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Design, synthesis, and evaluation of orally active inhibitors of plasminogen activator inhibitor-1 (PAI-1) production

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ABSTRACT

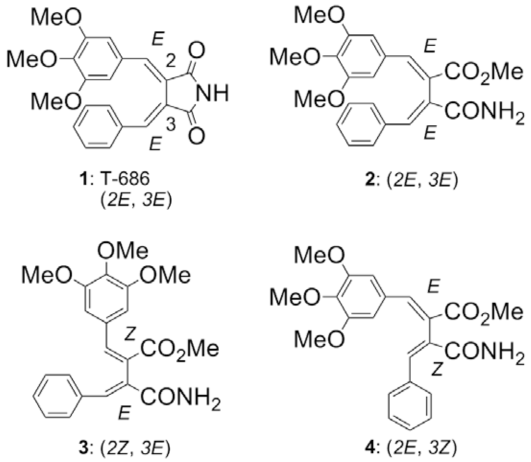
A novel series of butadiene-imide **1** (T-686) derivatives having an inhibitory activity against PAI-1 production was synthesized and evaluated their biological activities and DMPK profiles, in which **15k** (T-2639) was selected as the best compound based on its strong antithrombotic activity and good bioavailability.

Plasminogen activator inhibitor-1 (PAI-1), a specific inhibitor of both tissue-type plasminogen activator and urokinase-type plasminogen activator, plays an important role in the regulation of the fibrinolytic system.[1] 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 PAI-1 is the factor which disturbs fibrinolytic activity in the thrombotic and prethrombotic states.[4] Thus, inhibition of PAI-1 activity or reduction of PAI-1 production may shift the balance between thrombogenesis and thrombolysis towards thrombolysis. 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] Recently a number of small molecules have been reported to inhibit PAI-1[7] and show in vivo efficacy in animal thrombosis models.[8]

We previously described that a new butadiene-imide derivative **1** (T-686) inhibited the production of PAI-1 in cultured vascular endotherial cells[9] and could prevent the shutdown of fibrinolysis in an experimental hypofibrinolytic model.[10] Furthermore, **1** showed antithrombotic activity in both rat venous thrombosis and arterio-venous shunt models without affecting bleeding time.[11] These results suggest the possibility that inhibitors of PAI-1 production would provide safe antithrombotic agents.

Compound **1** has, however, some defects such as poor water solubility (9 µg/ml), low oral bioavailability (<3% in dog), and chromosomal aberration. In order to improve these defects, we started to identify alternative novel inhibitors that incorporated key structural features of the butadiene template. In this paper, we report the design, synthesis and biological activities of these butadiene analogues.

We have reported the syntheses of the butadiene derivatives (**1**–**4**, Fig. 1).[12] By using a similar method to that of **2**, butadiene analogues (**8**, **9**, **11**, and **12**) possessing methyl group on 1- or 4-position of the butadienes were synthesized (Scheme 1). The *E*- and *Z*-dimethyl benzylidenesuccinates (**6** and **7**) were synthesized by the first Stobbe condensation[13] of acetophenone with dimethyl succinate followed by esterification. These isomers were separated



1: T-686 (2*E*, 3*E*)

2: (2*E*, 3*E*)

3: (2*Z*, 3*E*)

4: (2*E*, 3*Z*)

Figure 1. T-686 and its derivatives.

