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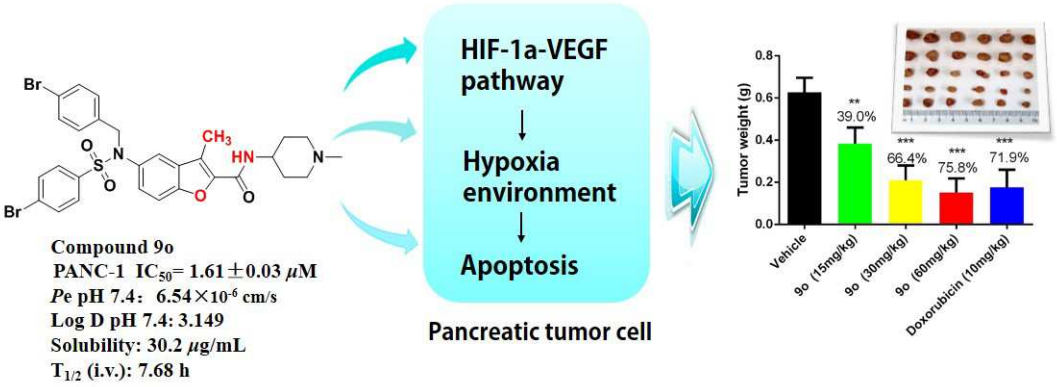

Design, synthesis, and evaluation of benzofuran derivatives as novel anti-pancreatic carcinoma agents via interfering the hypoxia environment by targeting HIF-1α pathway

Xiao-li Xu [a,b], Ying-rui Yang [a,b], Xiao-fei Mo[a,b], Jin-lian Wei[a,b], Xiao-jin Zhang [a,c], Qi-dong You [a,b*]

[a] State Key Laboratory of Natural Medicines, and Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 210009, China

[b] Department of Medicinal Chemistry, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China

[c] Department of Organic Chemistry, School of Science, China Pharmaceutical University, Nanjing, 210009, China

* *Corresponding author. e-mail: youqd@163.com (Q. You)*

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most common type of pancreatic cancer，and has still been the medicinal mystery. New drugs and treatment strategies are urgently needed. In this study, 32 benzofuran derivatives are designed, synthesized and evaluated as potential agents against the pancreatic cancer. Among them, compound **9o** with the best physicochemical and pharmacokinetic properties exhibited excellent cytotoxicity against many tumor cell lines. *In vivo* study showed that compound **9o** dramatically suppressed the tumor growth of nude mice. Furthermore, compound **9o** could affect the hypoxia environment through Hif-1α/VEGF pathway, resulting in the anti-angiogenic activity. These studies indicated that compound **9o** was a promising candidate for the treatment of PDAC, deserving further studies.

Key words Pancreatic cancer, Benzofuran derivatives, Anti-tumour activity, Structure-activity relationships, Apoptosis, HIF-1α pathway, Anti-angiogenesis

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC), as the most common form of pancreatic cancer (PC), is a kind of digestive-tract malignant tumor with highly invasive and metastatic features and has become the fourth most common cause of the lethal of malignancies in many countries[1, 2]. At the present, the 5-year survival rate of PDAC is lower than 5%[3], and it is estimated that PC will surpass the breast and colorectal cancer to become the second disease that cause the mortality within a decade in developed countries.[4] However, the management

of PDAC is still frustrating that mostly due to the poor detection on the early stages of pancreatic cancer and most patients with PC were only diagnosed at advanced-stage. Besides survival of PC patients over 5 years is only 15-20% [5], unclear actionable therapeutic targets, lack of effective drugs and inefficient therapeutic strategies also make PC become a great health burden for patients and medical researchers [6]. Thus, it is critical to identify novel and effective therapeutic drugs or strategies against pancreatic adenocarcinoma.

Administration of gemcitabine has been used as the standard therapy for patients with metastatic pancreatic cancer in the past decades [5, 7]. The investigational drugs are ongoing early clinical trials in metastatic pancreatic cancer including the cytotoxic agents such as irinotecan [8], PARP inhibitors such as iniparib [9, 10], Janus kinase inhibitors such as ruxolitinib [11], agents targeting *Kras* pathway including Mek or PI3K inhibitors such as trametinib and everolimus [12-16] and some monoclonal antibodies for immunotherpay and so on [17] (Fig.1).

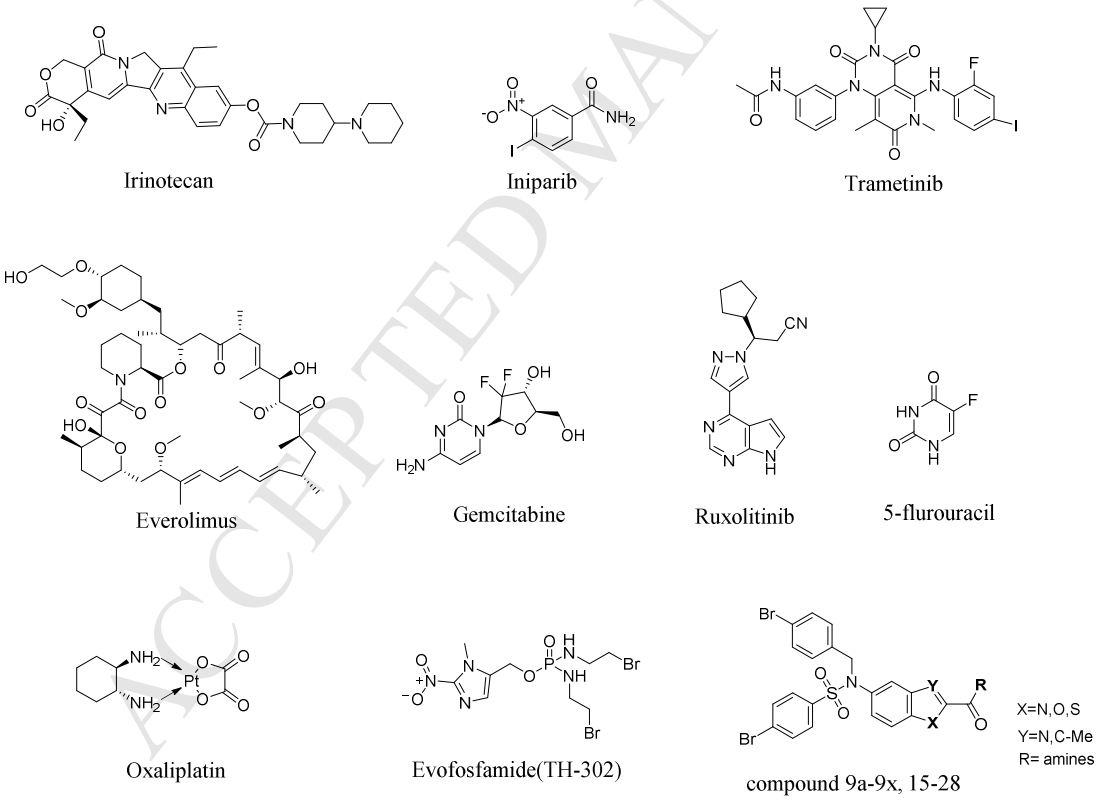


Fig.1 Drugs targeting pancreatic cancer cells and title compounds.

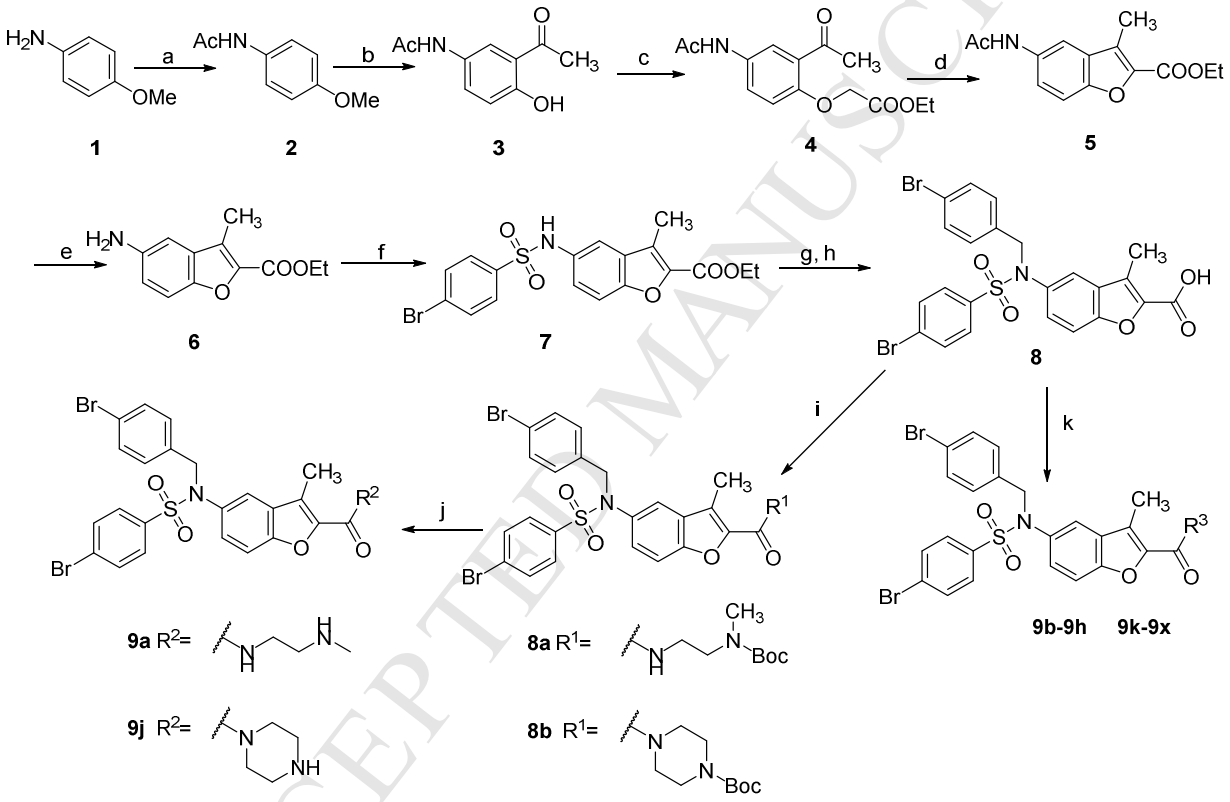
Cytotoxic agents with new formulation has been proved to be one of the effective drugs, holding the promise for targeting pancreatic cancer cells [18, 19]. Recently, both nab-paclitaxel

plus gemcitabine and FOLFIRINOX (folinic acid, 5-fluorouracil [5‑FU], irinotecan, and oxaliplatin) have performed better in the treatment of patients with advanced-stage metastatic pancreatic cancer [20-22]. Classical cytotoxic drugs with novel formulations such as MM-398 with nanoliposomal formulation of irinotecan has a longer duration of action and could aggregate at the tumor tissue [23]. DNA alkylating agent such as evofosfamide (knows as TH-320, hypoxia-activated prodrug of a nitrogen mustard derivative) is proved to increase the potency of gemcitabine alone for the advanced-stage pancreatic cancer (6.0 versus 3.6 months; P = 0.008) in the randomized trial [24](Fig.1). As a consequence, development of small molecules that could induce death of pancreatic cells is still urgent for the therapy of pancreatic cancer.

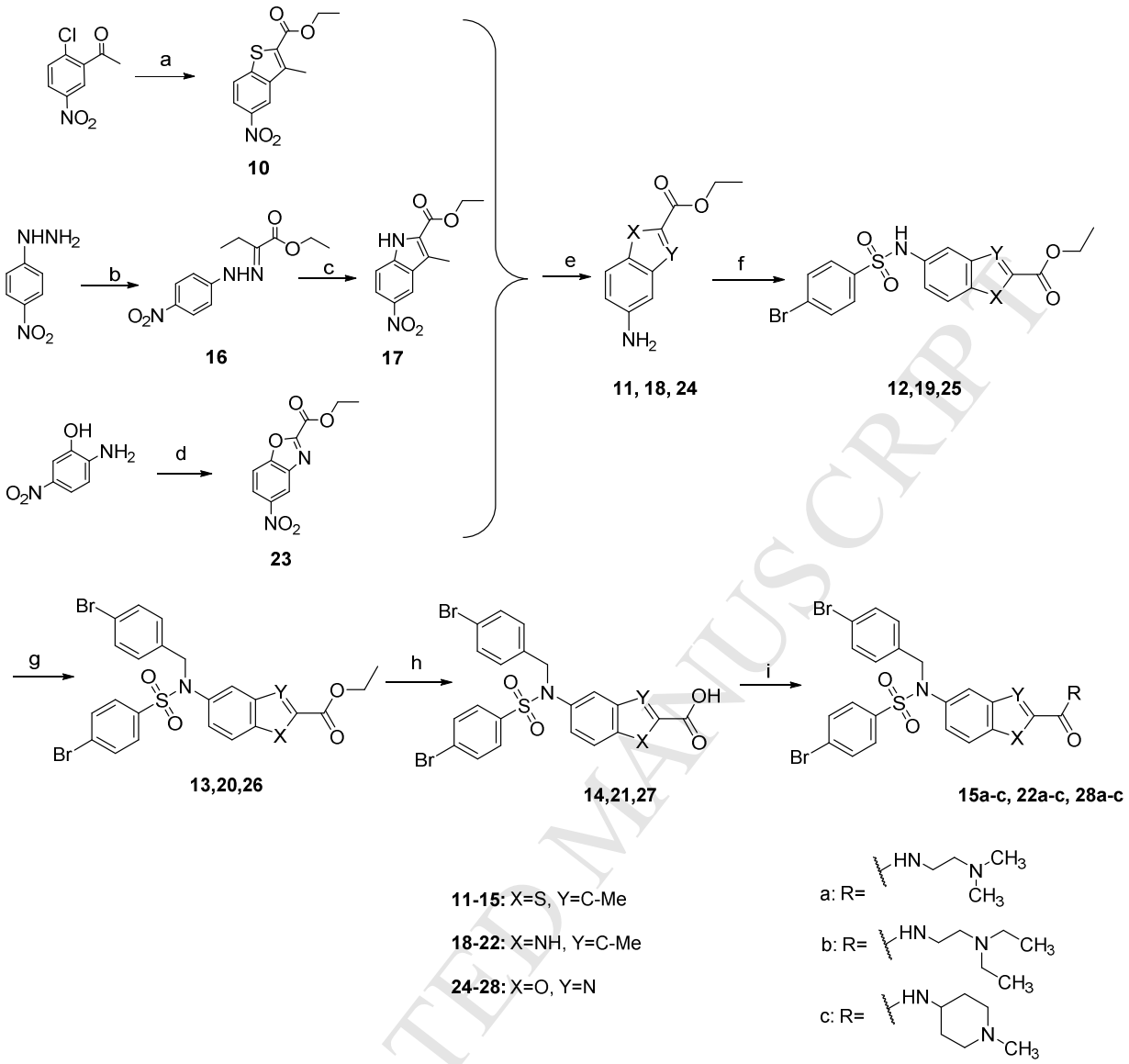
Although the clear mechanisms of PDAC remained vague and largely unknown, recent research achievements have shorten the distance between patients to PDAC and made its pathological mechanism more clear. It has been found that constitutively activation of oncogenes such as *Kras* and/or mutations or the loss of tumor suppressor genes (including p53, p16 and smad4) would trigger the PDAC tumors[25]. A profound role of *TP53* is related in cancer progression including pancreatic cancer [26, 27]. Therefore, molecules or chemotherapy against p53-independent pancreatic cancer cells could be a better therapeutic approach for pancreatic cancer. Additionally, the hypoxic tumor microenvironment and hypoxia-induced factors are critical drivers for metastasis and progression of PDAC [28]. Hypoxia appears in the microenvironment of many solid tumors, including pancreatic cancer cells with high level of HIF-1α and VEGF, angiopoietin-1 and so on [29, 30]. Therefore, targeting HIF-1α pathway of PDAC is an interesting attempt to improve the microenvironment for combating the resistance to chemotherapy and radiation.

