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Angiogenesis inhibitors identified by cell-based high-throughput screening: Synthesis, structure–activity relationships and biological evaluation of 3-[(*E*)-styryl]benzamides that specifically inhibit endothelial cell proliferation

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ABSTRACT

Proliferation of endothelial cells is critical for angiogenesis. We report orally available, in vivo active anti-angiogenic agents which specifically inhibit endothelial cell proliferation. After identifying human umbilical vein endothelial cell (HUVEC) proliferation inhibitors from a cell-based high-throughput screening (HTS), we eliminated those compounds which showed cytotoxicity against HCT116 and vascular endothelial growth factor receptor 2 (VEGFR-2) inhibitory activity. Evaluations in human Calu-6 xenograft model delivered lead compound **1**. Following extensive lead optimization and alteration of the scaffold we discovered **32f** and **32g**, which both inhibited the proliferation and tube formation of HUVEC without showing inhibitory activity against any of 25 kinases or cytotoxicity against either normal fibroblasts or 40 cancer cell lines. Upon oral administration, **32f** and **32g** had good pharmacokinetic profiles and potent antitumor activity and decreased microvessel density (MVD) in Calu-6 xenograft model. Combination therapy with a VEGFR inhibitor enhanced the in vivo efficacy. These results suggest that **32f** and **32g** may have potential for use in cancer treatment.

1. Introduction

Angiogenesis, the formation of new blood vessels from existing vasculature, plays an important role in tumor growth and metastasis.[1] The growth of new blood vessels involves the proliferation of endothelial cells in response to specific growth stimuli such as vascular endothelial growth factor (VEGF), one of the most potent tumor angiogenic factors, and the migration of these endothelial cells to the tumor site to form new capillaries supplying oxygen and nutrition to the growing tumor.[2] Evidence shows that inhibition of angiogenesis can suppress the progression of tumor growth. Indeed, the clinical benefit of angiogenesis inhibitors has been demonstrated by bevacizumab, a recombinant humanized monoclonal antibody to VEGF, which was approved for the treatment of colorectal cancer in combination with 5-FU/CPT-11 in 2004.[3] By binding to VEGF, bevacizumab prevents it from binding to the receptor (VEGFR), thus inhibiting endothelial cell proliferation and tube formation.[4] In other words, inhibiting endothelial cell proliferation can lead to antiangiogenesis.[5]

To date, a large number of small-molecule angiogenesis inhibitors have been reported. Among them, receptor tyrosine kinase inhibitors targeting VEGFRs, primarily VEGFR-2 (also known as KDR) have been the most studied and three multi-kinase inhibitors with potent VEGFR-2 inhibition, sunitinib,[6] sorafenib,[7] and pazopanib[8] have been approved for the treatment of advanced cancers. Despite their clinical benefits, drug resistance and on-target adverse events such as hypertension, proteinuria and hemorrhage are observed during treatment with VEGFR inhibitors.[9–13] Thus, there is still a need for angiogenesis inhibitors which could overcome these drawbacks through a different mode of action from that of VEGFR inhibitors. This premise prompted us to search for new small-molecule angiogenesis inhibitors.

Cell-based high-throughput screening (HTS) of our chemical library by applying human umbilical vein endothelial cell (HUVEC) antiproliferative assays followed by counter assays identified lead compound **1** (**RO0123743**), which inhibits angiogenesis both in vitro and in vivo and does not show cytotoxicity or VEGFR-2 inhibition. As a result of extensive chemical modifications, compounds **32f** and **32g** were identified as potent and specific endothelial proliferation inhibitors with good physicochemical properties, metabolic stability, and significant oral efficacy in a human xenograft model. Herein, we describe identifying lead compound **1** and optimizing it efficiently into **32f** and **32g**. The results of their biological evaluations are also described.

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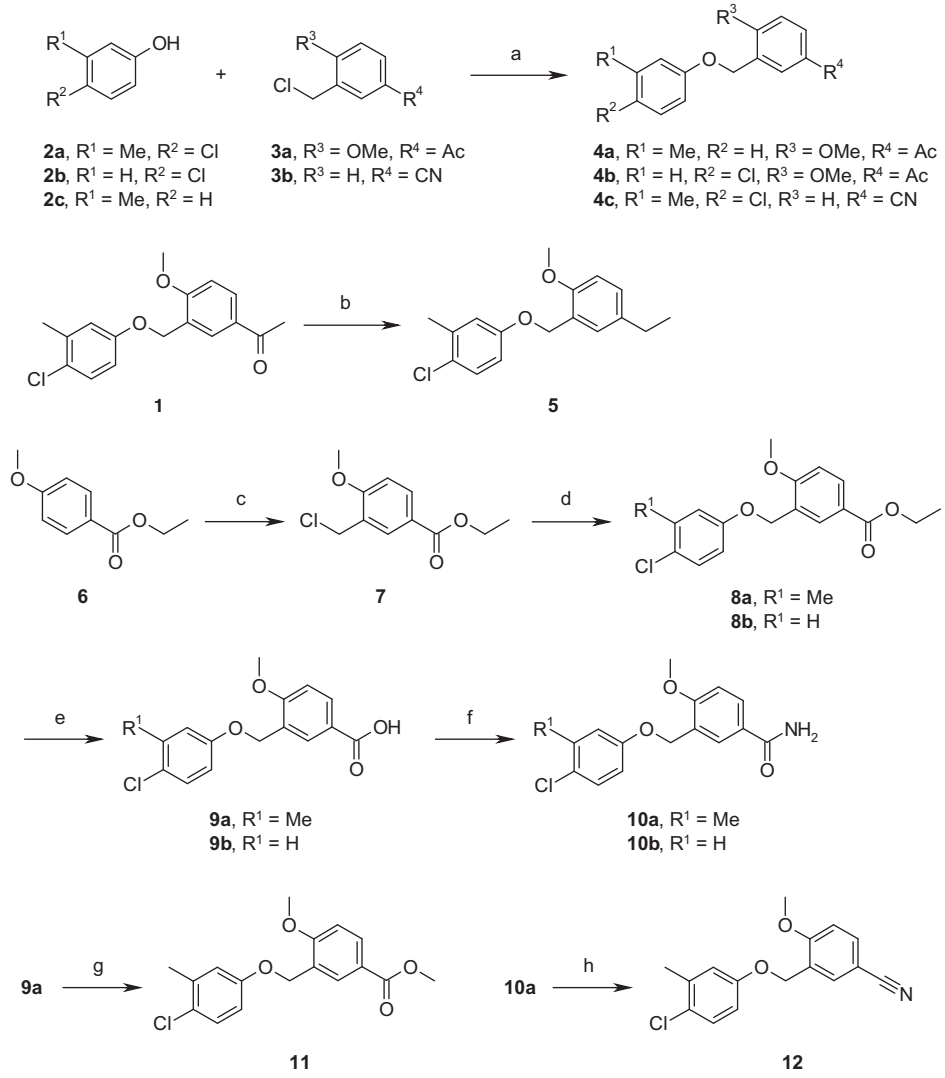
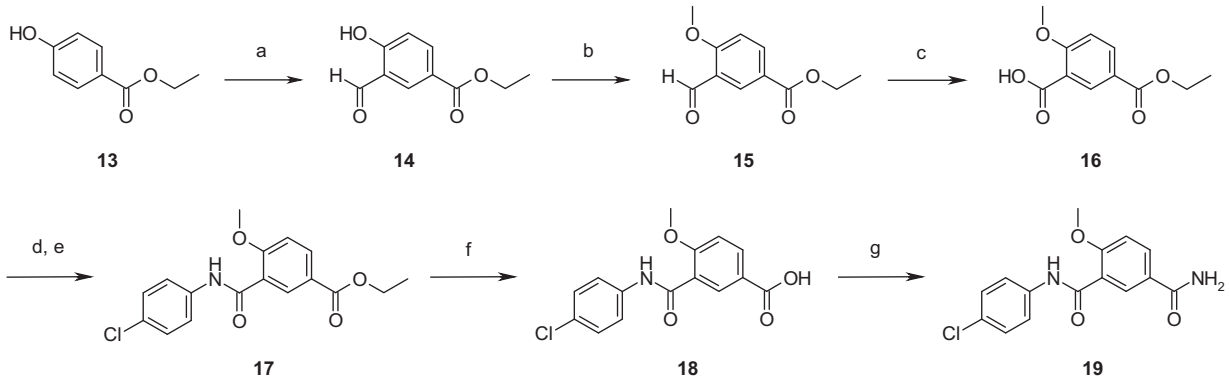


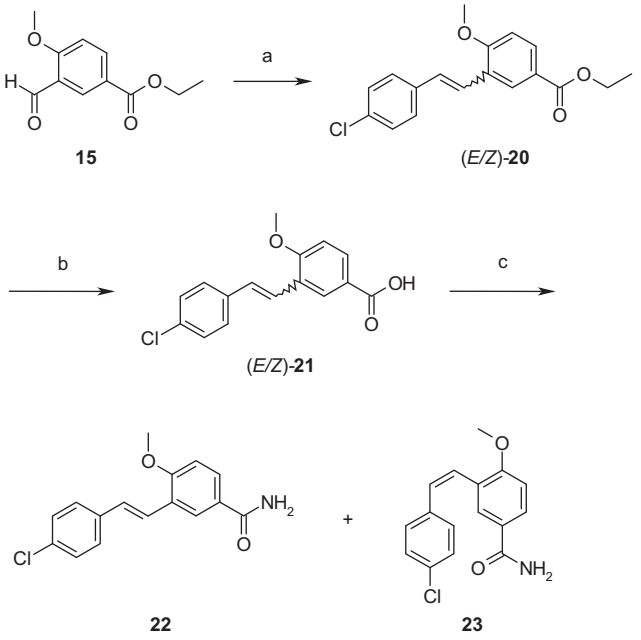
Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 80 °C; (b) Et_3SiH, TFA, rt, 6 h, 36%; (c) MOMCl, $SnCl_4$, CH_2Cl_2, 0 °C, 5 h, 60%; (d) **2a**–**b**, K_2CO_3, DMF, 80 °C; (e) 20% KOH, MeOH, reflux, 1 h; (f) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 1-hydroxybenzotriazole (HOBT), NH_4Cl, *N*,*N*-diisopropylethylamine (DIPEA), DMF, rt; (g) $TMSCHN_2$, toluene, MeOH, rt, 2.5 h, 95%; (h) $(HCHO)_n$, HCO_2H, CH_3CN, reflux, 14 h, 77%.



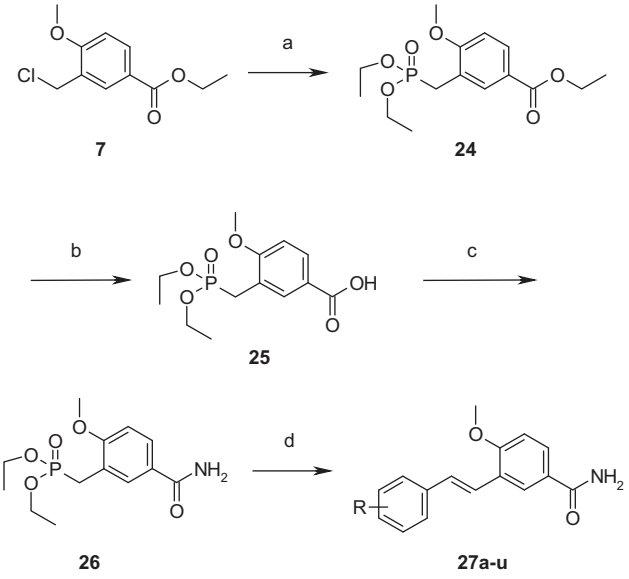
Scheme 2. Reagents and conditions: (a) $MgCl_2$, Et_3N, 1,2-dichloroethane, 40 °C, 1 h, then $(HCHO)_n$, 70 °C, 3 h, 76%; (b) Me_2SO_4, K_2CO_3, acetone, reflux, 1 h, 86%; (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-BuOH-H_2O, rt, 50 min, 100%; (d) $(COCl)_2$, cat.DMF, CH_2Cl_2, rt, 2.5 h. (e) 4-chloroaniline, DIPEA, CH_2Cl_2, rt, 1 h, 76% (2 steps); (f) 20% KOH, MeOH, reflux, 30 min, 94%; (g) EDC, HOBT, NH_4Cl, DIPEA, DMF, rt, 17 h, 93%.

2. Chemistry

The synthesis of analogues **4–12** bearing different functional groups from those of lead compound **1** on benzyl phenyl ether was conducted as outlined in Scheme 1. Compounds **4a**–**c** were prepared by coupling phenols **2a**–**c** with the corresponding benzyl chlorides **3a**–**b** under basic conditions. Reduction of the carbonyl group of **1** with Et_3SiH in trifluoroacetic acid (TFA) provided **5**.



Scheme 3. Reagents and conditions: (a) (4-chlorobenzyl)triphenylphosphonium chloride, NaOEt, EtOH, rt, 1 h, 92%; (b) 20% KOH, MeOH, reflux, 2 h, 95%; (c) EDC, HOBT, NH_4Cl, DIPEA, DMF, rt, 16 h, 33% for **22** and 62% for **23**, respectively.



Scheme 4. Reagents and conditions: (a) triethyl phosphite, 160 °C, 2.5 h, 95%; (b) 5 M NaOH, MeOH, 60 °C, 1.5 h, 96%; (c) EDC, HOBT, NH_4Cl, DIPEA, DMF, rt, 20 h, 62%; (d) substituted benzaldehydes, sodium *tert*-pentoxide, DMF, rt.

Compounds **9–12** were prepared from commercially available ethyl 4-methoxybenzoate **(6)** via 3–5 synthetic steps. Thus, reaction of **6** with methoxymethyl (MOM) chloride in the presence of $SnCl_4$ provided **7**.[14] Coupling of **7** with phenols **2a–b** in the presence of K_2CO_3 gave the corresponding benzyl phenyl ethers **8a–b**. Compounds **8a–b** were hydrolyzed under basic conditions to give **9a–b**. Esterification of the carboxylic acid **9a** with trimethylsilyldiazomethane afforded methyl ester **11**. Carboxylic acids **9a–b** were condensed with NH_4Cl to give the corresponding amides **10a–b**. Nitrile **12** was obtained from **10a** by direct conversion of the amide group by aldehyde-catalyzed water transfer.[15]

Amide derivative **19** was prepared as shown in Scheme 2. Formylation of **13** was performed in a similar way to the procedure of Skattebøl and co-workers.[16] Methylation of **14** using methyl iodide afforded **15**. Pinnick oxidation[17] of **15** afforded carboxylic acid **16**. Reaction of **16** with 4-chloroaniline via acid chloride provided **17**. Amide **19** was prepared by hydrolysis of ethyl ester in **17** followed by condensation of **18**. To obtain stilbene analogues, we adopted the synthetic methods shown in Scheme 3–6. Stilbenes **22** and **23** were synthesized as shown in Scheme 3. Wittig reaction of **15** with (4-chlorobenzyl)triphenylphosphonium chloride gave (*E*)-**20** and (*Z*)-**20** as a 1:2 mixture. Ester hydrolysis followed by condensation gave amides, which were separated into **22** [(*E*)-isomer] and **23** [(*Z*)-isomer].

4-Methoxy-3-[(*E*)-styryl]benzamide analogues **27a–u** described here were synthesized as outlined in Scheme 4. We selected **26** as a key intermediate to synthesize **27a–u** because Horner-Wadsworth-Emmons reaction with commercially available aldehydes gives derivatives with various substituents on the A phenyl ring. Arbuzov reaction of **7** with triethyl phosphite afforded **24**. Hydrolysis of the ethyl ester group in **24** under basic conditions provided acid **25**, which was converted to amide **26**. Horner-Wadsworth-Emmons reaction of **26** with different aldehydes gave compound **27a–u**.

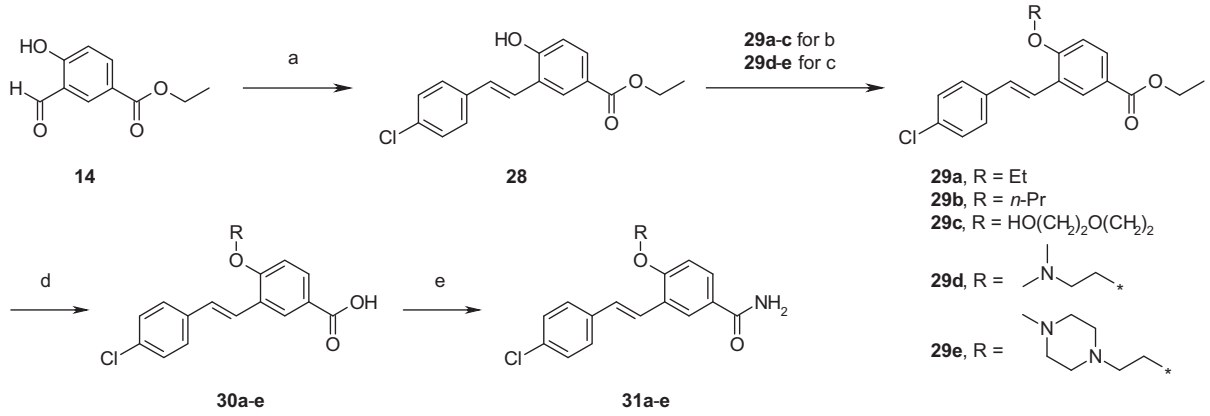
3-[(*E*)-2-(4-chlorophenyl)vinyl]benzamides **31a–e** were synthesized by the method shown in Scheme 5. We chose **28** as an intermediate to facilitate derivatization of the methoxy moiety of **22**. Horner-Wadsworth-Emmons reaction of **14** with diethyl (4-chlorobenzyl)phosphonate provided a stilbene **28**. Alkylation of **28** gave **29a–e**. Hydrolysis of **29a–e** followed by condensation furnished the target compounds **31a–e**.

