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Synthesis, discovery and preliminary SAR study of benzofuran derivatives as angiogenesis inhibitors

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ABSTRACT

A series of benzofuran derivatives were synthesized and evaluated against HUVEC proliferation. Among these compounds, compound **32** exhibited good inhibitory activity and remarkable selectivity to HUVEC. Our current data suggested that array order of methyl, acrylate and carboxylate groups in benzofuran scaffold is the basic requirement for inhibitory activity against HUVEC proliferation. These results demonstrated that benzofuran scaffold represents a promising structural core to discover a new class of active and selective angiogenesis inhibitors.

Cancer is one of the major mortal reasons all over the world. Reports from WHO shows that more than 7 millions of cancer patients died in 2007. Over last decades, many anticancer drugs were discovered and developed and being used in clinical treatment, but they are not met the needs for eradicating so incorrigible and crafty cancer cells. Discovery and development of new effective anticancer agents are still urgent for human's battling against cancer.

Many studies suggested that once a tumor grows beyond a critical size, which is of approximately 1 mm^3 and with about 10^6 cells, it has an ability to develop its own blood supply system for the gain of sufficient nutrients and oxygen and the removal of toxic wastes by angiogenesis-the process of new blood vessel formation.[1] On the situation, tumor cells can stimulate the transcription of vascular endothelial cell growth factor (VEGF) or/and basic fibroblast growth factor (bFGF), and these angiogenic factors, in turn, promote the development of new blood vessels from the preexisting vasculature.[2] Angiogenesis is not only responsible for the critical growth of a tumor but also for the recurrence and metastasis of a tumor.[3] Because of these reasons, the effective inhibition of tumor angiogenesis can block tumor progression and growth beyond a critical size or metastasis to other organs.[4]

It is known that dihydrobenzofuran scaffold actually exists in some anti-angiogenesis agents like cryptotanshinone[5], lignans[6] and A-3922.[7] Our current efforts to discover angiogenesis inhibitors from the Traditional Chinese Medicine (TCM) led to the identification of some benzofuran compounds with potential activity against HUVEC (Human umbilical vein endothelial cell) proliferation (data not shown here). Based on these facts, we designed and synthesized a series of benzofuran derivatives characterized as bi-substituted α,β-unsaturated carboxylic esters at different benzofuran positions. Among them, compound **32** showed good inhibitory activity meanwhile compounds **14**, **29** and **37** exhibited moderate inhibitory activity toward HUVEC proliferation. All of these active compounds have remarkable selectivity to HUVEC. Here, we report the chemical synthesis of these benzofuran compounds and their biological evaluation with the intention to reveal the structural elements that contribute to their inhibitory potency and selectivity against HUVEC proliferation. According to our current data, benzofuran compounds can be as lead compounds to discover more potent angiogenesis inhibitors.

Firstly, the benzofuran scaffold was constructed by Michael addition of quinone with activated propanal[8] or ethyl acetyl acetate and then cyclization (Scheme 1). It seemed that piperidine is the best amine to the preparation of **1** in our tries. Compound **2** was prepared by the catalysis of anhydrous zinc chloride.[9] Even different proportions of quinine and ethyl acetyl acetate were used for the reaction, **2** was always accompanied by 3-ring compound **3**[10] with almost same ratio. With the starting materials **1** and **2** in hand, we explored the introduction of bi-substituted α,β-unsaturated carboxylic ester and different substitutions at positions 1 and 2 of benzofuran core.

After phenol group of **1** was acetylated into **4**, **4** was bromated into **5** by NBS and AIBN. Treated **5** with KI in acetone and then by saturated $NaHCO_3$, **6** with hydroxyl group was got. Compound **6** was oxidized into an aldehyde **7** and then **7** was reacted with

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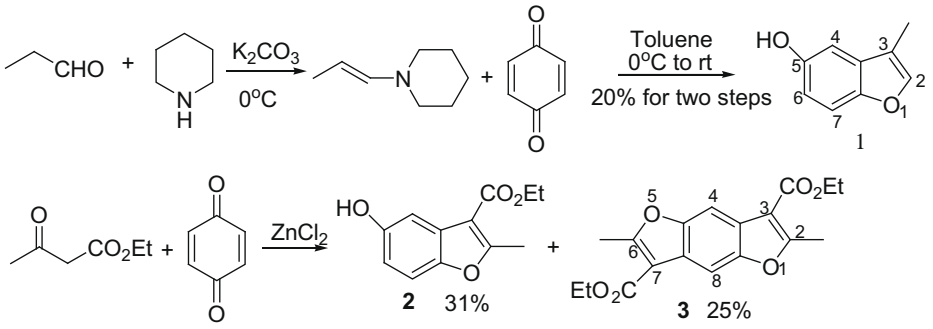


Scheme 1.

