48 (s, 1H),7.84 (d, J = 8.6 Hz, 2H),7.66 (d, J = 8.6 Hz, 2H),7.49 (dd, J = 5.3, 3.9 Hz, 2H), 7.14 (dd, J = 6.0, 3.1 Hz, 2H), 6.56 (s, 2H), 5.03 (s, 2H), 3.60 (d, J = 4.4 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ152.50, 149.59, 138.37, 137.43, 136.61, 134.36, 132.26, 131.82, 130.56, 129.71, 127.75, 127.39, 121.83, 114.92, 106.67, 59.96, 55.89, 49.45. HRMS (ESI) m/z calcd for $C_{23}H_{22}BrN_3O_5S$ [M+H] $^+$ 532.0542, found 532.0541.

5.3.16. 2-Chloro-N-((3a,7a-dihydro-1H-benzo[d]imidazol-2-yl) methyl)-N-(3,4,5- trimethoxyphenyl) benzenesulfonamide (14d)

Yellow solid, m.p. 163–164 °C, Yield, 52%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 7.89 (dd, J = 7.9, 1.5 Hz, 1H), 7.76 (dd, J = 8.0, 1.0 Hz, 1H), 7.68 (td, J = 7.7, 1.5 Hz, 1H), 7.55 (d, J = 7.7 Hz, 1H), 7.53–7.45 (m, 2H), 7.20–7.10 (m, 2H), 6.54 (s, 2H), 5.27 (s, 2H), 3.54 (d, J = 18.7 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 152.50,149.91, 137.32,135.97,134.77,133.59,132.35,132.08,131.11,127.70,121.78,115.04, 106.51,59.95,55.79,50.04. HRMS (ESI) m/z calcd for $C_{23}H_{22}ClN_3O_5S$ [M+H] $^+$ 488.1047, found 488.1046.

5.3.17. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-(tert-butyl)-N-(3,4,5-trimethoxyphenyl) benzenesulfonamide (14e)

White solid, m.p. 192–193 °C, Yield, 78%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.43 (s,1H), 7.56 (s,4H), 7.41 (dd, J = 5.8, 3.4 Hz, 2H), 7.06 (dd, J = 6.0, 3.2 Hz, 2H), 6.35 (s,2H), 4.94 (s,2H), 3.51 (s, 3H), 3.46 (s, 6H), 1.23 (s, 9H).^{13}C NMR (100 MHz, DMSO-d_6)δ156.58, 152.35, 149.76, 138.42, 137.17, 134.65, 134.11, 127.66, 126.01, 125.27, 124.28, 121.79, 114.96, 106.47, 59.94, 55.66, 49.65, 34.91, 31.04, 30.70. HRMS (ESI) m/z calcd for $C_{27}H_{31}N_3O_5S$ [M+H] $^+$ 510.2063, found 510.2062.

5.3.18. N-((1H-benzo[d]imidazol-2-yl)methyl)-3,4-dimethoxy-N-(3,4,5-trimethoxypheyl)benzenesulfonamide (14f)

White solid, m.p. 202–203 °C, Yield, 66%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s,1H), 7.46–7.37 (m,2H), 7.21 (dd, J = 8.5, 2.1 Hz, 1H), 7.07 (dd, J = 7.9, 5.3 Hz, 4H), 6.45 (s,2H), 4.93 (s,2H), 3.77 (s,3H), 3.67 (s,3H), 3.51 (d, J = 4.2 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ152.72, 152.35, 151.56, 150.01, 148.52, 137.12, 134.93, 128.42, 121.76, 111.11, 110.28, 106.42, 59.94, 55.91, 55.83, 55.68, 49.23. HRMS (ESI) m/z calcd for $C_{25}H_{27}N_3O_7S$ [M+H] $^+$ 514.1648, found 514.1649.

5.3.19. N-((1H-benzo[d]imidazol-2-yl)methyl)-N-(4-fluorophenyl)-4-methylbenzenesulfonamide(17a)

White solid, yield, 57%, m.p. 183–184 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.53 (s, 1H), 7.59–7.37 (m, 6H), 7.28–7.08 (m, 6H), 5.01 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.35, 159.91, 149.35, 143.94, 135.13, 135.10, 133.99, 130.43, 130.34, 129.79, 127.52, 121.75, 115.81, 115.59, 48.97, 21.03. HRMS (ESI) m/z calcd for $C_{21}H_{18}FN_3O_2S$ [M+H] $^+$ 396.1182, found 396.1181.