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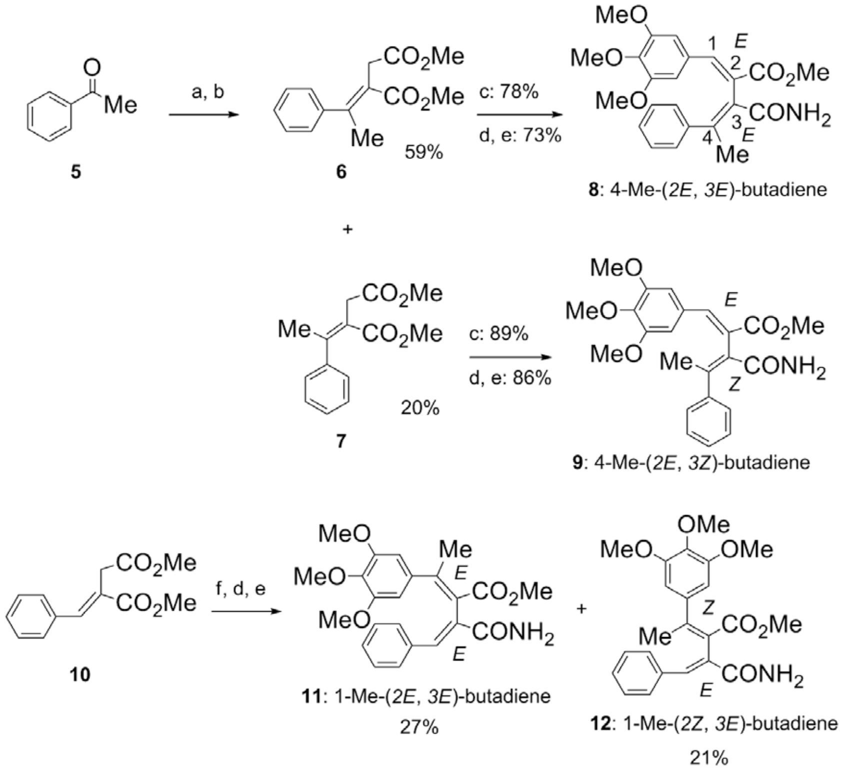
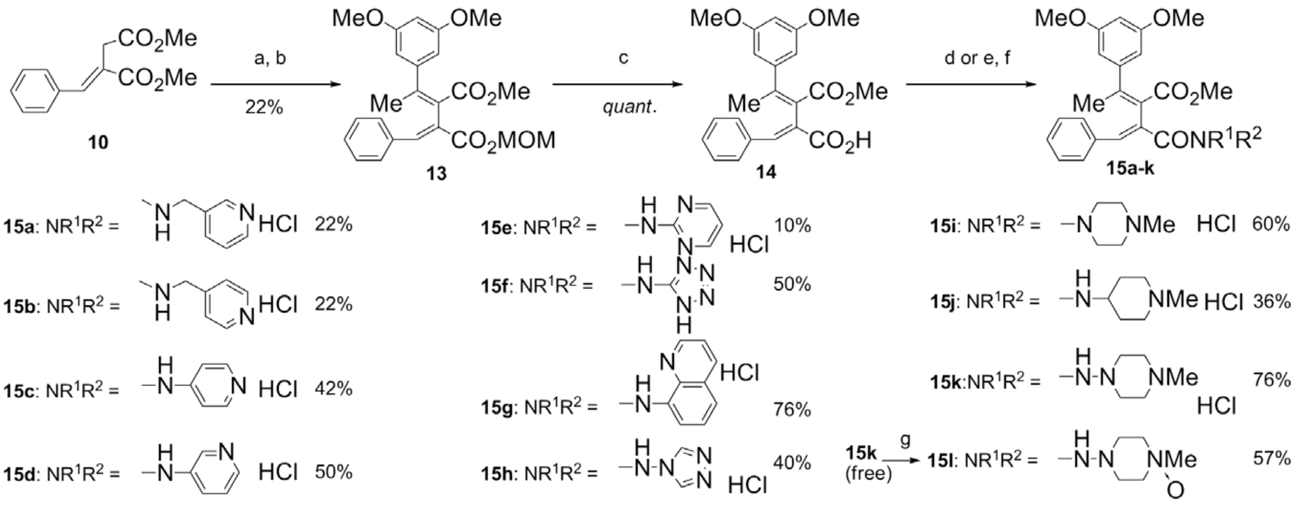


