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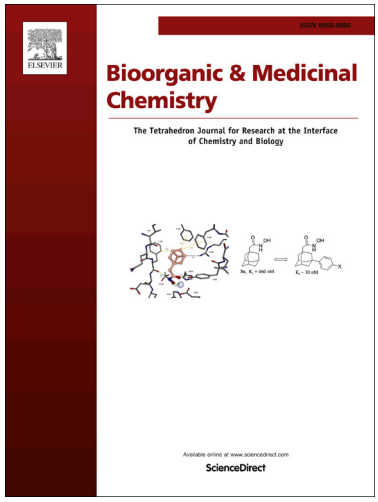

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Graphical Abstract

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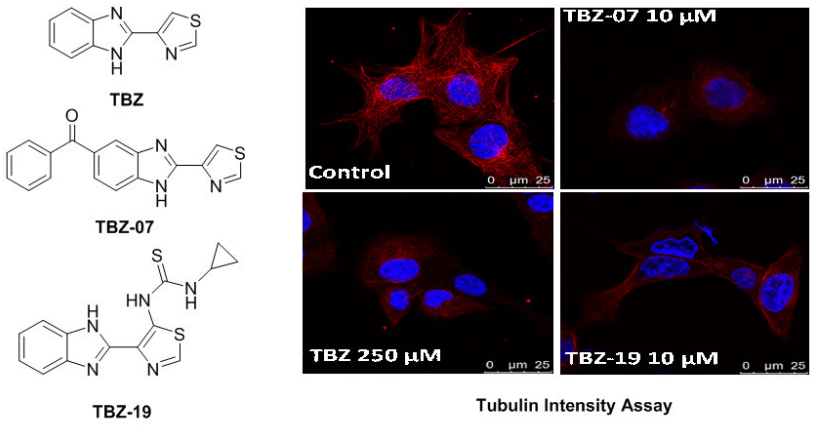

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Synthesis and Biological Evaluation of Thiabendazole Derivatives as Anti-angiogenesis and Vascular Disrupting Agents

Chao Zhang[a], Bo Zhong[b], Simin Yang [a], Liangkun Pan[a], Siwang Yu[a], Zhongjun Li[a], Shuchun Li[a], Bin Su[b], and Xiangbao Meng[a]*

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ABSTRACT

Thiabendazole, already approved by FDA for oral use as an anti-fungal and anti-helminthic drug since 1967, has recently been repurposed as a vascular disrupting agent. By optimization of the structure of the lead compound, we successfully identified compound TBZ-19 and the new derivative is over 100-fold more potent than the lead compound against the growth of four different cell lines (A549, HCT-116, HepG2 and HUVECs). The most potent two candidates **TBZ-07** and **TBZ-19**, exhibiting moderate inhibitory cell proliferation activity, were also verified as anti-angiogenesis and vascular disrupting agents. Therefore, TBZ-07 and TBZ-19 would be promising candidates with vasculature targeting activity and merit further development.

1. Introduction

As early as 1980s, Denekamp first proposed the concept that vascular targeting agents (VTAs) would be promising for the treatment of cancer.[1] Researchers observed that solid tumors degenerate as tumor blood vessels were naturally blocked and hypothesized that physically occlusion of blood vessels in tumor issues by means of VTAs to result in tumor regression. This hypothesis was later proved to be effective. VTAs are usually divided into two separate categories, [2-5] anti-angiogenic agents (AIAs), which halt the formation of new blood vessels, and vascular disrupting agents (VDAs) which mainly target endothelial cells and pericytes of the already established tumor vasculature, resulting tumor ischemia and necrosis. In addition, VDAs fall into two general classes referred to as ligand-directed VDAs and small molecule VDAs. [2] The former type takes advantage of antibodies, polypeptides or growth factors which selectively bind with endothelial cells of tumor issues and contribute to the necrosis of tumor. Rather than specifically localizing to the tumor endothelium, these agents take advantage of the pathophysiological differences between normal and tumor endothelium to induce selective occlusion of tumor vessels. While the latter, mainly consists of tubulin targeting agents, could either inhibit the polymerization of tubulin or stabilized the formation of microtubule. It is important to emphasize that there is a very clear distinction between these two approaches based on key three aspects: the physiologic target, the extension of disease that is likely to be susceptible to their activity, and the schedule of treatment. [2] Both approaches are proved to be successful in defense of cancer as there are several molecules which were validated to be effective both in preclinical and clinical trials. Among them, bevazizumab (AvastinTM) has been proved as an antiangiogenic VTA, which is a recombinant humanized monoclonal antibody that binds to vascular endothelial cell growth factor (VEGF) and blocks VEGF interaction with its corresponding receptors.[6,7] While biologics as VDAs is costly and proved to be unpractical, the small molecule approach has increased significantly over the past decade and today includes approximately a dozen compounds that are in human clinical trials. Many well-described antimitotic agents are derived from natural sources. Colchicine and Vinca alkaloids, the first tubulin binding agents discovered, cause destabilization of microtubules and, as a consequence, induce the cell to undergo apoptosis.[8] On the other hand paclitaxel, a standard antitumor agent originally extracted from *Taxus brevifolia*, binds to a different site within tubulin leading to the stabilization of microtubules.[9] Also, a large number of synthetic or semi-synthetic small molecules has been proved to be powerful microtubule targeting agents, including CA-4P[10], BNC 105[11], *iso*CA-4[12], Isoerianin[13], azaisoerianin[14], combreatabenzodiazepines[15], etc. Therefore, inhibition of microtubule function using tubulin targeting agents is a validated approach to anticancer therapy and merits further development to improve bioavailability, solubility and to overcome emerging drug resistance in clinic .[10,11, 16,17,18]

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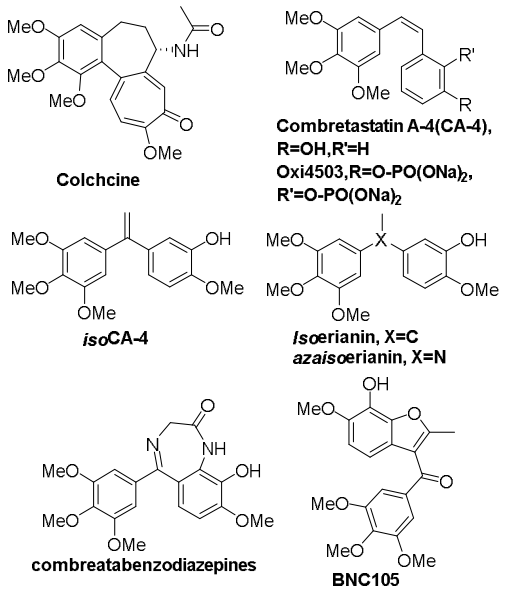


Figure 1. Structure of antimitotic agents with vascular-disrupting activities.

