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Synthesis and evaluation of 1,4-diphenylbutadiene derivatives as inhibitors of plasminogen activator inhibitor-1 (PAI-1) production

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ABSTRACT

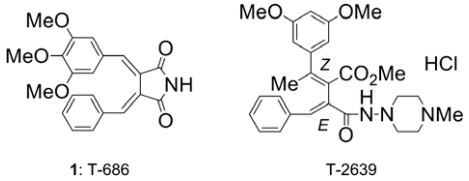
Butadiene-imide **1** (T-686) derivatives were synthesized and evaluated for their inhibitory activity against PAI-1 production and their ADMET (DMPK and toxicology) profiles. Among these derivatives, compound **15k** (T-2639) showed good antithrombotic activity in two rat thrombosis models without affecting bleeding time, indicating reduction of haemorrhagic risk. We also describe in this report a practical synthesis of **15k** suitable for scale-up using *Z*,*E*-selective Stobbe condensation.

1. Introduction

The first step in the healing of a bleeding wound is a natural physiologic response that causes blood at the injured site to clot. This response is controlled by a homeostatic balance between procoagulant forces and anticoagulant/fibrinolytic forces. However, under disease state 'thrombotic disorders', this balance is tipped in favor of coagulation, leading to the pathologic formation of thrombi in veins, arteries, or heart chambers. Thrombi can obstruct blood flow at the site of formation, causing atrial fibrillation, heart attack, myocardial infarction, unstable angina, deep vein thrombosis (DVT), pulmonary embolism, or acute ischemic stroke.

Plasminogen activator inhibitor-1 (PAI-1), a specific inhibitor of both tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), is a key negative regulator of the fibrinolytic system.[1] High levels of PAI-1 reduce fibrinolytic potential and contribute to the development of thrombosis. Indeed, elevated levels of PAI-1 in plasma have been observed in patients with deep vein thrombosis[2] and unstable angina.[3] Furthermore, a number of animal studies have shown that high levels of PAI-1 interfere with fibrinolytic activity in thrombotic and prethrombotic states.[4] Thus, inhibition of PAI-1 activity or reduction of its production may shift the balance between thrombogenesis and thrombolysis towards thrombolysis, leading to beneficial therapeutic effects for a number of thrombotic disorders. In fact, it has been reported that an antibody against PAI-1 can enhance clot lysis and decrease thrombus growth in animal models of venous thrombosis[5] and arterial thrombosis.[6] To date, small molecules have been reported to inhibit PAI-1[7] and show in vivo efficacy in animal thrombosis models.[8,9]

Among such small molecules, butadiene-imide compound **1** (T-686, Fig. 1),[8a] an inhibitor of PAI-1 production that prevents fibrinolysis shutdown,[10,11] was previously shown to exhibit antithrombotic activity in animal models.[12] Although compound **1** was believed to represent a new class of orally active antithrombotics, it showed several drawbacks, including low water solubility (9 μg/ml), poor oral bioavailability (<3% in dog), and chromosomal aberration toxicity. Further studies of butadiene-imide derivatives[8b] led us to the discovery of 1,4-diphenylbutadiene T-2639 as a strong in vitro and in vivo PAI-1 inhibitor with good ADMET profile. In this paper, we report our work on the discovery of T-2639 (drug design, synthesis, and evaluation of 1,4-diphenylbutadiene derivatives) and examine the beneficial effects of this compound in two rat thrombosis models. Moreover, we describe a practical synthesis of T-2639 using *Z*,*E*-selective Stobbe condensation.[13]



1: T-686 T-2639

Figure 1. **1** (T-686) and T-2639.

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2. Results and discussion

2.1. Synthesis

We have previously reported syntheses of the butadiene derivatives (**1**–**4**, Fig. 2).[8a] The butadiene analogues **8**, **9**, **11**, **12** were synthesized by conventional Stobbe condensation[14] as noted in a previous report[8b] (Scheme 1). In the case of Stobbe condensation between succinates and ketones, the condensation proceeded unselectively, leading to a mixture of two isomers. Due to difficulty to directly separate the two isomers as carboxylic acids, we proceeded with their separation after conversion into the corresponding methyl esters (**6**, **7**) or amides (**11**, **12**). Products **6** and **7** were transformed by Stobbe condensation and amidation into **8** and **9**.

Syntheses of **15a**–**n** are shown in Scheme 2. After conversion of Stobbe condensation product into the corresponding methoxymethyl (MOM) ester and separation of the two isomers by chromatography, the methoxymethyl (MOM) ester **13** was afforded. Treatment of **13** with HCl provided the key intermediate (2*Z*,3*E*)-**14**, whose stereochemistry was confirmed by X-ray crystallography (Fig. 3). (2*Z*,3*E*)-**14** was transformed into the desired amides and hydrazones **15a**–**l** using a conventional method, and the imide compounds **15m**,**n** were afforded by treatment of **15a**,**b** with sodium hydroxide solution followed by treatment with HCl.

The synthetic route of compound **21a**,**b** is shown in Scheme 3. Stobbe reaction between dimethyl succinate and 3′,5′-dimethoxyacetophenone proceeded selectively, and esterification of the crude product of this condensation predominantly provided the (*E*)-diester **17**. Isomerization of **17** by photo-irradiation and separation of the formed isomers by chromatography afforded the (*Z*)-diester **18**, which was transformed into **21a**,**b** in several conventional steps.

The *E* or *Z* configuration of the products in Scheme 1–3 could be confirmed by ^{1}H NMR. Assignments of their stereochemistry are in concordance with the previous reports.[15]

2.2. Design and evaluation of butadiene-imide 1 (T-686) derivatives

We first started our design of butadiene-imide **1** derivatives with optimization of the butadiene scaffold. The relative inhibitory activity against lipopolysaccharide (LPS)-induced PAI-1 production in cultured vascular endothelial cells (compound concentration: 10 μM) and the stability in dog blood are summarized in Table 1. As the good crystalline properties of the imide are believed to be responsible for low water solubility and poor bioavailability, the amide-ester type compounds **2**–**4** and compounds **8**, **9**, **11**, and **12**, where a methyl group was introduced, were synthesized and evaluated for their PAI-1 production inhibitory activity and stability in dog blood. Although **8**, **9**, and **11** showed decreased activity, **2**–**4** and **12** were found to possess moderate to high inhibitory activity of PAI-1 production. Compounds **2** and **4** showed a largely decreased stability in dog blood compared to **1**, and their ester moiety seemed to be unstable for hydrolysis (0% remains in dog blood). On the other hand, compounds **3** and **12** exhibited a largely increased stability in dog blood compared with **2** and **4** (**3**, 75%; **12**, 96%). It is believed that ester hydrolysis in these compounds is sterically blocked by the (*Z*)-positioned 3,4,5-trimethoxyphenyl ring. As expected, **12** exhibited improved solubility in water compared with **1** (**12**, 106 μg/ml; **1**, 9 μg/ml, Table 3) and enhanced bioavailability (**12**, 9%; **1**, 3% in dog). Moreover, **12** was negative in chromosomal aberration test.

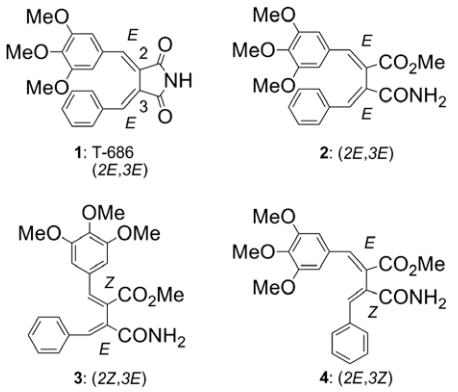
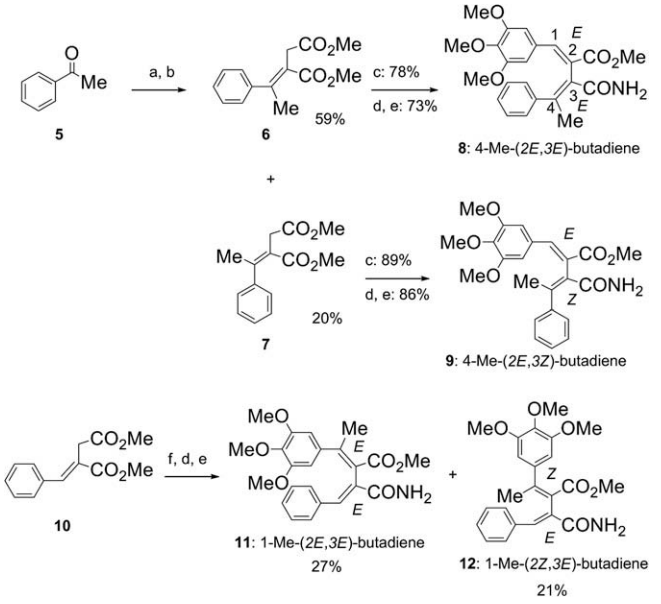


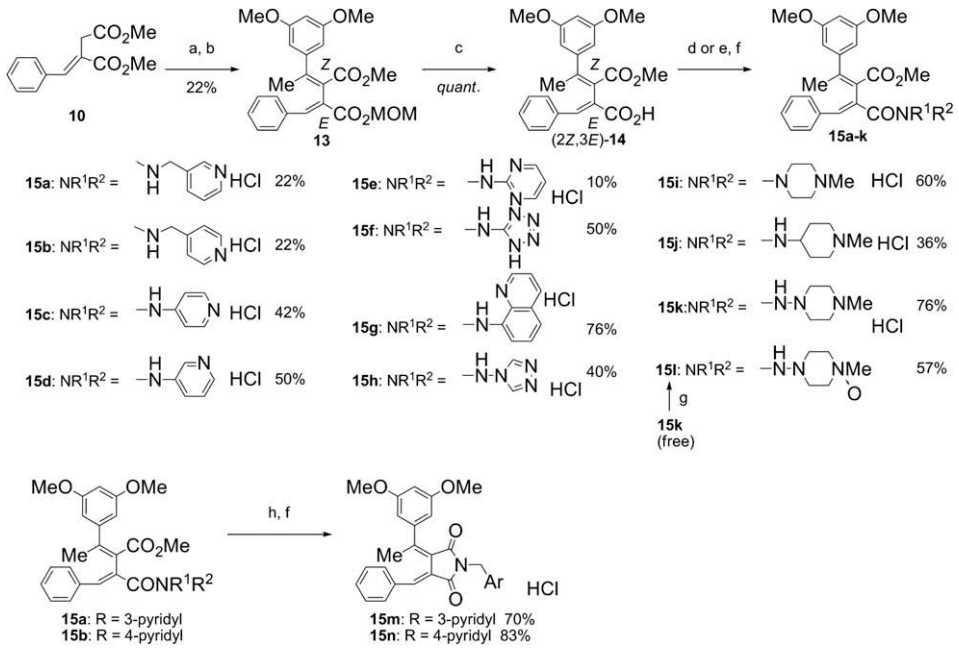
Figure 2. Compound **1** and its derivatives (**2**–**4**).