Scheme 1. Reagents and conditions: (a) dimethyl succinate, *t*-BuOK, *t*-BuOH, rt, 1 h; (b) $SOCl_2$, MeOH, rt, 16 h; (c) 3,4,5-trimethoxybenzaldehyde, *t*-BuOK, *t*-BuOH, rt, 1 h; (d) $SOCl_2$, $CHCl_3$, reflux, 30 min; (e) 28% NH_4OH, 0 °C–rt, 30 min; (f) 3′,4′,5′-trimethoxyacetophenone, *t*-BuOK,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

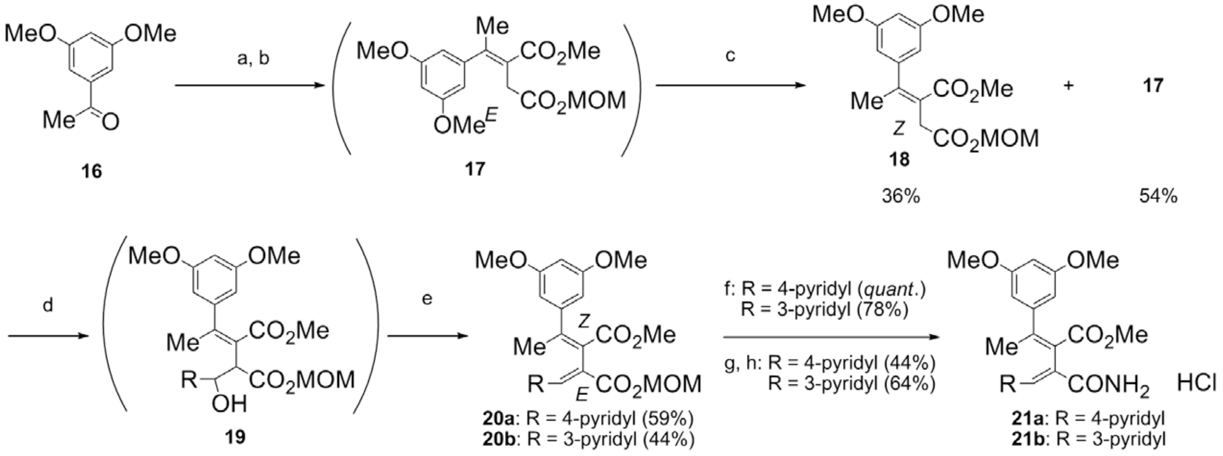
by chromatography and used for the next step. The second Stobbe condensation of succinate (**6** and **7**) with 3,4,5-trimethoxybenzaldehyde provided 2*E*-isomeric carboxylic acids as major products, which were respectively treated with $SOCl_2$ followed by aqueous NH_3 to afford the corresponding amides (**8** and **9**). Also, the butadiene analogues (**11** and **12**) were synthesized via the Stobbe condensation of **10**[12] with 3′,4′,5′-trimethoxyacetophenone, where the isomers were separated by chromatography after the amidation.

The syntheses of **15a**–**l** are summarized in Scheme 2. The key intermediate (2*Z*,3*E*)-carboxylic acid **14** was once isolated by chromatography as its methoxymethyl (MOM) ester **13** due to the difficulty in the separation of the initially produced carboxylic acid isomers. Treatment of **13** with HCl afforded **14**,[14] which was transformed into the desired amides and hydrazones **15a**–**k** by using the conventional method. The free form of **15k** was oxidized with *meta*-chloroperbenzoic acid (*m*CPBA) to give the corresponding *N*-oxide **15l**.

Compounds **21a** and **21b** were synthesized via the alternative method shown in Scheme 3. Esterification of the crude product of the Stobbe condensation of 3′,5′-dimethoxyacetophenone with dimethyl succinate provided the *E*-diester **17** predominantly. Photo-irradiation to the solution of *E*-isomer **17** afforded a mixture



Scheme 2. Reagents and conditions: (a) 3′,5′-dimethoxyacetophenone, *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, 1 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, THF, rt, 0.5 h; (f) 4 N-HCl/EtOAc, rt; (g) *m*CPBA, CH_2Cl_2, −50 °C–rt, 1 h.