Review of recent published literature reveals that many anti-helmintic drug on market have been verified to be anti-mitotic agents. Among them, albendazole and mebendazole are currently under development for the treatment of cancer. Albendazole is well tolerated, relatively safe and has evidence of anti-tumor effects in patients with advanced cancer.[19] While mebendazole is a potent antitumor agents both in vitro and in vivo due to its property of inducing mitotic arrest and apoptosis by depolymerizing tubulin.[20] Thiabendazole (TBZ;4-(1H-1,3-benzodiazol-2-yl)-1,3-thiazole), already approved by the U.S. Food and Drug Administration (FDA) for systemic oral use in humans (as an anti-fungal and anti-helminthic treatment), has recently been verified to be vascular disrupting agent and thus as a potential complementary therapeutic for use in combination with current anti-angiogenic therapies.[21] However, the IC_{50} value of TBZ was as high as over 250 μM to disrupt vascular, which seems limit its future application as an anti-cancer agent.

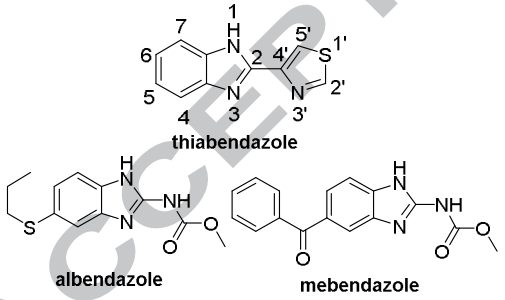


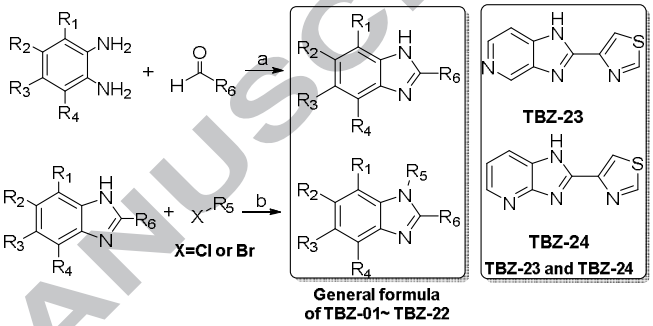
Figure 2. Structures of representative benzimidazoles with anti-helmintic activity.

Considering the unique selectivity and effectiveness of VTAs, we aimed to develop a new class of anticancer drugs bearing dual biological activity- anti-angiogenesis and vascular disruption, which is hypothesized to be synergistic for the treatment of cancer both in period of initiation and progression. Herein, we report the design, synthesis, and biological evaluation of thiabendazole derivatives as anticancer agents with vascular disrupting and anti-angiogenesis activity.

2. Results and discussion

2.1 Synthesis

The synthesis of twenty-four compounds (see **Table 1**) based on thiabenzole core was accomplished by condensations of o-phenyldiamine derivatives with thiazole-4-aldehyde, pydrine-2-aldehyde or tert-butyloxycarbonyl (4-formylthiazol-5-yl) carbamate catalysed by sodium pyrosulfite in DMF at 120ºC, respectively, which is described in **Scheme 1**. Among these aldehydes, the synthesis of tert-butyloxycarbonyl (4-formylthiazol-5-yl) carbamate would be relative complicated, the synthetic route[18] is described in **Scheme 2**. Synthesis of TBZ-19 was based on the former building block, tert-butyloxycarbonyl (4-formylthiazol-5-yl) carbamate. It was further condensed with o-phenyldiamine, deprotected, reacted with cyclopropylisothiocyanate to afford TBZ-19and the synthetic route was also described in **Scheme 2**.



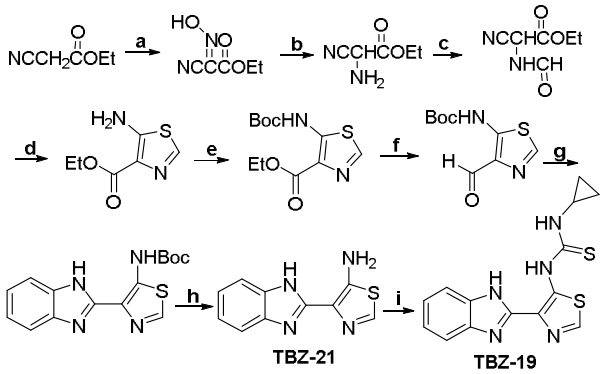
Scheme 1. Reagents and conditions: (a) $Na_2S_2O_5$, DMF, 120°C, 51-85%; (b) NaH, DMF, r.t. 68-92%.