Compounds **32a–h** were prepared by the synthetic route outlined in Scheme 6. Carboxylic acid (*E*)-**21** was adopted as a common intermediate to synthesize amides with various solubilizing groups. Horner-Wadsworth-Emmons reaction of **15** with diethyl (4-chlorobenzyl)phosphonates gave stilbene (*E*)-**20** as a sole isomer. Hydrolysis of the ester afforded carboxylic acid (*E*)-**21**. Compounds **32a–h** were prepared by condensation of (*E*)-**21** via acid chlorides with various amines.

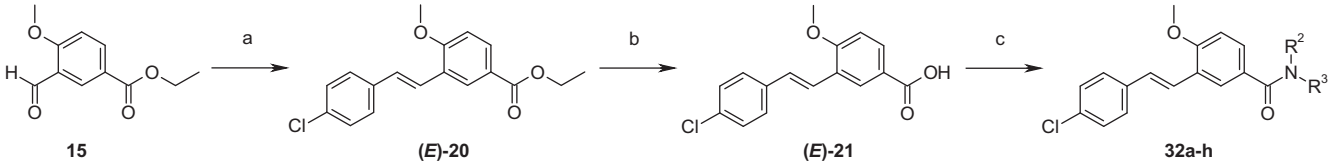
3. Results and discussion

3.1. Lead generation from cell-based HTS

The evaluation cascade used to obtain our lead compounds is shown in Figure 1. As a primary screening, high-throughput VEGF-stimulated HUVEC proliferation assays at 4 μM were performed on 280,000 compounds. The compounds which showed over 50% inhibition against HUVEC growth were further evaluated with a cell growth inhibition assay using a human colorectal cancer cell line, HCT116, and a VEGFR-2 inhibition assay to eliminate non-selective cytotoxics and VEGFR-2 inhibitors. We identified 14 lead candidates which have more than 5-fold selectivity (defined as the IC_{50} ratio of HCT116 to HUVEC) and no VEGFR-2 inhibition. Those candidates which showed tumor growth inhibition (TGI) in a human lung cancer (Calu-6) xenograft model (chosen for its sensitivity to antiangiogenic agents[18]) and microvessel density (MVD) reduction in the xenograft tissues were nominated as the lead compounds. We believe that this proof-of-concept confirmation in animal models is essential when generating leads from cell-based screening. Among the lead candidates, **1** (**RO0123743**) was the most promising lead compound showing antiproliferative activity against HUVEC (IC_{50} = 1.3 μM), weak antiproliferative activity against HCT116 (IC_{50} = 34 μM) and no VEGFR-2 inhibition (IC_{50} >50 μM) in vitro. In vivo, moderate activity (TGI = 34%) in the Calu-6 xenograft model was observed when **1** was orally administered once a day for 11 consecutive days (600 mg/kg), and antiangiogenic activity was confirmed by MVD reduction (15%) in the xenograft tissue (Fig. 2).



Scheme 5. Reagents and conditions: (a) diethyl (4-chlorobenzyl)phosphonate, sodium *tert*-pentoxide, DMF, 0 °C, 2 h, 71%; (b) alkyl halides, K_2CO_3, CH_3CN, 60 °C; (c) $Br(CH_2)_2Br$, K_2CO_3, DMF, 80 °C, then amines; (d) 20% KOH, MeOH, reflux; (e) EDC, HOBT, NH_4Cl, DIPEA, DMF, rt.



Scheme 6. Reagents and conditions: (a) diethyl (4-chlorobenzyl)phosphonates, sodium *tert*-pentoxide, toluene-THF, 0 °C; (b) 20% KOH, MeOH, 65 °C, 80% (2 steps); (c) $(COCl)_2$, cat.DMF, CH_2Cl_2, rt, then amines, DIPEA, CH_2Cl_2, rt.

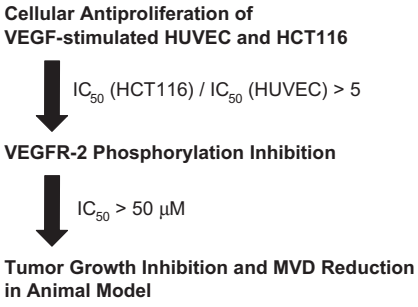


Figure 1. Evaluation cascade.

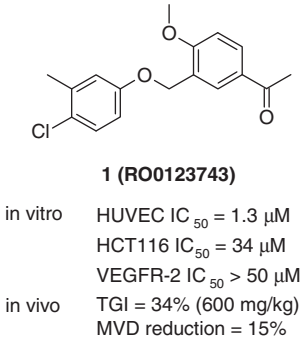


Figure 2. Structure and biological profiles of lead compound **1** (**RO0123743**).

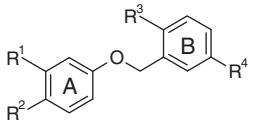
3.2. Lead optimization

We started structural optimization of lead compound **1** by optimizing functional groups around the benzyl phenyl ether moiety, using the same evaluation cascade as that of for lead identification (Table 1). The terminal acetyl group on the B phenyl ring was modified first. Replacement of the acetyl group (**1**) with a cyano group (**12**) or a methyl ester group (**11**) maintained the antiproliferative activity against HUVEC (IC_{50} = 1.5 and 2.3 μM for **12** and **11**, respectively) while an ethyl group (**5**) showed a reduction in activity (IC_{50} = 40 μM). Higher activity was obtained with an analogue carrying an amide (**10a**). These results suggest that a hydrogen acceptor at R^4-position is essential for the antiproliferative activity against HUVEC and a hydrogen donor enhances the activity. Compound **10a** also had no activity against HCT116 (IC_{50} >50 μM), resulting in high selectivity (IC_{50} ratio of HCT116 to HUVEC >78). Carboxylic acid (**9a**) did not inhibit the proliferation of HUVEC or HCT116, presumably due to low cell penetration.

The necessity for substituents at R^1-, R^2- and R^3-positions was examined by deletion studies. Deletion of the methyl group adjacent to the chloro group of the A phenyl ring did not affect antiproliferative activity on either HUVEC (IC_{50} = 1.2 and 0.66 μM for **4b** and **10b**, respectively) or HCT116 (IC_{50} = 39 and >50 μM for **4b** and **10b**, respectively) compared to those of **1** and **10a**. However, removal of the chloro group (**4a**) at R^2-position or methoxy group (**4c**) at R^3-position resulted in the reduction of antiproliferative activity on HUVEC (IC_{50} = 2.8 and 11 μM for **4a** and **4c**, respectively), indicating that substituents at both R^2- and R^3-positions were necessary for a potent inhibition of HUVEC proliferation.

Analogues **10a–b** were selected for a VEGFR-2 inhibition assay and were found to show no VEGFR inhibition (IC_{50} >50 μM), so we further evaluated their in vivo efficacy (Table 2). Compound **10b** showed improved antitumor and antiangiogenic activity (62% TGI and 27% MVD reduction, respectively) after once-daily oral administration for 11 consecutive days at 600 mg/kg. In contrast, compound **10a** displayed weak TGI (14%) and no MVD reduction. Mouse liver microsomal clearances of **10a** and **10b** (414 and

Table 1
Cell growth inhibition of benzyl phenyl ethers against HUVEC and HCT116



Compd	Substituents				Cell growth inhibition/IC_{50} (μM)		Selectivity[a]
	R^1	R^2	R^3	R^4	HUVEC	HCT116	HCT116/HUVEC
1	Me	Cl	MeO		1.3	34	26
4a	Me	H	MeO		2.8	24	8.6
4b	H	Cl	MeO		1.2	39	33
4c	Me	Cl	H	CN	11	45	4.1
5	Me	Cl	MeO	Et	40	43	1.1
9a	Me	Cl	MeO		>50	>50	ND[b]
10a	Me	Cl	MeO		0.64	>50	>78
10b	H	Cl	MeO		0.66	>50	>75
11	Me	Cl	MeO		2.3	23	10
12	Me	Cl	MeO	CN	1.5	>50	>33

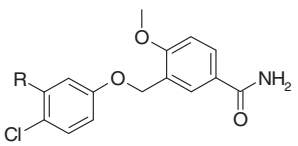
[a] IC_{50} ratio of HCT116 to HUVEC.
[b] Not determined.

97 μL/min/mg, respectively) might explain the weak in vivo efficacy of **10a**.

With the preferred amide, methoxy, and chloro groups kept in place on the A and B phenyl rings, our next effort focused on altering the benzyl phenyl ether bond. Compound **10b** still had weak antiproliferative activity against HUVEC (IC_{50} = 0.66 μM), presumably due to a high degree of conformational flexibility of the ether bond. Since the conformational restriction is one of the common practices for improvement of activity, we reduced the flexibility of **10b**. As shown in Table 3, replacement of the ether bond with a *trans*-double bond (**22**) significantly enhanced the antiproliferative activity against HUVEC (IC_{50} = 0.087 μM) while maintaining high selectivity (IC_{50} ratio of HCT116 to HUVEC = 82). A *cis*-double bond (**23**) and an amide bond (**19**) decreased inhibition of HUVEC proliferation. These results suggest that fixing position between A and B phenyl rings by hydrophobic *trans*-olefin is favorable for the potent inhibition of HUVEC proliferation. Compound **22** showed no VEGFR-2 inhibition (IC_{50} >50 μM) and improved in vivo antitumor and antiangiogenic activity (71% TGI and 60% MVD reduction, respectively) after once-daily oral administration for 11 consecutive days at 300 mg/kg.

To further improve the antiproliferative activity against HUVEC, intensive derivatization on A phenyl ring of **22** was performed (Table 4). Replacement of the chlorine atom at 4-position of **22** with bromine (**27c**), or fluorine (**27f**) resulted in a significant reduction of antiproliferative activity against HUVEC. Replacement of the chlorine atom with electron-withdrawing groups (**27d**, **27g** and **27h**) or electron-donating groups (**27b**, **27e** and **27i**) at 4-position also decreased inhibition of HUVEC proliferation while antiproliferative activity against HCT116 was less affected. As we expected from the result of the deletion studies, compounds carrying a substituent at 2- or 3-position (**27j**–**r**) decreased the antiproliferative activity on

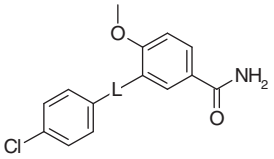
Table 2
VEGFR-2 inhibition and in vivo antitumor efficacy of **10a** and **10b**



Compd	R	VEGFR-2 inhibition/IC_{50} (μM)	Microsomal clearance[a] (μL/min/mg)	In vivo antitumor efficacy/TGI[b] (%)	MVD reduction[c] (%)
10a	Me	>50	414	14	0
10b	H	>50	97	62	27

[a] Clearance of test compound when incubated with mouse liver microsomes.
[b] Compound **10a** and **10b** were orally administered at 600 mg/kg in the Calu-6 xenograft model.
[c] Microvessel density reduction in the Calu-6 xenograft tissue.

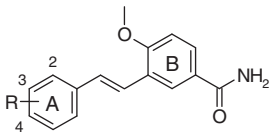
Table 3
Optimization of benzyl phenyl ether bond



Compd	L	Cell growth inhibition/IC_{50} (μM)		Selectivity[a]	Solubility in FaSSIF (μg/mL)	Microsomal clearance[b] (μL/mim/mg)	In vivo antitumor efficacy/TGI (%)	MVD reduction[c] (%)
		HUVEC	HCT116	HCT116/HUVEC				
19		5.5	>50	>9	5	16	ND[d]	ND[d]
22		0.087	7.1	82	16	110	71[e]	60[e]
23		2.9	29	10	137	544	ND[d]	ND[d]

[a] IC_{50} ratio of HCT116 to HUVEC.
[b] Clearance of test compound when incubated with mouse liver microsomes.
[c] Microvessel density reduction in the Calu-6 xenograft tissue.
[d] Not determined.
[e] Compound **22** was orally administered at 300 mg/kg in the Calu-6 xenograft model.

Table 4
In vitro structure–activity relationships on A phenyl ring of 3-[(*E*)-styryl]benzamides



Compd	R	Cell growth inhibition/IC_{50} (μM)		Selectivity[a]
		HUVEC	HCT116	HCT116/HUVEC
27a	H	2.4	36	15
27b	4-*t*-Bu	0.37	20	54
27c	4-Br	0.56	22	39
27d	4-CF_3	0.88	35	40
27e	4-Me	1.6	48	30
27f	4-F	2.1	24	11
27g	4-CN	3.1	16	5.2
27h	4-NO_2	4.7	22	4.7
27i	4-OMe	8.8	19	2.2
27j	3-Me	3.9	23	5.9
27k	3-F	8.3	>50	>6.0
27l	3-Cl	24	15	0.6
27m	3-CN	49	19	0.4
27n	2-F	2.0	26	13
27o	2-Me	2.3	11	4.8
27p	2-OMe	5.7	>50	>8.8
27q	2-OCF_3	8.9	36	4
27r	2-Cl	23	>50	2.2
27s	2,4-diCl	2.6	>50	>19
27t	3,4-diCl	0.56	11	20
27u	2-F-4-Cl	1.7	>10	>5.9

[a] IC_{50} ratio of HCT116 to HUVEC.

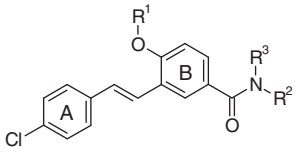
HUVEC. Larger substituents (**27l–m**, **27p–r**) at 2- or 3-position had a tendency to inhibit HUVEC proliferation less potently compared to the unsubstituted compound **27a** (IC_{50} = 2.4 μM), indicating limited spaces at 2- and 3-positions. The same tendency was observed in 2,4- and 3,4-disubstituted compounds **27s–u**. 3,4-Dichloro-substituted compound **27t** and 2,4-disubstituted compounds **27s** and **27u** were less potent than **22**. Overall, 4-monochloro substituent (**22**) was the most favorable for A phenyl ring.

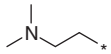
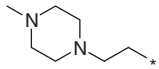
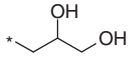
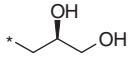
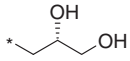
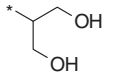
Despite its potent inhibition of HUVEC proliferation and good selectivity to HCT116, compound **22** had low solubility in fasted state simulated intestinal fluid (FaSSIF)[19] (16 μg/mL) and moderate mouse liver microsomal clearance (110 μL/min/mg), presumably due to high lipophilicity[20] (log*D* = 4.0 at pH 7.4) (Table 3). Further optimization of **22** to improve the solubility and the metabolic stability by introducing solubilizing groups led us eventually to identify **32f** and **32g**. We first tried to improve them by modifying the methoxy group on B phenyl ring (Table 5). An abrupt loss of activity was, however, observed with solubilizing groups (**31c–e**) as well as with substituents such as ethoxy (**31a**) or *n*-propoxy (**31b**) groups, suggesting that substituents at the position of the methoxy motif fit in a space limited in size. We explored the idea of introducing a solubilizing group at amide nitrogen. Amides **32a–c** were first prepared to test whether the modification of the amide moiety is tolerable. Mono-substituted amides **32a** and **32c** maintained antiproliferative activity against and selectivity to HUVEC, while *N*,*N*-dimethyl amide **32b** had diminished activity suggesting that a hydrogen donor is necessary for a potent inhibition of HUVEC proliferation. This observation is consistent with that of the R^4 on benzyl phenyl ether. Introduction of hydroxylated alkyl groups at amide nitrogen, as illustrated by ethanol **32d**, 1,2-propanediols **32e–g**, and 1,3-propanediol **32h** had moderate to good levels of antiproliferative activity against HUVEC (IC_{50} = 0.25–1.5 μM). Among them, 1,2-propanediols **32e–g** improved the solubility (317, 225, and 263 μg/mL in FaSSIF, respectively) and showed good stability in mouse liver microsomes (10, 7, and 12 μL/min/mg, respectively) while keeping antiproliferative activity against HUVEC and high selectivity (IC_{50} ratio of HCT116 to HUVEC = 30, 27, and 84, respectively). Chirality of 1,2-propanediols (**32f** and **32g**) did not affect antiproliferative activity against either HUVEC or HCT116. From these results, chiral **32f** and **32g** were selected for intensive in vitro and in vivo profiling.