Scheme 1. Reagents and conditions: (a) dimethyl succinate, *t*-BuOK, *t*-BuOH, rt, 1 h; (b) $SOCl_2$, MeOH, rt, 16 h; (c) 3,4,5-trimethoxybenzaldehyde, *t*-BuOK, *t*-BuOH, THF, rt, 1 h; (d) $SOCl_2$, $CHCl_3$, reflux, 30 min; (e) 28% NH_4OH, 0 °C to rt, 30 min; (f) 3′,4′,5′-trimethoxyacetophenone, *t*-BuOK, *t*-BuOH, THF, rt, 1 h.

We next intensified our efforts in optimizing compound **12**. We first considered metabolic stability, and thus tried to remove one of the three methoxy groups as these groups are generally known to be typical metabolic targets leading to demethylated phenol metabolite[16] (Segment A, Fig. 4). We also tried to replace the phenyl ring with a pyridine ring to enhance water solubility (Segment B). Moreover, we considered introducing other amide moieties that would form a salt, and thereby improve solubility (Segment C). Finally, we cycled the amide-ester part into an imide and introduced a pyridine ring to improve solubility (Segment D).

The relative inhibitory activity against LPS-induced and transforming growth factor (TGF)-β-induced PAI-1 production in cultured vascular endothelial cells is shown in Table 2 (LPS-induced: compound concentration 1 μM, TGF-β-induced: compound concentration 2 μM). Among the amide-ester type compounds, compounds **15a**–**d**, having a pyridine ring, and the hydrazone compound **15k** revealed equal or enhanced inhibitory activity against LPS-induced PAI-1 production compared with **12**. However, other amides (**15e**–**j**) and the hydrazone **15l** exhibited in general weak inhibitory activity. Replacement of the phenyl ring in the segment B with a 4-pyridyl ring (**21a**) led to an inhibitory activity equal to that of **12**, while the use of a 3-pyridyl ring (**21b**) led to decreased inhibitory activity. Finally, the imide type compounds **15m**,**n**, having a pyridine ring like **15a**,**b**, exhibited good inhibitory activity against LPS-induced PAI-1 production.



Scheme 2. Reagents and conditions: (a) 3′,5′-dimethoxyacetophenone **16**, *t*-BuOK, *t*-BuOH, THF, 50 °C, 4 h; (b) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 0.5 h; (c) concd HCl, THF, rt, 3 h; (d) CDI, CH_2Cl_2, rt, 2 h, then 3- or 4-picolylamine, rt, 8 h; (e) $(COCl)_2$, DMF (cat.), CH_2Cl_2, rt, 1 h, then HNR^1R^2, (Et_3N or pyridine), THF, rt; (f) 4*N* HCl/EtOAc, rt; (g) mCPBA, CH_2Cl_2, −50 °C to rt, 1 h; (h) NaOH aq, THF, rt, 30 min.

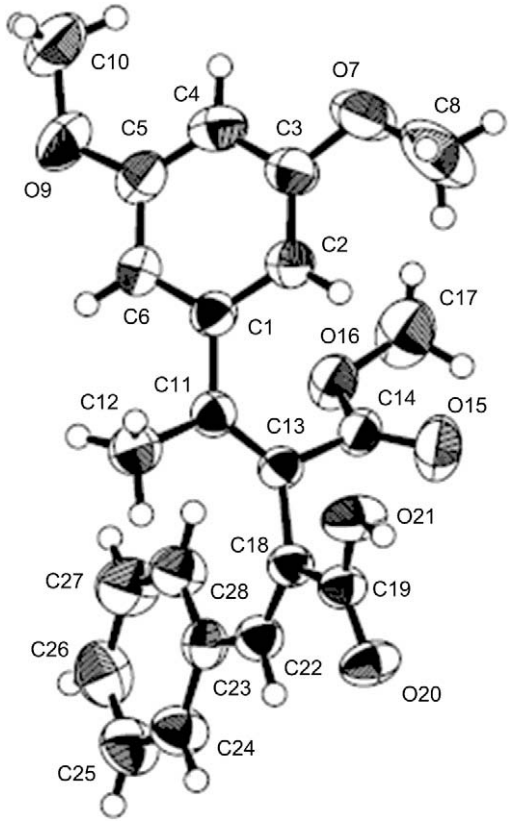


Figure 3. Perspective drawing of X-ray structure of (2*Z*,3*E*)-**14**.

As for the relative inhibitory activity against TGF-β-induced PAI-1 production, similar results as those obtained with inhibition of LPS-induced PAI-1 production were observed, with only few exceptions. Specifically, compounds **15a–d**,**k**,**m**,**n**, and **21a** showed moderate to good inhibitory activity against TGF-β-induced PAI-1 production.

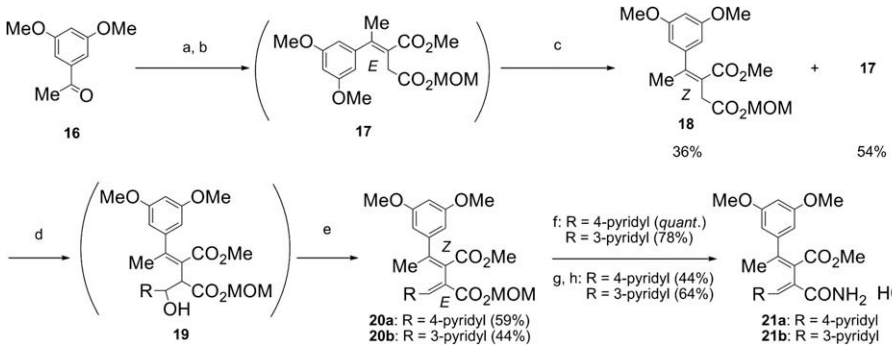
Next, **15a–d**,**k**,**m**,**n**, and **21a** were evaluated for their physicochemical and pharmacokinetic properties (Table 3). The solubility of amide-ester type compounds was largely enhanced compared to **1**, and stability of most compounds in dog blood was improved. This improvement in solubility and stability led to improved bioavailability with compounds **15a–c**,**k**, and **21a** satisfying our criterion (>20%). On the other hand, the imide type compounds **15m**,**n** exhibited a solubility lower than that of the amide-ester type compounds and their photostability proved to be remarkably scarce compared with that of **15a**,**b**. As the photostability of **1** is also poor, it is believed that imide type compounds are inherently unstable.

Finally, we examined compounds **15a–c**,**k**, and **21a** for chromosomal aberration, antithrombotic activity, and toxicity (300 mg/kg po) in male rats. Among the five compounds, only compound **15k** showed a good profile in all studies. Thus, we selected **15k** (T-2639) as the best compound.

2.3. Scale-up synthesis of 15k (T-2639)

In the synthetic route of **15k** in Scheme 2, Stobbe condensation between compound **10** and 3′,5′-dimethoxyacetphenone **16** proceeded unselectively, resulting in a mixture of two isomers (2*Z*,3*E*)- and (2*E*,3*E*)-**14**, which were difficult to separate. In order to improve selectivity in this Stobbe condensation, we examined the reaction conditions (Scheme 4).

The results of a previous report[13] have indicated that Stobbe condensation provides only a small amount of (2*Z*,3*E*)-**14** Na salt as crystals when 24% w/w NaOMe solution in MeOH[18] is used as a base and toluene as a solvent. This means that the crystallinity of (2*Z*,3*E*)-**14** Na salt in toluene is higher than that of (2*E*,3*E*)-**14** Na salt.[19] We have also confirmed that isomerization from (2*E*,3*E*)-**14** to (2*Z*,3*E*)-**14** occurs under basic conditions. Indeed, when 330 g of (2*E*,3*E*)-**14** was treated with *t*-BuOK (1.1–1.2 equiv) in *t*-BuOH at 50–60 °C for 10 min, 132 g (42%) of (2*Z*,3*E*)-**14** and 102 g (27%) of (2*E*,3*E*)-**14** were obtained. From these results, we



Scheme 3. Reagents and conditions: (a) dimethyl succinate, *t*-BuOK, *t*-BuOH, 50 °C, 4 h; (b) MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 16 h; (c) hν, MeCN, rt, 8 h; (d) LDA, THF, −78 °C, 0.5 h, then 3- or 4-pyridinecarboxaldehyde, THF, −100 °C, 20 min; (e) MsCl, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, then DBU, CH_2Cl_2, rt, 16 h; (f) concd HCl, THF, rt, 1 h; (g) CDI, THF, CH_2Cl_2, rt, 16 h, then 28% NH_4OH, 0 °C, 2 h; (h) 4*N* HCl/EtOAc, rt.

Table 1
Inhibitory activity on PAI-1 production and stability in dog blood

Compound	Relative inhibitory activity[a]	Stability in dog blood % of remain[b]
1 (T-686)	1.0	79
2	0.9	nd
3	0.7	75
4	0.7	nd
8	0.2	nt
9	0.3	nt
11	0.3	nt
12	1.0	96

nd: not detected.
nt: not tested.
[a] Relative inhibitory activity on LPS-induced PAI-1 production in cultured vascular endothelial cells (compound concentration: 10 μM). Compound **1** (T-686) is used as reference.[8a]
[b] % of remains shown in dog blood after 1 h at 37 °C.