We sought to develop inhibitors that can be used to improve the activity and drug-like ability against the pancreatic cancer. In our previous studies, we have described that benzofuran derivatives suppressed p53-independent malignant cancer cells through inhibition of HIF-1 pathway [31]. Preliminary SAR found that 4-halogen phenylsulfonyl and N containing alkyl chains contributed significantly to the antiproliferative activities [31]. In continuation with previous work, we intended to design and synthesize some novel series of benzofuran derivatives with new scaffolds and to evaluate their cell-based activities,

biochemical and physicochemical properties as well as *in vivo* activity against pancreatic cancer. Compared to previous compounds, new compound with heteroatom-containing group exhibited better physicochemical and pharmacokinetic properties. This work led to the identification of **9o** as the respective compound with the best cytotoxic activities against tumor cells. Moreover, the anti-tumor activity is related to anti-angiogenic activities against HIF-1α pathway. These inhibitors have potential to become drug candidates for the treatment of pancreatic cancer.



Scheme 1. Reagents and conditions: (a) Ac_2O, DCM, r.t., 6 h; (b) AcCl, $AlCl_3$, DCM, reflux, 12 h; (c) $BrCH_2COOEt$, K_2CO_3, KI, acetone, reflux, 6 h; (d) EtONa, DMF, microwave irradiation (350W), 145 ^oC, 7 min; (e) 3 M HCl, EtOH, reflux, 6 h; (f) pyridine, 4-bromobenzenesulfonyl chloride, DCM, 6 h; (g) p-bromobenzyl chloride, K_2CO_3, KI, acetone, reflux, 2 h; (h) NaOH, EtOH, reflux, 30 min; then diluted HCl; (i) EDCI, HOBt, DIPEA, corresponding amines, r.t., 12 h; (j) CF_3COOH, DCM, r.t., 12 h; (k) EDCI, HOBt, DIPEA, corresponding amines, r.t., 12 h.



Scheme 2. Reagents and conditions: (a) $HSCH_2COOC_2H_5$, K_2CO_3, C_2H_5OH, r.t., reflux, overnight; (b) ethyl 2-oxobutanoate, C_2H_5OH, reflux, 30 min; (c) PPA, dixoane, reflux, 48 h, (d) ethyl 2-oxoacetate, dioxane, 50 ^oC, then CAN, reflux, 6 h; (e) H_2, Pd/C, THF, r.t., overnight; (f) 4-bromobenzene-1-sulfonyl chloride, pyridine, TEA, DCM, r.t., overnight; (g) 1-bromo-4-(bromomethyl)benzene, K_2CO_3, KI, acetone, 4 h; (h) NaOH aqueous, C_2H_5OH, reflux, 3 h; (i) corresponding amines, EDCI, HOBT, DCM, r.t, overnight.

2. Results and Discussion

2.1 Chemistry

In previous study, it was found that a series of benzofuran derivatives as new chemotype exhibited potential inhibitory against p53-independent malignant tumor cells.[31] In this study,

in order to discover new chemical entity and improve the drug-like properties, a new series derivatives based on the main scaffold of benofuran were designed and synthesized as outlined in Scheme 1. In general, introduction of heteroatom-containing group is a good method to improve the physicochemical property for compounds. We changed the substitutes on the ring of benzofuran with heteroatom-containing group, including straight flexible side chains, and heterocyclic side chains.

The derivatives **9a-x** were designed and synthesized by utilizing the synthetic route shown in Scheme 1. First, acylation of *p*-methoxyaniline produced the amino-protected intermediate **2**. Friedel-crafts acylation and simultaneous demethylation of **2** afforded **3**. Intermediate **4** was prepared using bromoacetic acid ethyl ester and potassium carbonate in a polar aprotic solvent (acetone). Cyclization of **4** with sodium ethoxide under microwave irradiation gave intermediate **5** and then compound **6** was obtained by deacylation of **5**. Subsequently, sulfonation of **6** with 4-bromobenzenesulfonyl chloride in the presence of pyridine produced **7**, and then refluxed with *p*-bromobenzyl in presence of potassium carbonate and potassium iodide in acetone and followed by hydrolysis reaction to obtain the key intermediate **8**. Substitution of the carboxyl with corresponding amines produced the target compounds **9a**-**9x**.

At the same time, we attempted to identify the core that could server a main scaffold to extend the SAR. So the second series of derivatives where the benzofunan ring replaced with three kinds of benzoheterocyclic core were prepared and described in Scheme 2. Cyclization of 1-(2-chloro-5-nitrophenyl)ethan-1-one and 2-mercaptoacetate ethyl produced **10** with benzothiophene ring. The Fischer indole synthesis reaction afforded the derivatives with indole ring. Condensation between *p*-nitrophenyl hydrazine and ethyl 2-oxobutanoate in refluxing alcohol gave **16** and then cyclized with dixoane in the presence of PPA. Cyclization of 2-amino-5-nitrophenol with ethyl 2-oxoacetate in the presence of CAN afforded **23**. Reduction of **10**, **17** and **23** using Pd/C catalytic hydrogenation gave **11**, **18** and **24**, and then followed by sulfonylation using 4-bromobenzene-1-sulfonyl chloride to produce **12**, **19** and **25,** respectively. Bromobenzyl group was then introduced in the presence of TEA to give **13**, **20** and **26**, which were hydrolyzed to give the key intermediates **14**, **21**, **27**. Condensation of the carboxylic acid intermediates and corresponding hydrophilic amines produced the target compounds **15a-c,**

22a-c, 28a-c.

2.2 Biological Evaluation

2.2.1 Evaluation of Antiproliferative Activity

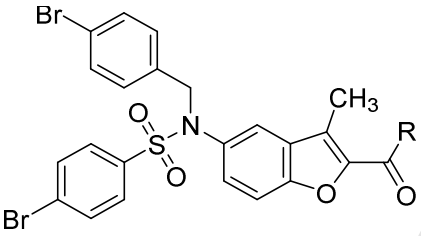
In order to evaluate the antiproliferative activity of the synthesized compounds **9a-9x**, **15a-c**, **22a-c**, **28a-c**, we chose a panel of pancreatic cancer cells including pancreatic (PANC-1, BxPC-3) and some other cell lines with high malignancy and prognosis such as colon (HTC116, p53 -/-), breast (MCF-7), lung (A549), and triple negative breast cancer cells (MDA-MB-231). Anti-proliferative activity was measured by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Doxorubicin was used as positive control and the results were shown in table **1-2**. In general, compounds with a long side chain such as **9a-9i** performed remarkable anti-proliferative activity with IC_{50} values between 1-10 μM, whereas the length of the side chains had little influence on the activity. However, the activity of compounds **9d**, **9f-9h** were much lower than **9a** and **9b**, suggesting that physicochemical property of the compounds might in part explain this behavior. Heterocyclic substituted compounds such as **9i-9o** kept the anti-proliferative activity, and heterocyclic aromatic substituted compounds such as **9p**, **9u-9x**, showed moderate or poor activity, indicating that flexible side chains were more suitable for the cytotoxic activity.

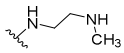
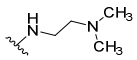
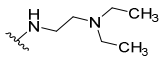
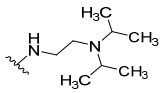
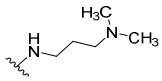
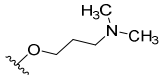
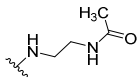
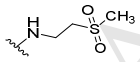
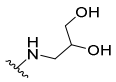
SAR analysis was also performed with three scaffolds made on the benzofunan ring. Exploration of these heterocyles at this position was expected to be a promising approach to increase the potency. However, replacement by benzothiophene, indole ring and benzoxazole resulted in a slightly decrease against three cancer cell lines.

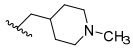
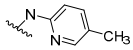
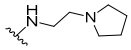
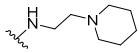
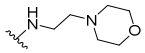
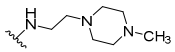
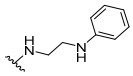
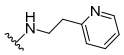
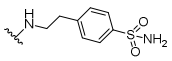
Based on the results of Table 2, replacement of benzofunan with benzothiophene, indole ring and benzoxazole, as in **15a-c**, **22a-c**, and **28a-c**, resulted in slightly lower activity than those of benzofunan compounds, as in **9b-9c** and **9o**. Compounds with methylpiperazine substitution (**9o**) were highly potent, regardless of whether the scaffold of benzoheterocycle were benzothiophene (**15c**), indole ring (**22c**) and benzoxazole (**28c**). In summary, although compounds with benzoheterocycle kept the cytotoxic activity, to some extent, the core of benzofunan provided superior potency. Moreover, among the series of benzofunan derivatives, **9c** and **9o** exhibited an outstanding activity against PANC-1, BxPC3, HCT116, HCT116

(p53-/-), MCF7, A549, MDA-MB-231 *in vitro*, with IC_{50} value of 1.07 ± 0.09, 0.65 ± 0.01, 1.81 ± 0.18, 1.61 ± 0.03, 2.39 ± 0.33, 2.68 ± 0.30, 1.90 ± 0.05, and 1.52 ± 0.12, 1.08 ± 0.07, 2.39 ± 0.08, 1.66 ± 0.05, 2.84 ± 0.35, 2.98 ± 0.73, 3.73 ± 0.10, respectively, which were chosen as representative for the further biological evaluation.

Table 1. Cell proliferation inhibition results of compound 9a-9x.

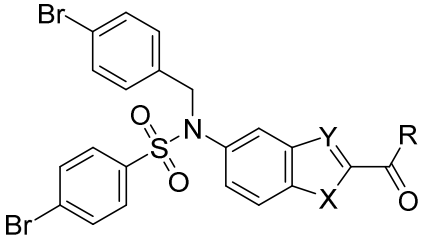


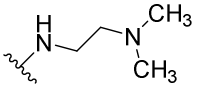
Compd	R	Anti-proliferation activities IC_{50} (μM)[a]						
		PANC-1	BxPC3	HCT116	HCT116 (p53-/-)	MCF7	A549	MDA-MB-231
9a		2.49±0.17	5.13±0.12	2.44 ±0.02	2.37±0.48	4.37±0.38	3.08±0.36	7.96±0.75
9b		5.94±0.16	4.79±0.78	3.64±1.07	1.20±0.13	3.66±0.15	5.40±0.13	8.24±0.54
9c		1.52±0.12	1.08±0.07	2.39±0.08	1.66±0.05	2.84±0.35	2.98±0.73	3.73±0.10
9d		6.25±0.18	28.3±4.6	10.4±0.6	3.11±0.68	10.9±0.19	5.50±0.23	9.44±0.45
9e		2.30±0.76	2.02±0.20	3.07±0.26	3.73±0.27	3.71±0.51	4.31±0.91	6.68±0.05
9f		24.6±0.6	24.2±0.8	11.2±0.1	7.05±1.03	39.7±4.8	>40	28.6±1.7
9g		34.8±6.6	>40	12.2±0.26	20.4±1.26	21.3±3.4	>40	>40
9h		>40	>40	>40	>40	>40	>40	>40
9i		29.3±1.19	27.56±1.3	27.8±1.1	11.5±0.2	36.5±2.09	30.5±2.09	16.5±0.69
9j		8.38±0.91	19.6±1.2	3.00±0.58	3.30±0.10	9.72±0.33	8.68±0.22	9.04±0.71
9k		>40	>40	8.55±0.26	5.47±0.18	>40	22.3±0.3	>40
9l		23.8±1.27	29.1±2.5	22.1±1.16	26.4±1.43	15.4±0.76	19.1±0.86	25.1±1.56

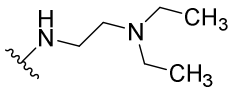
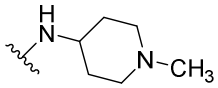
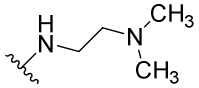
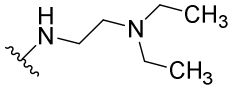
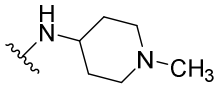
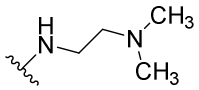
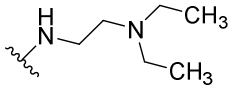
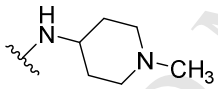
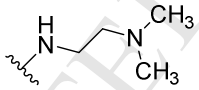
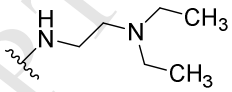
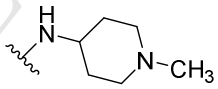
9m		>40	>40	7.88±1.24	21.9±0.8	>40	>40	>40
9n		26.22±1.4	16.65±1.4	23.12±1.1	28.3±1.2	32.78±2.2	33.69±1.7	19.4±0.7
9o		1.07±0.09	0.65±0.01	1.81±0.18	1.61±0.03	2.39±0.33	2.68±0.3	1.90±0.05
9p		>40	>40	>40	>40	>40	>40	>40
9q		2.78±0.31	2.36±0.15	2.67±0.10	2.29±0.35	5.44±0.84	5.29±0.40	8.17±0.15
9r		6.94±1.43	3.71±0.24	2.23±0.06	2.84±0.33	3.36±0.18	5.17±0.44	9.26±0.22
9s		32.12±1.6	27.2±1.28	10.5±0.79	13.28±1.1	17.2±0.69	21.37±1.4	23.5±1.19
9t		5.04±0.86	2.6±0.56	3.81±1.12	2.59±0.42	5.66±0.17	5.03±0.07	7.50±0.33
9u		>40	>40	>40	>40	>40	>40	>40
9v		>40	>40	>40	>40	>40	>40	>40
9x		>40	>40	5.59±1.07	19.2±2.3	>40	>40	>40
doxorubicin		0.41±0.04	0.56±0.06	1.01±0.09	1.23±0.11	0.67±0.08	0.89±0.1	0.49±0.04

[a] Values shown are the mean ± SD (n = 3).

Table 2. SAR exploration of the R position and the core.