3.3. Biological evaluation

As an indicator of in vitro antiangiogenic activity, the effect of **32f** and **32g** on tube formation was evaluated using an Angiogenesis Kit

Table 5
In vitro structure–activity relationships on B phenyl ring of 3-[(*E*)-styryl]benzamides



Compd	Substituents			Cell growth inhibition/IC_{50} (μM)		Selectivity[a]	Solubility in FaSSIF[b] (μg/mL)	Microsomal clearance[c] (μL/min/mg)
	R^1	R^2	R^3	HUVEC	HCT116	HCT116/HUVEC		
31a	Et	H	H	0.19	1.7	8.9	10	123
31b	*n*-Pr	H	H	1.3	>50	38	4	83
31c	$HO(CH_2)_2O(CH_2)_2$–	H	H	11	38	1.5	74	247
31d		H	H	8.4	4.9	0.6	109	288
31e		H	H	9.5	7.5	0.8	164	251
32a	Me	Me	H	0.19	16	84	24	331
32b	Me	Me	Me	2.5	35	14	ND[d]	ND[d]
32c	Me	Et	H	0.16	15	94	9	187
32d	Me	$(CH_2)_2OH$	H	0.91	17	19	16	73
32e	Me		H	0.40	12	30	317	10
32f	Me		H	0.48	13	27	225	7
32g	Me		H	0.25	21	84	263	12
32h	Me		H	1.5	14	9.3	251	8

[a] IC_{50} ratio of HCT116 to HUVEC.
[b] FaSSIF: fasted state simulated intestinal fluid.
[c] Clearance of test compound when incubated with mouse liver microsomes.
[d] Not determined.

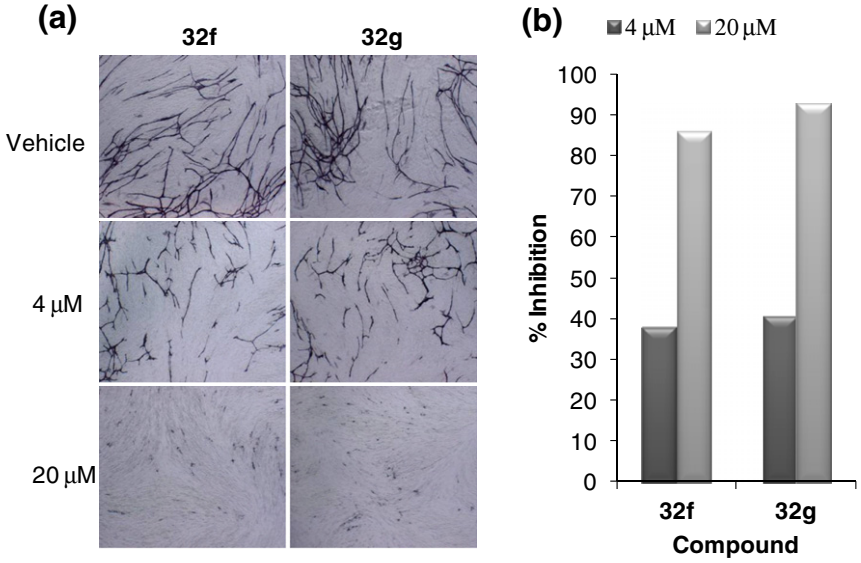


Figure 3. Inhibition of tube formation by **32f** and **32g** in a HUVEC and fibroblast co-culture system. (a) Images of **32f** and **32g** at concentrations of 0, 4, and 20 μM. (b) Percentage inhibition by **32f** and **32g** in a tube formation assay.

(Kurabo Industries Ltd.) which is composed of a co-culture of HUVEC and fibroblasts.[21] As shown in Figure 3, the tube formation was strongly inhibited by **32f** and **32g** at the concentrations of 4 and 20 μM (4 μM: 38% and 41%; 20 μM: 86% and 93% for **32f** and **32g**, respectively). No morphological damage of normal fibroblast cells was observed at either concentration. Both compounds also

Table 6
Cell growth inhibition of **32f** and **32g** against various cell lines

Cell line	Tumor type	IC_{50}/μM (selectivity[a])		Cell line	Tumor type	IC_{50}/μM (Selectivity[a])	
		32f	**32g**			**32f**	**32g**
HUVEC	Colon	0.48 (1)	0.25 (1)	DU145	Prostate	5.3 (11)	23 (92)
HCT116	Colon	13 (27)	27 (108)	PC3	Prostate	24 (50)	17 (68)
WiDr	Colon	7 (15)	15 (60)	22Rv1	Prostate	8.6 (18)	21 (84)
COLO205	Colon	18 (38)	18 (72)	AsPC-1	Pancreas	55 (115)	63 (252)
COLO320DM	Colon	46 (96)	61 (244)	Capan-1	Pancreas	47 (98)	67 (268)
DLD1	Colon	19 (40)	28 (112)	BxPC-3	Pancreas	27 (56)	51 (204)
HT29	Colon	10 (21)	16 (64)	Panc-1	Pancreas	39 (81)	49 (196)
HCT15	Colon	20 (42)	29 (116)	MKN45	Gastric	5.7 (12)	26 (104)
QG56	Lung	23 (48)	21 (84)	MKN-28	Gastric	15 (31)	23 (92)
NCI-H460	Lung	15 (31)	24 (96)	NCI-N87	Gastric	52 (108)	52 (208)
NCI-H460-PTX250	Lung	19 (40)	30 (120)	Huh-7	Liver	29 (60)	27 (108)
A549	Lung	13 (27)	18 (72)	Hep G2	Liver	16 (33)	28 (112)
Calu-6	Lung	40 (83)	54 (216)	HuH-1	Liver	30 (63)	30 (120)
NCI-H441	Lung	25 (52)	34(136)	C32	Melanoma	38 (79)	46 (184)
MDA-MB-231	Breast	39 (81)	38 (152)	10C9	Leukemia	38 (79)	50 (200)
KPL-4	Breast	4.9 (10)	15 (60)	CCRF-CEM	Leukemia	20 (42)	30 (120)
T47D	Breast	4.5 (9)	7.2 (29)	CEM/C2	Leukemia	30 (63)	30 (120)
MDA-MB-435s	Breast	74 (154)	69 (276)	K562	Leukemia	29 (60)	36 (144)
MCF7	Breast	65 (135)	63 (252)	JOK-1	Leukemia	31 (65)	38 (152)
IGROV1	Ovarian	0.92 (2)	1.4 (6)	KG-1a	Leukemia	17 (35)	20 (80)
IGROV1/T8	Ovarian	9.5 (20)	12 (48)				

[a] IC_{50} ratio of cancer cell line to HUVEC.

Table 7
Oral pharmacokinetic parameters in nude mice[a]

Compd	CL (mL/min/Kg)[b]	t_{max} (h)	C_{max} (ng/mL)	$t_{1/2}$ (h)	$AUC_{0-24\ h}$ (ng·h/mL)	*F* (%)
32f	5.8	1.0	8270	7.8	86400	100
32g	5.6	1.0	9370	8.5	90200	90[c]

[a] Compounds **32f** and **32g** were dosed at 30 mg/kg.
[b] CL/*F* (mL/min/kg).
[c] Oral bioavailability when **32g** was dosed at 10 mg/kg.

exhibit less antiproliferative activity against 40 cancer cell lines than against HUVEC (Table 6). These results indicate that **32f** and **32g** suppressed angiogenesis in vitro without showing cytotoxicity. Additional in vitro profiling revealed that **32f** and **32g** showed no inhibitory activity against 25 kinases (IC_{50} >50 μM) including VEGFR-2, platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor 2 (FGFR2), whose activities are related to angiogenesis (see Supplementary data). This result implies a different mode of action from that of kinase inhibitors.

Pharmacokinetic parameters in nude mice of **32f** and **32g** are shown in Table 7. Both compounds had low clearance (5.6–5.8 mL/min/kg) and high bioavailability (*F* = 90–100%), resulting in sustained exposure (AUC = 86400 and 90200 ng·h/mL for **32f** and **32g**, respectively) after single 30 mg/kg oral dosing.

The in vivo antitumor activity of **32f** and **32g** was evaluated in the Calu-6 xenograft model (Fig. 4). Once-daily oral administration of the compounds for 11 consecutive days inhibited the growth of tumor in a dose-dependent manner in the range of 1 to 10 mg/kg without apparent body weight loss (Fig. 4a and b). From the results shown in Figure 4a, the ED_{50} values of **32f** and **32g** for tumor growth inhibition were determined as 3.2 and 2.3 mg/kg, respectively. MVD in Calu-6 xenograft tissue was determined by immunohistochemistry 24 hours after the final administration. The result demonstrated a significant decrease of MVD in the tissue as compared to control (Fig. 4c). As shown in Table 6, **32f** and **32g** showed weak antiproliferative activity against the Calu-6 cancer cell line (IC_{50} = 40 and 54 μM for **32f** and **32g**, respectively). Taken together, these results demonstrate that **32f** and **32g** showed growth inhibition of the xenografted tumor through antiangiogenic activity.

As expected from the fact that **32f** has no VEGFR-2 inhibitory activity, **32f** in combination with sunitinib, a multi-kinase inhibitor with potent VEGFR-2 inhibition, enhanced the antitumor activity that either compound was capable of alone (Fig. 5). Add-on was possible to maximum tolerated dose (MTD) (80 mg/kg) of sunitinib without body weight loss. This result suggests that **32f** may be used for combination therapy with other antitumor agents including VEGFR inhibitors.

4. Conclusion

The orally available 3-[(*E*)-styryl]benzamides **32f** and **32g** have been shown to be potent HUVEC proliferation inhibitors both in vitro and in vivo. These compounds exemplify a unique type of angiogenesis inhibitor that specifically inhibits endothelial cell proliferation and does not show cytotoxicity or inhibition of angiogenesis-related kinases. In the lead generation and optimization stages, proof-of-concept confirmation of antiangiogenic activity in the animal model via oral administration prompted the identification of these unique compounds. By reason of their potent in vivo efficacy and their safety, **32f** and **32g** appear to have major potential for use in cancer treatment. The studies for clarifying the molecular targets are currently underway in our laboratories.

5. Experimental

5.1. Chemistry: instruments

Commercially available reagents and solvents were used without further purification. Reactions were monitored by TLC on silica

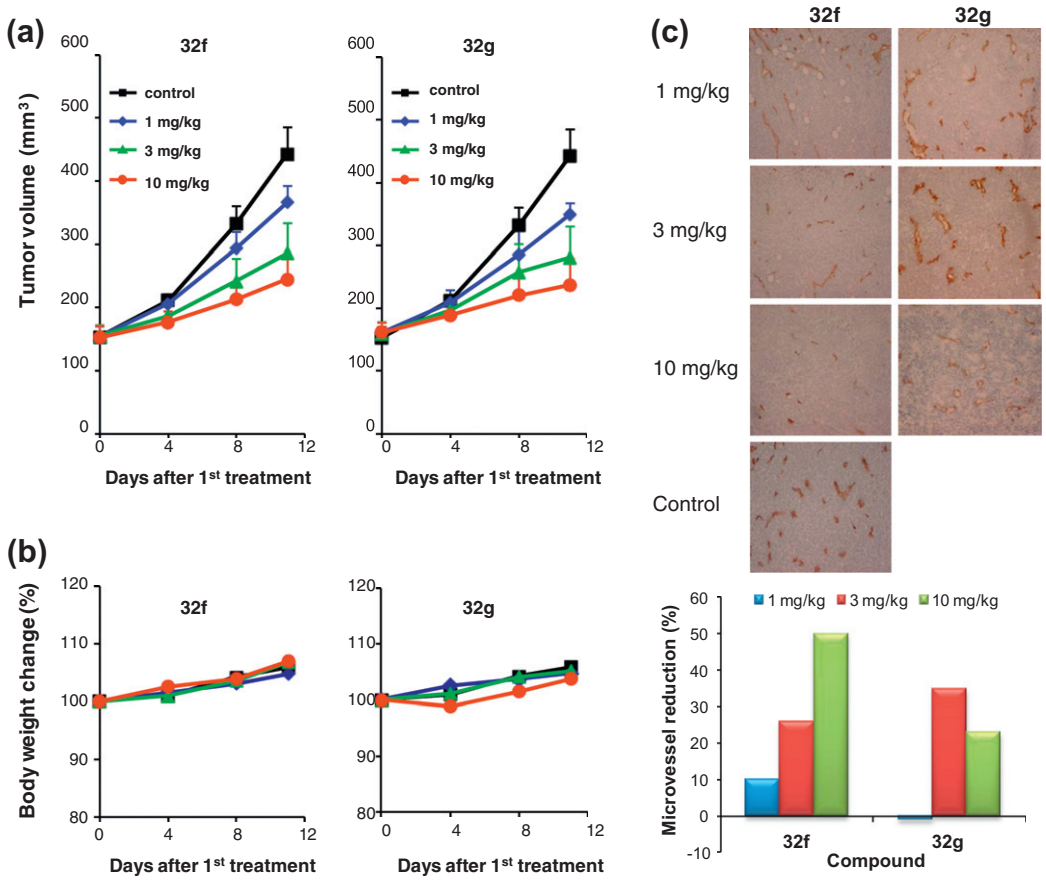


Figure 4. (a) In vivo antitumor activity in the Calu-6 xenograft model. Compounds **32f** and **32g** were orally administered once a day for 11 days at 1, 3, and 10 mg/kg to tumor bearing Balb/c nude mice. (b) Body weight change (%). (c) MVD reduction in the Calu-6 xenograft tissue. Photographs are immunohistochemical staining of tumor tissues by anti-mouse CD31 antibody (×10 magnification). Endothelial cells are stained brown.

gel 60 F254 precoated TLC plates (E. Merck) or NH TLC plates (E. Merck). Melting points were determined with a Yanaco MP-S3 apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained at 400 or 500 MHz on a JEOL JNM-ECA400 or a JEOL JNM-A500 spectrometer. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were obtained at 100 or 125 MHz on a JEOL JNM-ECA400 or a JEOL JNM-A500 spectrometer. All NMR chemical shifts are reported as δ values in parts per million (ppm), and coupling constants (J) are given in hertz (Hz). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; br s, broad singlet; br t, broad triplet; m, unresolved multiplet due to the field strength of the instrument; dd, doublet of doublet; dt, doublet of triplet; ddd, doublet of doublet of doublet; dddt, doublet of doublet of doublet of triplet, dtt; doublet of triplet of triplet. Chromatographic separations were carried out on prepacked silica gel columns (KP-Sil) or prepacked basic silica gel columns (KP-NH), supplied by Biotage. Yields are unoptimized. Purity of all final products was determined by LC–MS to be >95%. The LC–MS analyses were performed using a Waters LC–MS instrument equipped with a Waters ACQUITY SQD (ESI mode) and a Waters ACQUITY UPLC instrument. Elution was done with a gradient of 5–100% solvent B in solvent A (solvent A: 0.1% formic acid in water, solvent B: 0.1% of formic acid in acetonitrile) in 1 min followed by 0.4 min at 100% B through a Ascentis® Express C18 column (50 mm 2.1 mm, 2.7 μm particles) at 1 mL·min^{-1}. Area % purity was measured at 254 nm. High-resolution mass spectra (HRMS) were measured with a ThermoFisherScientific LTQ Orbitrap XL spectrometer. Optical rotations were measured at 25 °C on a JASCO DIP-1000 digital polarimeter using a 1.0-cm, 1-mL cell.

5.1.1. General procedure for the synthesis of compounds 4a–c

A mixture of **2a–c** (1.1 equiv), **3a–b** (1 equiv) and K_2CO_3 (1.1 equiv) in DMF was stirred at 80 °C for 3 h. After cooling to room temperature, the mixture was diluted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, n-hexane/EtOAc) to give the desired products **4a–c**.

5.1.2. 1-[4-Methoxy-3-[(3-methylphenoxy)methyl]phenyl]ethanone (4a)

Compound **4a** was obtained from **2c** and **3a** as a colorless oil in 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (3H, s), 2.57 (3H, s), 3.93 (3H, s), 5.09 (2H, s), 6.76–6.89 (3H, m), 6.95 (1H, d, J = 8.7 Hz), 7.18 (1H, t, J = 7.7 Hz), 7.96 (1H, dd, J = 8.7, 2.3 Hz), 8.11 (1H, d, J = 2.3 Hz). MS (ESI$^+$) 271 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{19}O_3$ [M+H]$^+$ 271.1329, found 271.1329.