Table 2
Inhibitory activity on LPS- or TGF-β-induced PAI-1 production in cultured vascular endothelial cells

Compound	Relative inhibitory activity[a]	
	LPS-induced	TGF-β-induced
1 (T-686)	1.0	1.0
12	1.5	0.7
(2*Z*,3*E*)-**14**	0.4	0.3
15a	1.3	1.8
15b	2.1	1.9
15c	1.8	1.1
15d	2.2	1.1
15e	0.1	nt
15f	0.1	nt
15g	0.4	nt
15h	0.6	nt
15i	0.3	0.3
15j	0.8	<0.1
15k	1.5	0.9
15l	0.2	nt
15m	1.0	1.4
15n	1.8	1.4
21a	1.5	1.1
21b	0.8	1.2

nt: not tested.
[a] Relative inhibitory activity on LPS- or TGF-β-induced PAI-1 production in cultured vascular endothelial cells (LPS-induced: compound concentration 1 μM, TGF-β-induced: compound concentration 2 μM). Compound **1** (T-686) is used as a reference. The IC_{50} values of **1** for LPS- or TGF-β-induced PAI-1 production were 0.9 μM or 4.3 μM, respectively.

anticipated that (2*Z*,3*E*)-**14** Na salt could predominantly be obtained if isomerization of (2*E*,3*E*)-**14** to (2*Z*,3*E*)-**14** takes place and the generated (2*Z*,3*E*)-**14** is separated as a solid from the reaction mixture under reaction conditions (Scheme 5).

Predominant precipitation and in situ isomerization were finally achieved by a combination of seeding (2*Z*,3*E*)-**14** Na salt (0.01 equiv) into the reaction mixture and removal of MeOH, which originated from NaOMe solution, from the Stobbe reaction system to precipitate the desired (2*Z*,3*E*)-**14** salt. As a result, (2*Z*,3*E*)-**14** was selectively obtained in 82% yield with 91:9 selectivity.[13]

As for the isomerization between (2*Z*,3*E*)-**14** and (2*E*,3*E*)-**14**, we speculate that the mechanism for this isomerization is conjugate addition of base (*t*-butoxide or methoxide) to the α,β-unsaturated ester group and isomerization of the resulting anion followed by re-elimination of the base as reported previously (Scheme 6).[15]

As described above, we could selectively prepare (2*Z*,3*E*)-**14**. This optimized method was also applicable to a scale-up synthesis of **15k**. In the synthesis of **15k**, the undesired isomer was removed by recrystallization in the final step. Four hundred and twenty gram of pure **15k** could be provided without chromatography in all steps. (Scheme 7).

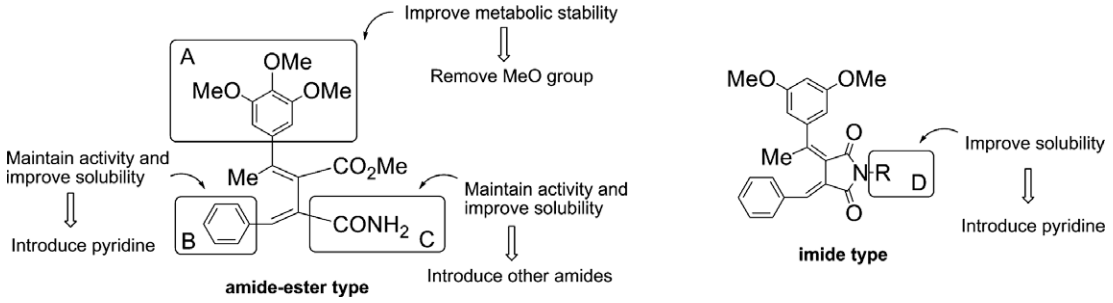
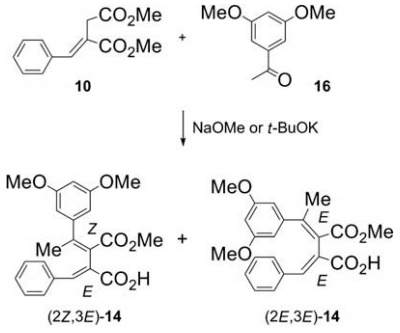


Figure 4. Optimization of **12**.

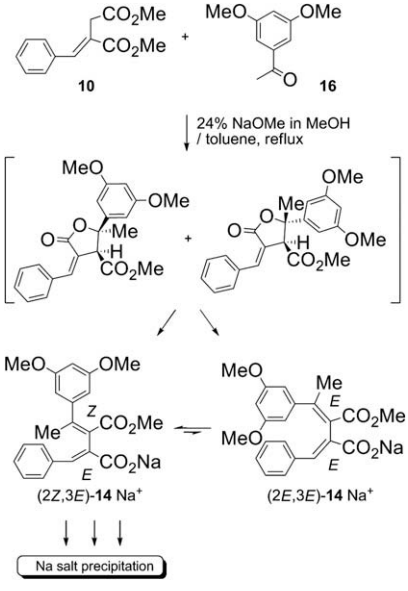
Table 3
Water solubility, stability in dog blood, bioavailability, photostability, geno-toxicity, antithrombotic activity, and toxicity in rats of selected compounds

Compound	Water solubility (μg/ml)	Stability in dog blood % remain[a]	Bioavailability (%) (dog)	Photostability% remain[b]	Chromosomal aberration test	Antithrombotic activity % decrease in thrombus weight[c]	Toxicity in male rats at 300 mg/kg po
1 (T-686)	9	79	3	12	+	nt	nt
12	106	96	9	91	–	nt	nt
15a	1600	87	21	90	–	53	Decrease in locomotor activity
15b	>2000	76	28	96	+	nt	nt
15c	>3000	87	52	85	–	58	Decrease in locomotor activity
15d	>2000	83	17	88	nt	nt	nt
15k	>4000	89	49	91	–	50	No toxicity
15m	38	65	nt	47	nt	nt	nt
15n	111	60	18	38	–	nt	nt
21a	>4000	88	78	97	+	nt	nt

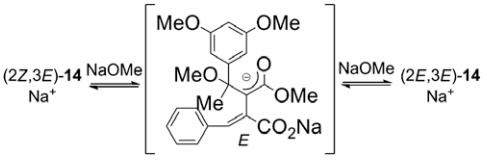
nt: not tested.
[a] % of remains shown in dog blood after 1 h at 37 °C.
[b] % of remains shown in 0.1*N* HCl solution under room light after 24 h.
[c] Antithrombotic effects in rat venous thrombosis model (3 mg/kg/day × 3 days). See Ref. 17.



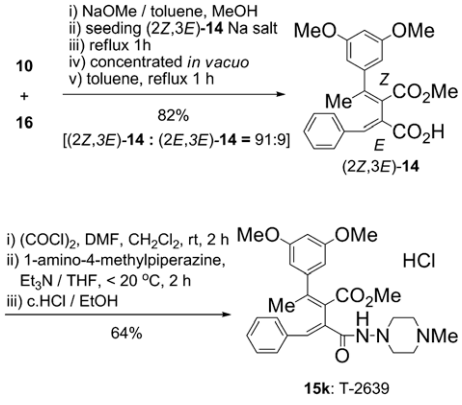
Scheme 4.



Scheme 5. Selective preparation of (2*Z*,3*E*)-**14** by predominant precipitation of (2*Z*,3*E*)-**14** Na salt and in situ isomerization of (2*E*,3*E*)-**14** to (2*Z*,3*E*)-**14**.



Scheme 6. Mechanism of isomerization between (2*Z*,3*E*)-**14** and (2*E*,3*E*)-**14**.



Scheme 7. Scale-up synthesis of **15k**.

2.4. Antithrombotic activity of 15k (T-2639)

15k also exhibited antithrombotic activity in a rat arterio-venous shunt model. In the rat arterio-venous shunt model,[12] treatment with **15k** (10 mg/kg/day) for eight consecutive days inhibited thrombus formation by 22% (Fig. 5).

Haemorrhagic risk of **15k** and Warfarin was evaluated using ED_{50} value of antithrombotic effects in the rat venous thrombosis model and ED_{100} value of bleeding effects in the rat tail incision bleeding time test[12] (Fig. 6, Table 4). Compound **15k** did not prolong bleeding time in the rat tail even at 125 times its effective dose in the venous thrombosis model, whereas Warfarin prolonged bleeding time at or near its antithrombotic dose.

3. Conclusion

We examined the structure–activity relationships of a series butadiene-imide **1** (T-686) derivatives and found **15k** (T-2639) as an orally active inhibitor of PAI-1 production. The synthesis of

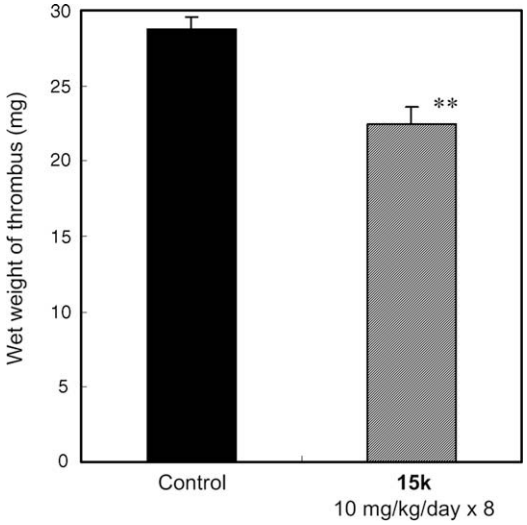


Figure 5. Antithrombotic effect of **15k** in the rat arterio-venous shunt model. Data are given as the mean ± SE of nine rats. $^{**}p$ <0.01 compared with the control.

15k was improved for pilot scale-up, and the biological effects of **15k** in rat arterio-venous shunt model and rat venous thrombosis model were evaluated. As a result, **15k** was proved not to possess the drawbacks seen in **1** and to show antithrombotic activity in the two experimental models without affecting bleeding time. These results suggest that **15k**, or its derivatives, might provide a safe treatment for thrombosis. Further research on the antithrombotic effects of **15k** is ongoing.

4. Experimental

4.1. Chemistry

Melting points were measured using a Büchi 535 capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer Spectrum One FT-IR spectrometer or Perkin-Elmer PARAGON1000. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200 spectrometer, Bruker AVANCE 400 spectrometer, or Varian UNITY INOVA500 with Me_4Si as an internal standard. Mass spectra were obtained on a Hitachi M-2000A spectrometer, ThermoQuest LCQ Advantage, ThermoFisher FINNIGAN LXQ or a Q-TOF Ultima API mass spectrometer. Elemental analyses were obtained on a Perkin-Elmer 2400 II (C, H, N) and Dionex DX-320 (Cl).