Compd	X	Y	R	Anti-proliferation activities IC_{50} (μM)[a]		
				PANC-1	BxPC3	HCT116
15a	S	C-Me		7.89 ± 0.52	9.0 ± 0.76	9.8 ± 0.86

15b	S	C-Me		7.65 ± 0.53	7.88 ± 0.81	10.9 ± 1.03
15c	S	C-Me		5.2 ± 0.78	7.1 ± 0.66	10.1 ± 0.87
22a	N	C-Me		11.2 ± 0.92	6.98 ± 0.4	16.6 ± 1.23
22b	N	C-Me		10.5 ± 0.9	17.4 ± 1.81	15.8 ± 1.72
22c	N	C-Me		3.67 ± 0.18	7.5 ± 0.63	14.5 ± 0.92
28a	O	C-Me		13.6 ± 1.09	13.7 ± 0.93	14.3 ± 1.09
28b	O	N		12.6 ± 1.10	16.3 ± 1.2	14.3 ± 0.78
28c	O	N		5.91 ± 0.5	10.16 ± 1.4	7.32 ± 0.66
9b	O	N		5.94 ± 0.16	4.79 ± 0.78	3.64 ± 1.07
9c	O	C-Me		1.52 ± 0.12	1.08 ± 0.07	2.39 ± 0.08
9o	O	C-Me		1.07 ± 0.09	0.65 ± 0.01	1.81 ± 0.18
Doxorubicin				0.41 ± 0.04	0.56 ± 0.06	1.01 ± 0.09

[a] Values shown are the mean ± SD (n = 3).

2.2.2 Physicochemical Properties and *in vivo* Pharmacokinetics Study

Parallel to the cytotoxicity evaluation, two representative compounds with better cytotoxic activity were selected for the evaluation of their potential drug-like properties. Solubility and permeability as two well-established markers of physicochemical properties were determined by PION and a standard parallel artificial membrane permeability assay (PAMPA). **9c** and **9o**

as the representative of the branched chain substituents exhibited similar properties. As the results shown in Fig.2A, compound **9o** with the moiety of methyl piperazine exhibited the better favorable Log D7.4 (3.149) and membrane permeability (6.54×10^{-6} cm/s) as well as the aqueous solubility of 30.2 μg/mL. Compound **9o** with the best cytotoxicity exhibited balanced physicochemical properties. Thus, compound **9o** was chosen for further *in vivo* pharmacokinetic studies in healthy rats. In general, oral and intraperitoneal administration showed considerable bioavailability, as shown in the Fig. 2B. For intravenous dosing, compound **9o** exhibited acceptable half-life (7.68 h) and clearance rate (1.34 L/h/kg), suggesting elimination was slow and widely distributed. After the treatment of **9o** for 36 h by intraperitoneal and intravenous administration, the concentration persisted in plasma was more than 10 μg/L (Fig. 2C), illustrating **9o** have possibility to keep higher concentration and longer retention in the tumor. Taken together, compound **9o** have a good pharmacokinetics data deserving the further study.

A

Compounds	Intrinsic solubility (μg/ml)	Log D, pH 7.4	*P*e pH 7.4(10^{-6} cm/s)[a]
9c	21.0	2.530	2.54 ± 0.84
9o	30.2	3.149	6.54 ± 1.38

B

	Compound 9o		
	p.o.	i.v.	i.p.
Dose (mg/kg)	10	10	10
Cmax(ug/L)	80.58	3102.6	549..22
$T_{1/2}$(h)	6.63	7.68	7.09
AUC_{0-t}(ug.h/L)	1081.40	7222.13	6220.28
F(%)	14.97	/	86.13

C

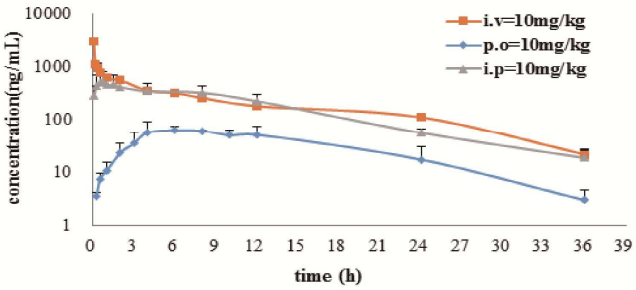


Fig.2 (A) Intrinsic solubility, Log D7.4 and permeability were determined. Ketoprofen ($4.5\times10\times6$ cm/s) and propranolol (112.8×10^{-6} cm/s) are internal standards in permeability determinations. (B) Pharmacokinetic analysis in healthy rats. (C) Plasma concentration vs time profile of compound **9o** in rat.

2.2.3 Compound 9o Induces Apoptosis of Pancreatic Cancer Cell

To further evaluate the cytotoxicity of compound **9o**, we detected whether **9o** could induce the cell death via apoptosis. PANC-1 cells were treated with compound **9o** for 24 h and the apoptotic cells were calculated by double staining of Annexin-V and PI. The results showed that apoptosis occurred in a dose-dependent manner, after the treatment of compound at 0.1, 1, 2 μM for 24 h, 9%, 45%, 51% apoptotic cells were induced, respectively (Fig.3A, B). In addition, **9o** also dramatically decreased colony formation of PANC-1 in a dose-dependent manner, suggesting the growth of PANC-1 cells were inhibited due to the apoptosis induced by **9o**. Moreover, compound **9o** led to the higher level of pro-apoptotic protein including cleaved Caspase-3, 9 and PARP, and decreased the anti-apoptotic protein such as Caspase-3, 9 and PARP, compared with control group (Fig.3D, E). Together, compound **9o** led to the apoptosis of PANC-1 cell via a mitochondrial apoptosis pathway.

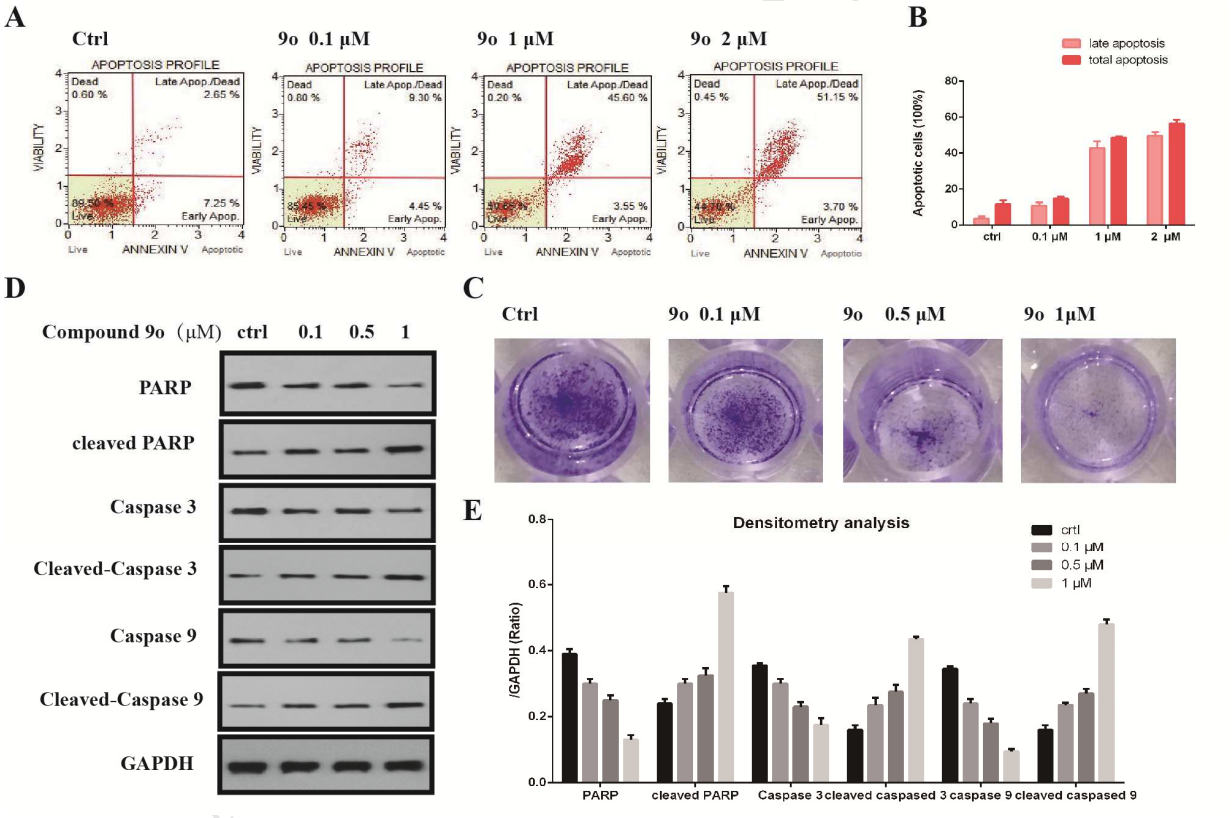


Fig.3 (A) Treatment of compound **9o** for 24 h induced the apoptosis of PANC-1 cell in a dose-dependent manner. (B) The rate of late apoptotic cell and total apoptosis were quantified by using flow cytometry. (C) Clonogenicity assay of PANC-1 in response to compound **9o** for two weeks. (D) The expression of apoptotic related proteins in PANC-1 cells treated with compound **9o** for 24 h. GAPDH is used as the control for protein loading. (E) Densitometry

analysis of the apoptotic related protein levels were shown as normalized (to GAPDH) ratios.

2.2.4 *In vivo* Anti-tumor Activity

Results of *in vivo* antitumor evaluation of compounds **9o** against mice bearing PANC-1 xenograft tumors were shown in Fig.4A. Doxorubicin was used as a standard agent. Compound **9o** was administered intraperitoneally after the tumor were established every two days for 21 days. As shown in Fig. 4C, twice-daily treatment with 15, 30, and 60 mg/kg **9o** caused significant tumor inhibition of the tumor weight by 39.0%, 66.4%, and 75.8%, respectively, compared with doxorubicin group (71.9%). Also, compound **9o** displayed considerable decrease in tumor volume. The 60 mg/kg dose showed equivalent tumor growth inhibition compared to the reference doxorubicin (10 mg/kg) (Fig. 4B). Meanwhile, all mice had normal weights and quality of life during the test. These results suggested that compound **9o** could effectively suppress the tumor development *in vivo*.

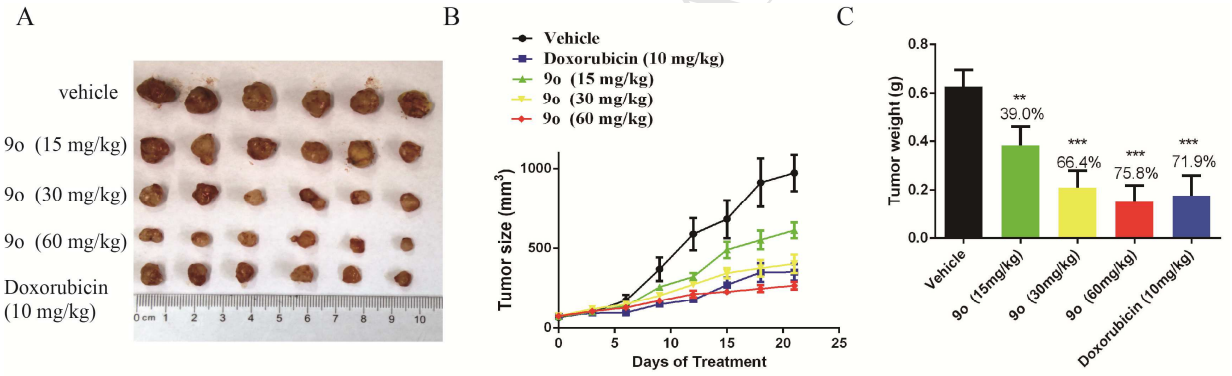


Fig.4 (A) Antitumor efficacy of compound **9o** in PANC-1 human pancreatic cancer xenografts. The PANC-1 exnograft-bearing mice were intraperitoneally injected with 15, 30, 60 mg/kg **9o** for 21 days. (B) Tumor volumes were calculated by measuring the tumor diameters. (C) Tumors were weighted when the mice were sacrificed after 21 days. **p < 0.01, ***p < 0.001; Student's t-test (n = 6).

2.2.5 Compound 9o Shows Anti-Angiogenesis Activity on the PANC-1 Cell Under Hypoxia

It has been reported that benzofuran derivatives displayed an inhibitory against malignant cancer cells through inhibition of HIF-1 pathway [31]. So we continued the mechanism studies of compound **9o** with anti-tumor activity based on the pathway of HIF-1α and angiogenesis.

Treatment of compound **9o** inhibited hypoxia-activated HIF-1α-driven expression of luciferase in a concentration dependent manner by a cell based luciferase reporter assay (Fig.5A). Using qRT-PCR assay, we identified that exposure of PANC-1 cells to hypoxia for 24 h with the treatment of compound **9o** resulted in the inhibition of VEGF transcriptional activity, which is a typical target of HIF-1α transcription (Fig.5B). Compound **9o** suppressed hypoxia-induced migration of Human umbilical vein endothelial cells (HUVECs) with the medium from PANC-1 cells in a time-dependent manner at 1 μM compared to the control group (Fig.5C). Tube-formation assay displayed that compound **9o** could impaired the tube-forming ability of HUVECs, suggesting that **9o** significantly suppressed the invasion activity of HUVECs (Fig.5D). Then we sought to examine whether HIF-1α was involved in **9o**-induced inhibition of *in vivo* tumor angiogenesis. CD31 is normally found on endothelial cells, so monitoring CD31 in histological tissue sections is used primarily to evaluate the degree of tumor angiogenesis [32]. Immunohistochemical study for HIF-1α, CD31 and VEGF in PANC-1 tumors showed that treatment of **9o** caused an inhibitory of tumor angiogenesis with low expression level of CD31 and VEGF. However, the expression of HIF-1α was not influenced much (Fig.5E). Altogether, these evidences suggested that compound **9o** showed an inhibitory on the function of HIF-1α transcription, resulting in an anti-angiogenesis activity which in part contributing the growth inhibitory on pancreatic tumor *in vivo*.