Scheme 3. Reagents and conditions: (a) dimethyl succinate, *t*-BuOK, *t*-BuOH, 50 °C, 4 h; (b) MOMCl, *i*-Pr$_2$NEt, CH$_2$Cl$_2$, rt, 0.5 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$_3$N, CH$_2$Cl$_2$, 0 °C, 0.5 h, then DBU, CH$_2$Cl$_2$, rt, 16 h; (f) concd HCl, THF, rt, 1 h; (g) CDI, THF, rt, 16 h, then 28% NH$_4$OH, 0 °C, 2 h; (h) 4 N-HCl/EtOAc, rt.

of *Z*- and *E*-isomers, which were separated by chromatography to give *Z*-isomer **18** in 36% yield. The adducts of the aldol reaction of **18** with 3- and 4-pyridinecarboxaldehydes were mesylated and treated with 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) to afford (2*Z*,3*E*)-isomers of pyridylbutadiene derivatives as major products, which were purified by chromatography to give **20a**,**b** respectively. Compounds **20a**,**b** were transformed into **21a**,**b** by using the method described above.

We started our research with the 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. During the course of the initial screening of the compounds related to imide **1**, it was proved that the amide-ester type compound **2** also showed good PAI-1 production inhibitory activity. Although the ester moiety seemed to be unstable to hydrolysis (0% remain in dog blood), this result encouraged us to synthesize the amide-ester type compounds because the good crystalline properties of imide led to low water solubility, which seemed to be one of the reasons for the low bioavailability of **1**. Next, our search for more attractive templates focused on the stereoisomeric structures of **2**. Although the corresponding (2*Z*,3*E*)- and (2*E*,3*Z*)-butadiene derivatives **3** and **4** exhibited decreased activity, it was found that **3** possessed largely increased stability in dog blood compared with **2** and **4** (**3**, 75%; **2** and **4**, 0% remain). It could be elucidated that the ester hydrolysis of **3** was sterically blocked by the (*Z*)-positioned 3,4,5-trimethoxyphenyl ring. We next introduced the methyl group onto the butadiene scaffolds. While **8**, **9**, and **11** were not effective, it was revealed that 1-Me-(2*Z*,3*E*)-butadiene derivative **12** possessed good potency and, as expected, excellent stability in dog blood (96% remain). Furthermore, it was found that **12** exhibited improved properties in comparison with **1** (Table 3. **1** vs **12**), that is, enhanced solubility in water (**12**, 106 μg/ml; **1**, 9 μg/ml), improved bioavailability (**12**, 9%; **1**, 3% in dog), and negative result in the chromosomal abnormality test.

Table 1
Inhibitory activities of PAI-1 production and stability in dog blood.

Compound	Relative potency[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 activities on LPS-induced PAI-1 production in cultured vascular endothelial cells (compound concentration: 10 μM) are shown using T-686 as a reference compound.

[b] % of remains are shown in dog blood after 1 h at 37 °C.

Encouraged by these fundamental data on the templates, we next intensified our efforts in optimizing the substituents of **12**. In the segment **A** (Fig. 2), we anticipated that removal of one of the three methoxy groups should improve metabolic stability because the methoxy group had been generally known to be the typical metabolic target leading to demethylated phenol metabolite.[15] In the segment **B**, we tried replacing the phenyl ring with the pyridine ring to enhance solubility. In the segment **C**, we planned to introduce other amide moieties that, if possible, could form a salt to improve solubility.

The relative inhibitory activity against LPS-induced PAI-1 production in cultured vascular endothelial cells (compound concen-

Table 2
Inhibitory activities on LPS-induced PAI-1 production in cultured vascular endothelial cells.