5.1.3. 1-[3-[(4-Chlorophenoxy)methyl]-4-methoxyphenyl]ethanone (4b)

Compound **4b** was obtained from **2b** and **3a** as a colorless oil in 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.56 (3H, s), 3.94 (3H, s), 5.08 (2H, s), 6.93 (2H, d, J = 9.3 Hz), 6.95 (1H, d, J = 8.8H), 7.23 (2H, d, J = 9.3 Hz), 7.96 (1H, dd, J = 8.8, 2.2 Hz), 8.07 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 291, 293 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{16}ClO_3$ [M+H]$^+$ 291.0782, found 291.0783.

5.1.4. 3-[(4-Chloro-3-methylphenoxy)methyl]benzonitrile (4c)

Compound **4c** was obtained from **2a** and **3b** as a colorless powder in 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (3H, s), 5.05 (2H,

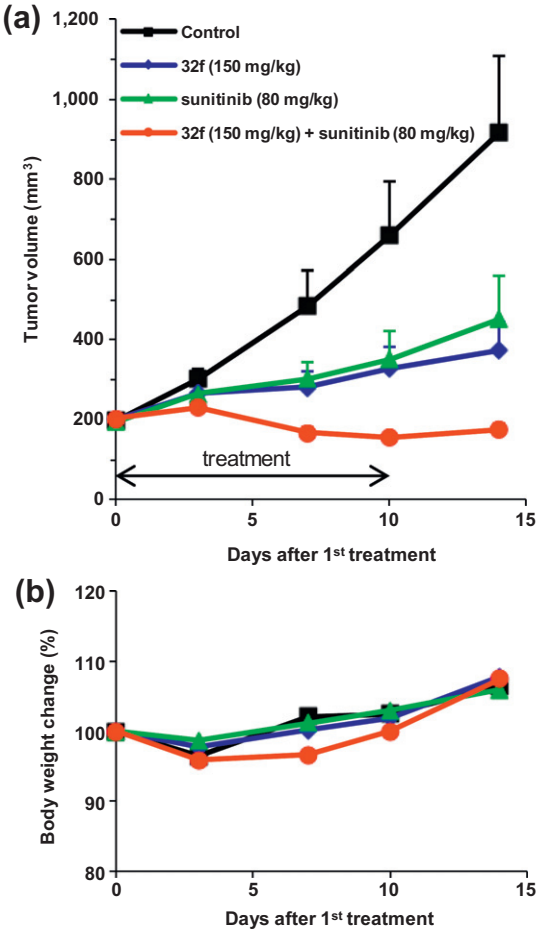


Figure 5. (a) In vivo antitumor activity of **32f** in the Calu-6 xenograft model in combination with sunitinib. Compound **32f** (150 mg/kg) and sunitinib (80 mg/kg) were orally administered once a day for 11 days to tumor bearing Balb/c nude mice. (b) Body weight change (%).

s), 6.72 (1H, dd, J = 8.7, 3.0 Hz), 6.85 (1H, d, J = 3.0 Hz), 7.24 (1H, d, J = 8.7 Hz), 7.50 (1H, dd, J = 7.7, 7.6 Hz), 7.61–7.66 (2H, m), 7.73 (1H, s). MS (ESI$^-$) 256, 258 (Cl isotope) [M−H]$^-$. HRMS (ESI$^-$) calcd for $C_{15}H_{11}ClNO$ [M−H]$^-$ 256.0524, found 256.0530.

5.1.5. 1-Chloro-4-[(5-ethyl-2-methoxyphenyl)methoxy]-2-methylbenzene (5)

To a solution of **1** (50 mg, 0.16 mmol) in TFA (1 mL) was added Et_3SiH (320 μL, 2.00 mmol) and the mixture was stirred for 6 h. The reaction mixture was concentrated in vacuo and the residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 10:1) to give **5** (17 mg, 36%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.17 (3H, t, J = 7.6 Hz), 2.48 (3H, s), 2.53 (2H, q, J = 7.6 Hz), 3.90 (3H, s), 3.97 (2H, s), 6.71 (1H, d, J = 8.7 Hz), 6.74 (1H, s), 6.83 (1H, d, J = 8.3 Hz), 6.99 (1H, d, J = 2.2 Hz), 7.03 (1H, dd, J = 8.3, 2.2 Hz), 7.13 (1H, d, J = 8.7 Hz). MS (ESI$^-$) 289, 291 (Cl isotope) [M−H]$^-$. HRMS (ESI$^-$) calcd for $C_{17}H_{16}ClO_2$ [M−H]$^-$ 289.0990, found 289.0989.

5.1.6. Ethyl 3-(chloromethyl)-4-methoxybenzoate (7)

To a solution of **6** (28.0 mL, 172 mmol) and methoxymethyl chloride (26.0 mL, 342 mmol) in CH_2Cl_2 (500 mL) at 0 °C was added $SnCl_4$ (10 mL, 85.5 mmol) dropwise over 15 min. The reaction mixture was stirred for 5 h at 0 °C and then poured into water (1 L). The organic layer was separated and the aqueous layer was extracted twice with CH_2Cl_2 (200 mL). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was recrystallized from *n*-hexane/EtOAc (30:1) to give **7** (23.8 g, 60%) as colorless plates. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, J = 7.0 Hz), 3.95 (3H, s), 4.36 (2H, q, J = 7.0 Hz), 4.66 (2H, s), 6.92 (1H, d, J = 8.3 Hz), 8.03 (1H, dd, J = 8.3, 2.1 Hz), 8.05 (1H, d, J = 2.1 Hz). MS (ESI$^+$) 229, 231 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{11}H_{14}ClO_3$ [M+H]$^+$ 229.0626, found 229.0628.

5.1.7. Ethyl 3-[(4-Chloro-3-methylphenoxymethyl)]-4-methoxybenzoate (8a)

A mixture of **7** (4.00 g, 17.5 mmol), **2a** (2.74 g, 19.2 mmol) and K_2CO_3 (2.66 g, 19.3 mmol) in DMF (40 mL) was stirred at 80 °C for 13 h. After cooling to room temperature, the solvent was removed in vacuo. The residue was diluted with EtOAc and the insoluble solid was filtered and the cake was washed with EtOAc. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 10:1 to 5:1) to give **8a** (5.91 g, quant.) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (3H, t, J = 7.1 Hz), 2.35 (3H, s), 3.92 (3H, s), 4.35 (2H, q, J = 7.1 Hz), 5.05 (2H, s), 6.78 (1H, dd, J = 8.7, 2.9 Hz), 6.90 (1H, d, J = 2.9 Hz), 6.93 (1H, d, J = 8.7 Hz), 7.23 (1H, d, J = 8.7 Hz), 8.03 (1H, dd, J = 8.7, 2.2 Hz), 8.14 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 335, 337 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{18}H_{20}ClO_4$ [M+H]$^+$ 335.1045, found 335.1042.

5.1.8. Ethyl 3-[(4-chlorophenoxy)methyl]-4-methoxybenzoate (8b)

Compound **8b** was prepared in a manner similar to that described for **8a** in quantitative yield as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.38 (3H, t, J = 7.1 Hz), 3.92 (3H, s), 4.35 (2H, q, J = 7.1 Hz), 5.06 (2H, s), 6.93 (1H, d, J = 8.5 Hz), 6.94 (2H, d, J = 9.3 Hz), 7.24 (2H, d, J = 9.3 Hz), 8.03 (1H, dd, J = 8.5, 2.2 Hz), 8.14 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 321, 323 (Cl isotope) [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{17}H_{16}ClO_4$ [M−H]$^-$ 319.0732, found 319.0734.

5.1.9. 3-[(4-Chloro-3-methylphenoxymethyl)]-4-methoxybenzoic acid (9a)

To a solution of **8a** (5.86 g, 17.5 mmol) in MeOH (50 mL) was added 20% aqueous solution of KOH (20 mL) and the mixture was stirred for 1 h under reflux. The reaction mixture was diluted in water (100 mL) and then cooled to 0 °C. The reaction mixture was adjusted to pH of 4 by addition of 37% aqueous solution of HCl. The resulting precipitate was filtered, washed with water to give **9a** (5.17 g, 96%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.29 (3H, s), 3.90 (3H, s), 5.07 (2H, s), 6.85 (1H, dd, J = 8.8, 3.1 Hz), 7.04 (1H, d, J = 3.1 Hz), 7.15 (1H, d, J = 8.8 Hz), 7.29 (1H, d, J = 8.8 Hz), 7.93 (1H, dd, J = 8.8, 2.2 Hz), 7.96 (1H, d, J = 2.2 Hz), 12.69 (1H, br s). MS (ESI$^+$) 307, 309 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{16}ClO_4$ [M+H]$^+$ 307.0732, found 307.0731.

5.1.10. 3-[(4-Chlorophenoxy)methyl]-4-methoxybenzoic acid (9b)

Compound **9b** was prepared in a manner similar to that described for **9a** in 99% yield as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.90 (3H, s), 5.09 (2H, s), 7.03 (2H, d, J = 9.1 Hz), 7.16 (1H, d, J = 8.5 Hz), 7.34 (2H, d, J = 9.1 Hz), 7.95 (1H, dd, J = 8.5, 2.2 Hz), 7.96 (1H, d, J = 2.2 Hz), 12.67 (1H, s). MS (ESI$^+$) 293, 295 (Cl isotope) [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{15}H_{12}ClO_4$ [M−H]$^-$ 291.0419, found 291.0420.

5.1.11. 3-[(4-Chloro-3-methylphenoxy)methyl]-4-methoxybenzamide (10a)

To a solution of **9a** (5.12 g, 16.7 mmol), NH_4Cl (1.34 g, 25.1 mmol), and 1-hydroxybenzotriazole (HOBT) (3.07 g,

20.0 mmol) in DMF (50 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (3.84 g, 20.0 mmol) and *N*,*N*-diisopropylethylamine (DIPEA) (8.7 mL, 49.9 mmol). The mixture was stirred at room temperature for 23 h and concentrated in vacuo. The residue was diluted with EtOAc. The organic layer was washed with a saturated aqueous solution of $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 1:1 to EtOAc) to give **10a** (4.48 g, 88%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.30 (3H, s), 3.88 (3H, s), 5.04 (2H, s), 6.85 (1H, dd, J = 8.8, 3.0 Hz), 7.04 (1H, d, J = 3.0 Hz), 7.10 (1H, d, J = 8.8 Hz), 7.21 (1H, br s), 7.30 (1H, d, J = 8.8 Hz), 7.87 (1H, br s), 7.89 (1H, dd, J = 8.8, 2.2 Hz), 7.95 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 306, 308 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{17}ClNO_3$ [M+H]$^+$ 306.0891, found 306.0889.

5.1.12. 3-[(4-Chlorophenoxy)methyl]-4-methoxybenzamide (10b)

Compound **10b** was prepared in a manner similar to that described for **10a** in 96% yield as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.88 (3H, s), 5.05 (2H, s), 7.04 (2H, d, J = 9.1 Hz), 7.11 (1H, d, J = 8.8 Hz), 7.22 (1H, br s), 7.34 (2H, d, J = 9.1 Hz), 7.88 (1H, br s), 7.90 (1H, dd, J = 8.8, 2.2 Hz), 7.94 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 292, 294 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{15}H_{15}ClNO_3$ [M+H]$^+$ 292.0735, found 292.0732.

5.1.13. Methyl 3-[(4-chloro-3-methylphenoxy)methyl]-4-methoxybenzoate (11)

To a solution of **9a** (20 mg, 0.065 mmol) in MeOH (1 mL) and toluene (3.5 mL) was added 10% $TMSCHN_2$ in hexane solution (0.34 mL, 0.21 mmol) and the mixture was stirred for 2.5 h at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 20:1) to give **11** (20 mg, 95%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (3H, s), 3.89 (3H, s), 3.93 (3H, s), 5.05 (2H, s), 6.78 (1H, dd, J = 8.8, 2.8 Hz), 6.90 (1H, d, J = 2.8 Hz), 6.94 (1H, d, J = 8.8 Hz), 7.23 (1H, d, J = 8.8 H z), 8.03 (1H, dd, J = 8.8, 2.0 Hz), 8.14 (1H, d, J = 2.0 Hz). MS (ESI$^+$) 321, 323 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{18}ClO_4$ [M+H]$^+$ 321.0888, found 321.0887.

5.1.14. 3-[(4-Chloro-3-methylphenoxy)methyl]-4-methoxybenzonitrile (12)

To a solution of **10a** (20 mg, 0.066 mmol) in CH_3CN (1 mL) at room temperature were successively added formic acid (0.25 mL) and paraformaldehyde (10 mg, 0.34 mmol). The reaction mixture was then refluxed for 12 h, and the resulting solution was cooled to room temperature. The crude mixture was concentrated in vacuo and the residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 15:1 to 1:1) to give **12** (15 mg, 77%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36 (3H, s), 3.94 (3H, s), 5.04 (2H, s), 6.75 (1H, dd, J = 8.8, 3.0 Hz), 6.87 (1H, d, J = 3.0 Hz), 6.96 (1H, d, J = 8.6 Hz), 7.24 (1H, d, J = 8.8 Hz), 7.62 (1H, dd, J = 8.6, 2.1 Hz), 7.77 (1H, d, J = 2.1 Hz). MS (ESI$^-$) 286, 288 (Cl isotope) [M−H]$^-$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}ClNO_2$ [M+H]$^+$ 288.0786, found 288.0786.

5.1.15. Ethyl 3-formyl-4-hydroxybenzoate (14)

To a solution of **13** (30.0 g, 197 mmol) and Et_3N (165 mL, 1.18 mol) in 1,2-dichloroethane was added $MgCl_2$ (93.9 g, 986 mmol) and the mixture was stirred for 1 h at 40 °C. Then paraformaldehyde (59.2 g, 1.97 mol) was added and the reaction mixture was stirred for 3 h at 70 °C. After cooling to 0 °C, 1 M HCl (1000 mL) was added. The mixture was filtered, and washed with CH_2Cl_2 (500 mL). The organic layer was separated, washed with 1 M HCl (500 mL) and brine (500 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude residue was recrystallized from MeOH-water (3:4) to give **14** (27.0 g, 76%) as colorless prisms. Melting point 67–68 °C (lit.[22] 69–70 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 4.39 (2H, q, J = 7.1 Hz), 7.03 (1H, d, J = 8.8 Hz), 8.19 (1H, dd, J = 8.8, 2,0 Hz), 8.31 (1H, d, J = 2.0 Hz), 9.95 (1H, s), 11.38 (1H, s). MS (ESI$^+$) 195 [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{10}H_9O_4$ [M−H]$^-$ 193.0495, found 193.0496.

5.1.16. Ethyl 3-formyl-4-methoxybenzoate (15)

To a solution of **14** (72.0 g, 371 mmol) in acetone were added K_2CO_3 (61.5 g, 445 mmol) and Me_2SO_4 (58.5 g, 463 mmol). The mixture was stirred for 1 h under reflux. After cooling to room temperature, the insoluble solid was filtered and the cake was washed with EtOAc (500 mL). The filtrate was diluted with EtOAc (500 mL) and saturated aqueous solution of $NaHCO_3$ (400 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (1000 mL × 2). The organic layers were combined, washed with brine (300 mL), dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The residue was recrystallized from acetonitrile–water (5:4) to give **15** (66.7 g, 86%) as colorless needles. Melting point 77–79 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.39 (3H, t, J = 7.1 Hz), 4.00 (3H, s), 4.37 (2H, q, J = 7.1 Hz), 7.04 (1H, d, J = 8.8 Hz), 8.25 (1H, dd, J = 8.8, 2.5 Hz), 8.49 (1H, d, J = 2.5 Hz), 10.45 (1H, s). MS (ESI$^+$) 209 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{11}H_{13}O_4$ [M+H]$^+$ 209.0808, found 209.0809.

5.1.17. 5-Ethoxycarbonyl-2-methoxybenzoic acid (16)

To a solution of **15** (500 mg, 2.40 mmol), NaH_2PO_4 (288 mg, 2.40 mmol), 2-methyl-2-butene (1.12 mL, 10.6 mmol) in *tert*-BuOH (15 mL) and water (4 mL) was added $NaClO_2$ (739 mg, 8.17 mmol) and the mixture was stirred for 50 min at room temperature. The reaction mixture was adjusted to pH of 4 by addition of 1 M HCl. The aqueous layer was extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **16** as a colorless powder (536 mg, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.32 (3H, t, J = 7.1 Hz), 3.91 (3H, s), 4.30 (2H, q, J = 7.1 Hz), 7.26 (1H, d, J = 8.8 Hz), 8.09 (1H, dd, J = 8.8, 2.2 Hz), 8.23 (1H, d, J = 2.2 Hz), 12.96 (1H, br s). MS (ESI$^+$) 225 [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{11}H_{11}O_5$ [M−H]$^-$ 223.0601, found 223.0604.