Wittig–Horner reagent to give **8**, which was easily de-acetylated by aq. Na_2CO_3 at room temperature to give compound **9** (Scheme 2).

Compound **14**[11] with 2-methyl substitution was designed to understand if methyl substitution at position 2 is important for anti-angiogenesis activity and selectivity as compared with **9**. Synthesis of **14** was achieved by general methods and using compound **2** as starting material. By substitution exchange of methyl group and acrylate group of compound **14**, target product **18** was expected. Phenol group in **1** was protected by benzyl group and then **15** was formylated into **16** with DMF and $POCl_3$. Wittig–Horner reaction of aldehyde **16** and deprotection of **17** by BBr_3 gave target compound **18** (Scheme 3).

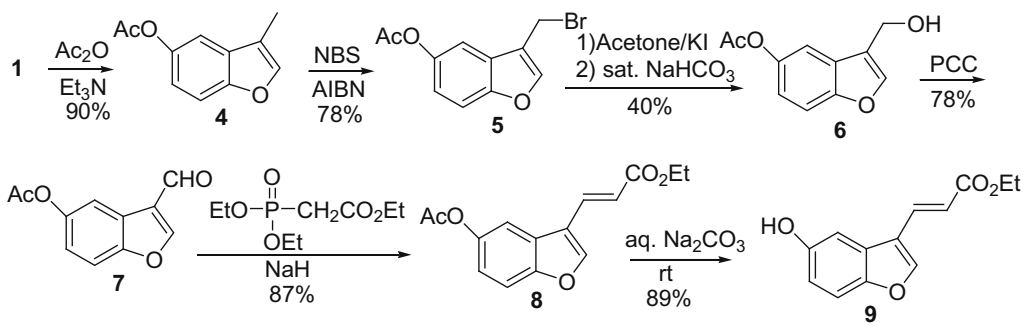
We also prepared compounds **29** and **32** (Scheme 4), which are corresponding to **18** and **14** by replacement of methyl with carboxylate, respectively. Starting with **10**, compound **20** was prepared after bromation of **10** and hydrolysis of **19** to give **20**. After protection of **20**, **21** was reduced into alcohol **22** by DIBAL-H and **22** was oxidized with PCC to produce aldehyde **23**. Wittig–Horner reaction of **23** and deprotection of **24** gave intermediate **25**. Compound **25** was selectively protected to yield **26** by acetylation at 0 °C. Later, free hydroxyl group of **26** was oxidized into carboxylic acid **28** through aldehyde **27**. Compound **29**[12] was at last prepared by esterification. Synthesis of **32** started with **20**, PCC oxidation of **20**, Wittig–Horner reaction of aldehyde **30**. After deprotection of **31**, compound **32**,[13] which is the counterpart of **29**, was prepared successfully.

After the exploration of incorporating one acrylate group into benzofuran core at position 1 or position 2, it was urged to introduce two acrylate groups into positions 1 and 2 simultaneously. The target compound **37**[14] was obtained by usual methods as shown in Scheme 5.