5.3.20. N-((1H-benzo[d]imidazol-2-yl)methyl)-N-(4-chlorophenyl)-4-methylbenzenesulfonamide(17b)

White solid, yield, 59%, m.p. 190–191 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.54 (s, 1H), 7.54 (d, J = 8.0 Hz, 2H), 7.44 (dd, J = 16.9, 5.6 Hz, 4H), 7.36 (d, J = 8.5 Hz, 2H), 7.22 (d, J = 8.5 Hz, 2H), 7.15–7.09 (m, 2H), 5.02 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.25, 144.05, 137.84, 133.86, 132.45, 129.84, 129.79, 128.90, 127.51, 121.92, 114.96, 48.59, 21.04. HRMS (ESI) m/z calcd for $C_{21}H_{18}ClN_3O_2S$ [M+H] $^+$ 412.0887, found 412.0888.

5.3.21. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-methyl-N-phenylbenzenesulfonamide(17c)

White solid, yield, 69%, m.p. 160–161 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.49–7.38 (m, 4H), 7.30–7.16 (m, 5H), 7.12 (s, 2H), 5.02 (s, 2H), 2.41 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 149.49, 143.85, 138.94, 134.21, 129.73, 128.81, 128.04, 127.89, 127.49, 121.71, 48.86, 21.02. HRMS (ESI) m/z calcd for $C_{21}H_{19}N_3O_2S$ [M+H] $^+$ 378.1276, found 378.13.

5.3.22. N-((1H-benzo[d]imidazol-2-yl)methyl)-N-(4-methoxyphenyl)-4-methylbenzenesulfonamide (17d)

White solid, yield, 69%, m.p. 121–122 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.49 (s, 1H), 7.56–7.35 (m, 6H), 7.18–7.02 (m, 4H), 6.79 (d, J = 8.7 Hz, 2H), 4.97 (s, 2H), 3.66 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.55, 149.56, 143.71, 134.33, 131.22, 129.71, 129.52, 127.53, 121.62, 113.96, 55.15, 49.20, 21.03. HRMS (ESI) m/z calcd for $C_{22}H_{21}N_3O_3S$ [M+H] $^+$ 408.1382, found 408.1381.

5.3.23. N-((1H-benzo[d]imidazol-2-yl)methyl)-N-(3,4-dimethoxyphenyl)-4-methylbenzenesulfonamide(17e)

White solid, yield, 69%, m.p. 112–113 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.49 (s, 1H), 7.59 (d, J = 7.9 Hz, 2H), 7.52–7.40 (m, 4H), 7.19–7.09 (m, 2H), 6.80 (d, J = 9.2 Hz, 1H), 6.68 (s, 2H), 4.98 (s, 2H), 3.67 (s, 3H), 3.53 (s, 3H), 2.42 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.67, 148.39, 148.12, 143.76, 134.38, 131.36, 129.66, 127.66, 121.63, 120.87, 112.34, 111.08, 55.37, 55.33, 49.41, 21.01. HRMS (ESI) m/z calcd for $C_{23}H_{23}N_3O_4S$ [M+H] $^+$ 438.1488, found 438.1488.

5.3.24. N-((1H-benzo[d]imidazol-2-yl)methyl)-N-(3,4,5-trimethoxyphenyl) benzamide(18a)

Yellow solid, m.p. 100–101 °C, Yield, 62%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.38 (s, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.41 (d, J = 6.8 Hz, 2H), 7.35–7.23 (m, 3H), 7.20–7.10 (m, 2H), 6.63 (s, 2H), 5.29 (s, 2H), 3.53 (d, J = 10.6 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.78, 152.46, 150.92, 143.00, 138.56, 136.06, 135.98, 134.34, 129.53, 128.03, 127.65, 121.99, 121.15, 118.50, 111.25, 105.79,

59.97, 55.82, 47.71. HR-MS (ESI) *m/z* calcd for $C_{24}H_{23}N_3O_4$ [M+H] $^+$ 418.1767, found: 418.1767.

5.3.25. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-fluoro-N-(3,4,5-trimethoxy phenyl) benzamide (18b)

White solid, m.p.152−153 °C, Yield 74%.^{1}H NMR (400 MHz, DMSO-d_6) δ 12.41 (s,1H), 7.62−7.45 (m, 4H), 7.20−7.08 (m,4H), 6.67 (s,2H), 5.30 (s,2H), 3.56 (d, J = 9.0 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 168.77, 163.64, 161.18, 152.57, 150.88, 143.03, 138.55, 136.13, 134.37, 132.46, 132.42, 130.75, 130.66, 121.98, 121.18, 118.50, 114.77, 114.55, 111.24, 105.80, 59.98, 55.85, 47.88. HR-MS (ESI) calcd for $C_{24}H_{22}FN_3O_4$ [M+H] $^+$ *m/z*:436.1673, found: 436.1674.

