Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 14 (2006) 7688–7705
Bioorganic & Medicinal Chemistry

Potent and selective TF/FVIIa inhibitors containing a neutral P1 ligand

Masanori Miura,* Norio Seki, Takanori Koike, Tsukasa Ishihara, Tatsuya Niimi, Fukushi Hirayama, Takeshi Shigenaga, Yumiko Sakai-Moritani, Tomihisa Kawasaki, Shuichi Sakamoto, Minoru Okada, Mitsuaki Ohta and Shin-ichi Tsukamoto

Institute for Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi, Ibaraki 305-8585, Japan

Received 2 June 2006; revised 9 August 2006; accepted 10 August 2006
Available online 30 August 2006

Abstract—Inhibition of tissue factor/factor VIIa complex (TF/FVIIa) is an attractive strategy for antithrombotic therapies. We began with an investigation of a non-amidine TF/FVIIa inhibitor based on a modification of amidine compound **1**. Optimization of the substituents on the P1 phenyl portion of the compound **1** led to a neutral or less basic alternative for the 4-amidinophenyl moiety. By further optimization of the substituents on the central phenyl ring, a highly potent and selective TF/FVIIa inhibitor **17d** was discovered.

1. Introduction

Thromboembolic disorders are the major cause of morbidity and mortality in the developed world.[1] Although current anticoagulant therapies for these diseases consist of low molecular weight heparin (LMWH)[2] and warfarin[3] in acute and chronic settings, respectively, the problems associated with these agents are well recognized.[4] Within the past decade, research efforts to identify small molecule inhibitors of blood coagulation enzymes as novel therapies for thromboembolic disorders have increased. The most widely studied targets for antithrombotic intervention have been trypsin-like serine proteases, such as thrombin[5] and factor Xa (FXa),[6] which play critical roles in the coagulation cascade, with thrombin initiating clot formation and FXa providing the sole mechanism for thrombin activation.[7] There is substantial evidence suggesting that FXa should be a more attractive target for inhibitor design;[8] however, there are currently no direct FXa inhibitors marketed as drugs.

More recently, much attention has also been directed toward the inhibitors of the tissue factor/factor VIIa complex (TF/FVIIa). The TF is an integral membrane protein not normally in contact with blood, but in a disease state or during injury it is exposed to the blood and comes in contact with FVIIa localized on the surface of subendothelial cells, forming the TF/FVIIa complex. TF/FVIIa is a serine protease complex that triggers the cascade of coagulation reactions by activating factors X and IX to the corresponding active serine protease forms, ultimately resulting in the generation of thrombin and a fibrin clot.[9] It is noteworthy that bleeding studies indicate that inhibition of the TF/FVIIa complex has the widest safety window with respect to therapeutic effectiveness and bleeding risk of any anticoagulant approach tested, such as the inhibition of thrombin and factor Xa.[10] Since there is a clear need for safe and effective anticoagulants to obtain maximum patient compliance, inhibitors of the TF/FVIIa complex have been of considerable interest to medicinal chemists.

Several groups have reported their efforts toward the design and synthesis of novel TF/FVIIa inhibitors.[11] Many of these inhibitors retain a highly basic amidine group bound in the S1 pocket of TF/FVIIa in the vicinity of Asp 189. It is generally accepted that inhibitors containing highly basic functions such as amidine groups are often poorly absorbed and/or are associated with undesirable side effects.[12] In addition, it is not easy to develop

Keywords: Thromboembolic disorders; Trypsin-like serine protease; Tissue factor/factor VIIa; Non-amidine inhibitor.
* Corresponding author. Tel.: +81 29 863 6691; fax: +81 29 852 5387; e-mail: masanori-miura@jp.astellas.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.08.010

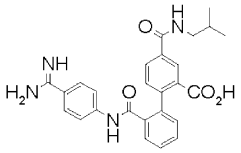


Figure 1. Structure of compound **1**.

highly selective compounds from inhibitors that possess amidine moiety, because other serine protease enzymes such as thrombin, FXa, and trypsin have Asp 189 at the base of the S1 pocket.