Table 1. Structures of TBZ derivatives

No.	R_1	R_2	R_3	R_4	R_5	R_6
TBZ	H	H	H	H	H	4-thiazole
TBZ-01	H	Me	H	H	H	4-thiazole
TBZ-02	Me	H	H	H	H	4-thiazole
TBZ-03	H	Me	Me	H	H	4-thiazole
TBZ-04	H	H	Cl	H	H	4-thiazole
TBZ-05	H	H	F	H	H	4-thiazole
TBZ-06[a]	H	H	OMe	H	H	4-thiazole
TBZ-07	H	Bz	H	H	H	4-thiazole
TBZ-08	H			H	H	4-thiazole
TBZ-09	H	H	NO_2	H	H	4-thiazole
TBZ-10	H	H	H	H	Me	4-thiazole
TBZ-11	H	H	H	H	Bn	4-thiazole
TBZ-12	H	H	H	H	pMB	4-thiazole
TBZ-13	H	H	H	H	mNB	4-thiazole
TBZ-14	Me	H	H	H	H	2-Py
TBZ-15	H		H	H	H	4-thiazole
TBZ-16	H	H	H		H	4-thiazole
TBZ-17	H	H	CF_3	H	H	4-thiazole
TBZ-18	H	H	H	H		4-thiazole
TBZ-19	H	H	H	H	H	
TBZ-20	H	H	H	H	Bn	benzene

TBZ-21	H	H	H	H	H	
TBZ-22	H	H	H	H	2-Py	2-Py

a. tautomer was determined by NMR spectroscopy analysis
b. Bn= benzyl; pMB= *p*-methoxyl benzyl; m-NB= *m*-nitro benzyl; 2-py= 2-pyridyl; Bz= benzoyl.



Scheme 2. Reagents and conditions: (a) $NaNO_2$, H_3PO_4, H_2O, 51%; (b) $Na_2S_2O_4$, aqueous $NaHCO_3$, CH_2Cl_2, 80%; (c) HCOOH, $(CH_3CO)_2O$, N_2, 0 ºC, 93%; (d) Lawesson's regent, toluene, reflux, 41%; (e) $(Boc)_2O$, DMAP, 0 ºC, anhydrous CH_2Cl_2, 24%; (f) DIBAL-H, -78ºC, Ar, anhydrous CH_2Cl_2, 62%; (g) *o*-phenylenediamine, $Na_2S_2O_5$, DMF, 120ºC, 94%; (h) 10% TFA, DCM, 99%; (i) cyclopropylisothiocyanate, pyrdine, reflux, 99%.

2.2 Biology

2.2.1 Cell viability assays

We firstly evaluated anti-proliferative activity of the newly synthesized compounds *in vitro*. These compounds were tested with human umbilical vein endothelial cells (HUVECs). Inhibitory data of compounds TBZ-01 to TBZ-24 at different concentrations (10 μM and 100 μM) to HUVECs are listed in **Table 2**.

Table 2. Inhibition rate(%) of TBZ derivatives against HUVECs[a]

Compd.	10μM	100μM
TBZ	0	11
TBZ-01	3	**56**
TBZ-02	8	**59**
TBZ-03	0	0
TBZ-04	7	**53**
TBZ-05	2	28
TBZ-06	3	19
TBZ-07	**50**	**58**
TBZ-08	2	**59**
TBZ-09	7	30
TBZ-10	0	3
TBZ-11	4	46
TBZ-12	0	41
TBZ-13	2	21
TBZ-14	7	10
TBZ-15	9	45
TBZ-16	10	25
TBZ-17	5	**57**
TBZ-18	2	**51**
TBZ-19	**63**	**59**
TBZ-20	7	38
TBZ-21	9	8
TBZ-22	8	0
TBZ-23	8	0
TBZ-24	7	0

[a]all assays were run in triplicate

Based on the above anti-proliferative data, a preliminary SAR was summarized. Compounds with methyl group at 6 or 7 position showed better potency at 100 μM compared with 10 μM. In addition, electron withdrawing group such as Cl or CF_3 at 6 position showed moderate inhibitory activity, whereas with benzoyl substitution the compound exhibited the best activity. 5, 6 fused benzene group derivative TBZ-08 also showed moderate inhibitory activity against HUVECs at 100 μM. 3, 4, 5-trimethoxy benzyl substitution at 1 position showed better activity compared with parent compound TBZ. Among all compounds, TBZ-19 bearing cyclopropanylthiourea substitution at 5 position of thiazole showed the best inhibitory activity in these assays.

It was encouraging that compound TBZ-07 and TBZ-19 displayed significant improvement in potency both at low and high concentration when compared with the parental compound TBZ. Furthermore, we determined the IC_{50} values of these two compounds against HUVECs, HCT116, HepG2, and A549 cell lines and the data was shown in **Table 3**.

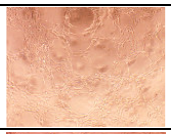
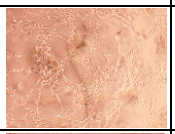
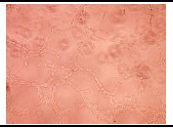
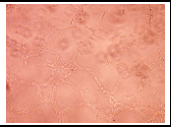
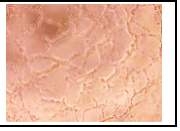
Table 3. IC_{50} (**μM**) values of selected compounds toward four cancer cell lines[a]

Compd.	HUVEC	HCT116	HepG2	A549
TBZ	>100	>100	>100	>100
TBZ-07	9.22±5.42	2.46±1.15	7.58±0.43	7.70±1.53
TBZ-19	1.23±0.61	0.43±0.08	0.79±0.12	1.02±0.17

[a] MTT assay was used for evaluation, and values were expressed as mean IC_{50} of the triplicate experiments.

2.2.2 Anti-angiogenesis Activity Assays

To further investigate the anti-cancer activity of TBZ-07 and TBZ-19, both compounds were determined to interfere with the angiogenesis process. One of the most widely used *in vitro* assays to model the reorganization stage of angiogenesis is the tube formation assay. This assay measures the ability of endothelial cells, plated at subconfluent densities with the appropriate extracellular matrix support, to form capillary-like structures.[19,20] Therefore, HUVEC tube formation had been used first as a model to test in vitro angiogenesis, as vascular endothelial tube network formed *in vitro* has many similarities with capillary vessels formed *in vivo*. Molecules significantly disturb the tube formation process are promising to be anti-angiogenic agents. If the process being disturbed, partially dissected tube remains or even totally blocked tubes could be observed. While the HUVECs were plated, elongated and robust tube-like structures were well established after incubation for 1 h in the negative control group; but treatment of HUVECs with compounds TBZ-07 and TBZ-19 at the concentration of 0.03, 0.1 0.33, 1.00, 3.00, 10.0 μM inhibited the formation of tubular structures in different ranks. Encouragingly, TBZ-07 and TBZ-19 could significantly inhibit the tube formation process of HUVECs at 0.33 μM, while positive control sunitinib partially inhibited its formation at 0.033 μM and completely blocked it at 0.33 μM (**Fig. 3**).