Table 4
Antithrombotic activity versus haemorrhagic risk in rats treated with **15k** or Warfarin for 8 days

	15k (T-2639)	Warfarin
ED_{50}VT (venous thrombosis)	0.8 mg/kg/day	0.1 mg/kg/day
ED_{100}BT (bleeding time)	>100 mg/kg/day	0.2 mg/kg/day
$ED_{100}BT/ED_{50}VT$	>125.0	2.0
Relative risk	1.0	>62.5

4.1.1. Dimethyl 2-[1-phenyl-(*E*)-ethylidene]succinate (6)

Acetophenone (15.5 g, 0.129 mol) and dimethyl succinate (27.1 g, 0.185 mol) were added dropwise to a suspension of *t*-BuOK (14.5 g, 0.129 mol) in *t*-BuOH (100 ml) below 40 °C, and the reaction mixture was stirred at room temperature for 1 h. Water was then added to the mixture and the resulting mixture was washed with *i*-Pr_2O. After the aqueous layer was acidified with concd HCl (pH 1), AcOEt was added. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting oil was dissolved with MeOH (150 ml), and thionyl chloride (12.5 ml, 0.194 mol) was slowly added at 0 °C. The reaction mixture was stirred at room temperature for 16 h, and then concentrated in vacuo. After the resulting oil was dissolved with AcOEt, water was added to the mixture. The organic layer was separated, washed with sat. $NaHCO_3$ aq and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel to give **6**[20] (18.6 g, 59%) and **7**[20] (6.2 g, 20%) as an oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.41 (3H, s), 3.20 (2H, s), 3.65 (3H, s), 3.79 (3H, s), 7.10–7.20 (2H, m), 7.25–7.40 (3H, m). IR (film) cm^{-1}: 2970, 2940, 2860, 1745, 1440, 1270, 1200, 1180, 1135, 1060, 770, 705. MS *m*/*z*: 248 ($[M]^+$).

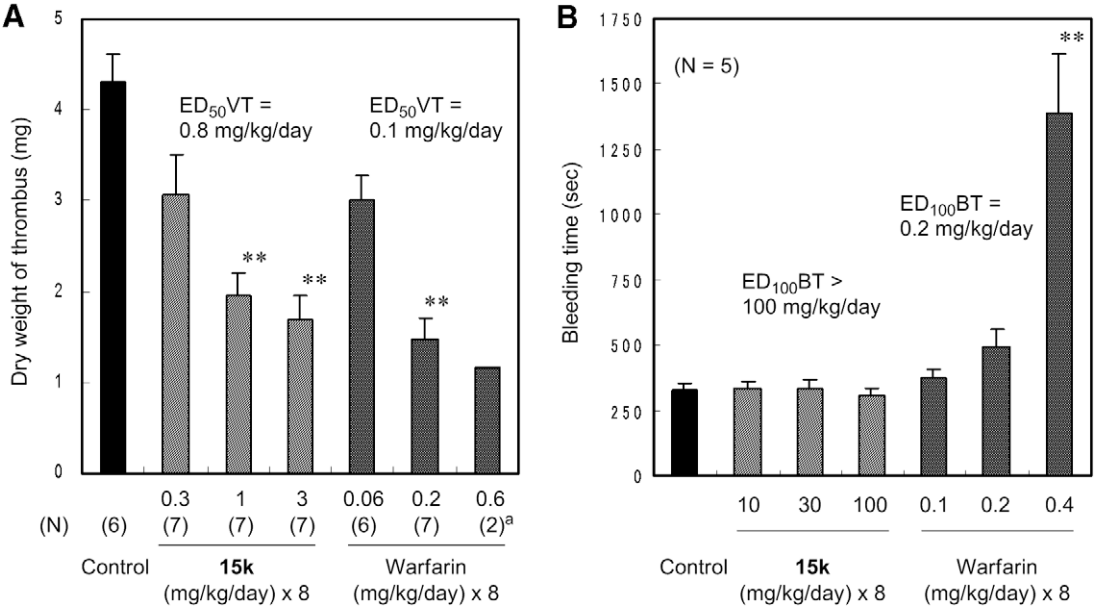


Figure 6. Antithrombotic effects of **15k** and Warfarin in the rat venous thrombosis model (A) and rat tail incision bleeding time test (B). $^{**}p$ <0.01 compared with the control.
[a]5 of 7 rats died under the test.

4.1.2. Dimethyl 2-[1-phenyl-(*Z*)-ethylidene]succinate (7)

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.14 (3H, s), 3.39 (3H, s), 3.54 (2H, s), 3.72 (3H, s), 7.10–7.20 (2H, m), 7.25–7.40 (3H, m). IR (film) cm^{-1}: 2960, 2920, 2850, 1740, 1710, 1440, 1320, 1250, 1195, 1170, 1140, 765, 700. MS *m*/*z*: 248 ([M]$^+$).

4.1.3. Methyl (*E*)-3-carbamoyl-4-phenyl-2-[1-(3′,4′,5′-trimethoxyphenyl)-(*E*)-ethylidene]-3-pentenoate (8)

A solution of **6** (5.0 g, 20.1 mmol) and 3,4,5-trimethoxybenzaldehyde (4.0 g, 20.1 mmol) in THF (20 ml) was added dropwise to a suspension of *t*-BuOK (2.26 g, 20.1 mmol) in *t*-BuOH (20 ml) below 40 °C, and the reaction mixture was stirred at room temperature for 1 h. Water was added to the mixture, and the resulting mixture was washed with *i*-Pr_2O. After the aqueous layer was acidified with concd HCl (pH 1), AcOEt was added. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on silica gel. After the obtained carboxylic acid (6.50 g, 15.8 mmol) was dissolved in $CHCl_3$ (30 ml), thionyl chloride (1.15 ml, 15.8 mmol) and DMF (three drops) were added, and the reaction mixture was stirred at reflux for 30 min. Twenty-eight percent NH_3 aq. (20 ml) was next added to the mixture at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. After water and $CHCl_3$ were added to the mixture, the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The resulting oil was chromatographed on silica gel to give **8** (4.7 g, 57%) as crystals.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.25 (3H, s), 3.62 (3H, s), 3.71 (3H, s), 3.76 (6H, s), 6.81 (2H, dd, J = 6.4, 2.9 Hz), 7.05 (2H, s), 7.06 (1H, br), 7.13–7.16 (3H, m), 7.24 (1H, br), 7.29 (1H, s). IR (Nujol) cm^{-1}: 3416, 3194, 2924, 2854, 1712, 1657, 1464, 1243, 1121. MS APCI *m*/*z*: 412 ([M+H]$^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.06; H, 6.13; N, 3.28.

4.1.4. Methyl (*Z*)-3-carbamoyl-4-phenyl-2-[1-(3′,4′,5′-trimethoxyphenyl)-(*E*)-ethylidene]-3-pentenoate (9)

This compound was prepared from **7** as described in the synthesis of **8**.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.74 (3H, s), 3.68 (3H, s), 3.77 (6H, s), 3.78 (3H, s), 6.58 (1H, br), 7.05 (1H, br), 7.23–7.38 (7H, m), 7.75 (1H, s). IR (Nujol) cm^{-1}: 3416, 2924, 2854, 1693, 1654, 1459, 1243, 1132. MS APCI *m*/*z*: 412 ([M+H]$^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.12; H, 5.90; N, 3.43.

4.1.5. Methyl (*E*)-2-((*E*)-1-carbamoyl-2-phenylvinyl)-3-(3′,4′,5′-trimethoxyphenyl)-2-butenoate (11)

A solution of **10**[8a] (22.5 g, 119 mmol) and 3′,4′,5′-trimethoxyacetophenone (25.0 g, 119 mmol) in THF (100 ml) was added dropwise to a suspension of *t*-BuOK (12.0 g, 119 mmol) in *t*-BuOH (125 ml) below 40 °C, and the reaction mixture was stirred at room temperature for 1 h. Water was added to the mixture, and the resulting mixture was washed with *i*-Pr_2O. After the aqueous layer was acidified with concd HCl (pH 1), AcOEt was added. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on silica gel. After the obtained carboxylic acids were dissolved in $CHCl_3$ (100 ml), thionyl chloride (7.5 ml, 103 mmol) was added to the mixture. The reaction mixture was then stirred at reflux for 30 min. Twenty-eight percent NH_3 aq (50 ml) was added to the mixture at 0 °C, and the reaction mixture was stirred at room temperature for 30 min. After water and $CHCl_3$ were added to the mixture, the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The resulting oil was chromatographed on silica gel to give **11** (13.2 g, 27%) and **12** (10.3 g, 21%) as crystals.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.32 (3H, s), 3.46 (6H, s), 3.58 (3H, s), 3.66 (3H, s), 6.05 (2H, s), 7.07 (1H, s), 7.12 (1H, br), 7.18–7.24 (2H, m), 7.27–7.34 (3H, m), 7.38 (1H, br). IR (Nujol) cm^{-1}: 3438, 3183, 2925, 2854, 1719, 1664, 1581, 1464, 1231, 1129. MS APCI *m*/*z*: 412 ([M+H]$^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.00; H, 5.73; N, 3.41.

4.1.6. Methyl (*Z*)-2-((*E*)-1-carbamoyl-2-phenylvinyl)-3-(3′,4′,5′-trimethoxyphenyl)-2-butenoate (12)

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.72 (3H, s), 3.39 (3H, s), 3.66 (3H, s), 3.76 (6H, s), 6.50 (2H, s), 7.26–7.37 (3H, m), 7.37–7.44 (2H, m), 7.52–7.59 (3H, m). IR (Nujol) cm^{-1}: 3426, 3322, 2924, 2853, 1709, 1581, 1459, 1129. MS APCI *m*/*z*: 412 ([M+H]$^+$). Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.06; H, 6.07; N, 3.38.