Compound	Relative potency[a]
1 (T-686)	1.0
12	1.5
14	0.4
15a	1.3
15b	2.1
15c	1.8
15d	2.2
15e	0.1
15f	0.1
15g	0.4
15h	0.6
15i	0.3
15j	0.8
15k	1.5
15l	0.2
21a	1.5
21b	0.8

[a] Relative inhibitory activities on LPS-induced PAI-1 production in cultured vascular endothelial cells (compound concentration: 1 μM) are shown using T-686 as a reference compound.

Table 3
Solubility, stability in dog blood, bioavailability, geno-toxicity, antithrombotic activity and toxicity in rats of selected compounds.

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

nt, not tested.

[a] % of remains are shown in dog blood after 1 h at 37 °C.

[b] Antithrombotic effects on the rat venous thrombosis model (3 mg/kg/day × 3 days) are shown. See Ref. 16.

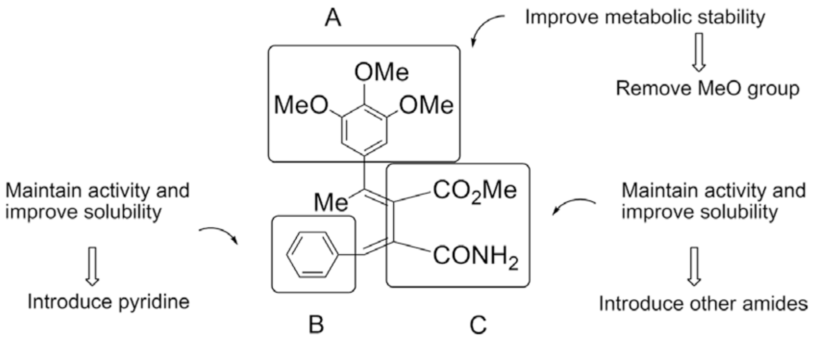


Figure 2. Optimization of the substituents of **12**.

tration: 1 μM) is summarized in Table 2. The synthetic intermediate carboxylic acid **14** showed poor potency. Among the amide derivatives, while 3- and 4-picolyl amides (**15a**,**b**) and 3- and 4-pyridyl amides (**15c**,**d**) were revealed to possess equal to enhanced potency compared with **12**, other amides and hydrazone (**15e**–**h**) exhibited no strong activity. Interestingly, hydrazone derivative **15k** exhibited good potency although the corresponding amide derivatives (**15i**,**j**) and *N*-oxide **15l** showed decreased potency. Replacement of the phenyl ring in the segment B with 4-pyridyl ring (**21a**) led to equal activity, and with 3-pyridyl ring (**21b**) to decreased activity compared with **12**.

In order to select the best compound, the physicochemical and pharmacokinetic properties of these potent compounds were evaluated and listed in Table 3. The solubility of these compounds was largely enhanced and stability of most compounds in dog blood was also improved compared with **1**. As a result, the bioavailability of the compounds was quite improved, and **15a**–**c**, **15k** and **21a** satisfied our criterion (>20%). Next, chromosomal aberration was tested for these five compounds and the negative result was shown in **15a**,**c** and **15k**. The antithrombotic activity of these three compounds was examined in a rat model of venous thrombosis. All three compounds inhibited the thrombus weight by around 50% in the rat venous thrombosis model. In the toxicity study in male rats at 300 mg/kg po, the two compounds **15a,c** showed decrease in locomotor activity as a side effect.

Based on these results, we selected **15k** (T-2639) as the best compound for further investigation.

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14. Crystal data of **14**; $C_{22}H_{22}O_6$, $M = 382.40$, monoclinic, space group $P2_1/c$, $a = 12.967\ (2)$, $b = 12.0214(2)$, $c = 13.360\ (1)$ Å, $\beta = 101.12\ (1)^\circ$, $V = 2043.4\ (5)$ Å^3, $Dc = 1.243$ mg m^{-3}, $Z = 4$, $R = 0.046$, $Rw = 0.165$, CCDC Reference No. 705024.
15. 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).
16. Compounds (3 mg/kg per 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. 11.