5.1.18. Ethyl 3-[(4-chlorophenyl)carbamoyl]-4-methoxybenzoate (17)

To a mixture of **16** (101 mg, 0.50 mmol) in CH_2Cl_2 (5 mL) and DMF (2 μL) at room temperature was added oxalyl chloride (59 μL, 0.68 mmol) and the mixture was stirred for 2.5 h at room temperature. The reaction mixture was concentrated in vacuo to give ethyl 3-chlorocarbonyl-4-methoxybenzoate (109 mg, quant.) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 4.00 (3H, s), 4.40 (2H, q, J = 7.1 Hz), 7.05 (1H, d, J = 8.8 Hz), 8.26 (1H, dd, J = 8.8, 2.2 Hz), 8.75 (1H, d, J = 2.2 Hz). To a solution of 4-chloroaniline (63 mg, 0.50 mmol) and DIPEA (235 μL, 1.35 mmol) in CH_2Cl_2 (2 mL) at room temperature was added a solution of ethyl 3-chlorocarbonyl-4-methoxybenzoate (109 mg, 0.50 mmol) in CH_2Cl_2 (3 mL) and the mixture was stirred for 1 h at room temperature. The reaction mixture was washed with saturated aqueous solution of NH_4Cl and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, *n*-hexane/CH_2Cl_2 = 1:1 to CH_2Cl_2) to give **17** (114 mg, 76%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.40 (3H, t, J = 7.1 Hz), 4.13 (3H, s), 4.38 (2H, q, J = 7.1 Hz), 7.10 (1H, d, J = 8.8 Hz), 7.33 (2H, dd, J = 8.8 Hz), 7.64 (2H, d, J = 8.8 Hz), 8.21 (1H, dd, J = 8.8, 2.5 Hz), 8.94 (1H, d, J = 2.5 Hz), 9.63 (1H, br s). MS (ESI$^+$) 334, 336 (Cl isotope) [M+H]$^+$.

HRMS (ESI^+) calcd for $C_{17}H_{17}ClNO_4$ $[M+H]^+$ 334.0841, found 334.0836.

5.1.19. 3-[(4-Chlorophenyl)carbamoyl]-4-methoxybenzoic acid (18)

To a suspension of **17** (50 mg, 0.15 mmol) in MeOH (1 mL) was added 20% aqueous solution of KOH (0.4 mL) and the mixture was stirred for 30 min under reflux. After cooling to room temperature, water (2 mL) and 1 M HCl (1.5 mL) was added. The resulting precipitate was filtered, washed with water to give **18** (43 mg, 94%) as a colorless powder. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.95 (3H, s), 7.28 (1H, d, J = 8.8 Hz), 7.41 (2H, d, J = 8.8 Hz), 7.76 (2H, d, J = 8.8 Hz), 8.07 (1H, dd, J = 8.8, 2.2 Hz), 8.12 (1H, d, J = 2.2 Hz), 10.32 (1H, s), 12.91 (1H, s). MS (ESI^+) 306, 308 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{15}H_{13}ClNO_4$ $[M+H]^+$ 306.0528, found 306.0528.

5.1.20. *N*3-(4-Chlorophenyl)-4-methoxybenzene-1, 3-dicarboxamide (19)

To a solution of **18** (46 mg, 0.15 mmol), NH_4Cl (12 mg, 0.23 mmol), and HOBT (28 mg, 0.18 mmol) in DMF (2 mL) were added EDC (35 mg, 0.18 mmol) and DIPEA (79 μL, 10.5 mmol) and the mixture was stirred at room temperature for 17 h. The solvent was removed and the resulting residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH = 50:1 to 20:1) to give **19** (43 mg, 93%) as a colorless powder. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.23 (1H, d, J = 8.8 Hz), 7.29 (1H, br s), 7.40 (2H, d, J=8.8 Hz), 7.76 (2H, d, J = 8.8 Hz), 7.96 (1H, br s), 8.02 (1H, dd, J = 8.8, 2.2 Hz), 8.10 (1H, d, J = 2.2 Hz), 10.32 (1H, s). MS (ESI^+) 305, 307 (Cl isotope) (M+H). HRMS (ESI^+) calcd for $C_{15}H_{14}ClN_2O_2$ $[M+H]^+$ 305.0687, found 305.0686.

5.1.21. Ethyl 3-[2-(4-chlorophenyl)vinyl]-4-methoxybenzoate ((*E*/*Z*)-20)

To a solution of **15** (101 mg, 0.48 mmol) and (4-chlorobenzyl)triphenylphosphonium chloride (215 mg, 0.51 mmol) in EtOH (5 mL) was added 20% ethanol solution of NaOEt (283 μL, 0.72 mmol). The mixture was stirred for 1 h at room temperature. 1 M HCl (0.8 mL) was added, and the mixture was extracted with EtOAc. The organic layer was separated, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 50:1 to 5:1) to give a colorless oil **(*E*/*Z*)-20** (141 mg, 92%) as a mixture of *E*/*Z* isomers in 1:2 ratio. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.29 (3H, t, J = 7.1 Hz), 1.41 (1.5H, t, J = 7.1 Hz), 3.84 (3H, s), 3.95 (1.5H, s), 4.25 (2H, q, J = 7.1 Hz), 4.38 (1H, q, J = 7.1 Hz), 6.62 (1H, d, J = 12.4 Hz), 6.66 (1H, d, J = 12.4 Hz), 6.89–6.93 (1.5H, m), 7.11–7.17 (4.5H, m), 7.32 (1H, d, J = 8.5 Hz), 7.41 (0.5H, d, J = 16.5 Hz), 7.47 (1H, d, J = 8.5 Hz), 7.83 (1H, d, J = 2.2 Hz), 7.94 (1H, dd, J = 8.5, 2.2 Hz), 7.96 (0.5H, d, J = 8.8, 2.2 Hz), 8.26 (0.5H, d, J = 2.2 Hz). MS (ESI^+) 317, 319 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{18}H_{18}ClO_3$ $[M+H]^+$ 317.939, found 317.936.

5.1.22. 3-[2-(4-chlorophenyl)vinyl]-4-methoxybenzoic acid ((*E*/*Z*)-21)

To a solution of **(*E*/*Z*)-20** (131 mg, 0.41 mmol) in MeOH (1.3 mL) was added 20% aqueous solution of KOH (0.26 mL) and the mixture was stirred for 2 h under reflux. After cooling to room temperature, 1 M HCl (1.5 mL) was added. The resulting precipitate was filtered, washed with water to give a colorless powder **(*E*/*Z*)-21** (113 mg, 95%) as a mixture of *E*/*Z* isomers in 1:2 ratio. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.84 (3H, s), 3.94 (1.5H, s), 6.63 (1H, d, J = 12.6 Hz), 6.66 (1H, d, J = 12.6 Hz), 7.13 (1H, d, J = 8.8 Hz), 7.14 (0.5H, d, J = 8.8 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.29 (2H, d, J = 8.5 Hz), 7.30 (0.5H, d, J = 16.5 Hz), 7.43 (1H, d, J = 8.5 Hz), 7.43 (0.5H, d, J = 16.5 Hz), 7.64 (1H, d, J = 2.2 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.85 (1H, dd, J = 8.8, 2.2 Hz), 7.88 (0.5H, dd, J = 8.8, 2.2 Hz), 8.21 (0.5H, d, J = 2.2 Hz), 12.7 (1.5H, br s). MS (ESI^+) 289, 291 (Cl isotope) $[M+H]^+$. HRMS (ESI^-) calcd for $C_{16}H_{12}ClO_3$ $[M+H]^+$ 287.0469, found 287.0469.

5.1.23. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-methoxybenzamide (22) and 3-[(*Z*)-2-(4-Chlorophenyl)vinyl]-4-methoxybenzamide (23)

To a solution of **(*E*/*Z*)-21** (96 mg, 0.33 mmol), NH_4Cl (27 mg, 0.51 mmol), and HOBT (51 mg, 0.33 mmol) in DMF (1.3 mL) were added EDC (96 mg, 0.50 mmol) and DIPEA (173 μL, 1.00 mmol). The mixture was stirred at room temperature for 16 h. The reaction mixture was diluted with EtOAc. The organic layer was washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 7:1 to EtOAc) to give **22** (31 mg, 33%) as a colorless powder and **23** (59 mg, 62%) as a colorless powder. Compound **22**: ^1H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.10 (1H, d, J = 8.8 Hz), 7.26 (1H, br s), 7.29 (1H, d, J = 16.5 Hz), 7.42 (1H, d, J = 16.5 Hz), 7.43 (2H, d, J = 8.5 Hz), 7.60 (2H, d, J = 8.5 Hz), 7.82 (1H, dd, J = 8.8, 2.2 Hz), 7.91 (1H, br s), 8.20 (1H, d, J = 2.2 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.8, 110.8, 123.1, 124.5, 125.7, 126.4, 127.9, 128.0, 128.6, 128.7, 131.8, 136.0, 158.5, 167.2. MS (ESI^+) 288, 290 (Cl isotope) $[M+H]^+$. HRMS calcd for $C_{16}H_{15}ClNO_2$ $[M+H]^+$ 288.0786, found 288.0787. Compound **23**: ^1H NMR (400 MHz, $CDCl_3$) δ: 3.86 (3H, s), 5.48 (2H, br s), 6.62 (1H, d, J = 12.1 Hz), 6.66 (1H, d, J = 12.1 Hz), 6.94 (1H, d, J = 8.8 Hz), 7.13 (2H, d, J = 8.8 Hz), 7.17 (2H, d, J = 8.8 Hz), 7.51 (1H, d, J = 2.2 Hz), 7.80 (1H, dd, J = 8.8, 2.2 Hz). MS (ESI^+) 288, 290 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{16}H_{15}ClNO_2$ $[M+H]^+$ 288.0786, found 288.0780.

5.1.24. Ethyl 3-(diethoxyphosphorylmethyl)-4-methoxybenzoate (24)

A mixture of **7** (25.0 g, 109 mmol) and triethyl phosphite (26.2 mL, 153 mmol) was stirred at 160 °C for 2.5 h. The reaction mixture was cooled to room temperature, and excess triethyl phosphite was removed by distillation to give **24** (34.2 g, 95%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.26 (6H, t, J = 7.1 Hz), 1.37 (3H, t, J = 7.1 Hz), 3.26 (2H, d, J = 21.4 Hz), 3.90 (3H, s), 4.04 (4H, quintet, J = 7.1 Hz), 4.33 (2H, q, J = 7.1 Hz), 6.88 (1H, d, J = 8.5 Hz), 7.94 (1H, ddd, J = 8.5, 2.2, 2.2 Hz), 7.98 (1H, dd, J = 2.2 Hz). MS (ESI^+) 331 $[M+H]^+$. HRMS (ESI^+) calcd for $C_{15}H_{24}O_6P$ $[M+H]^+$ 331.1305, found 331.1297.

5.1.25. 3-(Diethoxyphosphorylmethyl)-4-methoxybenzoic acid (25)

To a solution of **24** (2.01 g, 6.08 mmol) in MeOH (40 mL) was added 5 M NaOH aqueous solution (7.2 mL, 36.0 mmol), and the mixture was stirred at 60 °C for 1.5 h. After cooling to room temperature, the solvent was removed in vacauo. The residue was diluted with water and washed with diethyl ether. The aqueous solution was separated, acidified with 1 M HCl solution and extracted with EtOAc. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give **25** (1.76 g, 96%) as a colorless powder. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.16 (6H, t, J = 7.1 Hz), 3.22 (2H, d, J = 21.4 Hz), 3.86 (3H, s), 3.93 (4H, quintet, J = 7.1 Hz), 7.07 (1H, d, J = 8.8 Hz), 7.83 (1H, ddd, J = 8.8, 2.2, 2.2 Hz), 7.85 (1H, dd, J = 2.2, 2.2 Hz), 12.61 (1H, s). MS (ESI^+) 303 $[M+H]^+$. HRMS (ESI^+) calcd for $C_{13}H_{20}O_6P$ $[M+H]^+$ 303.0992, found 303.0985.

5.1.26. 3-(Diethoxyphosphorylmethyl)-4-methoxybenzamide (26)

To a solution of **25** (1.65 g, 5.45 mmol), NH_4Cl (439 mg, 8.21 mmol), and HOBT (838 mg, 5.45 mmol) in DMF (27 mL) were

added EDC (1.57 g, 8.18 mmol) and DIPEA (2.85 mL, 16.4 mmol). The mixture was stirred at room temperature for 20 h and concentrated in vacuo. The residue was diluted with an aqueous solution of NH_4Cl and extracted with EtOAc. The extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (basic-SiO_2, CH_2Cl_2/MeOH = 50:1 to 5:1) to give **26** (1.02 g, 62%) as a colorless powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.15 (6H, t, J = 7.1 Hz), 3.17 (2H, d, J = 22.0 Hz), 3.84 (3H, s), 3.92 (4H, quintet, J = 7.1 Hz), 7.02 (1H, d, J = 8.8 Hz), 7.15 (1H, br s), 7.76–7.82 (3H, m). MS (ESI$^+$) 302 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{13}H_{21}NO_5P$ [M+H]$^+$ 302.1152, found 302.1146.

5.1.27. General procedure for the synthesis of compounds 27a–u

To a solution of benzaldehydes (1.2 equiv) and **26** (1.0 equiv) in DMF was added sodium *tert*-pentoxide (2.0 equiv). The mixture was stirred at room temperature for 1 h. Two workup procedures were used. Procedure A: Saturated aqueous solution of NH_4Cl was added to the reaction mixture, and the resulting precipitate was filtered, washed with water, and dried in vacuo to give the desired products **27a–c**, **27e–f**, **27i–l**, **27n–p**, **27r**. Procedure B: The reaction mixture was diluted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude reaction mixture was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc) to give the desired products **27d**, **27g–h**, **27m**, **27q**, **27s–u**.

5.1.28. 4-Methoxy-3-[(*E*)-styryl]benzamide (27a)

The compound **27a** was obtained as a colorless powder according to the general procedure in 94% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.09 (1H, d, J = 8.5 Hz), 7.25 (1H, br s), 7.27–7.31 (2H, m), 7.36–7.43 (3H, m), 7.57 (2H, d, J = 7.7 Hz), 7.82 (1H, dd, J = 8.5, 2.2 Hz), 7.93 (1H, br s), 8.21 (1H, d, J = 2.2 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 55.7, 110.8, 122.7, 124.8, 125.5, 126.2, 126.3, 127.6, 128.5, 128.6, 129.3, 137.1, 158.4, 167.2. MS (ESI$^+$) 254 [M+H]$^+$. HRMS calcd for $C_{16}H_{16}NO_2$ [M+H]$^+$ 254.1176, found 254.1178.

5.1.29. 3-[(*E*)-2-(4-*tert*-Butylphenyl)vinyl]-4-methoxybenzamide (27b)

The compound **27b** was obtained as a colorless powder according to the general procedure in 99% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (9H, s), 3.91 (3H, s), 7.08 (1H, d, J = 8.8 Hz), 7.24 (1H, br s), 7.27 (1H, d, J = 16.8 Hz), 7.36 (1H, d, J = 16.8 Hz), 7.40 (2H, d, J = 8.2 Hz), 7.49 (2H, d, J = 8.2 Hz), 7.80 (1H, dd, J = 8.8, 2.2 Hz), 7.93 (1H, br s), 8.19 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 310 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{20}H_{24}NO_2$ [M+H]$^+$ 310.1802, found 310.1799.

5.1.30. 3-[(*E*)-2-(4-Bromophenyl)vinyl]-4-methoxybenzamide (27c)

The compound **27c** was obtained as a colorless powder according to the general procedure in 92% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.95 (3H, s), 7.09 (1H, d, J = 8.8 Hz), 7.26 (1H, br s), 7.27 (1H, d, J = 16.5 Hz), 7.43 (1H, d, J = 16.5 Hz), 7.53 (2H, d, J = 8.8 Hz), 7.56 (2H, d, J = 8.8 Hz), 7.82 (1H, dd, J = 8.8, 2.2 Hz), 7.91 (1H, br s), 8.20 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 332, 334 (Br isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}BrNO_2$ [M+H]$^+$ 332.0281, found 332.0279.

5.1.31. 4-Methoxy-3-[(*E*)-2-[4-(trifluoromethyl)phenyl]vinyl]benzamide (27d)

The compound **27d** was obtained as a colorless powder according to the general procedure in 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.93 (3H, s), 7.12 (1H, d, J = 8.8 Hz), 7.28 (1H, br s), 7.39 (1H, d, J = 16.4 Hz), 7.56 (1H, d, J = 16.4 Hz), 7.72 (2H, d, J = 8.2 Hz), 7.79 (2H, d, J = 8.2 Hz), 7.85 (1H, dd, J = 8.8, 2.2 Hz), 7.93 (1H, br s), 8.24 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 322 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{15}F_3NO_2$ [M+H]$^+$ 322.1049, found 322.1049.

5.1.32. 4-Methoxy-3-[(*E*)-2-(4-tolyl)vinyl]benzamide (27e)

The compound **27e** was obtained as a colorless powder according to the general procedure in 89% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.31 (3H, s), 3.91 (3H, s), 7.08 (1H, d, J = 8.8 Hz), 7.19 (2H, d, J = 8.2 Hz), 7.25 (1H, d, J = 16.8 Hz), 7.25 (1H, br s), 7.36 (1H, d, J = 16.8 Hz), 7.47 (2H, d, J = 8.2 Hz), 7.80 (1H, dd, J = 8.8, 2.2 Hz), 7.92 (1H, br s), 8.19 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 268 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{18}NO_2$ [M+H]$^+$ 268.1332, found 268.1332.