4.1.7. 1-Methyl 2-[1-(3′,5′-dimethoxyphenyl)-(*Z*)-ethylidene]-3-[1-phenyl-(*E*)-methylidene]-4-succinic Acid ((2*Z*,3*E*)-14)

A solution of 3′,5′-dimethoxyacetophenone (**16**) (4.8 g, 26.6 mmol) and **10** (6.3 g, 26.78 mmol) in THF (25 ml) was added dropwise to a suspension of *t*-BuOK (3.3 g, 29.4 mmol) in *t*-BuOH (20 ml) below 40 °C, and the reaction mixture was stirred at 50 °C for 4 h. Water was added to the mixture, and the resulting mixture was washed with *i*-Pr_2O. After the aqueous layer was acidified with concd HCl (pH 1), AcOEt was added. The organic layer was next separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2 (100 ml), and *N*,*N*-diisopropylethylamine (5.18 g, 40.0 mmol) and methoxymethyl chloride (2.59 g, 32.2 mmol) were slowly added to the mixture. The reaction mixture was stirred at room temperature for 30 min. Water and $CHCl_3$ were then added to the mixture. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel to give **13** (2.50 g, 22%) as an oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.75 (3H, s), 3.51 (3H, s), 3.51 (3H, s), 3.79 (6H, s), 5.40 (2H, s), 6.31 (2H, d, J = 2.2 Hz), 6.39 (1H, t, J = 2.2 Hz), 7.25–7.45 (3H, m), 7.50–7.70 (2H, m), 7.95 (1H, s). MS *m*/*z*: 426 ([M]$^+$).

Concd HCl (2.5 ml) was added dropwise to a solution of **13** (3.9 g, 9.1 mmol) in THF (25 ml). After the mixture was stirred at room temperature for 3 h, it was neutralized by adding 2*N* NaOH aq. After water and AcOEt were added to the mixture, the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue (2*Z*,3*E*)-**14** (foam, 3.5 g, quant.) was used for the next step without purification.

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.78 (3H, s), 3.53 (3H, s), 3.80 (6H, s), 6.33 (2H, d, J = 2.3 Hz), 6.40 (1H, t, J = 2.3 Hz), 7.30–7.45 (3H, m), 7.60–7.70 (2H, m), 8.00 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.6, 51.9, 55.3, 99.4, 104.6, 124.8, 128.0, 128.7, 129.8, 130.0, 134.7, 144.6, 144.9, 150.2, 160.5, 167.6, 172.1. IR (Nujol) cm^{-1}: 2924, 2853, 1710, 1685, 1603, 1589, 1451, 1424, 1274, 1206. MS ESI *m*/*z*: 381 ([M−H]$^-$). HR-MS ESI calcd for $C_{22}H_{23}O_6$ ([M+H]$^+$): 383.1495. Found: 383.1485.

5.1.7.1. X-ray crystallographic data of (2*Z*,3*E*)-14. The compound crystallized in the monoclinic system, space group $P2_1/c$ (No. 14), a = 12.967 (2), b = 12.0214 (2), c = 13.360 (1) Å, β = 101.12 (1)^0, V = 2043.4 (5) Å^3, Z = 4, D_{calc} = 1.243 mg m^{-3}, μ = 0.747 mm^{-1}. The intensity data were collected by $2\theta - \omega$ scan technique using graphite monochromated Cu-Kα radiation (λ = 1.54184 Å) on a Rigaku AFC5R diffractometer. The structure was solved by direct method using SHELXS-97[21] and subsequent difference Fourier method. The refinement of atomic parameters was carried out using SHELXL-97[21] with anisotropic thermal parameters for non-H atoms. All hydrogen atoms were located geometrically and fixed. The final R factors converged to R = 0.046, wR = 0.165. All calculations were performed using the CrystalStructure software package. Detailed crystallographic results (atomic coordinates, bond lengths, bond angles

and thermal parameters) have been deposited with the Cambridge Crystallographic Data Centre, UK, CCDC-705024.

4.1.8. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-{(*E*)-2-phenyl-1-[(pyridin-3-ylmethyl)carbamoyl]vinyl}-2-butenoate hydrochloride (15a)

This compound was prepared from (2*Z*,3*E*)-**14** as described in the synthesis of **15b**.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.71 (3H, s), 3.38 (3H, s), 3.74 (6H, s), 4.55 (2H, d, J = 5.7 Hz), 6.36 (2H, d, J = 2.0 Hz), 6.45 (1H, t, J = 2.2 Hz), 7.25–7.38 (1H, m), 7.38–7.44 (2H, m), 7.56 (1H, s), 7.58 (2H, s), 7.90 (1H, br), 8.27 (1H, br), 8.61 (1H, br), 8.75 (2H, s). IR (ATR) cm^{-1}: 3266, 2947, 2839, 2555, 2087, 1720, 1649, 1589, 1506, 1422, 1243, 1203, 1153, 1047, 683. MS APCI *m*/*z*: 473 ([M+H]$^+$). HR-MS ESI calcd for $C_{28}H_{28}N_2O_5$ ([M+H]$^+$): 473.2076. Found: 473.2070.

4.1.9. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-{(*E*)-2-phenyl-1-[(pyridin-4-ylmethyl)carbamoyl]vinyl}-2-butenoate hydrochloride (15b)

1,1′-Carbonyldiimidazole (700 mg, 4.32 mmol) was added to a solution of (2*Z*,3*E*)-**14** (1.5 g, 3.93 mmol) in CH_2Cl_2 (10 ml), and the reaction mixture was stirred at room temperature for 2 h. 4-Picolylamine (509 mg, 4.71 mmol) was then added at 0 °C to the mixture, and the reaction mixture was stirred for 8 h at room temperature. After AcOEt and water were added to the mixture, the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. 4*N* HCl in AcOEt (0.15 ml) was added to a solution of the obtained amide in AcOEt (10 ml), and the mixture was concentrated in vacuo. The residue was diluted with Et_2O, and filtered to give **15b** (440 mg, 22%).

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.75 (3H, s), 3.43 (3H, s), 3.74 (6H, s), 4.64 (2H, d, J = 4.3 Hz), 6.37 (2H, d, J = 2.1 Hz), 6.45 (1H, t, J = 2.1 Hz), 7.34–7.39 (1H, m), 7.43 (2H, t, J = 7.5 Hz), 7.56–7.64 (3H, m), 7.78 (2H, br), 8.64 (1H, br), 8.82 (2H, br). IR (ATR) cm^{-1}: 3366, 2947, 2839, 2599, 2088, 1708, 1639, 1589, 1505, 1243, 1203, 1153, 1048, 695. MS APCI *m*/*z*: 473 ([M+H]$^+$). HR-MS ESI calcd for $C_{28}H_{28}N_2O_5$ ([M+H]$^+$): 473.2076. Found: 473.2072.

4.1.10. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-2-phenyl-1-(pyridin-4-ylcarbamoyl)vinyl]-2-butenoate hydrochloride (15c)

Oxalyl chloride (2.0 g, 15.8 mmol) was added dropwise to a solution of (2*Z*,3*E*)-**14** (5.0 g, 13.0 mmol) and *N*,*N*-dimethylformamide (one drop) in CH_2Cl_2 (20 ml) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h and concentrated in vacuo. The resulting residue was dissolved in THF (10 ml) and the mixture was added dropwise to 4-aminopyridine (3.0 g, 32 mmol) in THF (20 ml) at 0 °C. Next, the reaction mixture was stirred at room temperature for 2 h, concentrated in vacuo, and AcOEt and sat $NaHCO_3$ aq were added to the residue. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on silica gel. 4*N* HCl in AcOEt (2.7 ml) was added to a solution of the obtained amide in $CHCl_3$ (10 ml), and the mixture was concentrated in vacuo. The residue was diluted with *i*-Pr_2O and filtered to give **15c** (2.7 g, 42%).

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.71 (3H, s), 3.39 (3H, s), 3.74 (6H, s), 6.29 (2H, d, J = 2.1 Hz), 6.45 (1H, t, J = 1.9 Hz), 7.38–7.45 (1H, m), 7.48 (2H, t, J = 7.6 Hz), 7.66 (2H, d, J = 7.6 Hz), 7.81 (1H, br), 8.21 (2H, br), 8.73 (2H, d, J = 6.9 Hz), 11.54 (1H, br). IR (Nujol) cm^{-1}: 2921, 2631, 1686, 1634, 1590, 1505, 1465, 1321, 1192, 1154. MS APCI *m*/*z*: 459 ([M+H]$^+$). HR-MS ESI calcd for $C_{27}H_{26}N_2O_5$ ([M+H]$^+$): 459.1920. Found: 459.1905.

4.1.11. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-2-phenyl-1-(pyridin-3-ylcarbamoyl)vinyl]-2-butenoate hydrochloride (15d)

This compound was prepared from (2*Z*,3*E*)-**14** as described in the synthesis of **15c**.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.72 (3H, s), 3.38 (3H, s), 3.74 (6H, s), 6.30 (2H, d, J = 2.1 Hz), 6.45 (1H, t, J = 2.1 Hz), 7.37–7.44 (1H, m), 7.46 (2H, t, J = 7.4 Hz), 7.64 (2H, d, J = 7.4 Hz), 7.72 (1H, br), 7.81 (1H, br), 8.53 (2H, br), 9.18 (1H, br), 10.84 (1H, br). IR (Nujol) cm^{-1}: 2921, 2852, 1706, 1671, 1589, 1545, 1458, 1375, 1204, 1155. MS APCI *m*/*z*: 459 ([M+H]$^+$). HR-MS ESI calcd for $C_{27}H_{26}N_2O_5$ ([M+H]$^+$): 459.1920. Found: 459.1900.

4.1.12. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-2-phenyl-1-(pyrimidin-2-ylcarbamoyl)vinyl]-2-butenoate hydrochloride (15e)

Oxalyl chloride (780 mg, 6.15 mmol) and *N*,*N*-dimethylformamide (100 μl) were added to a solution of (2*Z*,3*E*)-**14** (2.0 g, 5.24 mmol) in CH_2Cl_2 (20 ml) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h and concentrated in vacuo. The resulting residue was dissolved in THF (50 ml), and the mixture was slowly added to 2-aminopyrimidine (600 mg, 6.31 mmol) and pyridine (1 ml) in THF (20 ml) at 0 °C. Next, the reaction mixture was stirred at room temperature for 30 min, and AcOEt and water were added. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. 4*N* HCl in AcOEt (0.7 ml) was added to a solution of the obtained amide in $CHCl_3$ (20 ml), and the mixture was concentrated in vacuo. The residue was diluted with Et_2O and filtered to give **15e** (250 mg, 10%).