5.3.26. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-bromo-N-(3,4,5-trimethoxyphenyl) benzamide (18c)

White solid, m.p. 172−173 °C, Yield,70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 7.57 (d, J = 7.6 Hz, 1H), 7.50 (dd, J = 7.3, 5.5 Hz, 3H), 7.36 (d, J = 7.6 Hz, 2H), 7.20−7.12 (m,2H), 6.66 (s,2H), 5.27 (s,2H), 3.54 (d, J = 8.5 Hz, 9H).^{13}C NMR (100 MHz, DMSO-d_6) δ 168.78, 152.55, 150.76, 143.01, 138.27, 136.19, 135.27, 134.33, 130.72, 130.16, 123.00, 122.01, 121.16, 118.51, 111.22, 105.86, 60.00, 55.88, 47.74. HR-MS (ESI) *m/z* calcd for $C_{24}H_{22}BrN_3O_4$ [M+H] $^+$ 496.0872, found 496.0872.

5.3.27. N-((1H-benzo[d]imidazol-2-yl)methyl)-2-chloro-N-(3,4,5-trimethoxyphenyl) benzamide (18d)

White solid, m.p. 141−142 °C, Yield, 60%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.45 (s,1H), 7.59 (d, J = 7.6 Hz, 1H), 7.52 (d, J = 7.5 Hz, 1H), 7.48 (dd, J = 5.9, 3.3 Hz, 1H), 7.33 (ddd, J = 10.5, 5.8, 4.4 Hz, 1H), 7.29−7.22 (m,2H), 7.21−7.11 (m,2H), 6.67 (s,2H), 5.27 (s, 2H), 3.53 (d, J = 8.2 Hz, 6H), 3.51 (d, J = 4.5 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.18, 152.19, 150.34, 143.00, 136.90, 136.41, 136.24, 134.39, 130.22, 129.38, 129.04, 128.98, 126.56, 122.09, 121.18, 118.61, 111.33, 105.69, 59.91, 55.75, 46.59. HR-MS (ESI) *m/z* calcd for $C_{24}H_{22}ClN_3O_4$ [M+H] $^+$ 452.1377, found: 452.1376.

5.3.28. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-methyl-N-(3,4,5-trimethoxyphenyl) benzamide (18e)

White solid, M.p. 156−157 °C, Yield, 65%. ^{1}H NMR (400 MHz, DMSO) δ 12.39 (s, 1H), 7.58 (d, J = 6.9 Hz, 1H), 7.51 (d, J = 7.0 Hz, 1H), 7.33 (d, J = 7.6 Hz, 2H), 7.17 (d, J = 7.4 Hz, 2H), 7.08 (d, J = 7.9 Hz, 2H), 6.65 (s,2H), 5.29 (s,2H), 3.54 (d, J = 12.7 Hz, 9H), 2.23 (s,3H).^{13}C NMR (100 MHz, DMSO) δ 169.78, 152.51, 151.01, 143.02, 139.32, 138.81, 136.00, 134.37, 133.12, 128.24, 128.16, 121.96, 121.14, 118.49, 111.25, 105.78, 59.97, 55.82, 47.91, 20.81. HR-MS (ESI) calcd for $C_{25}H_{25}N_3O_4$ [M+H]$^+$*m/z*:432.1923, found: 432.1922.

5.3.29. N-((1H-benzo[d]imidazol-2-yl)methyl)-4-(tert-butyl)-N-(3,4,5-trimethoxyphenyl)benzamide (18f)

Yellow solid, M.p. 102−103 °C, Yield, 85%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.35 (s, 1H), 7.58−7.46 (m,2H), 7.32 (dd, J = 18.0, 8.2 Hz, 4H), 7.15 (s,2H), 6.62 (s,2H), 5.29 (s,2H), 3.53 (d, J = 16.1 Hz, 9H), 1.21 (s,9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ169.66, 152.50, 152.38, 138.72, 136.12, 133.10, 129.16, 128.07, 125.29, 124.32, 121.93, 121.12, 118.49, 111.26, 105.91, 59.99, 59.70, 55.89, 47.80, 34.41, 30.82. HR-MS (ESI) *m/z* calcd for $C_{28}H_{31}N_3O_4$ [M+H] $^+$ 474.2393, found: 474.2394.