5.1.33. 3-[(*E*)-2-(4-Fluorophenyl)vinyl]-4-methoxybenzamide (27f)

The compound **27f** was obtained as a colorless powder according to the general procedure in 57% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.09 (1H, d, J = 8.2 Hz), 7.20 (2H, dd, J = 8.8, 8.8 Hz), 7.25 (1H, br s), 7.28 (1H, d, J = 17.0 Hz), 7.35 (1H, d, J = 17.0 Hz), 7.62 (2H, dd, J = 8.8, 5.5 Hz), 7.81 (1H, dd, J = 8.2, 2.2 Hz), 7.91 (1H, br s), 8.18 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 272 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}FNO_2$ [M+H]$^+$ 272.1081, found 272.1083.

5.1.34. 3-[(*E*)-2-(4-Cyanophenyl)vinyl]-4-methoxybenzamide (27g)

The compound **27g** was obtained as a colorless powder according to the general procedure in 51% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.12 (1H, d, J = 8.8 Hz), 7.25 (1H, br s), 7.37 (1H, d, J = 16.5 Hz), 7.58 (1H, d, J = 16.5 Hz), 7.76 (2H, d, J = 8.2 Hz), 7.81 (2H, d, J = 8.2 Hz), 7.86 (1H, dd, J = 8.8, 2.2 Hz), 7.90 (1H, br s), 8.23 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 279 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{15}N_2O_2$ [M+H]$^+$ 279.1128, found 279.1130.

5.1.35. 4-Methoxy-3-[(*E*)-2-(4-nitrophenyl)vinyl]benzamide (27h)

The compound **27h** was obtained as a yellow powder according to the general procedure in 35% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.94 (3H, s), 7.13 (1H, d, J = 8.8 Hz), 7.27 (1H, br s), 7.46 (1H, d, J = 16.5 Hz), 7.65 (1H, d, J = 16.5 Hz), 7.85 (2H, d, J = 8.5 Hz), 7.87 (1H, dd, J = 8.8, 2.2 Hz), 7.91 (1H, br s), 8.23 (2H, d, J = 8.5 Hz), 8.26 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 299 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}N_2O_4$ [M+H]$^+$ 299.1026, found 299.1027.

5.1.36. 4-Methoxy-3-[(*E*)-2-(4-methoxyphenyl)vinyl]benzamide (27i)

The compound **27i** was obtained as a colorless powder according to the general procedure in quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.77 (3H, s), 3.90 (3H, s), 6.94 (2H, d, J = 8.5 Hz), 7.07 (1H, d, J = 8.2 Hz), 7.21 (1H, br s), 7.21 (1H, d, J = 17.0 Hz), 7.26 (1H, d, J = 17.0 Hz), 7.51 (2H, d, J = 8.5 Hz), 7.78 (1H, dd, J=8.2, 1.9 Hz), 7.89 (1H, br s), 8.16 (1H, d, J = 1.9 Hz). MS (ESI$^+$) 284 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{18}NO_3$ [M+H]$^+$ 284.1281, found 284.1279.

5.1.37. 4-Methoxy-3-[(*E*)-2-(3-tolyl)vinyl]benzamide (27j)

The compound **27j** was obtained as a colorless powder according to the general procedure in 55% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.34 (3H, s), 3.91 (3H, s), 7.09 (1H, d, J = 8.2 Hz), 7.09 (1H, d, J = 7.7 Hz), 7.22 (1H, br s), 7.25 (1H, d, J = 16.5 Hz), 7.26 (1H, t, J = 7.7 Hz), 7.35 (1H, d, J = 7.7 Hz), 7.39 (1H, d, J = 16.5 Hz), 7.39 (1H, s), 7.81 (1H, dd, J = 8.2, 2.2 Hz), 7.90 (1H, br s), 8.19 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 268 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{18}NO_2$ [M+H]$^+$ 268.1332, found 268.1333.

5.1.38. 3-[(*E*)-2-(3-Fluorophenyl)vinyl]-4-methoxybenzamide (27k)

The compound **27k** was obtained as a colorless powder according to the general procedure in 79% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.07–7.12 (2H, m), 7.25 (1H, br s), 7.30 (1H, d, J = 16.5 Hz), 7.38–7.43 (3H, m), 7.46 (1H, d, J = 16.5 Hz), 7.83 (1H, dd, J = 8.8, 2.2 Hz), 7.91 (1H, br s), 8.20 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 272 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}FNO_2$ [M+H]$^+$ 272.1081, found 272.1082.

5.1.39. 3-[(*E*)-2-(3-Chlorophenyl)vinyl]-4-methoxybenzamide (27l)

The compound **27l** was obtained as a colorless powder according to the general procedure in 57% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.10 (1H, d, J = 8.8 Hz), 7.24 (1H, br s), 7.28 (1H, d, J = 17.0 Hz), 7.31 (1H, d, J = 8.5 Hz), 7.40 (1H, dd, J = 8.5, 7.7 Hz), 7.46 (1H, d, J = 17.0 Hz), 7.54 (1H, d, J = 7.7 Hz), 7.63 (1H, s), 7.83 (1H, dd, J = 8.8, 2,2 Hz), 7.89 (1H, br s), 8.19 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 288, 290 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}ClNO_2$ [M+H]$^+$ 288.0786, found 288.0788.

5.1.40. 3-[(*E*)-2-(3-Cyanophenyl)vinyl]-4-methoxybenzamide (27m)

The compound **27m** was obtained as a colorless powder according to the general procedure in 53% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.11 (1H, d, J = 8.8 Hz), 7.26 (1H, br s), 7.33 (1H, d, J = 16.5 Hz), 7.54 (1H, d, J = 16.5 Hz), 7.58 (1H, dd, J = 7.7, 7.7 Hz), 7.72 (1H, d, J = 7.7 Hz), 7.85 (1H, dd, J = 8.8, 2.2 Hz), 7.91 (1H, br s), 7.92 (1H, d, J = 7.7 Hz), 8.06 (1H, s), 8.21 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 279 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{15}N_2O_2$ [M+H]$^+$ 279.1128, found 279.1129.

5.1.41. 3-[(*E*)-2-(2-Fluorophenyl)vinyl]-4-methoxybenzamide (27n)

The compound **27n** was obtained as a colorless powder according to the general procedure in 83% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.12 (1H, d, J = 8.8 Hz), 7.22–7.27 (3H, m), 7.33 (1H, ddd, J = 7.7, 6.6, 1.6 Hz), 7.38 (1H, d, J = 16.5 Hz), 7.51 (1H, d, J = 16.5 Hz), 7.75 (1H, ddd, J = 7.1, 6.6, 1.6 Hz), 7.85 (1H, dd, J = 8.8, 2.2 Hz), 7.99 (1H, br s), 8.22, (1H, d, J = 2.2 Hz). MS (ESI$^+$) 272 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}FNO_2$ [M+H]$^+$ 272.1081, found 272.1083.

5.1.42. 4-Methoxy-3-[(*E*)-2-(2-tolyl)vinyl]benzamide (27o)

The compound **27o** was obtained as a colorless powder according to the general procedure in 91% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.42 (3H, s), 3.91 (3H, s), 7.10 (1H, d, J = 8.8 Hz), 7.16–7.24 (3H, m), 7.27 (1H, d, J = 16.5 Hz), 7.27 (1H, br s), 7.46 (1H, d, J = 16.5 Hz), 7.60 (1H, d, J = 7.1 Hz), 7.83 (1H, dd, J = 8.8, 1.9 Hz), 7.96 (1H, br s), 8.19 (1H, d, J = 1.9 Hz). MS (ESI$^+$) 268 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{18}NO_2$ [M+H]$^+$ 268.1332, found 268.1331.

5.1.43. 4-Methoxy-3-[(*E*)-2-(2-methoxyphenyl)vinyl]benzamide (27p)

The compound **27p** was obtained as a colorless powder according to the general procedure in 97% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.86 (3H, s), 3.90 (3H, s), 6.97 (1H, dd, J = 7.7, 7.1 Hz), 7.04 (1H, d, J = 8.2 Hz), 7.08 (1H, d, J = 8.8 Hz), 7.24 (1H, br s), 7.27 (1H, ddd, J = 8.2, 7.1, 1.6 Hz), 7.38 (1H, d, J = 16.8 Hz), 7.48 (1H, d, J = 16.8 Hz), 7.60 (1H, dd, J = 7.7, 1.6 Hz), 7.81 (1H, dd, J = 8.8, 2.2 Hz), 7.98 (1H, br s), 8.15 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 284 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{18}NO_3$ [M+H]$^+$ 284.1281, found 284.1280.

5.1.44. 4-Methoxy-3-[(*E*)-2-[2-(trifluoromethoxy)phenyl]vinyl]benzamide (27q)

The compound **27q** was obtained as a colorless powder according to the general procedure in 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.13 (1H, d, J = 8.8 Hz), 7.27 (1H, br s), 7.40–7.46 (4H, m), 7.50 (1H, d, J = 16.8 Hz), 7.87 (1H, dd, J = 8.8, 2.2 Hz), 7.88–7.91 (1H, m), 7.98 (1H, br s), 8.16 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 338 [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{15}F_3NO_3$ [M+H]$^+$ 338.0999, found 338.0998.

5.1.45. 3-[(*E*)-2-(2-Chlorophenyl)vinyl]-4-methoxybenzamide (27r)

The compound **27r** was obtained as a colorless powder according to the general procedure in 63% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (3H, s), 7.12 (1H, d, J = 8.8 Hz), 7.27 (1H, br s), 7.30 (1H, ddd, J = 7.7, 7.7, 1.6 Hz), 7.37 (1H, dd, J = 7.7, 7.7 Hz), 7.41 (1H, d, J = 16.5 Hz), 7.49 (1H, d, J = 7.7 Hz), 7.55 (1H, d, J = 16.5 Hz), 7.81 (1H, dd, J = 7.7, 1.6 Hz), 7.86 (1H, dd, J = 8.8, 2.2 Hz), 7.99 (1H, br s), 8.18 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 288, 290 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{15}ClNO_2$ [M+H]$^+$ 288.0786, found 288.0788.

5.1.46. 3-[(*E*)-2-(2,4-Dichlorophenyl)vinyl]-4-methoxybenzamide (27s)

The compound **27s** was obtained as a colorless powder according to the general procedure in 51% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.12 (1H, d, J = 8.5 Hz), 7.26 (1H, br s), 7.43 (1H, d, J = 16.5 Hz), 7.44 (1H, dd, J = 8.5, 1.6 Hz), 7.50 (1H, d, J = 16.5 Hz), 7.65 (1H, d, J = 1.6 Hz), 7.84 (1H, d, J = 8.5 Hz), 7.86 (1H, dd, J = 8.5, 2.2 Hz), 7.98 (1H, br s), 8.17 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 322, 324 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{14}Cl_2NO_2$ [M+H]$^+$ 322.0396, found 322.0399.

5.1.47. 3-[(*E*)-2-(3,4-Dichlorophenyl)vinyl]-4-methoxybenzamide (27t)

The compound **27t** was obtained as a colorless powder according to the general procedure in 65% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.10 (1H, d, J = 8.8 Hz), 7.26 (1H, br s), 7.28 (1H, d, J = 16.5 Hz), 7.47 (1H, d, J = 16.5 Hz), 7.58 (1H, dd, J = 8.2, 2.2 Hz), 7.61 (1H, d, J = 8.2 Hz), 7.83 (1H, dd, J = 8.8, 2.2 Hz), 7.84 (1H, d, J = 1.6 Hz), 7.89 (1H, br s), 8.18 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 322, 324 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{14}Cl_2NO_2$ [M+H]$^+$ 322.0396, found 322.0395.

5.1.48. 3-[(*E*)-2-(4-Chloro-2-fluorophenyl)vinyl]-4-methoxybenzamide (27u)

The compound **27u** was obtained as a colorless powder according to the general procedure in 35% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.91 (3H, s), 7.11 (1H, d, J = 8.8 Hz), 7.26 (1H, br s), 7.30 (1H, dd, J = 8.2, 2.2 Hz), 7.32 (1H, d, J = 16.5 Hz), 7.46 (1H, dd, J = 11.0, 2.2 Hz), 7.50 (1H, d, J = 16.5 Hz), 7.78 (1H, dd, J = 8.2, 8.2 Hz), 7.85 (1H, dd, J = 8.8, 2.2 Hz), 7.96 (1H, br s), 8.20 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 306, 308 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{16}H_{14}ClFNO_2$ [M+H]$^+$ 306.0692, found 306.0692.

5.1.49. Ethyl 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-hydroxybenzoate (28)

To a solution of diethyl 4-chlorobenzylphosphonate (1.19 g, 4.54 mmol) in DMF (10 mL) at 0 °C was added sodium *tert*-pentoxide (1.37 g, 11.8 mmol) and the mixture was stirred for 20 min at 0 °C. Then a solution of **14** (842 mg, 4.33 mmol) in DMF (5 mL) was added and the reaction mixture was stirred for 2 h at 0 °C. The reaction mixture was poured into saturated aqueous solution of NH_4Cl (30 mL) and extracted with EtOAc (100 mL). The organic layer was separated, washed with water and brine, dried over

$MgSO_4$, filtered and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc = 3:1 to 2:1) to give **28** (925 mg, 71%) as a colorless powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.1 Hz), 5.74 (1H, br s), 6.84 (1H, d, J = 8.8 Hz), 7.14 (1H, d, J = 16.5 Hz), 7.31 (2H, d, J = 8.8 Hz), 7.32 (1H, d, J = 16.5 Hz), 7.45 (2H, d, J = 8.8 Hz), 7.85 (1H, dd, J = 8.8, 2.1 Hz), 8.23 (1H, d, J = 2.1 Hz). MS (ESI$^+$) 303, 305 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{16}ClO_3$ [M+H]$^+$ 303.0782, found 303.0778.

5.1.50. General procedure for the synthesis of compounds 29a–c

A mixture of **28** (1 equiv), alkyl halides (1.5 equiv), K_2CO_3 (2 equiv) in CH_3CN was stirred at 60 °C for 24 h. The insoluble solid was filtered and the cake was washed with CH_3CN. The filtrate was concentrated in vacuo and the residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc) to give the desired products **29a–c**.

5.1.51. Ethyl 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-ethoxybenzoate (29a)

The compound **29a** was obtained as a colorless powder according to the general procedure in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 1.52 (3H, t, J = 6.9 Hz), 4.18 (2H, q, J = 6.9 Hz), 4.38 (2H, q, J = 7.1 Hz), 6.90 (1H, d, J = 8.7 Hz), 7.17 (1H, d, J = 16.5 Hz), 7.33 (2H, d, J = 8.6 Hz), 7.42 (1H, d, J = 16.5 Hz), 7.47 (2H, d, J = 8.6 Hz), 7.93 (1H, dd, J = 8.7, 2.1 Hz), 8.26 (1H, d, J = 2.1 Hz). MS (ESI$^+$) 331, 333 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{19}H_{20}ClO_3$ [M+H]$^+$ 331.1095, found 331.1096.

5.1.52. Ethyl 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-propoxybenzoate (29b)

The compound **29b** was obtained as a colorless powder according to the general procedure in 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.11 (3H, t, J = 7.4 Hz), 1.41 (3H, t, J = 7.1 Hz), 1.92 (2H, tq, J = 7.4, 6.6 Hz), 4.06 (2H, t, J = 6.6 Hz), 4.38 (2H, q, J = 7.1 Hz), 6.91 (1H, d, J = 8.7 Hz), 7.18 (1H, d, J = 16.7 Hz), 7.33 (2H, d, J = 8.4 Hz), 7.42 (1H, d, J = 16.7 Hz), 7.46 (2H, d, J = 8.4 Hz), 7.93 (1H, dd, J = 8.7, 2.1 Hz), 8.26 (1H, d, J = 2.1 Hz). MS (ESI$^+$) 345, 347 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{20}H_{22}ClO_3$ [M+H]$^+$ 345.1252, found 345.1253.

5.1.53. Ethyl 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-[2-(2-hydroxyethoxy)ethoxy]benzoate (29c)

The compound **29c** was obtained as a colorless powder according to the general procedure in 95% yield. ^{1}H NMR (400 MHz, CD_3OD) δ: 1.40 (3H, t, J = 7.1 Hz), 3.64–3.75 (4H, m), 3.95 (2H, t, J = 4.6 Hz), 4.31 (2H, t, J = 4.6 Hz), 4.36 (2H, q, J = 7.1 Hz), 7.10 (1H, d, J = 8.8 Hz), 7.25 (1H, d, J = 16.5 Hz), 7.34 (2H, d, J = 8.5 Hz), 7.48 (1H, d, J = 16.5 Hz), 7.54 (2H, d, J = 8.5 Hz), 7.91 (1H, dd, J = 8.8, 1.9 Hz), 8.24 (1H, d, J = 1.9 Hz). MS (ESI$^+$) 391, 393 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{21}H_{24}ClO_5$ [M+H]$^+$ 391.1306, found 391.1309.