^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.95 (3H, s), 3.55 (3H, s), 3.81 (6H, s), 6.40 (2H, d, J = 2.1 Hz), 6.40–6.45 (1H, m), 7.04 (1H, t, J = 4.9 Hz), 7.25–7.50 (3H, m), 7.63–7.80 (2H, m), 7.94 (1H, s), 8.68 (2H, d, J = 4.9 Hz), 9.41 (1H, br). IR (ATR) cm^{-1}: 2948, 2840, 1725, 1687, 1572, 1533, 1201, 1154, 759, 696. MS APCI *m*/*z*: 460 ([M+H]$^+$). HR-MS ESI calcd for $C_{26}H_{25}N_3O_5$ ([M+H]$^+$): 460.1872. Found: 460.1856.

4.1.13. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-2-phenyl-1-(tetrazol-5-ylcarbamoyl)vinyl]-2-butenoate (15f)

Oxalyl chloride (390 mg, 3.07 mmol) and *N*,*N*-dimethylformamide (one drop) were added to a solution of (2*Z*,3*E*)-**14** (1.0 g, 2.61 mmol) in CH_2Cl_2 (10 ml) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h and concentrated in vacuo. The resulting residue was dissolved in THF (10 ml) and the mixture was slowly added to 5-aminotetrazol (232 mg, 2.73 mmol) and Et_3N (0.76 ml) in THF (10 ml) at 0 °C. Next, the reaction mixture was stirred at room temperature for 30 min, and AcOEt and water were added. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel and triturated with Et_2O to give **15f** (590 mg, 50%).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.72 (3H, s), 3.39 (3H, s), 3.74 (6H, s), 6.32 (2H, s), 6.44 (1H, s), 7.39 (1H, dd, J = 14.3, 7.4 Hz), 7.45 (2H, t, J = 7.4 Hz), 7.62 (2H, d, J = 7.4 Hz), 7.78 (1H, s), 11.42 (1H, br). IR (ATR) cm^{-1}: 2949, 2840, 1663, 1587, 1204, 1154, 1043, 767, 694. MS APCI *m*/*z*: 450 ([M+H]$^+$). HR-MS ESI calcd for $C_{23}H_{23}N_5O_5$ ([M+H]$^+$): 450.1777. Found: 450.1775.

4.1.14. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-2-phenyl-1-(quinolin-8-ylcarbamoyl)vinyl]-2-butenoate hydrochloride (15g)

This compound was prepared from (2*Z*,3*E*)-**14** as described in the synthesis of **15i**.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.01 (3H, s), 3.37 (3H, s), 3.83 (6H, s), 6.59 (1H, t, J = 2.1 Hz), 6.73 (2H, d, J = 2.1 Hz), 7.38–7.50

(3H, m), 7.63–7.77 (5H, m), 7.90 (1H, s), 8.48 (1H, dd, J = 8.4, 1.5 Hz), 8.83 (1H, dd, J = 3.4, 1.5 Hz), 8.85 (1H, d, J = 1.5 Hz), 10.80 (1H, s). IR (ATR) cm^{-1}: 2943, 2588, 1698, 1652, 1592, 1294, 1153, 829, 696, 458. MS APCI m/z: 509 ($[M+H]^+$). HR-MS ESI calcd for $C_{31}H_{28}N_2O_5$ ($[M+H]^+$): 509.2076. Found: 509.2091.

4.1.15. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-2-phenyl-1-([1,2,4]triazol-4-ylcarbamoyl)vinyl]-2-butenoate hydrochloride (15h)

This compound was prepared from (2*Z*,3*E*)-**14** as described in the synthesis of **15c**.

^1H NMR (400 MHz, DMSO-d_6) δ: 1.78 (3H, s), 3.43 (3H, s), 3.74 (6H, s), 6.36 (2H, d, J = 2.0 Hz), 6.45 (1H, t, J = 2.2 Hz), 7.38–7.50 (3H, m), 7.61 (2H, d, J = 7.2 Hz), 7.74 (1H, s), 8.84 (2H, s), 11.79 (1H, br). IR (ATR) cm^{-1}: 3105, 2952, 2301, 1871, 1727, 1680, 1595, 1249, 1209, 1191, 1152, 693. MS APCI m/z: 449 ($[M+H]^+$). HR-MS ESI calcd for $C_{24}H_{24}N_4O_5$ ($[M+H]^+$): 449.1825. Found: 449.1809.

4.1.16. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-1-(4-methylpiperazin-1-carbonyl)-2-phenylvinyl]-2-butenoate hydrochloride (15i)

Oxalyl chloride (2.0 g, 15.7 mmol) was added dropwise to a solution of (2*Z*,3*E*)-**14** (5.0 g, 13.0 mmol) and *N*,*N*-dimethylformamide (one drop) in CH_2Cl_2 (50 ml) at 0 °C. The reaction mixture was then stirred at room temperature for 1 h and concentrated in vacuo. The resulting residue was dissolved in THF (10 ml), and the mixture was added dropwise to *N*-methylpiperazine (1.57 g, 15.7 mmol) and Et_3N (2.21 ml, 15.7 mmol) in THF (50 ml) at 0 °C. Next, the reaction mixture was stirred at room temperature for 2 h. After the mixture was concentrated in vacuo, AcOEt and sat $NaHCO_3$ aq were added to the residue. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on silica gel. 4*N* HCl in AcOEt (2.7 ml) was added to a solution of the obtained amide in $CHCl_3$ (20 ml), and the mixture was concentrated in vacuo. The residue was diluted with Et_2O and filtered to give **15i** (3.9 g, 60%).

Mp: >219 °C (dec.). ^1H NMR (500 MHz, DMSO-d_6) δ: 1.74 (3H, s), 2.82 (3H, s), 3.05 (2H, br), 3.43 (3H, s), 3.25–3.50 (4H, br), 3.73 (6H, s), 4.39 (2H, br), 6.20 (2H, d, J = 2.0 Hz), 6.44 (1H, br), 6.99 (1H, s), 7.33–7.38 (1H, m), 7.42 (2H, t, J = 7.4 Hz), 7.54 (2H, d, J = 7.4 Hz), 10.76 (1H, br). IR (Nujol) cm^{-1}: 2924, 2854, 2345, 1708, 1606, 1460, 1208, 1160. MS APCI m/z: 465 ($[M+H]^+$). Anal. Calcd for $C_{27}H_{33}ClN_2O_5$: C, 64.73; H, 6.64; Cl, 7.08; N, 5.59. Found: C, 64.56; H, 6.75; Cl, 7.01; N, 5.57.

4.1.17. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-1-(1-methylpiperizin-4-ylcarbamoyl)-2-phenylvinyl]-2-butenoate hydrochloride (15j)

This compound was prepared from (2*Z*,3*E*)-**14** as described in the synthesis of **15i**.

Mp: >184 °C (dec.). ^1H NMR (500 MHz, DMSO-d_6) δ: 1.62 (3H, s), 1.81 (2H, br), 1.97 (2H, br), 2.74 (3H, s), 3.05 (2H, br), 3.36 (3H, s), 3.43 (2H, br), 3.73 (6H, s), 3.87 (1H, br), 6.28 (2H, d, J = 2.0 Hz), 6.43 (1H, br), 7.30–7.37 (1H, m), 7.37–7.44 (2H, m), 7.55 (2H, d, J = 7.3 Hz), 8.15 (1H, br), 9.93 (1H, br). IR (Nujol) cm^{-1}: 3273, 2923, 2853, 1695, 1641, 1595, 1463, 1376, 1153. MS APCI m/z: 479 ($[M+H]^+$). HR-MS ESI calcd for $C_{28}H_{34}N_2O_5$ ($[M+H]^+$): 479.2546. Found: 479.2543.

4.1.18. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-1-(4-methylpiperazin-1-ylcarbamoyl)-2-phenylvinyl]-2-butenoate hydrochloride (15k, T-2369)

This compound was prepared from (2*Z*,3*E*)-**14** as described in the synthesis of **15i**.

Mp: 235–240 °C. ^1H NMR (DMSO-d_6, 500 MHz) δ: 1.65 (3H, s), 2.79 (3H, s), 3.00–3.50 (8H, m), 3.37 (3H, s), 3.73 (6H, s), 6.29 (2H, d, J = 1.8 Hz), 6.43 (1H, s), 7.30–7.50 (4H, m), 7.54 (2H, d, J = 7.2 Hz), 9.48 (1H, s), 10.11 (1H, br). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 22.2, 43.2, 51.3, 52.4, 53.3, 55.5, 99.9, 104.7, 125.1, 128.6, 128.6, 129.5, 129.7, 134.7, 140.9, 143.5, 149.0, 160.8, 164.9, 168.9. IR (Nujol) cm^{-1}: 3196, 2924, 2854, 1698, 1662, 1595, 1464, 1262. MS APCI m/z: 480 ($[M+H]^+$). Anal. Calcd for $C_{27}H_{34}ClN_3O_5$: C, 62.84; H, 6.64; N, 8.14; Cl, 6.87. Found: C, 62.61; H, 6.71; N, 8.09; Cl, 6.79.

4.1.19. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-1-(4-methyl-4-oxy-piperazin-1-ylcarbamoyl)-2-phenylvinyl]-2-butenoate (15l)

Eighty percent mCPBA (248 mg, 1.15 mmol) was added at -50 °C to a free form solution of **15k** (500 mg, 1.05 mmol) in CH_2Cl_2 (25 ml),. The reaction mixture was stirred at room temperature for 1 h and concentrated in vacuo. The residue was chromatographed on silica gel to give **15l** (296 mg, 57%). ^1H NMR (400 MHz, $CDCl_3$) δ: 3.10 (2H, d, J = 10.8 Hz), 3.26 (2H, d, J = 10.8 Hz), 3.28 (3H, s), 3.51 (3H, s), 3.53–3.78 (4H, m), 3.81 (6H, s), 6.33 (2H, d, J = 2.3 Hz), 6.43 (1H, t, J = 2.3 Hz), 7.30–7.45 (3H, m), 7.57 (1H, s), 7.64 (2H, d, J = 6.9 Hz), 7.83 (1H, s). IR (ATR) cm^{-1}: 2949, 1715, 1655, 1590, 1204, 1154, 1049, 749, 696. MS APCI m/z: 496 ($[M+H]^+$). HR-MS ESI calcd for $C_{27}H_{33}N_3O_6$ ($[M+H]^+$): 496.2448. Found: 496.2445.