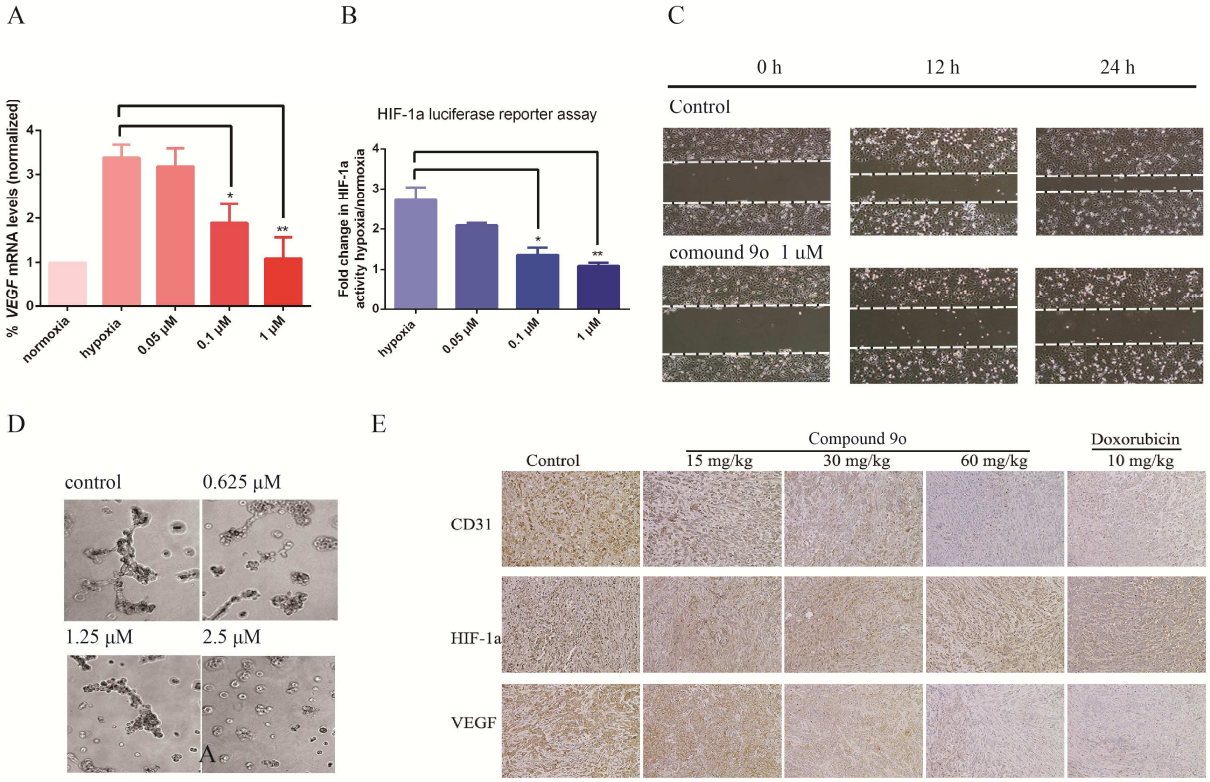


Fig.5 (A) Effects of Compound **9o** on HIF-1α–driven luciferase expression in HEK293 cells under hypoxic versus normoxic conditions (48 h). (B) Compound **9o** inhibited the ability of HUVECs on the formation of HUVECs after the treatment for 6 h. (C) Treatment of **9o** inhibited the motility of HUVECs for 6 h in the wound-healing assay. (D) The transcription of VEGF was inhibited by Compound **9o** in a dose-dependent way. (E) The expression of CD31, HIF-1 α , VEGF in tumor microenvironment were examined by immunochemistry using corresponding antibodies.

3. Conclusion

In conclusion, a series of benzofuran derivatives as anti-tumor agents were designed, synthesized and evaluated for their druggability and biological activity. All compounds were assayed by MTT assay against many cancer cell lines. The preliminary SAR analysis suggested that introduction of hydrophilic heteroatom-containing groups on the ring exhibited prominent antiproliferative activity, and replacement of the main scaffold basically kept the activity, especially benzofuran performed better. Finally, Compound **9o** with *N*-methylpiperazine group showed the most potent cytotoxicity as well as the balanced physicochemical property, illustrating the feasibility of inhibitor with heteroatom-containing groups. The flow-cytometry

revealed that treatment of **9o** induced the apoptosis of PANC-1 cells. WB analysis further confirmed that significant apoptotic proteins appeared after the treatment of **9o**, indicating the caspase-dependent way was triggered. In addition, it was found that **9o** inhibited the function of HIF-1α as transcription factor for VEGF. The wound and tube formation assay of HUVECs and histopathological examination provided insights into the evidence that the hypoxia environment of tumor was interfered by **9o**, laying the foundation for targeting the pancreatic cancer by interfering the hypoxic microenvironment through HIF-1/VEGF pathway. Overall, **9o** as the representative compound indicated that the series of benzofuran derivatives could be further studied for the therapeutic treatment of pancreatic cancer.

4. **Experimental section**

4.1 Chemistry

4.1.1 General materials and methods

All material and reagents were purchased from commercial sources and, unless otherwise stated, were used without further purification. Concentration of solutions from reactions and extractions in a rotary evaporator (Büchi Rotavapor) below 45°C at reduced pressure. Reactions were monitored by thin-layer chromatography (TLC) using Merck Kieselgel 60 F254 plates and visualized under UV light at 254 nm. Flash column chromatography was performed on silica gel (60e120 mesh size).Melting points were measured with a Melt-Temp II apparatus and were uncorrected. IR spectra were recorded by using a Nicolet iS10 Avatar FT-IR spectrometer with KBr film. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-300 instrument. Chemical shift (d) are in ppm, referenced to tetramethylsilane (TMS). Multiplicities were abbreviated as s (singlet), d (doublet), t (triplet) or m (multiplet), broad (br) or some combination of them. Coupling constants (J) are recorded in hertz and rounded to 0.5 Hz. High resolution mass spectra (HRMS) were recorded on a Water Q-Tof micro mass spectrometer. The purity (≥ 95%) of the compounds is verified by HPLC.

*4.1.2 N-(4-methoxyphenyl)acetamide (**2**)*

p-Methoxyaniline (12.3 g, 0.1 mol) was dissolved in 120 mL of CH_2Cl_2. Acetic anhydride (10.2 g, 9.44 mL, 0.1 mol) was added dropwise to this solution in an ice/water bath for 6 h at room temperature. The reaction mixture was quenched and filtered by petroleum ether. The

product is white power (14.4 g, 87.3% yield). m.p. 127-129 °C.

*4.1.3 N-(3-acetyl-4-hydroxyphenyl)acetamide (**3**)*

Intermediate **2** (4.2 g, 0.025 mol) was dissolved in 120 mL of CH_2Cl_2. $AlCl_3$ (13.3 g, 0.1 mol) and acetylchloride (7.9 g, 7.4 mL, 0.1 mol) were added slowly to this solution in an ice/water bath. The reaction mixture was then heated to reflux for 12 h. Then the reaction were quenched by ice water, and the supernatant was abandon. Then flake ice and 1 N HCl/ice water were added to the mixture for 30 mins with stir. The reaction mixture was concentrated under reduced pressure and the residue was purified via column chromatography to give the compound **3** as yellow-green solid (4.13 g, 84.1% yield). m.p. 162-164 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.55 (s, 1H), 9.93 (s, 1H), 8.06 (d, *J* = 2.58 Hz, 1H), 7.65 (dd, *J* = 2.62, 8.90 Hz, 1H), 6.91 (d, *J* = 8.90 Hz, 1H), 2.58 (s, 3H), 2.01 (s, 3H). HRMS(ESI): calcd for $C_{10}H_{12}NO_3$ $[M+H]^+$ 194.0812, found 194.0815.

*4.1.4 Ethyl 2-(4-acetamido-2-acetylphenoxy)acetate (**4**)*

Compound **3** (3.8 g, 19.7 mmol) was dissolved in 120 mL of acetone. Ethyl bromoacetate (4.35 mL，39.3 mmol), K_2CO_3 (5.44 g, 39.3 mmol) and KI (0.635 g, 3.93 mmol) were added to this solution. Heat the reaction mixture to reflux for 6 h. The reaction mixture was concentrated under reduced pressure and the residue was purified via column chromatography to give the compound **4** as white solid (5.0 g, 91.2% yield). m.p. 165-166 °C . ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.95 (s, 1H), 7.80 – 7.66 (m, 2H), 7.05 (d, *J* = 8.95 Hz, 1H), 4.89 (s, 2H), 4.17 (q, *J* = 7.10 Hz, 2H), 2.60 (s, 3H), 2.00 (s, 3H), 1.21 (t, *J* = 7.11 Hz, 4H). HRMS(ESI): calcd. for $C_{14}H_{18}NO_5$ $[M+H]^+$ 280.1179, found 280.1175.

*4.1.5 Ethyl 5-acetamido-3-methylbenzofuran-2-carboxylate (**5**)*

Add compound **4** (2.0 g, 7.16 mmol) and K_2CO_3 (1.4 g, 10.74 mmol) to microwave tube with 10 ml DMF. The reaction was carried out at 145 ^{o}C for 7 min under microwave irridiation. The reaction mixture was concentrated under reduced pressure and the residue was purified via column chromatography to give the compound **5** as white solid (1.1 g, 58.8% yield). m.p. 159-161 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.10 (s, 1H), 8.11 (d, *J* = 1.57 Hz, 1H), 7.65 – 7.46 (m, 2H), 4.34 (q, *J* = 7.09 Hz, 2H), 2.50 (s, 3H), 2.06 (s, 3H), 1.33 (t, *J* = 7.10 Hz, 3H).

HRMS(ESI): calcd. for $C_{14}H_{16}NO_4$ $[M+H]^+$ 262.1074; found 262.1072.

*4.1.6 Ethyl 5-amino-3-methylbenzofuran-2-carboxylate (**6**)*

Compound **5** (1.2 g, 4.59 mmol) was dissolved in the mixture solution of 3 M HCl and ethanol. Heat the reaction to reflux with the protection of N_2 for 6 h. The reaction mixture was concentrated under reduced pressure and the residue was purified via column chromatography to give the compound **6** as yellow solid (0.74 g, 63.0 % yield). m.p. 218-220 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.56 (brs, 2H), 7.84 – 7.71 (m, 2H), 7.56 – 7.44 (m, 1H), 4.35 (q, J = 7.09 Hz, 2H), 2.53 (s, 3H), 1.34 (t, J = 7.10 Hz, 3H). HRMS(ESI): calcd for $C_{12}H_{14}NO_3$ $[M+H]^+$ 220.0968, found 220.0964.

*4.1.7 Ethyl 5-(4-bromophenylsulfonamido)-3-methylbenzofuran-2-carboxylate (**7**)*

Compound **6** (1.1 g, 4.30 mmol), *p*-bromobenzenesulfonyl chloride (1.36 g, 6.45 mmol) pyridine (1.7 g, 1.74 ml, 21.5 mmol) were added to the anhydrous dichloromethane. The reaction mixture was stirred for 12 h at room temperature, and monitored by TLC, and washed by diluted hydrochloric acid and saturated brines. The reaction were dried over anhydrous Na_2SO_4, concentrated under reduced pressure and the residue was purified via column chromatography to give the compound **7** as yellow solid (1.54 g, 81.7% yield). m.p. 167-169 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.39 (s, 1H), 7.80 – 7.69 (m, 2H, Ar-H), 7.68 – 7.52 (m, 3H), 7.47 – 7.39 (m, 1H), 7.17 (dd, J = 2.23, 8.89 Hz, 1H), 4.33 (q, J = 7.10 Hz, 2H,), 2.46 (s, 3H), 1.32 (t, J = 7.10 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.30, 150.95, 141.21, 138.44, 133.03, 132.31, 128.87, 128.68, 126.75, 124.91, 122.74, 113.65, 112.70, 60.84, 14.08, 8.98; HRMS(ESI): calcd. for $C_{18}H_{17}BrNO_5S$ $[M+H]^+$ 438.0005; found 437.9998;. IR (KBr, cm-1):3415, 3237, 2923, 2847, 2310, 1714, 1581, 1467, 1456, 1401, 1337, 1308, 1159,1136,1087,1009,821,748,539; HPLC (75% methanol in water), t_R = 4.5 min, 96.8%.

*4.1.8 Ethyl-5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methylbenzofuran-2-carboxylate (**8**)*

To a stirring solution of compound **7** (282 mg, 0.64 mmol) in acetone (10 mL) was added bromobenzyl bromide (241 mg, 0.97 mmol), K_2CO_3 (266 mg, 1.9 mmol) and KI (100 mg, 0.06 mmol). The reaction mixture was heated to reflux for 2 h, and then filtered. The filtration was concentrated under reduced pressure. The residue was added to a mixture of NaOH/EtOH (v:v=1:1, 20 mL). Heat it to reflux and regulate pH to 4-5 with diluted HCl, the solid appeared

was filtered and purified via column chromatography to give compound **8** (1.5 g, 84.6% yield). m.p. 191-193 ^{o}C.^{1}H NMR (300 MHz, DMSO-d_6): δ 7.82 (d, J = 8.57 Hz, 2H), 7.60 – 7.38 (m, 6H), 7.22 (d, J = 8.37 Hz, 2H), 7.13 (dd, J = 2.09, 8.85 Hz, 1H), 4.81 (s, 2H), 2.40 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.72, 152.32, 142.39, 136.51, 135.44, 133.72, 132.43, 131.28, 130.40, 129.50, 128.98, 128.37, 127.34, 124.26, 121.77, 120.65, 112.25, 53.23, 8.98. HRMS (ESI): calcd for $C_{23}H_{18}Br_2NO_5S$ [M+H] $^{+}$ 577.9267; found 577.9268; IR (KBr, cm^{-1}):3376, 3111, 3095, 1718, 1604, 1573, 1469, 1336, 1163, 1148, 1016, 1007, 879, 818, 762, 654; HPLC (0.1% TFA in methanol) : t_R = 3.4 min, 94.9%.

*4.1.9 Tert-butyl (2-(5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-3-methylbenzofuran-2-carboxamido) ethyl)(methyl)carbamate (**8a**)*

To a stirring solution of compound **8** (140 mg, 0.24 mmol) in DCM (10 mL) was added EDCI (91 mg, 0.47 mmol), HOBt (64 mg, 0.47 mmol) and *N*,*N*-Dimethylethylenediamine (42 mg, 0.47 mmol) with a drop of DIPEA. The reaction mixture was stirred for 12 h at room temperature, then washed with diluted HCl and saturated brines. The organic layer were combined and dried over anhydrous Na_2SO_4, concentrated in vacuo, and then purified by flash chromatography to give compound **8a** as solid. (87 mg, 52.3% yield).

*4.1.10 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(2 (methylamino)ethyl)benzofuran-2-carboxamide (**9a**)*

To a stirring solution of compound **8a** (93 mg, 0.13 mmol) in DCM (10 mL) was added 1 mL of trifluoroacetic (1 mL). Then heated the reaction to reflux for 12 h. The reaction mixture was washed with saturated $NaHCO_3$ and extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, concentrated in vacuo, and then purified by flash chromatography to give 9a as yellow solid (58 mg, 72.2% yield). m.p. 80-83 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (s, 1H), 7.84 (d, J = 8.08 Hz, 2H), 7.55 (d, J = 8.22 Hz, 2H), 7.51 – 7.36 (m, 4H), 7.23 (d, J = 8.09 Hz, 2H), 7.10 (d, J = 8.77 Hz, 1H), 4.82 (s, 2H), 2.61 (t, J = 6.31 Hz, 2H), 2.38 (s, 3H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 158.97, 151.39, 135.48, 133.63, 132.40, 131.26, 130.40, 129.47, 127.33, 120.64, 120.34, 111.85, 53.33, 50.45, 38.05, 35.72, 8.48. HRMS (ESI): calcd for $C_{26}H_{26}Br_2N_3O_4S$ $[M + H]^+$ 634.0005, found 634.0011. IR (KBr, cm^{-1}): 3414, 2920, 2849, 1654, 1612, 1573, 1522, 1487, 1467, 1388, 1351, 1280, 1164, 1089, 1068, 1009, 857, 821, 747, 734, 649; HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.6 min, 95.3 %.

General procedure for preparation of **9b-9h**, **9k-9x**

To a stirring solution of compound **8** (200 mg, 0.35 mmol) in DCM (10 mL) was added EDCI

(91 mg, 0.47 mmol), HOBt (64 mg, 0.47 mmol) and the corresponding diamine derivatives (0.35 mmol) with a drop of DIPEA. The reaction mixture was stirred for 12 h at room temperature, then washed with diluted HCl and saturated brines. The organic layer were combined and dried over anhydrous Na_2SO_4, concentrated in vacuo, and then purified by flash chromatography to give product **9b-9h, 9k-9v**, respectively.