5.1.54. Ethyl 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-(2-dimethylaminoethyloxy)benzoate (29d)

A mixture of **28** (151 mg, 0.50 mmol), 1,2-dibromoethane (0.43 mL, 4.97 mmol), K_2CO_3 (344 mg, 2.49 mmol) in DMF (3 mL) was stirred at 80 °C for 1 h. To the mixture was added dimethylamine solution (2.0 M in THF, 2.5 mL, 5.0 mmol), and the mixture was stirred at 80 °C for 1 h. The reaction mixture was diluted with EtOAc (30 mL). The organic layer was washed with water and brine, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH = 50:1 to 10:1) to give **29d** (109 mg, 58%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 2.39 (6H, s), 2.84 (2H, t, J = 5.8 Hz), 4.20 (2H, t, J = 5.8 Hz), 4.38 (2H, q, J = 7.1 Hz), 6.91 (1H, d, J = 8.2 Hz), 7.17 (1H, d, J = 16.5 Hz), 7.32 (2H, d, J = 8.2 Hz), 7.39 (1H, d, J = 16.5 Hz), 7.44 (2H, d, J = 8.2 Hz), 7.93 (1H, dd, J = 8.2, 1.8 Hz), 8.25 (1H, d, J = 1.8 Hz). MS (ESI$^+$) 374, 376 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{21}H_{25}ClNO_3$ [M+H]$^+$ 374.1517, found 374.1507.

5.1.55. Ethyl 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-[2-(4-methylpiperazin-1-yl)ethoxy]benzoate (29e)

Compound **29e** was prepared in a manner similar to that described for **29d** in 66% yield as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.1 Hz), 2.30 (3H, s), 2.49 (4H, br s), 2.68 (4H, br s), 2.92 (2H, t, J = 5.8 Hz), 4.23 (2H, t, J = 5.8 Hz), 4.38 (2H, q, J = 7.1 Hz), 6.90 (1H, d, J = 8.5 Hz), 7.17 (1H, d, J = 16.5 Hz), 7.32 (2H, d, J = 8.2 Hz), 7.38 (1H, d, J = 16.5 Hz), 7.44 (2H, d, J = 8.2 Hz), 7.93 (1H, dd, J = 8.5, 1.9 Hz), 8.25 (1H, d, J = 1.9 Hz). MS (ESI$^+$) 429, 431 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{24}H_{30}ClN_2O_3$ [M+H]$^+$ 429.1939, found 429.1930.

5.1.56. General procedure for the synthesis of compounds 30a–d

To a solution of **29a–d** (0.14 mmol) in MeOH (3 mL) was added 20% aqueous solution of KOH (0.6 mL) and the mixture was stirred for 1 h under reflux. After removal of MeOH, 1 M HCl (2.4 mL) was added. The resulting precipitate was filtered, washed with water to give the desired products **30a–d**.

5.1.57. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-ethoxybenzoic acid (30a)

The compound **30a** was obtained as a colorless powder according to the general procedure in 87% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.44 (3H, t, J = 7.1 Hz), 4.20 (2H, q, J = 7.1 Hz), 7.13 (1H, d, J = 8.8 Hz), 7.33 (1H, d, J = 16.6 Hz), 7.42 (1H, d, J = 16.6 Hz), 7.43 (2H, d, J = 8.8 Hz), 7.63 (2H, d, J = 8.8 Hz), 7.86 (1H, dd, J = 8.8, 2.0 Hz), 8.20 (1H, d, J = 2.0 Hz), 12.73 (1H, br s). MS (ESI$^+$) 303, 305 (Cl isotope) [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{17}H_{14}ClO_3$ [M−H]$^-$ 301.0626, found 301.0620.

5.1.58. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-propoxybenzoic acid (30b)

The compound **30b** was obtained as a colorless powder according to the general procedure in 97% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.05 (3H, t, J = 7.3 Hz), 1.84 (2H, tq, J = 7.3, 6.4 Hz), 4.11 (2H, t, J = 6.4 Hz), 7.14 (1H, d, J = 8.8 Hz), 7.34 (1H, d, J = 16.6 Hz), 7.43 (1H, d, J = 16.6 Hz), 7.44 (2H, d, J = 8.8 Hz), 7.62 (2H, d, J = 8.8 Hz), 7.85 (1H, dd, J = 8.8, 2.0 Hz), 8.20 (1H, d, J = 2.0 Hz). MS (ESI$^+$) 317, 319 (Cl isotope) [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{18}H_{16}ClO_3$ [M−H]$^-$ 315.0782, found 315.0775.

5.1.59. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-[2-(2-hydroxyethoxy)ethoxy]benzoic acid (30c)

The compound **30c** was obtained as a colorless powder according to the general procedure in 87% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.52–3.58 (4H, m), 3.86 (2H, t, J = 4.7 Hz), 4.27 (2H, t, J = 4.7 Hz), 4.64 (1H, t, J = 4.7 Hz), 7.16 (1H, d, J = 8.8 Hz), 7.37 (1H, d, J = 16.6 Hz), 7.43 (2H, d, J = 8.8 Hz), 7.44 (1H, d, J = 16.6 Hz), 7.63 (2H, d, J = 8.8 Hz), 7.85 (1H, dd, J = 8.8, 2.4 Hz), 8.20 (1H, d, J = 2.4 Hz), 12.75 (1H, br s). MS (ESI$^+$) 363, 365 (Cl isotope) [M+H]$^+$. HRMS (ESI$^-$) calcd for $C_{19}H_{18}ClO_5$ [M−H]$^-$ 361.0837, found 361.0832.

5.1.60. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-(2-dimethylaminoethoxy)benzoic acid (30d)

The compound **30c** was obtained as a colorless powder according to the general procedure in 92% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.28 (6H, s), 2.76 (2H, t, J = 5.8 Hz), 4.22 (2H, t, J = 5.8 Hz), 7.15 (1H, d, J = 8.5 Hz), 7.37 (1H, d, J = 17.0 Hz), 7.42 (1H, d, J = 17.0 Hz), 7.44 (2H, d, J = 8.5 Hz), 7.60 (2H, d, J = 8.5 Hz),

7.84 (1H, dd, J = 8.5, 2.2 Hz), 8.18 (1H, d, J = 2.2 Hz). MS (ESI^+) 346, 348 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{19}H_{21}ClNO_3$ $[M+H]^+$ 346.1204, found 346.1194.

5.1.61. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-[2-(4-methylpiperazin-1-yl)ethoxy]benzoic acid (30e)

To a solution of **29e** (59 mg, 0.14 mmol) in MeOH (3 mL) was added 20% aqueous solution of KOH (0.6 mL) and the mixture was stirred for 2.5 h under reflux. After removal of MeOH, 1 M HCl (2.4 mL) was added and the aqueous layer was extracted with EtOAc. The organic layers were combined, washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give **30e** (50 mg, 90%) as a colorless powder. ^1H NMR (400 MHz, DMSO-d_6) δ: 2.14 (3H, s), 2.33 (4H, br s), 2.54 (4H, br s), 2.80 (2H, t, J = 5.5 Hz), 4.22 (2H, t, J = 5.5 Hz), 7.13 (1H, d, J = 8.8 Hz), 7.34 (1H, d, J = 17.0 Hz), 7.42 (1H, d, J = 17.0 Hz), 7.43 (2H, d, J = 8.5 Hz), 7.60 (2H, d, J = 8.5 Hz), 7.83 (1H, dd, J = 8.8, 2.2 Hz), 8.18 (1H, d, J = 2.2 Hz). MS (ESI^+) 401, 403 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{22}H_{26}ClN_2O_3$ $[M+H]^+$ 401.1626, found 401.1616.

5.1.62. General procedure for the synthesis of compounds 31a–e

To a solution of **30a–e** (1 equiv), NH_4Cl (2 equiv), and HOBT (1 equiv) in DMF were added EDC (1.5 equiv) and DIPEA (4 equiv) and the mixture was stirred at room temperature for 8 h. Water was added, and the resulting precipitate was filtered and washed with water to give the desired products **31a–e**.

5.1.63. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-ethoxybenzamide (31a)

The compound **31a** was obtained as a colorless powder according to the general procedure in 96% yield. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.42 (3H, t, J = 6.9 Hz), 4.17 (2H, q, J = 6.9 Hz), 7.09 (1H, d, J = 8.6 Hz), 7.27 (1H, br s), 7.31 (1H, d, J = 16.7 Hz), 7.44 (1H, d, J = 16.7 Hz), 7.45 (2H, d, J = 8.6 Hz), 7.60 (2H, d, J = 8.6 Hz), 7.81 (1H, dd, J = 8.6, 2.2 Hz), 7.92 (1H, br s), 8.21 (1H, d, J = 2.2 Hz). MS (ESI^+) 302, 304 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{17}H_{17}ClNO_2$ $[M+H]^+$ 302.0942, found 302.0938.

5.1.64. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-propoxybenzamide (31b)

The compound **31b** was obtained as a colorless powder according to the general procedure in 85% yield. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.04 (3H, t, J = 7.4 Hz), 1.83 (2H, tq, J = 7.4, 6.5 Hz), 4.08 (2H, t, J = 6.5 Hz), 7.09 (1H, d, J = 8.6 Hz), 7.26 (1H, br s), 7.32 (1H, d, J = 16.5 Hz), 7.44 (1H, d, J = 16.5 Hz), 7.45 (2H, d, J = 8.6 Hz), 7.59 (2H, d, J = 8.6 Hz), 7.81 (1H, dd, J = 8.6, 2.2 Hz), 7.92 (1H, br s), 8.20 (1H, d, J = 2.2 Hz). MS (ESI^+) 316, 318 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{18}H_{19}ClNO_2$ $[M+H]^+$ 316.1099, found 316.1092.

5.1.65. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-[2-(2-hydroxyethoxy)ethoxy]benzamide (31c)

The compound **31c** was obtained as a colorless powder according to the general procedure in 83% yield. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.51–3.58 (4H, m), 3.85 (2H, t, J = 4.7 Hz), 4.24 (2H, t, J = 4.7 Hz), 4.63 (1H, t, J = 4.7 Hz), 7.11 (1H, d, J = 8.8 Hz), 7.25 (1H, br s), 7.35 (1H, d, J = 16.6 Hz), 7.43 (1H, d, J = 16.6 Hz), 7.44 (2H, d, J = 8.8 Hz), 7.59 (2H, d, J = 8.8 Hz), 7.80 (1H, dd, J = 8.8, 2.2 Hz), 7.90 (1H, br s), 8.19 (1H, d, J = 2.2 Hz). MS (ESI^+) 362, 364 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{19}H_{21}ClNO_4$ $[M+H]^+$ 362.1154, found 362.1147.

5.1.66. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-(2-dimethylaminoethoxy)benzamide (31d)

The compound **31d** was obtained as a colorless powder according to the general procedure in 76% yield. ^1H NMR (400 MHz, DMSO-d_6) δ: 2.27 (6H, s), 2.73 (2H, t, J = 5.9 Hz), 4.19 (2H, t, J = 5.9 Hz), 7.11 (1H, d, J = 8.8 Hz), 7.24 (1H, br s), 7.35 (1H, d, J = 16.4 Hz), 7.42 (1H, d, J = 16.4 Hz), 7.45 (2H, d, J = 8.8 Hz), 7.57 (2H, d, J = 8.8 Hz), 7.80 (1H, dd, J = 8.8, 2.2 Hz), 7.90 (1H, br s), 8.18 (1H, d, J = 2.2 Hz). MS (ESI^+) 345, 347 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{19}H_{22}ClN_2O_2$ $[M+H]^+$ 345.1364, found 345.1355.

5.1.67. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-[2-(4-methylpiperazin-1-yl)ethoxy]benzamide (31e)

The compound **31e** was obtained as a colorless powder according to the general procedure in 82% yield. ^1H NMR (400 MHz, DMSO-d_6) δ: 2.13 (3H, s), 2.31 (4H, br s), 2.53 (4H, br s), 2.79 (2H, t, J = 5.7 Hz), 4.21 (2H, t, J = 5.7 Hz), 7.12 (1H, d, J = 8.8 Hz), 7.24 (1H, br s), 7.34 (1H, d, J = 16.6 Hz), 7.42 (1H, d, J = 16.6 Hz), 7.45 (2H, d, J = 8.3 Hz), 7.58 (2H, d, J = 8.3 Hz), 7.80 (1H, dd, J = 8.8, 2.0 Hz), 7.89 (1H, br s), 8.19 (1H, d, J = 2.0 Hz). MS (ESI^+) 400, 402 (Cl isotope) $[M+H]^+$. HRMS (ESI^+) calcd for $C_{22}H_{27}ClN_3O_2$ $[M+H]^+$ 400.1786, found 400.1778.

5.1.68. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-methoxybenzoic acid ((*E*)-21)

To a solution of diethyl 4-chlorobenzylphosphonate (13.3 g, 50.4 mmol) in toluene (150 mL) at 0 °C was added sodium *tert*-pentoxide (8.35 g, 72.1 mmol) and the mixture was stirred for 20 min at 0 °C. Then a solution of **15** (10.0 g, 48.0 mmol) in THF (40 mL) was added dropwise over 20 min and the reaction mixture was stirred for 1.5 h at 0 °C. The reaction mixture was poured into saturated aqueous solution of NH_4Cl (300 mL) and extracted with EtOAc (350 mL × 2). The organic layers were combined, washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give crude (***E***)-**20** (16.4 g). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.41 (3H, t, J = 7.2 Hz), 3.98 (3H, s), 4.38 (2H, q, J = 7.2 Hz), 6.92 (1H, d, J = 8.8 Hz), 7.15 (1H, d, J = 16.4 Hz), 7.32 (2H, d, J = 8.6 Hz), 7.41 (1H, d, J = 16.4 Hz), 7.47 (2H, d, J = 8.6 Hz), 7.95 (1H, dd, J = 8.8, 2.4 Hz), 8.26 (1H, d, J = 2.4 Hz). To a solution of crude **(*E*)-20** (16.0 g) in MeOH (100 mL) was added 20% aqueous solution of KOH (30 mL). The mixture was stirred for 2 h at 65 °C, and then cooled to 0 °C. The reaction mixture was adjusted to pH of 3 by addition of 1 M HCl (170 mL). The resulting precipitate was filtered, washed with water and *n*-hexane/EtOAc (10:1). The residue was recrystallized from EtOH (700 mL) to give **(*E*)-21** (11.2 g, 80%) as colorless needles. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.94 (3H, s), 7.15 (1H, d, J = 8.8 Hz), 7.30 (1H, d, J = 16.6 Hz), 7.43 (2H, d, J = 8.4 Hz), 7.43 (1H, d, J = 16.6 Hz), 7.64 (2H, d, J = 8.4 Hz), 7.88 (1H, dd, J = 8.8, 2.1 Hz), 8.20 (1H, d, J = 2.1 Hz), 12.8 (1H, br s). MS (ESI^+) 289, 291 (Cl isotope) $[M+H]^+$. HRMS (ESI^-) calcd for $C_{16}H_{12}ClO_3$ $[M-H]^-$ 287.0469, found 287.0470.

5.1.69. General procedure for the synthesis of compounds 32a–h

To a mixture of **(*E*)-21** (501 mg, 1.74 mmol) in CH_2Cl_2 (15 mL) and DMF (7 μL) at 0 °C was added oxalyl chloride (331 mg, 2.60 mmol) and the mixture was stirred for 13 h at room temperature. The reaction mixture was concentrated in vacuo to give 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-methoxy-benzoyl chloride (535 mg, quant.) as a colorless powder. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.99 (3H, s), 6.97 (1H, d, J = 8.8 Hz), 7.14 (1H, d, J = 16.5 Hz), 7.33 (2H, d, J = 8.8 Hz), 7.36 (1H, d, J = 16.5 Hz), 7.47 (2H, d, J = 8.8 Hz), 8.05 (1H, dd, J = 8.8, 2.2 Hz), 8.30 (1H, d, J = 2.2 Hz). To a solution of amines (2.5 equiv) and DIPEA (5 equiv) in CH_2Cl_2 at room temperature was added a solution of 3-[(*E*)-2-(4-chlorophenyl)vinyl]-4-methoxy-benzoyl chloride (1 equiv) in CH_2Cl_2 and the mixture was stirred for 1 h at room temperature. The reaction mixture was washed with saturated aqueous solution of NH_4Cl and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo.

The residue was purified by flash chromatography (SiO_2, *n*-hexane/EtOAc) to give the desired products **32a–h**.