4.1.20. 3-[1-(3′,5′-Dimethoxyphenyl)-(*Z*)-ethylidene]-4-[1-phenyl-(*E*)-methylidene]-1-pyridin-3-ylmethylpyrrolidine-2,5-dione hydrochloride (15m)

2*N* NaOH aq (2 ml, 4 mmol) was added dropwise to a free form (1.1 g, 2.33 mmol) solution of **15a** in THF (20 ml), and the mixture was stirred for 30 min at room temperature. After the mixture was neutralized with 2*N* HCl aq, AcOEt was added. The organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was next chromatographed on silica gel. 4*N* HCl in AcOEt (2 ml) was added to a solution of the obtained imide in AcOEt (50 ml), and the mixture was concentrated in vacuo. The residue was diluted with Et_2O, and filtered to give **15m** (720 mg, 70%). ^1H NMR (400 MHz, DMSO-d_6) δ: 1.70 (3H, s), 3.74 (6H. s), 4.83 (2H, s), 6.51 (1H, t, J = 2.0 Hz), 6.54 (2H, d, J = 2.0 Hz), 7.35–7.44 (1H, m), 7.45–7.52 (2H, m), 7.57–7.64 (3H, m), 7.87 (1H, dd, J = 7.9, 5.4 Hz), 8.28 (1H, d, J = 7.9 Hz), 8.76 (1H, dd, J = 5.4, 1.4 Hz), 8.79 (1H, d, J = 1.4 Hz). IR (ATR) cm^{-1}: 3406, 2939, 2410, 2087, 1754, 1698, 1587, 1421, 1396, 1333, 1203, 1153, 1037, 768, 693, 586, 503, 477. MS APCI m/z: 441 ($[M+H]^+$). HR-MS ESI calcd for $C_{27}H_{24}N_2O_4$ ($[M+H]^+$): 441.1814. Found: 441.1805.

4.1.21. 3-[1-(3′,5′-Dimethoxyphenyl)-(*Z*)-ethylidene]-4-[1-phenyl-(*E*)-methylidene]-1-pyridin-4-ylmethylpyrrolidine-2,5-dione hydrochloride (15n)

This compound was prepared from the free form of **15b** as described in the synthesis of **15m**.

Mp: 127 °C. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.73 (3H, s), 3.74 (6H. s), 4.91 (2H, s), 6.51 (1H, t, J = 2.3 Hz), 6.55 (2H, d, J = 2.3 Hz), 7.38–7.45 (1H, m), 7.45–7.53 (2H, m), 7.59–7.67 (3H, m), 7.83 (2H, d, J = 6.4 Hz), 8.80 (2H, d, J = 6.4 Hz). IR (ATR) cm^{-1}: 2960, 2314, 2093, 2030, 1987, 1758, 1704, 1629, 1605, 1583, 1411, 1389, 1335, 1156, 1041, 924, 770, 699, 476, 467. MS APCI m/z: 441 ($[M+H]^+$). HR-MS ESI calcd for $C_{27}H_{24}N_2O_4$ ($[M+H]^+$): 441.1814. Found: 441.1801.

4.1.22. 4-Methoxymethyl 1-methyl 2-[1-(3′,5′-dimethoxyphenyl)-(*Z*)-ethylidene]-3-[1-pyridin-4-yl-(*E*)-methylidene]succinate (20a)

A solution of 3′,5′-dimethoxyacetophenone (**16**) (30 g, 166 mmol) and dimethyl succinate (29.1 g, 199 mmol) in *t*-BuOH (100 ml) was added to a suspension of *t*-BuOK (18.7 g, 166 mmol) in *t*-BuOH (200 ml) below 40 °C and the reaction mixture was stirred at 50 °C for 4 h. Water was then added to the mixture, and the

resulting mixture was washed with *i*-Pr_2O. After the aqueous layer was acidified with concd HCl (pH 1), AcOEt was added. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was diluted with CH_2Cl_2 (500 ml), and *N*,*N*-diisopropylethylamine (30 ml, 169 mmol) and methoxymethyl chloride (16 ml, 172 mmol) were added to the mixture. The reaction mixture was then stirred overnight at room temperature and concentrated in vacuo. After AcOEt and water were added to the mixture, the organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel to give **17**. Compound **17** was dissolved in acetonitrile (500 ml) and the solution was illuminated with a mercury lamp for 8 h. The solution was then concentrated in vacuo and the residue was chromatographed on silica gel to give **18** (20.2 g, 36%). (30.3 g of **17** was recovered (54%).)

^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.13 (3H, s), 3.45 (3H, s), 3.48 (3H, s), 3.56 (2H, s), 3.77 (6H, s), 5.28 (2H, s), 6.30 (2H, d, J = 2.2 Hz), 6.38 (1H, t, J = 2.2 Hz). IR (film) cm^{-1}: 2950, 1739, 1593, 1424, 1156, 1092, 929. MS *m*/*z*: 338 ([M]$^+$).

1.6 M *n*-BuLi in hexane (17.7 ml, 28.4 mmol) was added below −70 °C to a solution of diisopropylamine (4.0 ml, 28.4 mmol) in THF (60 ml). After the mixture was stirred at −78 °C for 30 min, **18** (8.0 g, 23.6 mmol) in THF (80 ml) was added dropwise to the solution below −70 °C. The reaction mixture was again stirred at −78 °C for 30 min, and then a solution of 4-pyridinecarboxaldehyde (3.0 g, 28.4 mmol) in THF (30 ml) was added dropwise to the mixture at −100 °C. After the mixture was stirred at −100 °C for 20 min, water and AcOEt were added to the mixture. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was dissolved in CH_2Cl_2 (60 ml), and triethylamine (4.0 ml, 28.4 mmol) and methanesulfonyl chloride (2.2 ml, 28.4 mmol) were added to the mixture at 0 °C. After the reaction mixture was stirred for 30 min at 0 °C, DBU (4.2 ml, 24.8 mmol) was added to the mixture. The mixture was then stirred overnight at room temperature. After AcOEt and water were added to the mixture, the organic layer was separated, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel to give **20a** (6.0 g, 59%).

^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.76 (3H, s), 3.51 (3H, s), 3.52 (3H, s), 3.79 (6H, s), 5.41 (2H, s), 6.25 (2H, d, J = 2.3 Hz), 6.40 (1H, t, J = 2.3 Hz), 7.50 (2H, dd, J = 4.6, 1.3 Hz), 7.85 (1H, s), 8.67 (2H, dd, J = 4.6, 1.6 Hz). IR (film) cm^{-1}: 2952, 1724, 1592, 1423, 1157, 1050, 928, 821, 698, 538. MS *m*/*z*: 427 ([M]$^+$).

4.1.23. Methyl (*Z*)-2-((*E*)-1-carbamoyl-2-pyridin-4-ylvinyl)-3-(3′,5′-dimethoxyphenyl)-2-butenoate hydrochloride (21a)

Concd HCl (6.0 ml) was added to a solution of **20a** (6.0 g, 14 mmol) in THF (60 ml). The mixture was stirred at room temperature for 1 h, diluted with water, and neutralized with 2*N* NaOH aq After AcOEt was added to the mixture, the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. The obtained carboxylic acid was dissolved in THF (300 ml), and 1,1′-carbonyldiimidazole (3.4 g, 21.0 mmol) was added to the mixture. After the reaction mixture was stirred at room temperature for 2 h, THF (200 ml), CH_2Cl_2 (50 ml), and 1,1′-carbonyldiimidazole (1.7 g, 10.5 mmol) were added again. The mixture was then stirred overnight at room temperature. Twenty-eight percent NH_3 aq (5.0 ml) was added to the mixture at 0 °C and the resulting mixture was stirred at 0 °C for 2 h. After water and $CHCl_3$ were added to the mixture, the organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was chromatographed on silica gel. Next, 4*N* HCl in AcOEt (1.5 ml) was added to a solution of the obtained amide in $CHCl_3$ (20 ml), and then the mixture was concentrated in vacuo. The residue was diluted with $CHCl_3$, filtered and washed with Et_2O to give **21a** (2.58 g, 44%) as pale yellow crystals.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.73 (3H, s), 3.40 (3H, s), 3.74 (6H, s), 6.35 (2H, d, J = 2.1 Hz), 6.44 (1H, t, J = 2.1 Hz), 7.53–7.65 (3H, m), 7.87 (2H, br), 8.83 (2H, br). IR (Nujol) cm^{-1}: 2921, 2853, 1699, 1592, 1461, 1373, 1153. MS APCI *m*/*z*: 383 ([M+H]$^+$). HR-MS ESI Calcd for $C_{21}H_{22}N_2O_5$ ([M+H]$^+$): 383.1607. Found: 383.1605.

4.1.24. 4-Methoxymethyl 1-methyl 2-[1-(3′,5′-dimethoxyphenyl)-(*Z*)-ethylidene]-3-[1-pyridin-3-yl-(*E*)-methylidene]succinate (20b)

This compound was prepared from **18** as described in the synthesis of **20a**.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.78 (3H, s), 3.51 (6H, s), 3.79 (6H, s), 5.41 (2H, s), 6.29 (2H, d, J = 2.2 Hz), 6.40 (1H, t, J = 2.2 Hz), 7.35 (1H, dd, J = 7.9, 4.8 Hz), 7.92 (1H, S), 8.02 (1H, d, J = 7.9 Hz), 8.58 (1H, d, J = 3.4 Hz), 8.81 (1H, s). IR (film) cm^{-1}: 2952, 1713, 1592, 1424, 1158, 1051, 928, 709. MS *m*/*z*: 427 ([M]$^+$).

4.1.25. Methyl (*Z*)-2-((*E*)-1-carbamoyl-2-pyridin-3-ylvinyl)-3-(3′,5′-dimethoxyphenyl)-2-butenoate hydrochloride (21b)

This compound was prepared from **20b** as described in the synthesis of **21a**.