4.1.11

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(dimethylamino)ethyl)-3-methylbenzofuran-2-carboxamide (**9b**)*

(87 mg, 63.3% yield). m.p. 89-91 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.22 Hz, 2H), 7.49 (d, *J* = 8.31 Hz, 2H), 7.40 – 7.25 (m, 3H), 7.20 – 7.05 (m, 4H), 6.89 (d, *J* = 8.62 Hz, 1H), 4.71 (s, 2H), 3.52 (q, *J* = 5.37 Hz, 2H), 2.56 – 2.50 (m, 2H), 2.49 (s, 3H), 2.29 (s, 6H). ^{13}C NMR (75 MHz, DMSO) δ 159.35, 151.88, 144.65, 136.98, 135.97, 134.15, 132.90, 131.76, 130.89, 129.97, 128.26, 127.83, 121.72, 121.13, 120.94, 112.37, 58.39, 53.80, 45.60, 36.90, 8.98.HRMS (ESI): calcd for $C_{27}H_{28}Br_2N_3O_4S$ $[M + H]^+$ 648.0162, found 648.0157. IR (KBr, cm^{-1}): 3413, 2942, 2856, 2820, 2772, 2359, 2342, 1649, 1600, 1575, 1522, 1466, 1388, 1352, 1263, 1164, 1068, 1009, 855, 819, 649, 603, 550. HPLC:(0.1% Et_3N and 90% methanol in water), t_R = 6.0 min, 94.8 %

4.1.12

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(diethylamino)ethyl)-3-methylbenzofuran-2-carboxamide (**9c**)*

(108 mg, 57.7% yield). m.p. 105-107 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69- 7.48 (m, 4H), 7.41 -7.28 (m, 3H), 7.22 – 7.06 (m, 4H), 6.90 (d, *J* = 8.88 Hz, 1H), 4.72 (s, 2H), 3.56 – 3.43 (m, 2H), 2.70 – 2.54 (m, 6H), 2.51 (s, 3H), 1.06 (t, *J* = 7.07 Hz, 6H). HRMS (ESI): calcd for $C_{29}H_{32}Br_2N_3O_4S$ $[M + H]^+$ 676.0475, found 676.0462. IR (KBr, cm^{-1}): 3413, 2967, 2809, 2361, 1647, 1617, 1575, 1521, 1466, 1389, 1351, 1280, 1164, 1069, 1010, 819, 647, 604, 551. HPLC:(0.1% Et_3N and 90% methanol in water), t_R =7.8min, 96.0 %.

4.1.13

*5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-N-(2-(diisopropylamino)ethyl)-3-methylbenzofuran-2-carboxamide (**9d**)*

(94 mg, 61.9% yield). m.p.129-131 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 - 7.46 (m, 4H), 7.37 (d, *J* = 8.45 Hz, 3H), 7.21- 7.06 (m, 3H), 6.89 (dd, *J* = 8.79, 2.18 Hz, 1H), 4.72 (s, 2H),

3.50 – 3.34 (m, 2H), 3.13 – 3.02 (m, 2H), 2.69 (t, *J* = 5.89 Hz, 2H), 2.51 (s, 3H), 1.06 (d, *J* = 6.55 Hz, 12H). HRMS (ESI): calcd for $C_{31}H_{36}Br_2N_3O_4S$ $[M + H]^+$ 704.0788, found 704.0790. IR (KBr, cm^{-1}):75, 2963, 2922, 2849, 1661, 1608, 1573, 1505, 1486, 1467, 1386, 1352, 1284, 1171, 1122, 1087, 1068, 1054, 1008, 930, 871, 836, 750, 703, 651. HPLC :(0.1% Et_3N and 90% methanol in water), t_R =12.7 min, 97.3 %.

4.1.14

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(3-(dimethylamino)propyl)-3-methylbenzofuran-2-carboxamide (**9e**)*

(134 mg, 67.1% yield). m.p.125-127 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (t, *J* = 5.05 Hz, 1H), 7.69 -– 7.45 (m, 4H), 7.42-7.32 (m, 2H), 7.20-7.06 (m, 3H), 6.89 (dd, *J* = 8.74, 2.18 Hz, 1H), 4.72 (s, 2H), 3.54 (q, *J* = 5.86 Hz, 2H), 2.50 (s, 3H), 2.49-2.43 (m, 2H), 2.30 (s, 6H), 1.85-1.70 (m, 3H). HRMS (ESI): calcd for $C_{28}H_{30}Br_2N_3O_4S$ $[M + H]^+$ 662.0318, found 662.0327. IR (KBr, cm^{-1}): 3415, 2942, 2858, 2817, 2770, 1655, 1611, 1572, 1522, 1466, 1405, 1388, 1352, 1263, 1164, 1089, 1068, 1009, 856, 822, 749, 648, 603. HPLC:(0.1% Et_3N and 90% methanol in water), t_R =6.4 min, 95.4 %.

*4.1.15 3-(dimethylamino)propyl 5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-3-methylbenzofuran-2-carboxylate (**9f**)*

(112 mg, 62.4% yield). m.p.167-169 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 7.83 (d, *J* = 8.37 Hz, 2H), 7.62 – 7.47 (m, 4H), 7.43 (d, *J* = 8.08 Hz, 2H), 7.27 – 7.07 (m, 3H), 4.82 (s, 2H), 4.31 (t, *J* = 6.45 Hz, 2H), 2.42 (s, 3H), 2.31 (t, *J* = 7.02 Hz, 2H), 2.12 (s, 6H), 1.88 – 1.77 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.27, 152.46, 141.42,136.49, 135.40, 133.89, 132.43, 131.27, 130.40, 129.48, 128.77, 128.72, 127.36, 125.04, 121.90, 120.66, 112.35, 63.27, 55.43, 53.21, 45.07, 26.21, 8.98. HRMS (ESI): calcd for $C_{28}H_{29}Br_2N_2O_5S$ $[M + H]^+$ 663.0158, found 663.0167. IR(KBr, cm^{-1})： 3416, 3101, 2924, 2855, 2818, 2762, 2367, 2334, 1723, 1575, 1464, 1397, 1349, 1315, 1284, 1163, 1144, 1087, 1068, 1011, 861, 751, 649, 602. HPLC:(0.1% Et_3N and 90% methanol in water), t_R =7.9 min, 96.3 %.

4.1.16

*N-(2-acetamidoethyl)-5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methylbenzofuran-2-carboxamide (**9g**)*

(98 mg, 68.1% yield). m.p. 187-190 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 8.03 Hz, 2H), 7.49 (d, *J* = 8.14 Hz, 2H), 7.39 – 7.20 (m, 4H), 7.09 (d, *J* = 8.12 Hz, 2H), 6.88 (d, *J* = 8.04

Hz, 1H), 6.30 (s, 1H), 4.70 (s, 2H), 3.84 – 3.27 (m, 4H), 2.49 (s, 3H), 1.98 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.72, 160.37, 151.86, 143.19, 136.80, 134.00, 133.28, 131.80, 131.19, 130.41, 129.86, 129.78, 128.72, 127.57, 126.99, 122.04, 121.71, 121.47, 111.76, 54.39, 39.71, 38.98, 22.70, 8.37. HRMS (ESI): calcd for $C_{27}H_{26}Br_2N_3O_5S$ $[M + H]^+$ 661.9954, found 661.9946. IR (KBr, cm^{-1}):3413, 3318, 2365, 2342, 1646, 1617, 1535, 1487, 1466, 1389, 1346, 1284, 1157, 1089, 1069, 1010, 857, 600, 543. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =4.9min, 94.9 %.

4.1.17

*5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-3-methyl-N-(2-(methylsulfonyl)ethyl)benzofuran-2-carboxamide (**9h**)*

(116 mg, yield, 49.1%). mp:182-184 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.45 (m, 4H), 7.41 – 7.30 (m, 3H), 7.23 (d, *J* = 8.23 Hz, 1H), 7.10 (d, *J* = 8.50 Hz, 2H), 6.89 (dd, *J* = 8.83, 2.16 Hz, 1H), 4.72 (s, 2H), 4.00 (q, *J* = 6.14 Hz, 2H), 3.42 – 3.31 (m, 2H), 3.00 (s, 3H), 2.51 (s, 3H). HRMS (ESI): calcd for $C_{26}H_{24}Br_2N_2NaO_6S$ $[M + Na]^+$ 704.9335, found 704.9340. IR (KBr, cm^{-1}):3412, 2920, 2849, 2363, 2342, 1654, 1615, 1573, 1521, 1487, 1466, 1388, 1352, 1294, 1163, 1131, 1089, 1068, 1009, 858, 749, 735, 603. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =4.6 min, 95.9 %.

4.1.18

*5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-N-(2,3-dihydroxypropyl)-3-methylbenzofuran-2-carboxamide (**9i**)*

Compound **8** (300 mg, 0.52 mmol) was dissolved in 7 mL of $SOCl_2$ and refluxed for 1 h. Then add the anhydrous acetone (10 mL) to the mixture for cooling to -10 ^{o}C. The pre-cooling mixture of 3-amino-1,2-propanediol (188 mg, 1.55 mmol) and water (5 mL H_2O) was added to the reaction for 30 min at -10 ^{o}C. Acetone was removed by reduced pressure distillation. The reaction mixture was extracted with EA/water, and the organic extracts were combined, purified by flash chromatography to afford white solid (100 mg, 24.4% yield). mp:117-120 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.60 (m, 2H), 7.55 – 7.46 (m, 2H), 7.41 – 7.32 (m, 2H), 7.20 (d, *J* = 1.84 Hz, 1H), 7.10 (d, *J* = 8.48 Hz, 2H), 6.93 (dd, *J* = 8.79, 2.20 Hz, 1H), 4.72 (s, 2H), 3.69 – 3.53 (m, 4H), 2.51 (s, 3H). HRMS (ESI): calcd for $C_{26}H_{25}Br_2N_2O_6S$ $[M + H]^+$ 650.9795, found 650.979791. IR (KBr, cm^{-1}): 3414, 3088, 2921, 2360, 2342, 1653, 1609, 1573, 1527, 1487, 1466, 1388, 1351, 1279, 1195, 1163, 1110, 1089, 1069, 1009, 917, 857, 822, 748, 649, 602. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =4.6 min, 95.0 %.

*4.1.19 Tert-butyl 4-(5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-3-methylbenzofuran-2-carbonyl)piperazine-1-carboxylate (**8b**)*

The compound was prepared by a procedure identical to the preparation of **8a**.

*4.1.20 4-bromo-N-(4-bromobenzyl)-N-(3-methyl-2-(piperazine-1-carbonyl)benzofuran-5-yl)benzenesulfonamide (**9j**)*

The compound **9j** was prepared by a procedure identical to the preparation of **9a** (143 mg, 64.0 % yield over three steps). m.p. 95-97 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (d, *J* = 8.70 Hz, 2H), 7.61 – 7.43 (m, 4H), 7.43 – 7.35 (m, 2H), 7.23 (d, *J* = 8.48 Hz, 2H), 7.04 (dd, *J* = 8.84, 2.14 Hz, 1H), 4.81 (s, 2H), 3.48 – 3.40 (m, 4H), 2.77 – 2.61 (m, 4H), 2.19 (s, 3H).HRMS (ESI): calcd for $C_{27}H_{26}Br_2N_3O_4S$ $[M + H]^+$ 646.0005, found 646.0006. IR (KBr, cm^{-1})： 3415, 3086, 2917, 2850, 2359, 2338, 1629, 1573, 1458, 1388, 1350, 1267, 1164, 1109, 1089, 1068, 1009, 920, 856, 820, 797, 735, 703, 642, 600. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.0 min, 94.9 %.

*4.1.21 4-bromo-N-(4-bromobenzyl)-N-(3-methyl-2-(4-methylpiperazine-1-carbonyl)benzofuran-5-yl)benzenesulfonamide (**9k**)*

The compound **9k** was prepared by a procedure identical to the preparation of **9a** (97 mg, 42.5% yield). m.p.119-121 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.46 (m, 4H), 7.36 (d, *J* = 7.21 Hz, 2H), 7.29 – 7.23 (m, 1H), 7.20 – 7.05 (m, 3H), 6.86 (d, *J* = 8.57 Hz, 1H), 4.71 (s, 2H), 3.97 – 3.48 (m, 4H), 2.48 (s, 4H), 2.34 (s, 3H), 2.31 (s, 3H). HRMS (ESI): calcd for $C_{28}H_{28}Br_2N_3O_4S$ $[M + H]^+$ 660.0162, found 660.017. IR (KBr, cm-1): 3415, 2928, 2789, 2360, 2341, 1636, 1574, 1463, 1350, 1295, 1262, 1163, 1088, 1069, 1007, 924, 881, 860, 824, 757, 738, 639. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.7 min, 96.0 %.

*4.1.22 4-bromo-N-(4-bromobenzyl)-N-(3-methyl-2-(morpholine-4-carbonyl)benzofuran-5-yl)benzenesulfonamide (**9l**)*

The compound **9l** was prepared by a procedure identical to the preparation of **9a** (158 mg, 70.6%

yield). m.p. 92-94 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 – 7.46 (m, 4H), 7.41 – 7.28 (m, 3H), 7.20 – 7.03 (m, 3H), 6.87 (dd, *J* = 8.78, 2.17 Hz, 1H), 4.72 (s, 2H), 4.03 – 3.54 (m, 8H), 2.34 (s, 3H).HRMS (ESI): calcd for $C_{27}H_{25}Br_2N_2O_5S$ $[M + H]^+$ 646.9845, found 646.9836. IR (KBr, cm^{-1})： 3420, 3087, 2961, 2919, 2853, 2359, 2338, 1635, 1573, 1487, 1456, 1436, 1388, 1352, 1277, 1256, 1164, 1115, 1089, 1068, 1009, 855, 822, 752, 736, 643, 601. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.6 min, 95.1 %.

4.1.23

*4-bromo-N-(4-bromobenzyl)-N-(3-methyl-2-(3-oxopiperazine-1-carbonyl)benzofuran-5-yl)benzenesulfonamide (**9m**)*

(107 mg, 46.9% yield). m.p. 207-210 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 – 7.45 (m, 4H), 7.36 (d, *J* = 6.92 Hz, 2H), 7.20 (s, 1H), 7.10 (d, *J* = 7.12 Hz, 2H), 6.96 – 6.68 (m, 2H), 4.71 (s, 2H), 4.42 (s, 2H), 3.95 (brs, 2H), 3.52 (brs, 2H), 2.37 (s, 3H). HRMS (ESI): calcd for $C_{27}H_{24}Br_2N_3O_5S$ $[M + H]^+$ 659.9798, found 659.9786. IR (KBr, cm^{-1})：3414, 2922, 2363, 2342, 1679, 1662, 1628, 1573, 1487, 1458, 1388, 1352, 1261, 1226, 1193, 1163, 1110, 1089, 1068, 1009, 857, 822, 752, 737, 640, 551. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =4.6 min, 94.6 %.