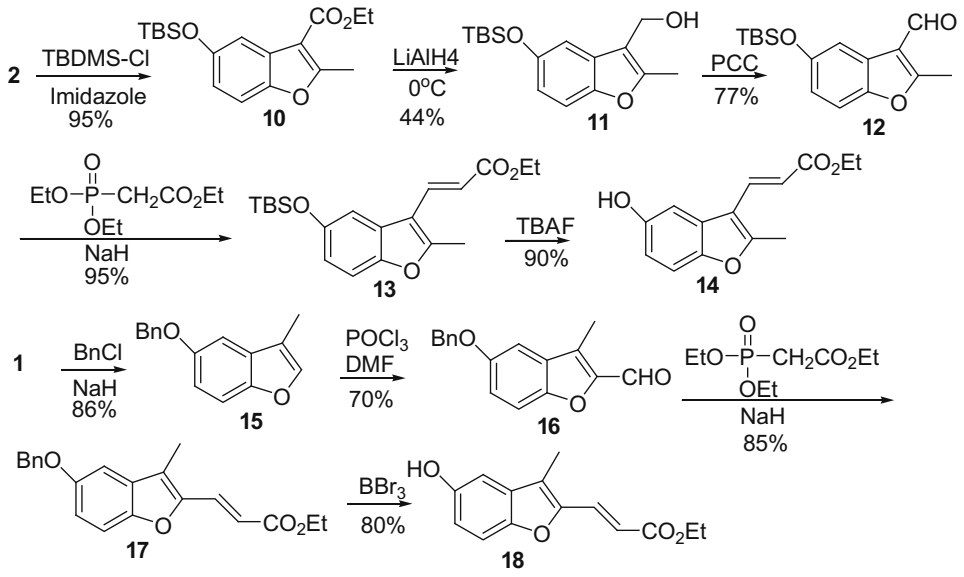
The inhibitory activity of these compounds against cell proliferation of HUVEC (human umbilical vein endothelial cell) and three cancer cell lines of A549 (non-small cell lung cancer cell line), Bel-7402 (hepatocarcinoma cell line) and MCF-7 (breast cancer cell line) was assayed by MTT method. Briefly, 1000 HUVECs/well or 1500 cancer cells/well were seeded in 96-well plate and allowed to attach for 20 h before treated with a compound in 0.1% DMSO at different concentrations or same volume of carrier. Every concentration of each compound or carrier is in triplicate. After incubated for 96 h, cells were treated with MTT reagents and relative cell viability of each well was calculated. IC_{50} value of the compound was calculated based on the relationship of cell viability versus the corresponding concentration. The assay data are summarized in Table 1.

In our study streamline, compound **9** from **1** was the first compound to be tested for inhibitory activity against HUVEC proliferation but **9** did not show the activity of anti-HUVEC proliferation. This fact made us shift to functionate position 2 of benzofuran core and compounds **2** and **14** were prepared for the evaluation against HUVEC proliferation. It was fortune that compound **2** showed potential activity and compound **14** has moderate activity for anti-HUVEC proliferation. These initial testing data provided us a clue that methyl, acrylate and carboxylate can be introduced into benzofuran core at different position to tune benzofuran's activity against HUVEC proliferation. Based on this initiation, compounds **18**, **25**, **29** and **32** were synthesized and evaluated. After exchange of methyl and acrylate in **14**, **18** lost anti-HUVEC proliferation activity which exhibited in original compound **14**; conversion of methyl into hydroxymethyl made **25** become an inactive compound to HUVEC proliferation. With different substitution at position 2 from compounds **9**, **14** and **25**, compound **29** displayed moderate inhibitory activity like compound **14**. Reciprocation of carboxylate and acrylate in **29**, compound **32** showed surprisingly good inhibitory potency (4.3 μM) against HUVEC proliferation. Comparison of **32** with **2** implied that acrylate group in **32** enhanced anti-HUVEC activity.