^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.73 (3H, s), 3.39 (3H, s), 3.74 (6H, s), 6.36 (2H, d, J = 2.1 Hz), 6.43 (1H, t, J = 2.1 Hz), 7.47 (2H, br), 7.59 (1H, s), 7.75 (1H, br), 8.26 (1H, br), 8.67 (1H, br), 8.85 (1H, br). IR (Nujol) cm^{-1}: 2918, 1743, 1699, 1671, 1589, 1523, 1462, 1204, 1155, 1037. MS APCI *m*/*z*: 383 ([M+H]$^+$). Anal. Calcd for $C_{21}H_{23}ClN_2O_5$: C, 60.22; H, 5.53; Cl, 8.46; N, 6.69. Found: C, 60.23; H, 5.40; Cl, 8.44; N, 6.66.

4.2. Scale-up synthesis of 15k (T-2639)

4.2.1. 1-Methyl 2-[1-(3′,5′-dimethoxyphenyl)-(*Z*)-ethylidene]-3-[1-phenyl-(*E*)-methylidene]-4-succinic acid ((2*Z*,3*E*)-14): *Z*,*E*-selective Stobbe condensation

Twenty-four percent w/w NaOMe solution in MeOH (20.5 g, 91.5 mmol) was added to a solution of **10** (17.1 g, 73 mmol) in toluene (50 ml), and the mixture was stirred at 30–40 °C for 30 min. After 3′,5′-dimethoxyacetophenone (**16**) (11.0 g, 61 mmol) was added to the reaction mixture at 30–40 °C, powder of (2*Z*,3*E*)-**14** Na salts (233 mg, 0.61 mmol) was added as seeds to the reaction mixture, and the resulting mixture was stirred at reflux for 1 h. After the salts were precipitated, the mixture was concentrated in vacuo until the volume was about 22 ml (2 v/w of 3′,5′-dimethoxyacetophenone (**16**)). The mixture was again stirred at reflux for 1 h. Acetic acid (2.9 g, 49 mmol) was then added to the mixture below 15 °C, and ice-water was poured into the mixture. The organic and aqueous layers were separated, and the organic layer was washed with 5% aqueous $NaHCO_3$ solution (100 ml). AcOEt (250 ml) was added to the aqueous layer, and the mixture was acidified with concd HCl below 15 °C (pH 2). The organic and aqueous layers were again separated, and the organic layer was washed with 10% brine (100 ml) and saturated brine (100 ml) and dried over $MgSO_4$. After activated charcoal was added to the solution, the suspension was filtered, and the filtrate was concentrated in vacuo. The residue was diluted with *i*-Pr_2O and filtered to give **14** (19.1 g, 82%, (2*Z*,3*E*)-**14**:(2*E*,3*E*)-**14** = 91:9) as a solid.

4.2.2. 1-Methyl 2-[1-(3′,5′-dimethoxyphenyl)-(*E*)-ethylidene]-3-[1-phenyl-(*E*)-methylidene]-4-succinic Acid ((2*E*,3*E*)-14)

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.44 (3H, s), 3.54 (6H, s), 3.79 (3H, s), 5.85 (2H, d, J = 2.3 Hz), 6.22 (1H, t, J = 2.3 Hz), 7.25–7.40 (5H, m), 7.50 (1H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 23.1, 52.0, 55.1, 100.4, 103.9, 124.0, 128.3, 129.2, 129.4, 129.9, 135.0, 143.4, 144.8, 154.7, 160.1, 167.5, 172.6. IR (ATR) cm^{-1}: 2841, 2524, 1711,

1658, 1592, 1426, 1316, 1205, 1157. MS ESI *m*/*z*: 381 ([M-H]$^-$). HR-MS ESI calcd for $C_{22}H_{23}O_6$ ([M+H]$^+$): 383.1495. Found: 383.1485.

4.2.3. Methyl (*Z*)-3-(3′,5′-dimethoxyphenyl)-2-[(*E*)-1-(4-methylpiperazin-1-ylcarbamoyl)-2-phenylvinyl]-2-butenoate hydrochloride (15k, T-2369)

Oxalyl chloride (194 g, 133.3 ml, 1.52 mol) was added dropwise over 20 min to (2*Z*,3*E*)-**14** (487 g, 1.27 mol, containing <10% of (2*E*,3*E*)-**14**) solution in CH_2Cl_2 (1 l) and DMF (1 l). The reaction mixture was then stirred for 2 h and concentrated in vacuo. After the residue was dissolved in THF (4 l), a mixture of triethylamine (214 ml, 1.52 mol) and 1-amino-4-methylpiperazine (168 ml, 1.52 mol) was added dropwise to the mixture while maintaining the temperature below 20 °C. After 2 h, the reaction mixture was diluted with AcOEt (4 l) and washed with water (3 l) twice and brine (2 l). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting oil was diluted with CH_2Cl_2 (3 l), and *i*-Pr_2O (1 l) was added. The mixture was next concentrated in vacuo until the volume was about 1.5 l. After the mixture was stirred for 5 h at room temperature, the crystals were collected by filtration and washed with *i*-Pr_2O. The crystals obtained were next dissolved in EtOH (4.9 l), and concd HCl (86 ml) was added dropwise at room temperature. The reaction mixture was left at room temperature for 1 h and then cooled in a refrigerator (−25 °C) for 14 h. Crystals were collected by filtration and washed with Et_2O (1 l) to provide 420 g of **15k** (64%).

4.3. Inhibitory effect on elevation of PAI-1 antigen induced by LPS or TGF-β in bovine endothelial cells (BCaEs)

BCaEs (passages 8 and 9) were grown to confluence on a 24-well culture plate in E'MEM supplemented with 10% fetal calf serum (FCS). The cells were exposed to test compounds (1 or 2 μM) in the presence or absence of LPS (1 μg/ml) or TGF-β (0.3 ng/ml) after serum starvation for 24 h. Twenty-four fours after incubation with test compounds, the conditioned medium was collected and centrifuged at 3000 rpm for 10 min at 4 °C to remove cellular debris. PAI-1 antigen in the conditioned medium was determined using a specific enzyme-linked immunosorbent assay (ELISA) kit obtained from Biopool AB (Umea, Sweden).

4.4. Antithrombotic activity of 15k (T-2639) in a rat arteriovenous shunt model

4.4.1. Administration of 15k

Compound **15k** was orally administered at a dose of 10 mg/kg once a day for 8 days. Vehicle was administered to the control group.

4.4.2. Thrombus formation

Rats were anesthetized with Nembutal® (Abbot, 50 mg/kg ip). A cervical incision was made in the mid-line to expose the right carotid artery and left jugular vein. A 24-cm-long polyethylene tube with 6-cm-long silk thread fixed in the lumen was filled with saline and one end of the tube was inserted into the left jugular vein and tied. The proximal side of the right carotid artery was clamped to block blood flow temporarily, while the free end of the tube was inserted into the artery and tied.

Two hours after the last administration of the test compound or its vehicle, the clamp was removed and the tube was perfused with arterial blood. After 30 min perfusion, the silk thread covered with thrombus was removed from the tube, and the wet weight of thrombus was immediately determined using a Sartorius A120S (Sartorius, Germany).

4.5. Antithrombotic activity of 15k (T-2639) and Warfarin in a rat venous thrombosis model

4.5.1. Administration of 15k and Warfarin

15k (0.3, 1, and 3 mg/kg) or Warfarin (0.06, 0.2, and 0.6 mg/kg) were orally administered to rats once a day for 8 days. Vehicle was administered to the control group.

4.5.2. Thrombus formation

Rats were anesthetized with Nembutal® (Abbot, 50 mg/kg ip) and the abdomen was surgically opened to expose the vena cava. All branches of the vena cava between the left renal vein and the bifurcation were ligated with a medical suture (Matsuda Sutures, Tokyo, Japan). Two hours after the last administration of each compound or its vehicle, 0.1 ml of diluted rabbit brain thromboplastin (1:10, SIGMA Chemicals, St. Louise, MO, USA) was injected into the dorsal vein of the penis. Ten seconds after injection of thromboplastin, the vena cava was ligated beneath the left renal vein. The abdominal cavity was provisionally closed. After 15 min of stasis, the abdominal cavity was reopened and the vena cava was ligated near the bifurcation (2 cm beneath the left renal vein) and was longitudinally opened by a surgical blade. The formed thrombus was removed and its dry weight was measured using Sartorius Supermicro S4 (Sartorius).

4.6. Effects of 15k (T-2639) and Warfarin in the rat tail incision bleeding time test

4.6.1. Administration of 15k and Warfarin

15k (10, 30, and 100 mg/kg) or Warfarin (0.1, 0.2, and 0.4 mg/kg) were orally administered to rats once a day for 8 days. Vehicle was administered to the control group.

4.6.2. Determinations of bleeding time

Rats were anesthetized with Nembutal® (Abbot, 50 mg/kg ip). Two hours after the last administration of each compound or its vehicle, an incision 2 mm in depth was made with a surgical blade 3.5 cm from the tip of the tail. Blood was blotted in filter paper (No. 2) every 30 s and bleeding time was determined by measuring the time until no blood was seen on the filter paper.

4.7. Stability in dog blood

One ml of stock solution (100 μg/ml in acetonitrile) was diluted with saline to a final volume of 10 ml. Next, 0.3 ml of the solution was added to 3 ml of a heparinized dog blood, which was pre-incubated at 37 °C, and the whole was incubated at 37 °C. After 1 h, the plasma was separated with diethylether by centrifugation and test compounds plasma concentrations were determined by HPLC.

4.8. Photostability

To 0.1 ml of stock solution (100 μg/ml in acetonitrile) was added 0.9 ml of 0.1*N* HCl aq. The solution was stored at room temperature for 24 h with or without aluminum foil cover to protect from light. The covered and uncovered solutions were analyzed by HPLC.

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16. We saw during another project that the 4-methoxy group was predominantly demethylated to phenol metabolite in the 3,4,5-trimethoxyphenyl group (unpublished result).
17. Compounds (3 mg/kg/day) were orally administered for three consecutive days. Two hours after the last administration, thrombi were induced and their dry weights were measured. See Ref. 12.
18. NaOMe solution was used instead of powder NaOMe because it was easy to handle in consideration of application to process chemistry. The reaction will take place similarly even if powder NaOMe is used.
19. Because such salt precipitation was not observed with *t*-BuOK, the effect of Na may be related to precipitation.
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