5.3.30. N-((1H-benzo[d]imidazol-2-yl)methyl)-3,4-dimethoxy-N-(3,4,5-trimethoxypheyl)benzamide (18 g)

Yellow solid, M.p. 80−81 °C, Yield, 70%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.34 (s,1H), 7.51 (s,2H), 7.11 (dd, J = 28.3, 4.0 Hz, 2H), 6.03 (t, J = 20.3 Hz, 2H), 5.19 (t, J = 44.5 Hz, 1H), 4.59 (d, J = 98.3 Hz, 2H), 3.85−3.60 (m,6H), 3.61−3.39 (m,9H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.17, 153.63, 153.37, 152.65, 151.02, 149.98, 148.31, 147.41, 145.03, 139.16, 136.17, 129.18, 127.67, 123.16, 122.10, 121.35, 112.34, 111.98, 111.00, 110.59, 105.94, 90.55, 60.10, 55.95, 55.54, 55.43, 55.30. HR-MS (ESI) *m/z* calcd for $C_{26}H_{27}N_3O_6$ [M+H] $^+$ 478.1978, found 478.1980.

5.4. Cell culture

All the cancer cells were cultured at 37 °C in an atmosphere with 5% CO_2. And using RPMI-1640 medium with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 0.1 mg/ml streptomycin as culture medium.

5.5. MTT assay

Cells were seeded 5×10^3 per well in 96-well plates and incubated for 24 h, then treated with different concentrations of compound as shown in the figures. After 48 h, 20 μL MTT solution each well was added, and incubated at 37 °C for 4 h 150 μL DMSO was added to each well to dissolve the formazan after discarding the supernatant liquid, the absorbance was determined at 490 nm IC_{50} values are calculated with SPSS version 10.0 (SPSS, Inc., Chicago, IL, USA).

5.6. Colony formation assay

5000 per well MGC-803 cells, SGC-7901 cells and HGC-27 cells were seeded in a 6-well plate and incubated at 37 °C in 5% CO_2 for 24 h, then treated with different concentrations of 10e. After 7 days, remove the culture medium, the cells were washed with PBS twice, fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Cells' images were captured with camera.

5.7. Cell cycle distribution assay

MGC-803 cells, SGC-7901 cells and HGC-27 cells were seeded in 6-well culture plate, then treated with certain concentration of compound **13g** for 48 h. Then cells were harvested and fixed at 4 °C with 70% cold ethanol for 8 h. The fixed cells were washed and resuspended with PBS containing 10 mg/mL RNaseA and 50 mg/mL PI for 0.5 h in the dark. Then, samples were analyzed for DNA content with flow cytometry (Becton, Dickinson and Company, NJ).

5.8. Cell apoptosis assay

MGC-803 cells, SGC-7901 cells and HGC-27 cells were seeded in 6-well culture plate and treated with certain concentrations of compound **13g** for 48 h. Then cells were harvested and suspended in binding buffer containing Annexin V-FITC (0.5 mg/mL) and PI (0.5 mg/mL). After that, samples were incubated for 0.5 h in dark and analyzed with flow cytometry (Becton, Dickinson and Company, NJ).

5.9. Western blot analysis

Human gastric cancer cell lines (MGC-803, SGC-7901 and HGC-27) were treated with different concentrations of compound **13g** or DMSO for 48 h. And then cells were harvested and lysed. Protein lysates were denatured and resolved by SDS-PAGE and transferred to nitrocellulose membrane. The membranes were incubated with appropriate antibodies at 4 °C for 8 h after blocking with 5% skimmed milk. After conjugated with secondary antibodies, the detection of proteins was carried out with an ECL kit.

5.10. Combination index calculation

MTT was used to determine respective and associated activity of compound **13g** and piperlongumine against MGC-803 cells [41]. Then the combination index (CI) was calculated using the CompuSyn software and the Chou-Talalay method, in which the combination index (CI) theorem offers a quantitative definition for an additive (CI = 1), synergistic (CI < 1), or antagonistic (CI > 1) effect of a drug combination [42].

5.11. Statistical analysis

Data from three independent experiments are presented as mean ± SD. IC_{50} values and SD values were calculated by SPSS version 10.0 (SPSS, Inc., Chicago, IL, USA).

Competing financial interests

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111731.

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