Con. μM	compounds		
	TBZ-07	**TBZ-19**	**sunitinib**
0			
0.03			

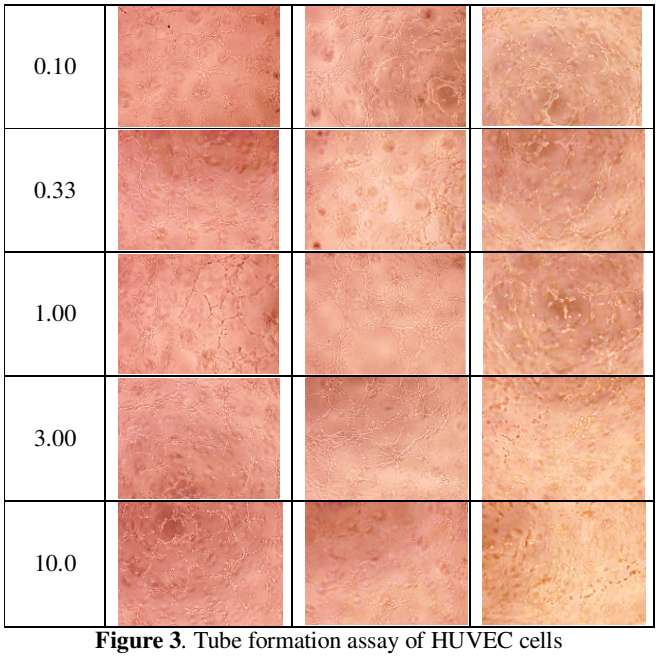
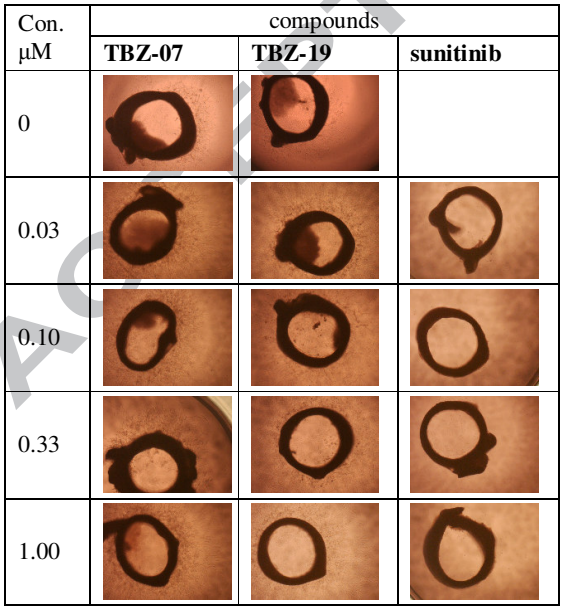


Figure 3. Tube formation assay of HUVEC cells

Considering that rat thoracic aorta rings (TARs) model is more close to in vivo condition compared with HUVEC tube formation model in view of multi-steps involved in angiogenesis: sprouting, proliferation, migration and differentiation, TARs assay was then employed to evaluate the antiangiogenic activity of compounds.[21,22] In this assay, aortic rings cultured in collagen gel give rise to microvascular networks composed of branching endothelial channels. Compounds with anti-angiogenic activity could significantly inhibit the formation and number of branches in the aortic rings. According to the experiment, TBZ-07 and TBZ-19 significantly inhibited the outgrowth of microvessels at 3 to 10 μM in a concentration-dependent manner, while positive control sunitinib demonstrated almost complete inhibition of angiogenesis at 0.33 μM (**Fig.4**).



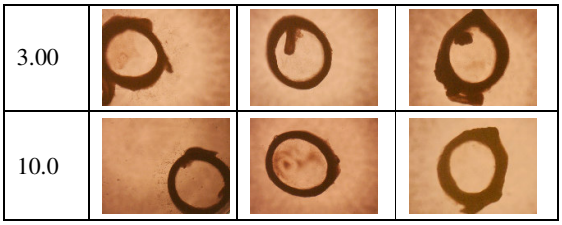


Figure 4. Rat aortic ring assay

2.2.3 **Tubulin Intensity Assays**

We then move on to examine the effects of these two compounds on microtubule polymerization. We began by assessing the integrity of the microtubule network by immunofluorescence microscopy, using an antibody against α-tubulin. Tubulin intensity assays were carried on to examine the ability of TBZ-07 and TBZ-19 to interfere with microtuble assembly. As shown in **Fig. 5**, the DMSO-treated control cells displayed a well – developed array of microtubules radiating from the juxtanuclear microtubule organizing center. In contrast, both TBZ-07 can TBZ-19 can inhibit the formation of tubulin at 10 μM as they both exhibited a tubulin staining pattern that was diffuse and disorganized. In addition, TBZ at 250μM and vinblastine at 50 nM showed similar effect on microtubule organization of A549 cell line.

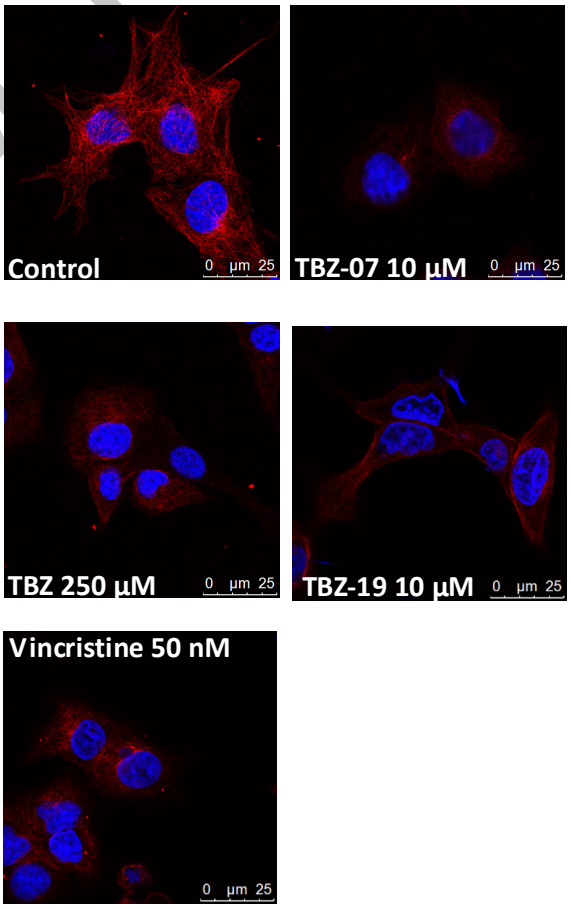


Figure 5. Effect of selective compounds on tubulin intensity in A549 cells.