4.1.24

*4-bromo-N-(4-bromobenzyl)-N-(3-methyl-2-(3-methylpiperidine-1-carbonyl)benzofuran-5-yl)benzenesulfonamide (**9n**)*

(151 mg, 66.2% yield). m.p. 160-161 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.46 (m, 4H), 7.42 – 7.28 (m, 3H), 7.19 – 7.06 (m, 3H), 6.84 (dd, *J* = 8.75, 2.17 Hz, 1H), 4.72 (s, 2H), 4.54 – 4.37 (m, 1H), 4.13 – 3.62 (m, 1H), 3.27 – 2.48 (m, 2H), 2.28 (s, 3H), 1.96 – 1.51 (m,5H), 1.38 – 1.28 (m, 2H). HRMS (ESI): calcd for $C_{29}H_{29}Br_2N_2O_4S$ $[M + H]^+$ 659.0209, found 659.0202. IR (KBr, cm^{-1}): 3415, 2955, 2930, 2851, 1625, 1589, 1488, 1469, 1440, 1388, 1352, 1277, 1165, 1121, 1087, 1069, 1015, 1007, 920, 877, 823, 754, 604, 551, 472. HPLC:(0.1% Et_3N and 90% methanol in water), t_R =8.7 min ,94.3 %.

4.1.25

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(1-methylpiperidin-4-yl)benzofuran-2-carboxamide (**9o**)*

(118 mg, 50.6% yield). m.p. 195-197 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 – 7.45 (m, 4H), 7.41 – 7.29 (m, 3H), 7.18 (d, *J* = 2.06 Hz, 1H), 7.10 (d, *J* = 8.36 Hz, 2H), 6.91 (dd, *J* = 8.77, 2.17 Hz, 1H), 6.43 (d, *J* = 8.09 Hz, 1H), 4.72 (s, 2H), 4.07 – 3.84 (m, 1H), 2.92 – 2.76 (m, 2H), 2.50 (s, 3H), 2.32 (s, 3H), 2.16 (t, *J* = 11.50 Hz, 2H), 2.08 – 1.95 (m, 2H), 1.70 – 1.51 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 159.23, 152.19, 143.63, 137.11, 134.49, 133.71, 132.28, 131.63, 130.35, 130.25, 129.19, 128.07, 127.46, 122.46, 122.11, 121.89, 112.21, 54.85, 54.17, 45.10, 44.85, 30.91, 8.86.HRMS (ESI): calcd for $C_{29}H_{30}Br_2N_3O_4S$ $[M + H]^+$ 674.0318, found 674.0329. IR (KBr, cm^{-1})： 3338, 2925, 2852, 2778, 1643, 1615, 1575, 1525, 1487, 1466, 1389, 1377, 1344, 1344, 1285, 1200, 1157, 1110, 1089, 1069, 1011, 857, 843, 735, 649, 605. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =6.6 min, 96.9 %.

4.1.26

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(5-methylpyridin-2-yl)benzofuran-2-carboxamide (**9p**)*

(157mg, 67.9% yield). m.p. 148-150 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.08 (s, 1H), 8.20 (s, 1H), 8.02 (d, *J* = 8.02 Hz, 1H), 7.84 (d, *J* = 6.66 Hz, 2H), 7.67 – 7.38 (m, 7H), 7.23 (d, *J* = 6.77 Hz, 2H), 7.14 (d, *J* = 8.86 Hz, 1H), 4.82 (s, 2H), 2.44 (s, 3H), 2.25 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.53, 151.64, 148.69, 147.92, 143.33, 138.61, 136.48, 135.40, 133.82, 132.44, 131.28, 130.42, 129.48, 129.34, 128.25, 127.37, 122.62, 121.50, 120.67, 113.87, 112.27, 53.29, 17.26, 8.67; HRMS (ESI): calcd for $C_{29}H_{24}Br_2N_3O_4S$ $[M + H]^+$ 667.9849, found 667.9839. IR (KBr, cm^{-1})： 3417, 2918, 2360, 2342, 1670, 1515, 1466, 1384, 1356, 1302, 1166, 1147, 1085, 1069, 1007, 846, 753, 736, 701, 653. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =9.7 min, 95.5 %.

4.1.27

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(2-(pyrrolidin-1-yl)ethyl)benzofuran-2-carboxamide (**9q**)*

(122 mg, 52.3% yield). m.p. 152-154 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 – 7.47 (m, 4H), 7.41 – 7.29 (m, 3H), 7.19 (d, *J* = 2.04 Hz, 1H), 7.14 – 7.04 (m, 2H), 6.89 (dd, *J* = 8.78, 2.20 Hz, 1H), 4.72 (s, 2H), 3.67 – 3.59 (m, 2H), 2.87 – 2.79 (m, 2H), 2.78 – 2.61 (m, 4H), 2.50 (s, 3H), 2.01 – 1.70 (m, 4H). HRMS (ESI): calcd for $C_{29}H_{30}Br_2N_3O_4S$ $[M + H]^+$ 674.0318, found 674.0311. IR (KBr, cm^{-1}): 3423, 2965, 2796, 2367, 2342, 1664, 1609, 1574, 1516, 1462, 1342, 1280, 1157, 1094, 1068, 858, 751, 652, 547 HPLC: (0.1% Et_3N and 90% methanol in water), t_R =7.1 min, 97.4 %.

4.1.28

*5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-3-methyl-N-(2-(piperidin-1-yl)ethyl)benzofuran-2-carboxamide (**9r**)*

(129 mg, 54.2% yield). m.p. 135-138 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 – 7.46 (m, 5H),

7.41 – 7.29 (m, 3H), 7.18 (d, *J* = 2.03 Hz, 1H), 7.11 (d, *J* = 8.46 Hz, 2H), 6.90 (dd, *J* = 8.77, 2.18 Hz, 1H), 4.72 (s, 2H), 3.59 – 3.50 (m, 2H), 2.64 – 2.53 (m, 2H), 2.51 (s, 3H), 2.49 – 2.45 (m, 2H), 1.66 – 1.56 (m, 6H), 1.53 – 1.44 (m, 2H). HRMS (ESI): calcd for $C_{30}H_{32}Br_2N_3O_4S$ $[M + H]^+$ 688.0475, found 688.0464. IR (KBr, cm^{-1}): 3422, 2922, 2851, 2360, 2341, 1664, 1608, 1575, 1533, 1488, 1464, 1388, 1342, 1263, 1163, 1121, 1090, 1070, 1009, 849, 744, 647, 602. HPLC:(0.1% Et_3N and 90% methanol in water), t_R =8.4 min ,95.2 %.

4.1.29

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(2-morpholinoethyl)benzofuran-2-carboxamide (**9s**)*

(144 mg, 60.3% yield). m.p. 163-165 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.70 – 7.46 (m, 4H), 7.41 – 7.29 (m, 3H), 7.19 (d, *J* = 1.90 Hz, 1H), 7.11 (d, *J* = 8.44 Hz, 3H), 6.91 (dd, *J* = 8.81, 2.14 Hz, 1H), 4.72 (s, 2H), 3.81 – 3.71 (m, 4H), 3.61 – 3.52 (m, 2H), 2.62 (t, *J* = 6.00 Hz, 2H), 2.57 – 2.44 (m, 7H).HRMS (ESI): calcd for $C_{29}H_{30}Br_2N_3O_5S$ $[M + H]^+$ 690.0267, found 690.0262. IR (KBr, cm^{-1})：3364, 2923, 2853, 2809, 2355, 1652, 1612, 1573, 1528, 1486, 1467, 1389, 1342, 1265, 1158, 1115, 1089, 1068, 1010, 861, 821, 651, 548. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.8 min, 96.7 %.

4.1.30

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(2-(4-methylpiperazin-1-yl)ethyl)benzofuran-2-carboxamide (**9t**)*

(99 mg, 40.7% yield). m.p.142-145 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.66 – 7.47 (m, 4H), 7.36 (d, *J* = 8.40 Hz, 2H), 7.22 – 7.03 (m, 4H), 6.91 (dd, *J* = 8.74, 2.14 Hz, 1H), 4.72 (s, 2H), 3.73 (q, *J* = 7.02 Hz, 2H), 3.60 – 3.49 (m, 2H), 2.68 – 2.55 (m, 8H), 2.50 (s, 3H), 2.37 (s, 3H), 1.28 – 1.20 (m, 4H). HRMS (ESI): calcd for $C_{30}H_{33}Br_2N_4O_4S$ $[M + H]^+$ 703.0548, found 703.0592. IR (KBr, cm^{-1})：3414, 2933, 2791, 2342, 1659, 1609, 1573, 1515, 1465, 1344, 1261, 1162, 1087, 1069, 1010, 918, 877, 820, 743, 703, 647. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.9 min, 95.7 %.

4.1.31

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(2-(phenylamino)ethyl)benzofuran-2-carboxamide (**9u**)*

(78 mg, 32.4% yield). m.p. 178-180 °C. ^1H NMR (300 MHz, DMSO-d_6) δ 8.63 (t, *J* = 5.90 Hz, 1H), 7.88 – 7.79 (m, 2H), 7.59 – 7.50 (m, 2H), 7.50 – 7.37 (m, 4H), 7.22 (d, *J* = 8.45 Hz, 2H),

7.15 – 7.00 (m, 3H), 6.59 (d, *J* = 7.64 Hz, 2H), 6.51 (t, *J* = 7.25 Hz, 1H), 5.71 (t, *J* = 5.73 Hz, 1H), 4.82 (s, 2H), 3.45 – 3.35 (m, 2H), 3.23 – 3.12 (m, 2H), 2.41 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.19, 151.42, 148.53, 144.15, 136.49, 135.49, 133.68, 132.42, 131.28, 130.41, 129.50, 128.90, 127.86,127.34, 121.26, 120.65, 120.62, 115.62, 111.82, 53.29, 42.21, 37.80, 8.53. HRMS (ESI): calcd for $C_{31}H_{28}Br_2N_3O_4S$ $[M + H]^+$ 696.0162, found 696.0157. IR (KBr, cm^{-1})：3380, 1647, 1603, 1574, 1515, 1486, 1466, 1430, 1350, 1271, 1159, 1089, 1068, 1010, 854, 748, 650, 602. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =7.0 min, 95.3 %.

4.1.32

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(2-(pyridin-2-yl)ethyl)benzofuran-2-carboxamide (**9v**)*

(113 mg, 47.9% yield). m.p. 172-174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59 (d, *J* = 4.37 Hz, 1H), 7.70 – 7.47 (m, 6H), 7.36 (d, *J* = 8.34 Hz, 2H), 7.25 – 7.04 (m, 5H), 6.89 (dd, *J* = 8.72, 2.06 Hz, 1H), 4.71 (s, 2H), 3.88 (q, *J* = 5.89 Hz, 2H), 3.12 (t, *J* = 6.29 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (75 MHz, DMSO) δ 159.18, 151.41, 148.52, 144.14, 136.48, 135.48, 133.67, 132.41, 131.27, 130.40, 129.49, 128.89, 127.86, 127.34, 121.26, 120.64, 120.62, 115.62, 111.82, 42.21, 37.79, 8.53. HRMS (ESI): calcd for $C_{30}H_{26}Br_2N_3O_4S$ $[M + H]^+$ 682.0005, found 682.002. IR (KBr, cm^{-1})：3412, 2920, 1662, 1609, 1588, 1575, 1533, 1486, 1465, 1389, 1339, 1279, 1263, 1159, 1120, 1089, 1071, 1010, 918, 883, 853, 763, 703, 648, 601, 545. HPLC:(0.1% Et_3N and 90% methanol in water), t_R =6.0 min, 94.5 %.

4.1.33

*N-(3-(1H-imidazol-1-yl)propyl)-5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methylbenzofuran-2-carboxamide (**9w**)*

(126 mg, 53.2% yield). m.p. 97-100 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.69 – 8.62 (m, 1H), 7.83 (d, *J* = 8.37 Hz, 2H), 7.56 – 7.39 (m, 6H), 7.22 (d, *J* = 8.29 Hz, 3H), 7.19 – 7.05 (m, 2H), 6.89 (s, 1H), 4.82 (s, 2H), 3.98 (t, *J* = 6.89 Hz, 2H), 3.26 – 3.17 (m, 2H), 2.40 (s, 3H), 1.97 – 1.90 (m, 2H). HRMS (ESI): calcd for $C_{29}H_{27}Br_2N_4O_4S$ $[M + H]^+$ 685.0014, found 685.012. IR (KBr, cm^{-1})：3388, 3088, 2925, 1655, 1608, 1573, 1510, 1487, 1467, 1388, 1351, 1280, 1163, 1109, 1088, 1068, 1009, 918, 857, 703, 649, 602. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =5.0 min, 95.8 %.

4.1.34

5-((4-bromo-N-(4-bromobenzyl)phenyl)sulfonamido)-3-methyl-N-(4-sulfamoylphenethyl)benzof

*uran-2-carboxamide(**9x**)*

The compound was prepared by a procedure identical to the preparation of **9i**. (181mg, 45.6% yield). m.p. 168-170 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.36 Hz, 2H), 7.70 – 7.60 (m, 2H), 7.55 – 7.46 (m, 2H), 7.45 – 7.32 (m, 4H), 7.24 – 7.16 (m, 1H), 7.10 (d, *J* = 8.37 Hz, 2H), 6.90 (dd, *J* = 8.77, 2.16 Hz, 1H), 6.65 (t, *J* = 6.06 Hz, 1H), 4.86 (s, 2H), 4.71 (s, 2H), 3.73 (q, *J* = 6.81 Hz, 2H), 3.03 (t, *J* = 7.02 Hz, 2H), 2.51 (s, 3H); HRMS (ESI): calcd for $C_{31}H_{28}Br_2N_3O_6S_2$ $[M + H]^+$ 759.9781, found 759.9777. IR (KBr, cm^{-1})**:** 3373, 3088, 2923, 1653, 1609, 1573, 1525, 1487, 1467, 1341, 1280, 1161, 1089, 1068, 1009, 858, 749, 649, 602, 549. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =4.5 min, 95.0 %.

*4.1.35 ethyl 3-methyl-5-nitrobenzo[b]thiophene-2-carboxylate (**10**)*

Ethyl 2-mercaptoacetate (8.21 mL, 7.52 mmol) was added into a solution of 1-(2-chloro-5-nitrophenyl)ethanone (1.0 g, 5.01 mmol) in dry ethanol (25 mL), followed by addition of K_2CO_3 (830.93 mg, 6.01 mmol) with stirring overnight at room temperature. The reaction was monitored by TLC (PE:EA=10:1).Then the mixture solution was poured into 100 mL water with stirring, and the precipitate was filtered and dried to afford a solid of green. (1.3 g, 99% yield). m.p. 164.0-166.1 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.76 (d, *J* = 1.95 Hz 1H), 8.32 (dd, *J* = 8.92, 2.16 Hz, 1H), 7.96 (d, *J* = 8.90, 1H), 4.44 (q, *J* = 7.15 Hz, 2H), 2.86 (s, 3H), 1.45 (t, *J* = 7.13 Hz, 3H). HRMS(ESI): calcd, for $C_{12}H_{12}NO_4S$ $[M+H]^+$ 266.0485, found 266.0482.