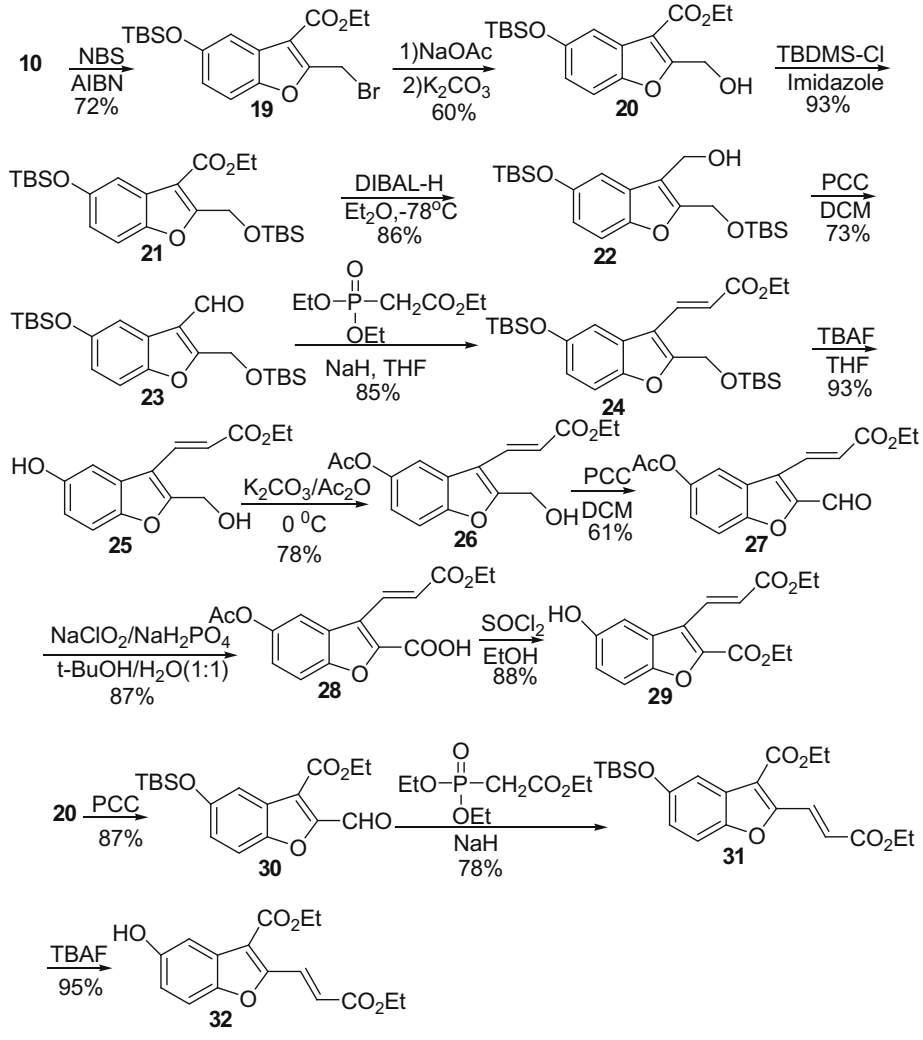
Because acrylate group is important for the activity of **32**, **37** with two acrylate groups was synthesized and used to explore whether two acrylate groups may make interaction be stronger



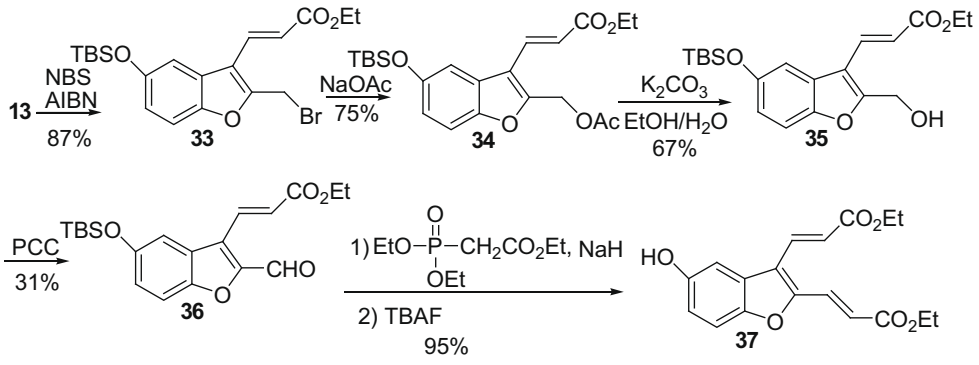
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

Table 1
Inhibitory activity of compounds

Compd	IC_{50}, μM			
	HUVEC	A549	Bel-7402	MCF-7
2	>50	NA	NA	NA
9	NA	NA	NA	NA
14	27.6	NA	NA	NA
18	>50	NA	NA	NA
25	NA	NA	NA	NA
29	32.6	NA	NA	NA
32	4.3	NA	NA	NA
37	32.9	NA	NA	NA

Notes: 'NA' means 'not active'; '>50' represents 'approaching 50% inhibition at 50 μM'.

than **32**. Due to the bulkiness of **37** or/and the possibility that two acrylate groups make the interaction become complicated, **37** expressed much weaker inhibitory activity than **32** to HUVEC proliferation.