2.2.4 **Tubulin polymerization assays**

Tubulin can be affected in two different manners. Taxanes and epothilones stabilize tubulin polymerization, whereas vinca alkaloids, halichondrins, and colchicine inhibit tubulin polymerization.[23-26] In order to have a better understanding of the binding manner, tubulin inhibitory activity of TBZ-07 and TBZ-19 was determined with tubulin polymerization assay by monitoring the absorptions at 340 nm. In this assay, we observed here that TBZ-07 and TBZ-19 can act as microtubule depolymerizing agents time-dependently at 5 μM concentration. As illustrated in **Fig.6**, the curve of TBZ-07 and TBZ-19 under the curve of DMSO demonstrated that these two compounds acted in a similar mechanism with Nacodazole, a well-known tubulin inhibitor used as a positive control for the assay, to inhibit tubulin polymerization.[27] While Taxol at 10 μM which appears above the control line means its microtubule stabilization effect. Therefore, this study indicates that TBZ-07 and TBZ-19 bind at the colchicine site on tubulin. To sum up, the above results indicate that these compounds play anti-angiogenic and vascular-disrupting role through inhibition of the proliferative and tube formation of HUVECs, and microtubule depolymerization during mitosis, respectively.

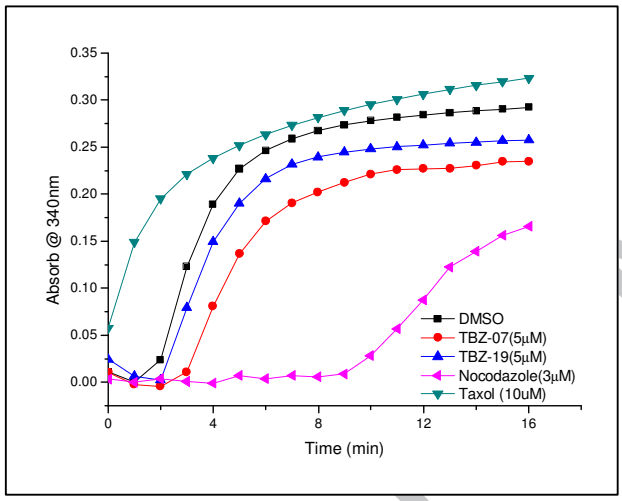


Figure 6. Tubulin polymerization in the presence of **TBZ-07**, **TBZ-19**, **Nocodazole**, and **Taoxl**

3. Conclusion

In conclusion, we successfully synthesized 24 derivatives of lead compound TBZ and optimized the anti-angiogenic and tumor vasculature disruption effect. Among all the synthesized compounds, TBZ-07 and TBZ-19 showed superiority compared to the parental TBZ. In particularly, TBZ-19 was proved to be the most potent one, with nearly 100-fold improvement of potency against the growth of HUVECs, A549, HCT116, and HepG2 cell lines. Biological evaluations also confirm their mechanism of anti-angiogenic and tumor vasculature disruption effect. Consequently, compound TBZ-07 and TBZ-19 would be promising anticancer agents and merit further investigation.

4. Experiment Section

4.1 Chemistry

4.1.1. General

Unless otherwise noted, all reagents were obtained from commercial suppliersand used without further purification. Reactions were monitored by thin-layer chromatography (TLC) using commercial silica gel HSGF254 plates. Column chromatography was performed on Silica Gel 60(E. Merck, 230-400 mesh). Melting points were measured on an X-5 micro-melting point apparatus and were uncorrected. The ^{1}H (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded with BrukerAM-400 spectrometer in $CDCl_3$ and DMSO-d_6 solution. Chemical shifts were referenced with tetramethylsilane (TMS). The HR-ESI-MS data were measured on a Bruker Apex IV FTMS.

4.1.2. General procedure for the synthesis of TBZ compounds (**TBZ-1-9**, **14-17**, **23-24**).

A solution of substituted o-phenyldiamine (1.0eq), thiazole-4-aldehyde or pydrine-2-aldehyde (1.0eq) with sodium pyrosulfite in DMF was stirred at 120 ℃ overnight. On completion of the reaction monitored by TLC, the solvent was evaporated and the residue was purified by silica gel chromatography by DCM/MeOH system to afford the final product. If necessary, the crude product could be recrystallized in DCM or dichloroethane to afford pure sample.

4.1.3. General procedure for the synthesis of TBZ compounds (**TBZ-10**, **11**, **12**, **13**, **18**, **20**).

To a solution of TBZ (1.0eq) (for TBZ-10,11,12,13,18) or 2-phenyl-1H-benzo[d]imidazole (1.0eq) (for TBZ-20) with sodium hydride (1.2eq) in DMF for 15 minutes, then corresponding iodomethane, benzyl bromide, 4-methoxybenzylchloride, 3-nitrobenzyl bromide, 5-(chloromethyl)-1,2,3-trimethoxybenzene (1.0eq) was added slowly at room temperature. On completion of the reaction monitored by TLC, the solvent was evaporated and the residue was purified by silica gel chromatography by DCM/MeOH system to afford the final product.