*4.1.36 ethyl 2-(2-(4-nitrophenyl)hydrazono)butanoate (**16**)*

A mixture of (4-nitrophenyl)hydrazine (1.0 g, 6.53 mmol) and ethyl 2-oxobutanoate (2.0 g, 13.06 mmol) in ethanol (50 mL) was refluxed 30 min with stirring. Then the solution was concentrated in vacuo and purified by silica gel column chromatography (PE:EA=10:1) to give solid. (346 mg, 20% yield). m.p. 212.5-215.6 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.36 (s, 1H), 8.19 (d, *J* = 9.12 Hz 2H), 7.21 (d, *J* = 9.90 Hz 2H), 4.32 (q, *J* = 7.11 Hz, 2H), 2.38 – 2.15 (m, 5H), 1.39 (t, *J* = 7.13 Hz, 3H). HRMS(ESI): calcd, for $C_{12}H_{12}NO_4S$ [M+H]+ 265.0409, found 266.0482.

*4.1.37 ethyl 3-methyl-5-nitro-1H-indole-2-carboxylate (**17**)*

A solution of **16** (300 mg, 1.13 mmol) in dioxane (20 mL) was added polyphosphoric acid (1 g) and refluxed 48 h with vigorously stirring. Then the solution was poured into water (100 mL) and the precipitate was filtered and dried to give solid (157 mg, 57% yield). m.p. 237.1-240.4 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.15 (s, 1H), 8.68 (d, *J* = 2.22 Hz, 1H), 8.47 (dd, *J* = 9.13, 2.67 Hz, 1H), 8.00 (d, *J* = 9.14 Hz, 1H), 4.55 (q, *J* = 7.05 Hz, 2H), 2.67 (s, 3H), 1.46 (t, *J* =

7.05 Hz 3H).

*4.1.38 ethyl 5-nitrobenzo[d]oxazole-2-carboxylate (**23**)*

To a solution of 2-amino-4-nitrophenol (2.0 g, 12.98 mmol) in dioxane (50 mL) was added ethyl 2-oxoacetate (50% in toluene, 5.30 g, 25.95 mmol) in two portion per hour, the mixture was heated at 50 °C with vigorously stirring for 2 h. CAN (8.54 g, 15.57 mmol) was then added into the resulting solution and the mixture was refluxed for 6 h. The reaction mixture was then filtered using Celite. After the filtrate was concentrated, the product was isolated by silica gel column chromatography (PE:EA=5:1) to give a pale yellow solid. (1.2 g, 40% yield). m.p. 174.2-176.6 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (d, *J* = 2.28 Hz, 1H), 8.55 (dd, *J* = 9.06, 2.27 Hz, 1H), 7.88 (d, *J* = 9.09 Hz, 1H), 4.66 (q, *J* = 7.16 Hz, 3H), 1.33 (d, *J* = 6.63 Hz, 2H). HRMS (ESI): calcd, for $C_{10}H_9N_2O_5$ $[M+H]^+$ 237.0506, found 237.0515.

*General procedure for preparation of **11, 18 and 24***

0.1g Pd/C (10%, wet) was add into a solution of **10, 17** or **23** in dry tetrahydrofuran (30 mL). The solution was stirred at room temperature under the protection of H_2 overnight and the reaction was monitored by TLC. The solution was filtered out Pd/C by diatomite and the solvent was evaporated under reduced pressure to give **10, 17** or **23** as yellow liquid (70%~90% yield). The crude residue could be used to next step without further purification.

*4.1.39 Ethyl 5-amino-3-methylbenzo[b]thiophene-2-carboxylate (**11**)*

(87 mg, 84.7% yield).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (d, *J* = 9.96 Hz, 1H), 7.06 (dd, *J* = 11.19, 1.95 Hz, 1H), 6.92 (d, *J* = 2.01 Hz, 1H), 5.17(s, 2H), 4.39 (q, *J* = 7.13, 2H), 2.73 (s, 3H), 1.42 (t, *J* = 7.08, 3H). HRMS(ESI): calcd for $C_{12}H_{14}NO_2S$ $[M+H]^+$ 236.0735, found 236.074.

*4.1.40 Ethyl 5-amino-3-methyl-1H-indole-2-carboxylate (**18**)*

(92 mg, 85.9% yield).^{1}H NMR (300 MHz, DMSO-d_6) δ 11.0(s, 1H), 7.11(d, *J* = 8,52 Hz, 1H), 6.70-6.66(m, 2H), 4.64(s, 2H), 4.29(t, *J* = 7.07 Hz, 2H), 2.43(s, 3H), 1.30(t, *J* = 6.18 Hz,3H). HRMS(ESI): calcd. for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ 219.1128; found 219.1131.

*4.1.41 Ethyl 5-(4-bromophenylsulfonamido)-3-methyl-1H-indole-2-carboxylate (**24**))*

(104 mg, 83.5% yield).^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (d, *J* = 8.61 Hz, 1H), 6.53 (d, J =

1.84 Hz, 1H), 6.00-5.98 (m, 1H), 4.64 (s, 2H), 4.14 (q, *J* = 7.20, 2H), 1.24 (t, *J* = 7.05, 3H). HRMS(ESI): calcd. for $C_{10}H_{10}N_2O_3$ $[M-H]^+$ 422.9656, found 422.9653.

General procedure for preparation of 12, 19 and 25

4-Bromobenzene-1-sulfonyl chloride (1.2 equiv) was added into a solution of **11, 18** or **24** in dry dichloromethane (25 mL) in portion. Then three drops pyridine and trimethylamine (1 equiv) were added into the resulting solution. The mixture was stirred overnight at room temperature, and was washed with dilute HCl and water. The organic phase was dried over Na_2SO_4, concentrated in vacuo and purified by silica gel column chromatography (PE:EA=4:1) to give light orange solid (70%~79% yield) .

*4.1.42 Ethyl 5-(4-bromophenylsulfonamido)-3-methylbenzo[b]thiophene-2-carboxylate (**12**)*

(92 mg, 72.5% yield). m.p. 130.4-133.7 ^oC.^1H NMR (300 MHz, DMSO-d_6) δ 10.56 (s, 1H), 7.89 (d, *J* = 8.73 Hz, 1H), 7.80 – 7.62 (m, 4H), 7.58 (d, *J* = 2.03 Hz, 1H), 7.25 (dd, *J* = 8.79, 2.03 Hz, 1H), 4.31 (q, *J* = 7.08 Hz, 2H), 2.61 (s, 3H), 2.53 (s, 1H), 1.30 (t, *J* = 7.08 Hz, 3H). HRMS(ESI): calcd. for $C_{18}H_{17}BrNO_4S_2$ $[M+H]^+$ 453.9787, found 453.9777.

*4.1.43 Ethyl 5-(4-bromophenylsulfonamido)-3-methyl-1H-indole-2-carboxylate (**19**)*

(63 mg, 76% yield). m.p. 147.9-149.3 ^oC.^1H NMR (300 MHz, DMSO-d_6) δ 11.53(s, 1H), 10.07 (s, 1H), 7.74(d, *J* = 8.37 Hz, 2H), 7.59(d, *J* = 8.28 Hz, 2H), 7.28 – 7.26 (m, 2H), 6.97 – 6.94(m, 1H), 4.32 (q, *J* =7.12, 2H), 2.43 (s, 3H), 1.34 (t, *J* =7.02, 3H). HRMS(ESI): calcd for $C_{18}H_{18}BrN_2O_4S$ $[M+H]^+$ 436.0092, found 436.9951.

4.1.44 Ethyl 5-(4-bromophenylsulfonamido)benzo[d]oxazole-2-carboxylate (**25**)

(87 mg, 78.1% yield). m.p. 158.5-160.2 ^oC.^1H NMR (300 MHz, $CDCl_3$) δ 7.67 – 7.52 (m, 5H), 7.32 (dd, *J* = 8.91, 2.07 Hz 1H), 7.12 (m, 1H), 4.55 (q, *J* = 7.18 Hz, 2H), 1.49 (t, *J* = 7.14 Hz, 3H). HRMS(ESI): calcd for $C_{16}H_{13}BrN_2O_5$ $[M-H]^+$ 422.9656, found 422.9653.

General procedure for preparation of 13, 20 and 26

To a solution of 1**2, 19** or **25** in acetone was added potassium carbonate (1.5 equiv), potassium

iodide (0.1 equiv) and 1-bromo-4-(bromomethyl)benzene (1.2 equiv). The mixture was refluxed for 4 h. The hot solution was filtered out to remove insoluble impurities. The filtrate was removed solvent under reduced pressure and recrystallization by PE and DCM to give 1**2, 19** or **25** as white solid (93%~99% yield).

*4.1.45 Ethyl 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methylbenzo[b]thiophene-2-carboxylate (**13**)*

(79 mg, 95.7% yield).^{1}H NMR (300 MHz, DMSO-d_6) δ 7.90 (d, *J* = 8.61 Hz, 1H), 7.85 (d, *J* = 8.58 Hz, 2H), 7.58 – 7.55 (m, 3H), 7.43 (d, *J* =8.34 Hz, 2H), 7.24 (d, *J* =8.40 Hz, 2H), 7.20 (d, *J* = 1.95 Hz, 1H), 4.85 (s, 2H), 4.32 (q, *J* = 7.07 Hz, 2H), 2.58 (s, 2H), 1.30 (t, *J* = 7.07 Hz, 3H). HRMS(ESI): calcd for $C_{25}H_{22}Br_2NO_4S_2$ $[M+H]^+$ 621.9352, found 621.9358.

*4.1.46 Ethyl 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-1H-indole-2-carboxylate (**20**)*

(155 mg, 97.8% yield). m.p. 189.9-191.3 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.60 (s, 1H), 7.84 (d, *J* = 8.49 Hz, 2H), 7.56 (d, *J* = 8.40 Hz, 2H), 7.45 (d, *J* =8.13 Hz, 2H), 7.27 – 7.23 (m, 4H), 6.87 (d, *J* = 8.19 Hz, 1H), 4.76 (s, 2H), 4.32 (q, *J* = 6.93 Hz, 2H), 2.40 (s, 2H), 1.34 (t, *J* = 7.02 Hz, 3H). HRMS(ESI): calcd for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ 219.1128, found 219.1131.

*4.1.47 Ethyl 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)benzo[d]oxazole-2-carboxylate (**26**)*

(91 mg, 98.1% yield). m.p. 153.5-156.2 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 – 7.60 (m, 2H), 7.60 – 7.44 (m, 3H), 7.39 – 7.29 (m, 3H), 7.31 – 7.16 (m, 1H), 7.14 – 7.04 (m, 2H), 4.71 (s, 2H), 4.55 (q, *J* = 7.14 Hz, 2H), 1.47 (d, *J* = 7.16 Hz, 2H). HRMS(ESI): calcd for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ 219.1128, found 219.1131.

General procedure for preparation of 14, 21 and 27

A mixture of **13, 20** or **26** and 10 % sodium hydroxide solution/ethanol solution (50 mL) was refluxed for 3 hour. The clear solution was acidified while at room temperature with

concentrated hydrochloric acid. The suspension was cooled to room temperature and filtered out to give the **13, 20** or **26** as white solid product (yield 80%~83%).

*4.1.48 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methylbenzo[b]thiophene-2-carboxylic acid (**14**)*

(98 mg, 81% yield). m.p. 172-175 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (d, *J* = 8.58 Hz 2H), 7.69 (d, *J* = 8.51 Hz, 1H), 7.56 (d, *J* = 8.61 Hz, 2H), 7.43 (d, *J* = 8.34 Hz, 2H), 7.24 – 7.21 (m, 3H), 6.97 (dd, *J* = 8.56, 2.02 Hz, 1H), 4.82 (s, 2H). HRMS(ESI): calcd for $C_{23}H_{16}Br_2NO_4S_2$ $[M-H]^+$ 591.8893, found 591.8901.

*4.1.49 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-1H-indole-2-carboxylic acid (**21**)*

(103 mg, 82.2% yield). m.p. 153.5-156.2 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$) δ 8.81 (s, 1H), 7.67 (d, *J* = 8.38 Hz, 2H), 7.56 (d, *J* = 8.41 Hz, 2H), 7.39 (d, *J* = 8.08 Hz, 2H), 7.28 (d, *J* = 7.83 Hz, 2H), 7.16 (d, *J* = 8.09 Hz, 2H), 6.87 – 6.82 (m, 1H), 4.75 (s, 2H), 2.55 (s, 3H). HRMS(ESI): calcd for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ 219.1128, found 219.1131.

*4.1.50 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)benzo[d]oxazole-2-carboxylic acid (**27**)*

(123 mg, 85.7% yield). m.p. 253.5-256.2 ^{o}C.^{1}H NMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H), 7.88 – 7.78 (m, 3H), 7.58 (d, *J* = 8.36 Hz, 2H), 7.51 – 7.41 (m, 2H), 7.19 (d, *J* = 8.29 Hz, 2H), 6.75 (d, *J* = 8.62 Hz, 1H), 6.61 (dd, *J* = 8.66, 2.52 Hz, 1H), 4.65 (s, 2H). HRMS(ESI): calcd for $C_{12}H_{15}N_2O_2$ $[M+H]^+$ 219.1128, found 219.1131.

General procedure for preparation of 15a-15c, 22a~22c and 28a~28c

To a solution of **14, 21** or **27** and amine (1.5 equiv) in dry dichloromethane (20 mL) were

added HoBt (1.5 equiv) and EDCI (1.5equiv). The reaction mixture was stirred at room temperature overnight, then washed with brine more than 5 times, and dried with anhydrous sodium thiosulfate. Then the solution was concentrated and recrystallized by PE and DCM to give **14, 21** or **27** as white solid (83%-90% yield).

4.1.51

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(dimethylamino)ethyl)-3-methylbenzo [b]thiophene-2-carboxamide (**15a**)*

(198 mg, 84.8 yield). m.p. 167.1-170.3 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (dd, *J* = 8.55, 1.76 Hz, 3H), 7.50 (d, *J* = 8.16Hz, 2H), 7.42 – 7.29 (m, 3H), 7.12 (d, *J* = 8.15 Hz, 2H), 6.93 (dd, *J* = 8.57, 2.04 Hz, 1H), 4.72 (s, *2*H), 3.51 (d, *J* = 6.37 Hz, 2H), 2.74 – 2.46 (m, 6H), 1.08 (t, *J* = 7.10 Hz, 6H). HRMS(ESI): calcd. for $C_{27}H_{28}Br_2N_3O_3S_2$ $[M+H]^+$ 663.9933, found 663.9933. HPLC: (0.1% Et_3N and 90% methanol in water), t_R = 5.9 min, 96.6 %.

4.1.52

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(diethylamino)ethyl)-3-methylbenzo[b]thiophene-2-carboxamide (**15b**)*

(103 mg, 92.7% yield). m.p. 96.6-99.7 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (dd, *J* = 8.58, 1.76 Hz, 3H), 7.60 – 7.45 (m, 2H), 7.42 – 7.29 (m, 3H), 7.12 (d, *J* = 8.15 Hz, 2H), 6.93 (dd, *J* = 8.57, 2.04 Hz, 1H), 4.72 (s, *2*H), 3.51 (d, *J* = 6.37 Hz, 2H), 2.74 – 2.46 (m, 6H), 2.57 (s, 3H), 1.08 (t, *J* = 7.10 Hz, 6H). IR (cm^{-1}, KBr film):3386, 2967, 2930, 2811, 1637, 1573, 1488, 1166, 1089, 802, 638. HRMS(ESI): calcd for $C_{29}H_{32}Br_2N_3O_3S_2$ $[M+H]^+$ 692.0246, found 692.0241. HPLC: (0.1% Et_3N and 90% methanol in water), t_R = 6.1 min, 95.2 %.