It is important to note that all of these compounds showed remarkable preference for their inhibitory activity against HUVEC proliferation and absence of the inhibitory activity to cancer cells of A549, Bel-7402 and MCF-7.

In summary, a series of benzofuran derivatives were synthesized and tested against the proliferation of HUVEC, A549, Bel-7402 and MCF-7. Compound **32** exhibited remarkable selectivity against HUVEC proliferation with IC_{50} in low micromolar range. These data suggested that array order of methyl, acrylate and carboxylate groups in benzofuran scaffold is the basic requirement for inhibitory activity against HUVEC proliferation. The results demonstrated that benzofuran scaffold represents a promising structural core to discover a new class of active and selective angiogenesis inhibitors.

Acknowledgements

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11. *Spectral data for compound* **14**: ESI-MS: *m/z* 247 $[M+H]^+$, 245 $[M-H]^+$. ^1H NMR ($CDCl_3$): δ 7.75 (dd, J = 1.6, 16 Hz, 1H), 7.28 (dd, J = 1.6, 6.8 Hz, 1H), 7.22 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 2.8, 6.0 Hz, 1H), 6.42 (d, J = 16 Hz, 1H), 4.28 (q, J = 7.2 Hz, 2H), 2.54 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 166.8, 160.5, 154.6, 148.1, 135.5, 126.6, 116.1, 113.2, 112.2, 111.9, 105.6, 60.4, 14.7, 12.9.
12. Spectral data for compound **29**: ESI-MS: *m/z* 303 $[M-H]^+$, 305 $[M+H]^+$, 327 $[M+Na]^+$, 607 $[2M-H]^+$. ^1H NMR ($CDCl_3$): δ 8.46 (d, J = 16.4 Hz, 1H), 7.48 (d, J = 8.8 Hz, 1H), 7.35 (d, J = 2.4 Hz, 1H), 7.05 (dd, J = 2.4, 6.4 Hz, 1H), 6.67 (d, J = 16.4 Hz, 1H), 4.49 (q, J = 7.2 Hz, 2H), 4.31 (q, J = 7.2 Hz, 2H), 1.47 (t, J = 7.2 Hz, 3H), 1.37 (t, J = 7.2 Hz, 3H).
13. Spectral data for compound **32**: ESI-MS: *m/z* 303 $[M-H]^+$, 607 $[2M-H]^+$, 631 $[2M+Na]^+$, 911 $[3M-H]^+$. ^1H NMR (CD_3OD): δ 8.25 (d, J = 16 Hz, 1H), 7.38 (d, J = 6.0 Hz, 1H), 6.83 (dd, J = 2.4, 6.0 Hz, 1H), 6.68 (d, J = 16 Hz, 1H), 4.43 (q, J = 7.2 Hz, 2H), 4.27 (q, J = 7.2 Hz, 2H), 1.46 (t, J = 7.2 Hz, 3H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 165.6, 163.0, 155.9, 155.2, 148.7, 129.9, 126.7, 123.0, 117.2, 113.7, 112.5, 106.9, 61.3, 61.1, 14.5, 14.4.
14. Spectral data for compound **37**: ESI-MS: *m/z* 329 $[M-H]^+$, 331 $[M+H]^+$, 353 $[M+Na]^+$, 659 $[M-H]^+$, 683 $[2M+Na]^+$. ^1H NMR (DMSO-d_6): δ 9.46 (s, 1H), 7.84 (d, J = 16.0 Hz, 1H), 7.73 (d, J = 16.0 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.20 (d, J = 2.4 Hz, 1H), 6.89 (dd, J = 2.4, 6.4 Hz, 1H), 6.48 (dd, J = 6.0, 10.0 Hz, 1H), 4.17 (m, 4H), 1.21 (m, 6H). ^{13}C NMR (DMSO-d_6): δ 166.3, 165.8, 155.2, 153.2, 149.2, 133.4, 128.3, 126.2, 120.7, 120.6, 119.0, 117.2, 112.8, 106.3, 61.0, 60.8, 14.7, 14.6.