4.1.4. Procedure for the synthesis of **TBZ-20**

A solution of TBZ (1.0eq), 2-bromo-pyridine (2.0eq), Cs_2CO_3 (2.0eq), CuI (1.0eq), 1,3-di(pyridin-2-yl)propane-1,3-dione (1.0eq) in DMF was stirred at room temperature under Ar for 30 minutes, then the solution was heated to 110℃ for 48 hours. The precipitate was removed off by celite, the filtrate was diluted with EtOAc for 3 times, washed by brine, dried over $MgSO_4$, purified by silica gel chromatography to afford final product **TBZ-20**.

4.1.5. Procedure for the synthesis of **TBZ-21**

Following the former mentioned procedure for the synthesis of TBZ compounds, TBZ-21 was synthesized by the general procedure, the Boc group was deprotected by 5% TFA in DCM, the solvent was evaporated off and the remaining was neutralized by aqueous $NaHCO_3$, extracted by DCM, the organic layer was combined and dried over $MgSO_4$. DCM was evaporated to afford **TBZ-21** without further purification and used directly in the next step.

4.1.6. 4-(1H-benzo[d]imidazol-2-yl) thiazole (**TBZ**). White solid; yield 85%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.32 (d, J = 1.9 Hz, 1H), 8.45 (d, J = 1.9 Hz, 1H), 7.61 – 7.54 (m, 2H), 7.22 – 7.14 (m, 2H).

4.1.7. 4-(5-methyl-1H-benzo[d]imidazole-2-yl) thiazole (**TBZ-01**). Brown solid; yield 58%; ^{1}H-NMR (400 MHz, DMSO-d_6+5%CF_3COOH) δ 9.51 (d, J = 1.4 Hz, 1H), 8.93 (d, J = 1.4 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.63 (s, 1H), 7.43 (d, J = 8.4 Hz, 1H). note : the signal of methyl group was buried in DMSO.

4.1.8. 4-(4-methyl-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-02**). Brown solid; yield 63%; ^{1}H-NMR (400 MHz, DMSO-d_6+5%CF_3COOH) δ 9.48 (d, J = 1.8 Hz, 1H), 8.98 (d, J = 1.9 Hz,

1H), 7.60 (d, J = 8.2 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.35 (d, J = 7.4 Hz, 1H), 2.64 (s, 3H).

4.1.9. 4-(5, 6-dimethyl-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-03**). Brown solid; yield 80%; ^{1}H-NMR (400 MHz, DMSO-d_6+5%CF_3COOH) δ 9.48 (dd, J = 7.7, 1.8 Hz, 1H), 8.86 (d, J = 1.8 Hz, 1H), 7.57 (s, 2H), 2.39 (s, 6H).

4.1.10. 4-(6-chloro-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-04**). Grey solid; yield 62%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 9.34 (s, 1H), 8.49 (d, J = 1.4 Hz, 1H), 7.58 (s, 2H), 7.23 (dd, J = 8.5, 1.6 Hz, 1H).

4.1.11. 4-(6-fluoro-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-05**). Brown solid; yield 67%; ^{1}H-NMR (400 MHz, DMSO-d_6-5%TFA) δ 9.48 (d, J = 1.8 Hz, 1H), 8.90 (d, J = 1.8 Hz, 1H), 7.84 (dd, J = 9.0, 4.5 Hz, 1H), 7.66 (dd, J = 8.5, 2.4 Hz, 1H), 7.43 (td, J = 9.4, 2.5 Hz, 1H).

4.1.12.4-(6-methoxy-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-06**). Brown solid; yield 65%; ^{1}H-NMR (400 MHz, DMSO-d_6-5%TFA) δ 9.48 (d, J = 1.8 Hz, 1H), 8.86 (d, J = 1.8 Hz, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.22 (d, J = 2.3 Hz, 1H), 7.18 (dd, J = 9.0, 2.4 Hz, 1H), 3.87 (s, 3H).; ^{13}C-NMR (101 MHz, DMSO-d_6) δ 167.18, 156.33, 150.26, 149.52, 147.79, 146.91, 143.87, 138.43, 134.56, 124.26, 124.13, 123.91, 123.41, 121.43, 121.07, 120.95, 119.05, 114.05, 112.27, 52.42.; HR-EI-MS: Calcd for $C_{11}H_{10}N_3OS[M+H]^+$: 232.05391; found: 232.053892.

4.1.13.Phenyl(2-(thiazol-4-yl)-1H-benzo[d]imidazol-5-yl)methanone (**TBZ-07**). Yellow solid; yield 51%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.35 (s, 1H), 8.14 (s, 1H), 7.84 (d, J = 7.5 Hz, 3H), 7.72 (s, 1H), 7.59 (t, J = 7.4 Hz, 1H), 7.49 (t, J = 7.5 Hz, 2H).

4.1.14. 4-(1H-naphtho[2,3-d]imidazol-2-yl) thiazole (**TBZ-08**). Off-white solid; yield 65%; ^{1}H-NMR (400 MHz, DMSO-d_6-5%TFA) δ 9.49 (d, J = 1.2 Hz, 1H), 9.05 (d, J = 1.4 Hz, 1H), 8.30 (s, 2H), 8.13 (dd, J = 6.3, 3.3 Hz, 2H), 7.52 (dd, J = 6.5, 3.2 Hz, 2H).; ^{13}C-NMR (101 MHz, DMSO-d_6-5%TFA) δ 159.0, 158.6, 158.2, 157.8, 146.9, 139.3, 131.2, 130.7, 128.8, 127.9, 125.6, 116.4, 113.5, 110.; HR-EI-MS: Calcd for $C_{14}H_{10}N_3S[M+H]^+$: 252.05899, found: 252.05902.

4.1.15. 4-(6-nitro-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-09**). Yellow solid; yield 62%; ^{1}H-NMR (400 MHz, DMSO-d_6-5%TFA) δ 9.43 (s, 1H), 8.79 (s, 1H), 8.52 (s, 1H), 8.24 (d, J = 8.9 Hz, 1H), 7.85 (d, J = 8.8 Hz, 1H).