*4.1.53 5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(1-methylpiperidin-4-yl) benzo[b]thiophene-2-carboxamide (**15c**)*

(104 mg, 95.6% yield). m.p. 169.1-170.3 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.62 Hz, 3H), 7.49 (d, *J* = 8.64 Hz ,2H), 7.48 – 7.31 (m, 3H), 7.10 (d, *J* = 8.34 Hz, 2H), 6.95 (dd, *J*

= 8.61, 2.08 Hz, 1H), 4.73 (s, 2H), 3.99 (m, 1H), 2.85 (m, 2H), 2.52 (s, 3H), 2.33 (s, 3H), 2.28 – 2.12 (m, 2H), 2.09 – 2.04 (m, 2H), 1.61 (m, 2H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 161.90, 140.59, 137.63, 136.81, 134.93, 134.60,133.99, 132.06, 131.78, 131.18, 129.78, 128.71, 127.57, 126.05 , 123.46, 122.73, 121.48, 54.21, 53.82, 46.27, 45.58, 31.60, 12.47. IR (cm^{-1}, KBr film):3404, 1573, 1389, 1341, 1157, 1090, 642. HRMS(ESI): calcd for $C_{29}H_{30}Br_2N_3O_3S_2$ $[M+H]^+$ 690.009, found 690,0089. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =6.7 min, 99.1 %.

*4.1.545-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(dimethylamino)ethyl)-3-methy l-1H-indole-2-carboxamide (**22a**)*

(102 mg, 96.8% yield). m.p. 229.7-232.0 °C.^{1}H NMR (300 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.53 (d, *J* = 8.20 Hz, 2H), 7.42 (d, *J* = 8.24 Hz, 2H), 7.25 (d, *J* = 8.22 Hz, 1H), 7.17 – 6.98 (m, 5H), 6.60 (dd, *J* = 8.37 Hz, *J* =1.5 Hz, 1H), 4.63 (s, 2H), 3.50 (q, *J* = 5.32 Hz, 2H), 2.53 (t, *J* = 5.81 Hz, 2H), 2.30 (s, 3H), 2.28 (s, 6H). IR (cm^{-1}, KBr film):3414, 3256, 2948, 1632, 1574, 1407, 1347, 1162, 1089. HRMS(ESI): calcd for $C_{27}H_{29}Br_2N_4O_3S$ $[M+H]^+$ 647.0322; found 647.0325. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =14.8 min, 96.7 %.

4.1.55

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(diethylamino)ethyl)-3-methyl-1H-ind ole-2-carboxamide (**22b**)*

(100 mg, 74.5% yield). m.p. 169.4-170.1 °C.^{1}H NMR (300 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.53 (d, *J* = 8.49 Hz, 2H), 7.41 (d, *J* = 8.56 Hz, 2H), 7.26 (d, *J* = 8.40 Hz, 2H), 7.15 – 6.98 (m, 4H), 6.59 (dd, *J* = 8.75, 1.99 Hz, 1H), 4.62 (s, 2H), 3.63 – 3.54 (m, 2H), 2.84 – 2.81 (m, 2H), 2.73 (q, *J* = 7.65, 4H), 1.07 (t, *J* = 7.20, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 163.61, 137.10, 134.66, 134.61, 131.66, 130.54, 129.87, 128.79, 128.16, 127.26, 126.99, 124.71, 121.14, 118.30, 112.04, 110.32, 106.99, 47.25, 36.85, 36.31, 36.26, 9.35, 9.06. IR (cm^{-1}, KBr film):3126, 2967, 1653, 1613, 1480, 1400, 1330, 1229. HRMS(ESI): calcd for $C_{29}H_{33}Br_2N_4O_3S$ $[M+H]^+$ 675.0635; found 679.0645. HPLC: (0.1% Et_3N and 90% methanol in water), t_R = 13.6 min, 95.6 %.

4.1.56

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-3-methyl-N-(1-methylpiperidin-4-yl)-1H-indole-2-carboxamide (**22c**)*

(100 mg, 84.0 yield). m.p. 229.7-230.5 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$) δ 9.93 (s, 1H), 7.64 (d, *J* = 8.49 Hz, 2H), 7.52 (d, *J* = 8.63 Hz, 2H), 7.45 – 7.31 (m, 2H), 7.24 – 7.15 (m, 1H), 7.13 (d, *J* = 8.29 Hz, 2H), 6.89 (d, *J* = 7.24 Hz, 1H), 6.76 (dd, *J* = 8.64, 2.00 Hz, 1H), 4.73 (s, 2H), 4.17 – 4.15 (m, 1H), 3.15 (d, *J* = 11.69 Hz, 2H), 2.94 – 2.90 (m, 2H), 2.55 (s, 3H), 2.28 (s, 3H), 2.17 (d, *J* = 12.45 Hz, 2H), 2.08 – 1.93 (m, 2H). IR (cm^{-1}, KBr film):3449, 3524, 2938, 2731, 1637, 1486, 1164, 735. HRMS (ESI): calcd for $C_{29}H_{31}Br_2N_4O_3S$ $[M+H]^+$ 673.0478, found 673.0487. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =14.4 min, 96.1 %.

4.1.57

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(dimethylamino)ethyl)benzo[d]oxazole-2-carboxamide (**28a**)*

(93 mg, 82.7% yield). m.p. 146.8-148.6 ^{o}C.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.66 (d, *J* = 8.70 Hz, 2H), 7.58 – 7.46 (m, 3H), 7.38 – 7.34 (m, 3H), 7.12 – 7.07 (m, 3H), 4.72 (s, 2H), 3.57 (q, *J* = 5.54 Hz, 2H), 2.54 (t, *J* = 5.91 Hz, 2H), 2.30 (s, 6H). ^{13}C NMR (300 MHz, $CDCl_3$) δ 156.19, 154.67, 149.78, 140.17, 136.53, 135.35, 133.62, 131.98, 131.27, 129.77, 128.62, 128.07, 127.77, 121.59, 121.34, 111.70, 56.85, 54.32, 44.66, 36.65. IR (cm^{-1}, KBr film):3389, 2973, 2819, 1574, 1390, 1166, 1011, 636. HRMS(ESI): calcd for $C_{25}H_{25}Br_2N_4O_4S$ $[M+H]^+$ 634.9958, found 634.9976. HPLC: (0.1% Et_3N and 90% methanol in water), t_R = 3.3 min, 95.2 %.

4.1.58

*5-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(2-(diethylamino)ethyl)benzo[d]oxazole-2-carboxamide (**28b**)*

(100 mg, 75.0 % yield). m.p. 149.6-150.7 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.66

(d, *J* = 8.34 Hz, 2H), 7.56 – 7.46 (m, 3H), 7.45 – 7.31 (m, 3H), 7.15 – 7.04 (m, 3H), 4.73 (s, 2H), 3.58 – 3.50 (m, 2H), 2.74 – 2.65 (m, 2H), 2.61 (q, *J* = 7.33 Hz, 4H), 1.08 (t, *J* = 8.64 Hz, 6H). IR (cm^{-1}, KBr film):3405, 2967, 1692, 1552, 1355, 1166, 747. HRMS(ESI): calcd for $C_{27}H_{29}Br_2N_4O_4S$ $[M+H]^+$ 663.0271, found 663.0275. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =3.6 min, 96.4 %.

*4.1.595-(4-bromo-N-(4-bromobenzyl)phenylsulfonamido)-N-(1-methylpiperidin-4-yl)benzo[d]oxazole-2-carboxamide (**28c**)*

(85 mg, 76.3 % yield). m.p. 161.8-163.4 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.10 Hz, 2H), 7.54 – 7.49 (m, 3H), 7.37 – 7.34 (m, 3H), 7.15 – 7.06 (m, 3H), 4.72 (s, 2H), 4.05 – 3.96 (m, 1H), 2.86 (d, *J* = 11.54 Hz, 2H), 2.33 (s, 3H), 2.18 (t, *J* = 11.71 Hz, 2H), 2.05 (d, *J* = 12.72 Hz, 2H), 1.67 (t, *J* = 12.15 Hz, 2H). IR (cm^{-1}, KBr film):3126, 2967, 1653, 1613, 1480, 1400, 1330, 1229. HRMS(ESI): calcd for $C_{27}H_{27}Br_2N_4O_4S$ $[M+H]^+$ 661.0114, found 661.0136. HPLC: (0.1% Et_3N and 90% methanol in water), t_R =3.4 min, 99.1 %.

4.2 Biological evaluation

4.2.1 Cell culture

The colon (HTC116, p53 -/-), breast (MCF-7), lung (A549), pancreatic (PANC-1, BxPC-3), and triple negative breast cancer cells MDA-MB-231, human umbilical vein endothelial cell (HUVEC) were obtained from Cell Culture Center at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. L-15, RPMI 1640 and DMEM mediums, FBS were obtained from life technologies (Carlsbad, CA). The cells were maintained in a humidified atmosphere with 5% CO_2 at 37°C. The hypoxia environment is formed by 1% O_2, 94% N_2 and 5% CO_2 at 37°C.

4.2.2 Anti-proliferation assay

MTT method was used to evaluate the anti-proliferative activity as previous reported [33]. Tumor cells are cultured in 96-plate at 5×10^5 cell/mL overnight. Compounds with different concentrations were added to the cells for 48 h. Then MTT (20 μL, 5 mg/mL, Sigma) were added to the cells for 4 h at 37 °C. The supernatant in the well was removed and formazon crystals were dissolved in 150 μL of DMSO. The absorbance was measured at 570 nm

(Thermo Scientific Varioskan Flash, USA). The IC_{50} was calculated by Graphpad Prism 6.0.

4.2.3 Colony formation assay

100 cells/well was cultured in the 12-well plate for overnight. Compounds at different concentrations are added to the well for two weeks. The medium was changed every three days. After 14 days the colony in the well were washed with PBS buffer and fixed with 10% CH_3CH_2OH for 10 min. Then the colonies were stained by 0.5% crystal violet (C0775, Sigma) in solution of 25% CH_3CH_2OH for another 10 min. After many washes with PBS, the colonies in plates were dried and images were scanned for studies.

4.2.4 Flow-cytometry analysis

The apoptotic cells induced by compounds were determined by Fow-cytometry (Becton Dichinso, USA). PANC-1 cells were treated with compounds and collected by trypsinized. The cells at 1×10^6 cells/mL were incubated with Annexin V and PI staining (556547, BD) for 10 min in the dark at 37°C. The cells in binding buffer were regulated to 500 μL and calculated using a FacScan analyzer (Becton Dickinson, USA). Then the data were analyzed by using Flow software.

4.2.5 Western blotting

The expression of apoptotic proteins were evaluated by Western blotting. PANC-1 cells with treatment of compound in different concentration were collected and lysed. The supernatants containing protein in SDS-PAGE was transferred onto PVDF menbranes. Block the membrane and incubate it with primary antibody followed the secondary antibody in the dark for 1 h. The results were detected by Odyssey infrared imaging system (LI-COR, Lincoln, Nebraska, USA). The antibodies used from Cell Signaling Technology (Beverly, MA, USA) were PARP (#9542), cleaved PARP (#5625), Caspase-3(#9665), cleaved caspase-3 (#9664) Caspase-9 (#9508), cleaved caspase-9 (#7237) antibodies.

4.2.6 Pharmacokinetics study

The pharmacokinetics properties of compounds were evaluated in rats (n = 6 per group).The rats were administered with compounds at 10 mg/kg, intravenously, orally and intraperitoneally. At predetermined time points (0, 0.083, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 h) the blood samples were collected into heparinized Eppendorf tubes and centrifuged at 4 °C and 8000 rpm for 5 min. The plasma samples were analyzed by LC−MS/MS.

4.2.7 Physicochemical Properties

The procedures are conducted as the previous reported [34]. The partition coefficient (CLogP) values were obtained with the soft of ChemBioDraw. Intrinsic aqueous solubility and the distribution coefficients (Log D7.4) were measured with the help of Gemini Profiler instrument (pION) as the previous reported method. Permeability coefficients were determined thought double-sink PAMPA on a PAMPA Explorer instrument (pION).

4.2.8 In vivo xenograft study and Immunohistochemical Experiment

The animal procedure was according to the Institutional Animal Care and use Committee of China Pharmaceutical University. Female nude mice are used to evaluate the in vivo antitumor activity of compound **9o**. PANC-1 (1×10^7 cells) were injected subcutaneously in the right flank. After 4 weeks, the mice with tumor of average 100 mm^3 were randomly divided into five groups. Mice were injected intraperitoneally with 15, 30, 60 mg/kg of compound **9o** every two days for 21days. Mice were monitored for the toxicity, the tumor growth and body weight. When the mice were sacrificed, the volume and weight of tumor were record. And the formula is volume = $(\text{width})^2 \times \text{length}/2$. Immunohistochemical staining assay were carried out with the CD31 antibody (#28364, Abcam), HIF-1a antibody (#ab51608, Abcam) and VEGF antibody (sc-53462, santa cruz).

4.2.9 Would healing and tube formation assay of HUVECs

1×10^5 cells of HUVECs were seeded in 6-well plate overnight. When the cells were converged to 90%, the medium were changed without FBS. Then monolayers of cells were scratched with sterile pipette tip. And compound **9o** were added to the medium under hypoxia condition. The results were obtained at 12 h and 24 h with an inverted-phase microscope. The tube formation assay were did according the previously reported. Compound **9o** at 0.625, 0.25, 1.25 μM were added into the 6-well with HUVECs for 4 h. Then the HUVECs were seeded into 48-well plate with matrigel (200 μL/well, #356234, Becton Dickinson) under hypoxia conditions. The results were obtained by microscope after 6 h.

4.2.10 Luciferase reporter assay

The detailed procedures were according to the previous reported. HEK293 cells were cultured in 24 well plates. The plasmid HRE-luciferase (#26731, addgene) and pRL-SV40 (E2231, Promega) were co-transfected into the cells by Lipofectamine™ 2000 (Invitrogen) for

6 h. Then the cells are treated wtih compound 9o under hypoxia conditions for another 24 h. Luciferase activity was assessed by dual-luciferase reporter assay system (E1910, Promega) by using a luminometer (Thermo Scientific LuminoskanAscent).

4.2.11 Real-time qRT-PCR

Real-time qRT-PCR was used to determine the effect of **9o** on VEGF in the PANC-1 cell line under hypoxia. The detailed procedures were conducted as the previous reported [31]. GAPDH expression was used as control. VEGF: forward primer 5'-TCTGCAGCTCTGTGTGAAGG-3', reverse primer 5'-TGAATTCTCAGCCCTCTTCAA-3') and GAPDH (forward primer 5'-TTCCTCTTGTGCTCTTGCTGG-3', reverse primer 5'-CCCTCAACGACCACTTTGTCA-3'). The real-time were performed with Step one system Fast real time PRC system (ThermoFisher).

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Appendix A. Supplementary data

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Highlights

- A series of benzofuran derivatives were designed, synthesized and evaluated as novel pancreatic carcinoma inhibitors.
- Physicochemical properties and pharmacokinetic property were studied.
- **9o** exerted significant *in vivo* activity and anti-angiogenesis activity.
- **9o** targeted the hypoxia environment of tumor cells through Hif-1α-mediated VEGF production.