5.1.70. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-methoxy-*N*-methylbenzamide (32a)

Compound **32a** was obtained from **(*E*)-21** and methylamine hydrochloride as a colorless powder in 84% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.79 (3H, d, J = 4.4 Hz), 3.91 (3H, s), 7.10 (1H, d, J = 8.8 Hz), 7.27 (1H, d, J = 16.5 Hz), 7.42 (1H, d, J = 16.5 Hz), 7.43 (2H, d, J = 8.5 Hz), 7.61 (2H, d, J = 8.5 Hz), 7.78 (1H, dd, J = 8.8, 2.2 Hz), 8.14 (1H, d, J = 2.2 Hz), 8.35 (1H, br q, J = 4.4 Hz). MS (ESI$^+$) 302, 304 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{17}H_{17}ClNO_2$ [M+H]$^+$ 302.0942, found 302.0943.

5.1.71. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-4-methoxy-*N*,*N*-dimethylbenzamide (32b)

Compound **32b** was obtained from **(*E*)-21** and dimethylamine hydrochloride as a yellow oil in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.08 (6H, br s), 3.91 (3H, s), 6.89 (1H, d, J = 8.5 Hz), 7.06 (1H, d, J = 16.5 Hz), 7.30 (2H, d, J = 8.8 Hz), 7.33 (1H, dd, J = 8.5, 2.2 Hz), 7.40 (1H, d, J = 16.5 Hz), 7.44 (2H, d, J = 8.8 Hz), 7.67 (1H, d, J = 2.2 Hz). MS (ESI$^+$) 316, 318 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{18}H_{19}ClNO_2$ [M+H]$^+$ 316.1099, found 316.1097.

5.1.72. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-ethyl-4-methoxybenzamide (32c)

Compound **32c** was obtained from **(*E*)-21** and ethylamine hydrochloride as a colorless powder in 86% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.13 (3H, t, J = 7.1 Hz), 3.25–3.31 (2H, m), 3.90 (3H, s), 7.09 (1H, d, J = 8.8 Hz), 7.26 (1H, d, J = 16.5 Hz), 7.41 (1H, d, J = 16.5 Hz), 7.42 (2H, d, J = 8.5 Hz), 7.60 (2H, d, J = 8.5 Hz), 7.78 (1H, dd, J = 8.8, 2.2 Hz), 8.13 (1H, d, J = 2.2 Hz), 8.37 (1H, brt, J = 5.5 Hz). MS (ESI$^+$) 316, 318 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{18}H_{19}ClNO_2$ [M+H]$^+$ 316.1099, found 316.1098.

5.1.73. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-(2-hydroxyethyl)-4-methoxybenzamide (32d)

Compound **32d** was obtained from **(*E*)-21** and 2-aminoethanol as a pale yellow powder in 81% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.30 (2H, dt, J = 5.8, 5.5 Hz), 3.52 (2H, dt, J = 5.8, 5.8 Hz), 3.91 (3H, s), 4.74 (1H, t, J = 5.8 Hz), 7.10 (1H, d, J = 8.5 Hz), 7.28 (1H, d, J = 16.5 Hz), 7.43 (1H, d, J = 16.5 Hz), 7.43 (2H, d, J = 8.5 Hz), 7.61 (2H, d, J = 8.5 Hz), 7.81 (1H, dd, J = 8.5, 2.2 Hz), 8.17 (1H, d, J = 2.2 Hz), 8.39 (1H, t, J = 5.5 Hz). MS (ESI$^+$) 332, 334 (Cl isotope) [M+H]$^+$. HRMS calcd for $C_{18}H_{19}ClNO_3$ [M+H]$^+$ 332.1048, found 332.1048.

5.1.74. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-(2,3-dihydroxypropyl)-4-methoxybenzamide (32e)

Compound **32e** was obtained from **(*E*)-21** and 3-aminopropane-1,2-diol as a colorless powder in 93% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.21 (1H, ddd, J = 13.6, 5.5, 5.5 Hz), 3.35 (2H, dd, J = 5.5, 5.5 Hz), 3.41 (1H, ddd, J = 13.6, 5.5, 5.5 Hz), 3.63 (1H, dddt, J = 6.0, 5.5, 5.5, 5.5 Hz), 3.91 (3H, s), 4.58 (1H, t, J = 5.5 Hz), 4.83 (1H, d, J = 6.0 Hz), 7.13 (1H, d, J = 8.8 Hz), 7.29 (1H, d, J = 16.5 Hz), 7.43 (1H, d, J = 16.5 Hz), 7.43 (2H, d, J = 8.5 Hz), 7.61 (2H, d, J = 8.5 Hz), 7.82 (1H, dd, J = 8.8, 2.0 Hz), 8.19 (1H, d, J = 2.0 Hz), 8.38 (1H, t, J = 5.5 Hz). MS (ESI$^+$) 362, 364 (Cl isotope) [M+H]$^+$. HRMS calcd for $C_{19}H_{21}ClNO_4$ [M+H]$^+$ 362.1154, found 362.1152.

5.1.75. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-[(2*R*)-2,3-dihydroxypropyl]-4-methoxybenzamide (32f)

Compound **32f** was obtained from **(*E*)-21** and (*R*)-3-aminopropane-1,2-diol as a colorless powder in 90% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.21 (1H, ddd, J = 13.6, 5.5, 5.5 Hz), 3.36 (2H, dd, J = 5.5, 5.5 Hz), 3.41 (1H, ddd, J = 13.6, 5.5, 5.5 Hz), 3.65 (1H, dddt, J = 6.0, 5.5, 5.5, 5.5 Hz), 3.92 (3H, s), 4.59 (1H, t, J = 5.5 Hz), 4.83 (1H, d, J = 6.0 Hz), 7.12 (1H, d, J = 8.7 Hz), 7.30 (1H, d, J = 16.5 Hz), 7.44 (1H, d, J = 16.5 Hz), 7.44 (2H, d, J = 8.5 Hz), 7.62 (2H, d, J = 8.5 Hz), 7.83 (1H, dd, J = 8.7, 2.3 Hz), 8.19 (1H, d, J = 2.3 Hz), 8.38 (1H, t, J = 5.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 42.9, 55.8, 63.8, 70.4, 110.9, 123.3, 124.6, 125.4, 126.6, 128.0, 128.1, 128.5, 128.7, 131.9, 136.1, 158.5, 166.0. MS (ESI$^+$) 362, 364 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{19}H_{21}ClNO_4$ [M+H]$^+$ 362.1154, found 362.1152. $[\alpha]_D^{25}$ +14.3° (*c* 0.2, MeOH).

5.1.76. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-[(2*S*)-2,3-dihydroxypropyl]-4-methoxybenzamide (32g)

Compound **32g** was obtained from **(*E*)-21** and (*S*)-3-aminopropane-1,2-diol as a colorless powder in 92% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.21 (1H, ddd, J = 13.6, 5.5, 5.5 Hz), 3.35 (2H, dd, J = 5.5, 5.5 Hz), 3.41 (1H, ddd, J = 13.6, 5.5, 5.5 Hz), 3.65 (1H, dddt, J = 6.0, 5.5, 5.5, 5.5 Hz), 3.92 (3H, s), 4.59 (1H, t, J = 5.5 Hz), 4.84 (1H, d, J = 6.0 Hz), 7.12 (1H, d, J = 8.7 Hz), 7.29 (1H, d, J = 16.5 Hz), 7.44 (1H, d, J = 16.5 Hz), 7.44 (2H, d, J = 8.5 Hz), 7.62 (2H, d, J = 8.5 Hz), 7.82 (1H, dd, J = 8.7, 2.3 Hz), 8.19 (1H, d, J = 2.3 Hz), 8.39 (1H, t, J = 5.5 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 42.9, 55.8, 63.8, 70.4, 110.9, 123.3, 124.6, 125.4, 126.6, 128.0, 128.1, 128.5, 128.7, 131.9, 136.1, 158.5, 166.0. MS (ESI$^+$) 362, 364 (Cl isotope) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{19}H_{21}ClNO_4$ [M+H]$^+$ 362.1154, found 362.1154. $[\alpha]_D^{25}$ −14.5° (*c* 0.2, MeOH).

5.1.77. 3-[(*E*)-2-(4-Chlorophenyl)vinyl]-*N*-[2-hydroxy-1-(hydroxymethyl)ethyl]-4-methoxybenzamide (32h)

Compound **32h** was obtained from **(*E*)-21** and 2-aminopropane-1,3-diol as a colorless powder in 95% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.54 (4H, dd, J = 6.0, 6.0 Hz), 3.92 (3H, s), 3.98 (1H, dtt, J = 8.2, 6.0, 6.0 Hz), 4.68 (2H, t, J = 6.0 Hz), 7.11 (1H, d, J = 8.7 Hz), 7.30 (1H, d, J = 16.5 Hz), 7.44 (1H, d, J = 16.5 Hz), 7.44 (2H, d, J = 8.5 Hz), 7.62 (2H, d, J = 8.5 Hz), 7.83 (1H, dd, J = 8.7, 2.3 Hz), 7.93 (1H, d, J = 8.2 Hz), 8.18 (1H, d, J = 2.3 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 53.8, 55.8, 60.3, 110.7, 123.4, 124.5, 125.6, 126.9, 128.0, 128.1, 128.7, 128.7, 131.9, 136.1, 158.4, 166.0. MS (ESI$^+$) 362, 364 (Cl isotope) [M+H]$^+$. HRMS calcd for $C_{19}H_{21}ClNO_4$ [M+H] 362.1154, found 362.1152.

5.2. HUVEC proliferation assay

For the serial dilution of the compound, 5 μL of a solution was repeatedly diluted with the equal volume of DMSO onto 96-well plates. Human umbilical vein endothelial cells (HUVECs) were cultured in RPMI1640 supplemented with 10% FCS, containing 50 μg/mL heparin and 30 μg/mL endothelial cell growth supplement (ECGS). Cells treated with a range of concentrations of the compounds and seeded at 3000 cells/well in RPMI1640 supplemented with 5% FCS, containing 20 ng/mL vascular endothelial growth factor (VEGF) and culture medium as a reference. The plates were incubated for 4 days at 37 °C in a humidified incubator containing 5% CO_2. On the last day, 10–20 μL of Cell Counting Kit-8 (Dojindo Laboratories, Tokyo, Japan) was added to the culture in each well and the plates were further incubated for a few hours at 37 °C in a humidified incubator containing 5% CO_2. After incubation, absorbance in each well was measured at 450 nm using a micro-plate reader (BIO-RAD Model 3550). The antiproliferative activity of each compound for VEGF-induced condition was calculated by the formula (1-T/C) × 100 (%), where T and C represent mean difference absorbance at 450 nm of the cells treated with compounds (T) and that of the untreated control cells (C). The IC_{50} values were calculated using an Excel function entitled mod100LinearIC_{50} developed by Roche Palo Alto Research Center (CA, USA).

5.3. Tube formation assay

The Angiogenesis Kit (Kurabo Industries Ltd.) was charged with the test compound at final concentration of 4 μM and 20 μM, and incubated in a CO_2 incubator (37 °C, 5%). After 11 days of incubation, capillary-like tubes formed were fixed with 70% ethanol and visualized with a CD31 Staining Kit (also produced by Kurabo Industries Ltd.). Under a microscope, stain images of the wells were photographed, stored as an image file, and measured quantitatively for the area of capillary-like tube formation with Kurabo angiogenesis image analysis software. The inhibition rates (%) of the test compound-charged wells were calculated, with the control taken as 100%.

5.4. Solubility assay (LYSA)

Samples were prepared in triplicate from 10 mM dimethylsulfoxide stock solutions. After evaporation (1 h) of dimethylsulfoxide with a centrifugal vacuum evaporator, the compounds were dissolved in FaSSIF, and shaken for 2 h. The solutions were filtered using a microtiter filter plate (Whatman UNIFILTER) and the filtrate was analyzed by UV measurement or HPLC-UV. In addition, a four point calibration curve is prepared from the 10 mM stock solutions and used to determine the solubility of the compounds. The results are expressed in μg/mL. Starting from a 10 mM stock solution, the measurement range for MW 500 was 0–666 μg/mL.

5.5. Liver microsomal stability assay

Mouse or human microsome incubations were conducted by an automated procedure implemented on a Biomek FX (Beckman Coulter). Compounds (5 μM) were incubated in microsomes at 1.0 mg protein/mL in a 50 mM potassium phosphate buffer, pH 7.4, at 37 °C. Cofactor (NADPH) was produced by a generating system (glucose 6-phosphate, 3.2 mM; β-NADP, 2.6 mM; $MgCl_2$, 6.5 mM). Addition of the NADPH generating system to the prewarmed microsomes containing the test compound started the reaction. Aliquots (50 μL) were taken at four defined time points within 60 min and transferred into 100 μL of MeOH containing an internal standard. Concentration of each compound was analyzed by LC–MS/MS using an ODS-3 RP 18 column (GL Sciences). Quantitative detection was achieved on a API 365 instrument (AB Sciex) using electron spray ionization. Concentrations were determined by ratio of test compound and internal standard peaks and given as a percentage of the concentration measured at the first time point (substrate depletion). Intrinsic clearance (CL, in μL/min/mg microsomal protein) is the rate constant of the first-order decay of the test compound, normalized for the protein concentration in the incubation.

5.6. Pharmacokinetic studies

Pharmacokinetic studies of **32f** and **32g** were performed in female nude mice following an oral dose of 30 mg/kg. The compounds were dissolved in DMSO/Cremophor EL/1% Tween 80 (1:1:8 v/v/v) and orally administered to animals through a stomach sonde. Blood of mice (n = 2/time point) was collected from the retro-orbital vein using a heparinized capillary or by heart puncture using a disposable syringe with a 25G needle under light isoflurane anesthesia at the scheduled time points of 30 min, 1, 2, 4, 7 and 24 h after administration. Blood samples were immediately centrifuged by a hematocrit centrifuge (Model 3220, Kubota) at 12,000 rpm for 3 min or by a centrifuge at 3000 rpm for 10 min at 4 °C (LX-130, Tomy Seiko) to separate the plasma. Plasma samples were immediately frozen on dry ice and stored in a deep freezer set at −80 °C until analysis. The plasma sample (25 μL) was added with three volumes of CH_3CN/MeOH (1:1 v/v) including an internal standard, then thoroughly mixed to precipitate plasma protein. The suspensions were transferred to 96-well MultiScreen filtration plates (0.45 μm) and filtrated by centrifugation at 1000×g for 5 min at 4 °C (LX-130, Tomy Seiko). The filtrate was applied to a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system (API3000, Applied Biosystems/MDS SCIEX) to measure concentrations of the compounds. The lower limit of quantification (LLOQ) was 20 ng/mL for each compound. The pharmacokinetic parameters were calculated by non-compartmental analysis using Watson ver. 6.3 (ThermoFisher Scientific).

5.7. Antitumor test

Cell suspension of human lung carcinoma cell line Calu-6 was prepared using Hanks' balanced salt solution implanted subcutaneously in the flank region of female Balb/c nude mice, respectively at 5.0×10^6 cells per mouse. When the volume of the tumor reached 150 mm^3, the test compound was orally administered once daily for 11 days. The tumor volume was calculated from the equation $1/2 \times (\text{long diameter} \times \text{short diameter}^2)$. The tumor growth inhibition rate was calculated from changes in the tumor volume in the test compound treatment group relative to changes in that of the control group.

5.8. Measurement of the number of blood vessels in the tumor

5.0×10^6 Cells of human lung carcinoma cell line Calu-6 were implanted subcutaneously in the flank region of female Balb/c nude mice. When the tumor volume reached 150 mm^3, the test compound was orally administered once daily for 11 days. Twenty-four hours after the final administration, xenograft tissue was removed from the mice, and a middle portion of the long diameter of the tumor was embedded, as a block 2 to 3 mm thick, in O.C.T. Compound, and preserved as a frozen tissue specimen. Frozen sections were prepared, and blood vessels in the tumor tissue were stained by an immunohistological method using anti-mouse CD31 antibody. The stained tissue was photographed under a microscope, and the images were stored as an image file. The number of the stained blood vessels was measured by Image-Pro Plus (Nippon Roper K.K.). The rate of blood vessel density decrease was calculated as a rate compared to the blood vessel density decrease in the control group.

5.9. Antitumor test of 32f in combination with sunitinib

Cell suspension of human lung carcinoma cell line Calu-6 was prepared using Hanks' balanced salt solution and implanted subcutaneously in the flank region of female and Balb/c nude mice at 5.0×10^6 cells per mouse. When the volume of the tumor reached 200 mm^3, the mice were randomized into 4 groups (n = 5), and the following treatment was implemented orally once daily for 11 days: (1) vehicle, (2) **32f** (150 mg/kg), (3) sunitinib (80 mg/kg), (4) combination of both test compounds. The tumor volume was calculated from the equation $1/2 \times (\text{long diameter} \times \text{short diameter}^2)$. The tumor growth inhibition rate was calculated from changes in the tumor volume in the test compounds treatment group relative to changes in that of the control group.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.058.

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