4.1.16. 4-(1-methyl-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-10**). White solid; yield 90%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.31 (s, 1H), 7.80 (d, J = 7.4 Hz, 1H), 7.42 (d, J = 7.1 Hz, 1H), 7.36 – 7.28 (m, 2H), 4.23 (s, 3H).

4.1.17. 4-(1-benzyl-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-11**). White solid; yield 87%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.87 (d, J = 2.0 Hz, 1H), 8.32 (d, J = 2.1 Hz, 1H), 7.82 (d, J = 7.8 Hz, 1H), 7.34 – 7.28 (m, 2H), 7.26 – 7.21 (m, 4H), 7.15 (d, J = 6.6 Hz, 2H), 6.08 (s, 2H).

4.1.18.4-(1-(4-methoxybenzyl)-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-12**). White solid; m.p. 150-152 ºC; yield 82%;^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.86 (d, J = 2.1 Hz, 1H), 8.30 (d, J = 2.1 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.34 – 7.20 (m, 3H), 7.10 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 8.7 Hz, 2H), 5.97 (s, 2H), 3.70 (s, 3H).; ^{13}C-NMR (101 MHz, $CDCl_3$) δ 158.98, 152.98, 147.89, 146.86, 143.08, 135.96, 129.16, 128.12, 123.22, 122.77, 121.32, 119.74, 114.05, 110.71, 77.43, 77.11, 76.79, 55.21, 47.98.; HR-EI-MS : Calcd for $C_{18}H_{16}N_3OS[M+H]^+$: 322.10086; found: 322.10142.

4.1.19.4-(1-(3-nitrobenzyl)-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-13**). Light brown solid; yield 88%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.87 (d, J = 2.0 Hz, 1H), 8.39 (d, J = 2.0 Hz, 1H), 8.16 (s, 1H), 8.09 (d, J = 7.4 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.45 – 7.38 (m, 2H), 7.35 – 7.27 (m, 3H), 6.16 (s, 2H).; ^{13}C-NMR (101 MHz, $CDCl_3$) δ 153.26, 148.45, 146.63, 143.06, 139.37, 135.62, 132.78, 129.75, 123.68, 123.24, 122.65, 122.04, 121.70, 120.05, 110.01, 77.38, 77.06, 76.74, 47.94. HR-EI-MS : Calcd for $C_{17}H_{13}N_4O_2S$ $[M+H]^+$: 337.07537; found: 337.07515.

4.1.20.5-methyl-2-(pyridin-2-yl)-1H-benzo[d]imidazole (**TBZ-14**). Brown solid; yield 62%; ^{1}H-NMR(400 MHz, DMSO-d_6-5%TFA, ppm): 2,51 (3H, s), 7,42 (1H, d, J=8.2 Hz), 7,63 (1H, s), 7,72-7,77 (2H,m), 8,22 (1H, td, J=7.9, 1.6 Hz), 8,41 (1H, d, J=7.9 Hz), 8,91 (1H, d, 4.1 Hz).

4.1.21. Methyl 2-(thiazol-4-yl)-1H-benzo[d]imidazole-5-carboxylate (**TBZ-15**). Pale yellow solid; m.p. 110-111 ºC; yield 62%; ^{1}H-NMR (400 MHz, DMSO) δ 13.36 (s, 1H), 9.37 (s, 1H), 8.56 (s, 1H), 8.20 (s, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.67 (s, 1H), 3.88 (s, 3H).; ^{13}C-NMR (101 MHz, DMSO) δ 155.88, 155.39, 147.23, 118.48, 111.84, 55.41. ; HR-EI-MS: Calcd for $C_{12}H_{10}N_3O_2S$ $[M+H]^+$: 260.04882, found: 260.04898.

4.1.22. Methyl 2-(thiazol-4-yl)-1H-benzo[d]imidazole-7-carboxylate (**TBZ-16**). Light brown solid ; m.p. 141-143 ºC; yield 65%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.05 (s, 1H), 8.91 (d, J = 1.5 Hz, 1H), 8.31 (d, J = 1.5 Hz, 1H), 7.97 (dd, J = 19.3, 7.8 Hz, 2H), 7.34 (t, J = 7.8 Hz, 1H), 4.03 (s, 3H).; ^{13}C-NMR (101 MHz, DMSO) δ 165.72, 155.64, 148.32, 146.04, 144.71, 133.33, 124.66, 124.42, 121.82, 121.05, 113.92, 52.15.; HR-EI-MS: calcd for $C_{12}H_{10}N_3O_2S[M+H]^+$: 260.04882, found: 260.04905.

4.1.23.4-(5-(trifluoromethyl)-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-17**). Light brown solid; m.p. 153-154 ºC; yield 55%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J = 1.9 Hz, 1H), 8.36 (d, J = 1.9 Hz, 1H), 7.95 (s, 1H), 7.63 (d, J = 51.3 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H). ; ^{13}C-NMR (101 MHz, DMSO) δ 208.47, 156.39, 149.93, 146.78, 129.69, 126.85, 124.15, 123.45, 123.14, 121.40, 119.35; HR-EI-MS: Calcd for $C_{11}H_7F_3N_3S$ $[M+H]^+$: 270.03073; found: 270.03044.

4.1.24.4-(1-(3, 4, 5-trimethoxybenzyl)-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-18**). Pale yellow solid; m.p. 147-149 ºC; yield 68%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.92 (d, J = 2.1 Hz, 1H), 8.34 (d, J = 2.1 Hz, 1H), 7.87 – 7.75 (m, 1H), 7.36 (dd, J = 6.8, 1.2 Hz, 1H), 7.33 – 7.24 (m, 2H), 6.41 (s, 2H), 5.98 (s, 2H), 3.78 (s, 3H), 3.69 (s, 6H).; 13C-NMR (101 MHz, CDCl3) δ 153.53, 153.17, 136.05, 132.77, 123.46, 123.03, 121.58, 119.89, 110.75, 104.06, 60.91, 56.14, 48.77. HR-EI-MS : Calcd for $C_{20}H_{20}N_3O3S$ $[M+H]^+$: 382.12199; found: 382.12128.

4.1.25.1-(4-(1H-benzo[d]imidazol-2-yl) thiazol-5-yl)-3-cyclopropylthiourea (**TBZ-19**). Light yellow solid; m.p. 244-246 ºC; yield 83%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 13.31 (s, 1H), 13.12 (s, 1H), 9.34 (s, 1H), 8.64 (s, 1H), 7.68 – 7.46 (m, 2H), 7.24 (s, 2H), 2.91 (d, J = 16.8 Hz, 1H), 1.17 (s, 2H), 0.79 (s, 2H).; 13C-NMR (101 MHz, DMSO-d_6) δ 178.13, 148.69, 145.31, 142.38, 139.62, 133.19, 128.63, 122.73, 121.99, 117.86, 111.71, 24.63, 7.54. ; HR-EI-MS : Calcd for $C_{14}H_{14}N_5S_2$ $[M+H]^+$: 316.06851; found: 316.06893.

4.1.26. 1-benzyl-2-phenyl-1H-benzo[d]imidazole (**TBZ-20**). White solid; yield 92%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.87 (d, J = 8.0 Hz, 1H), 7.72 – 7.65 (m, 2H), 7.48 – 7.41 (m, 3H), 7.35 – 7.26 (m, 4H), 7.25 – 7.19 (m, 2H), 7.13 – 7.06 (m, 2H), 5.44 (s, 2H).

4.1.27. 4-(1H-benzo[d]imidazol-2-yl) thiazol-5-amine (**TBZ-21**). Light yellow solid; yield 99%; ^{1}H-NMR (400 MHz, DMSO) δ

12.47 (s, 1H), 8.22 (s, 1H), 7.55 (s, 1H), 7.41 (s, 3H), 7.20 – 7.07 (m, 2H). ; HR-EI-MS：Calcd for $C_{10}H_9N_4S$ $[M+H]^+$: 217.05424; found 217.05412.

4.1.28. 4-(1-(pyridin-2-yl)-1H-benzo[d]imidazol-2-yl) thiazole (**TBZ-22**). Brown solid; 177-178 ºC; yield 73%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.64 – 8.59 (m, 2H), 8.09 (d, J = 2.0 Hz, 1H), 7.86 (ddd, J = 13.1, 6.7, 2.7 Hz, 2H), 7.42 – 7.36 (m, 2H), 7.36 – 7.27 (m, 3H). ; 13C-NMR (101 MHz, DMSO) δ 154.52, 149.81, 149.16, 146.55, 146.12, 142.35, 138.93, 135.67, 123.77, 123.75, 123.11, 122.42, 121.77, 119.38, 111.09. ; HR-EI-MS：Calcd for $C_{15}H_{11}N_4S$ $[M+H]^+$: 279.06989; found: 279.06971 .

4.1.29. 4-(1H-imidazo[4,5-c]pyridin-2-yl) thiazole (**TBZ-23**). White solid; yield 62%; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.39 (t, J = 9.8 Hz, 1H), 8.93 (s, 1H), 8.60 (t, J = 7.9 Hz, 1H), 8.32 (d, J = 5.5 Hz, 1H), 7.56 (s, 1H).

4.1.30. 4-(1H-imidazo[4,5-b]pyridin-2-yl) thiazole (**TBZ-24**). Purple solid; yield 55%; ^{1}H-NMR (400 MHz, DMSO-d_6-5%TFA) δ 9.40 (s, 1H), 8.84 (s, 1H), 8.63 (d, J = 5.3 Hz, 1H), 8.45 (d, J = 7.9 Hz, 1H), 7.75 – 7.37 (m, 1H).

4.2. **Biological evaluation**

4.2.1. In vitro cytotoxicity evaluation

All cells used in the research were prepared at 3.5×10^4 cells/mL concentration and each 100 mL cells suspension was seeded in 96-well cell imcroplate for 24 h (37 ºC, 5%CO_2). Then each solution was added and incubated for another 72 h. For the control group, equivalent concentration of DMSO (final concentration 0.5%) was added. MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide) method was employed to measure the number of surviving cells and recorded the OD value at 492nm/620 nm. The IC_{50} values were calculated using Prism Graphpad software of the triplicate experiment.

4.2.2. HUVEC tube formation assay

Thawed matrigel (Becton Dickinson Labware, Bedford, MA, USA), 60 μL per well, was added to a prechilled 96-well sterile plate and incubated at 37 ºC for 1 h. Then, 1.5×10^4 HUVEC per well suspended in M199 culture medium containing 20% FBS were added into each well, together with various concentrations of test agents. After incubation for 8 h at 37 ºC, cells were imaged using a high magnification field (Olympus Optical Co., Ltd.).

4.2.3. Rat aortic ring assay.

The aorta of Sprague Dawley rats (6 weeks) was isolated under ether anesthesia, rinsed with serumfree M199, and cut into 1-mm ring sections. The sections were then placed in a 96-well plate, embedded with 70 μL Matrigel for each well, and incubated at 37 ºC for 1 h. The serum-free M199 medium was subsequently added into each well, with or without 20% FBS, and various concentrationsof test agents. On the sixth day, images were taken through an inverse microscope.

4.2.4. Tubulin polymerization assay.

Microtubule-associated protein-rich tubulin (2 mg/mL, bovine brain, Cytoskeleton) in buffer containing 80 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 0.5 mM EGTA, and 5% glycerol was placed in cuvettes, 200 μL/assay, and incubated respectively with DMSO, 5μM compound TBZ-07 and TBZ-19 respectively. Polymerization was started by adding 1 mM GTP and incubating at 37 °C, followed by absorption readings at 340 nm with a Varian Cary 50 series spectrophotometer (every 5 s/min 0 to min 3, every 10 s/min 3 to min 5, every 30 s/min 5 to min10, and every 60 s/min 10 to min 17).

4.2.5. Tubulin intensity assay

A549 cells were treatment for 24h in the presence of 10μM, 25μM of TBZ-19 and TBZ-07, 50nM vinblastine, 250μM TBZ, and stained for α-tubulin(red) with TRITC and nucleus(green) with Hoechst 33258, images were taken by Operetta(20×).

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