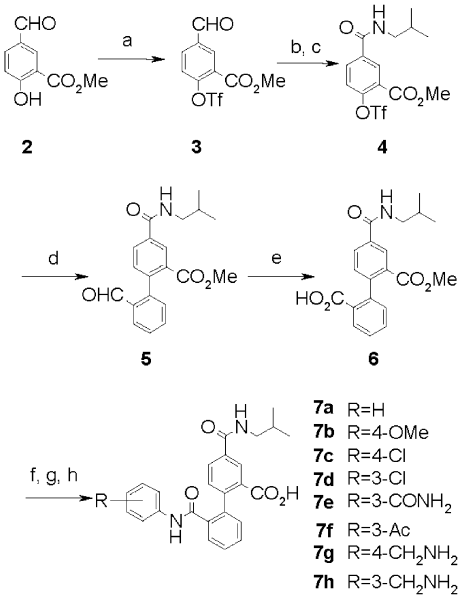
Our strategy was to first find a less basic or neutral alternative for this amidine moiety, and then to convert the inhibitor to potent and selective TF/FVIIa inhibitors by exploiting the potential structure based drug design. With the above considerations in mind, we had initiated the TF/FVIIa inhibitor development program using a compound **1** recently reported by Senokuchi and coworkers as a starting point (Fig. 1).[11a] Herein, we wish to report our progress on the P1 group optimization of **1** and the identification of 3-aminocarbonylphenyl and 4-aminomethylphenyl moieties as important replacements for the 4-amidinophenyl moiety at the P1 group. In addition, we describe our progress on the central phenyl group optimization, which has led to the identification of **17d** as a potent and selective TF/FVIIa inhibitor (IC_{50} = 0.69 μM).

2. Chemistry

The synthesis of compounds (**7a**–**h**) is shown in Scheme 1. Treatment of trifluoromethanesulfonic anhydride with phenol **2** and oxidation of aldehyde **3** by $NaClO_2$ afforded an acid, which was converted to acyl chloride with oxalyl chloride and then reacted with 2-methyl-1-propylamine to afford amide **4**. Suzuki cross-coupling reaction with (2-formylphenyl)boronic acid afforded biphenyl **5**. Oxidation of aldehyde **5** gave compound **6**. After condensation of carboxylic acid **6** with corresponding aniline derivatives, hydrolysis of the methyl ester under basic conditions (NaOH/water) gave the desired carboxylic acid (**7a**–**f**). The benzylamine analogues (**7g** and **7h**) were achieved by deprotection of the Boc group.

Compounds **12a**–**f** were synthesized in seven steps from 2-bromobenzoic acid derivatives (**8a**–**f**) in the same manner as described in Scheme 1 (Scheme 2). In the case of 4′-methoxy analogue **12g**, biaryl intermediate **10g** was prepared by cross-coupling reaction of methyl 2-bromobenzoate with commercially available 2-formyl-4-methoxybenzenboronic acid.

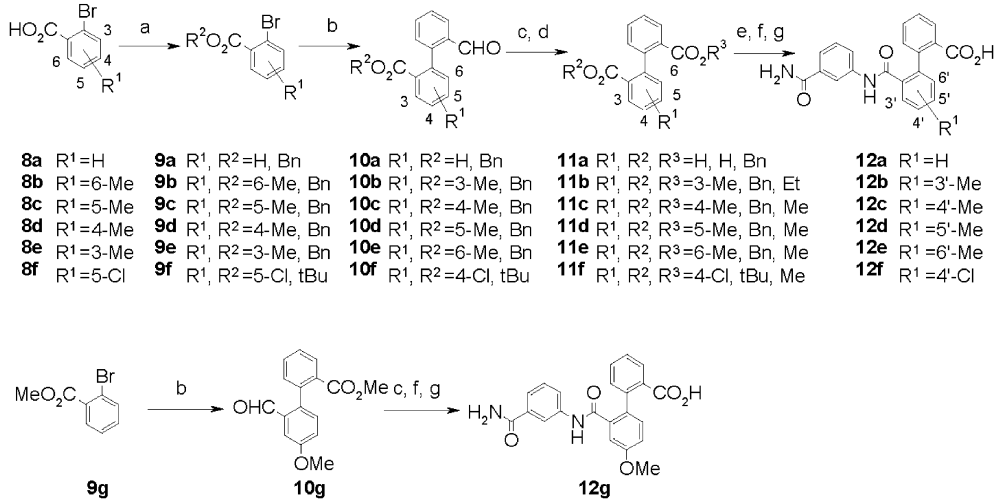
The synthesis of compound **17a** was accomplished by the use of bis(pinacolato)diboron;[13] however, with only unsatisfactory yield (34%), the cross-coupling product **16** was obtained. We thus synthesized the compounds with other 4′-substituents (**17b** and **17c**) by the use of boronic acid **22** (Scheme 3). Bromination of **18** using



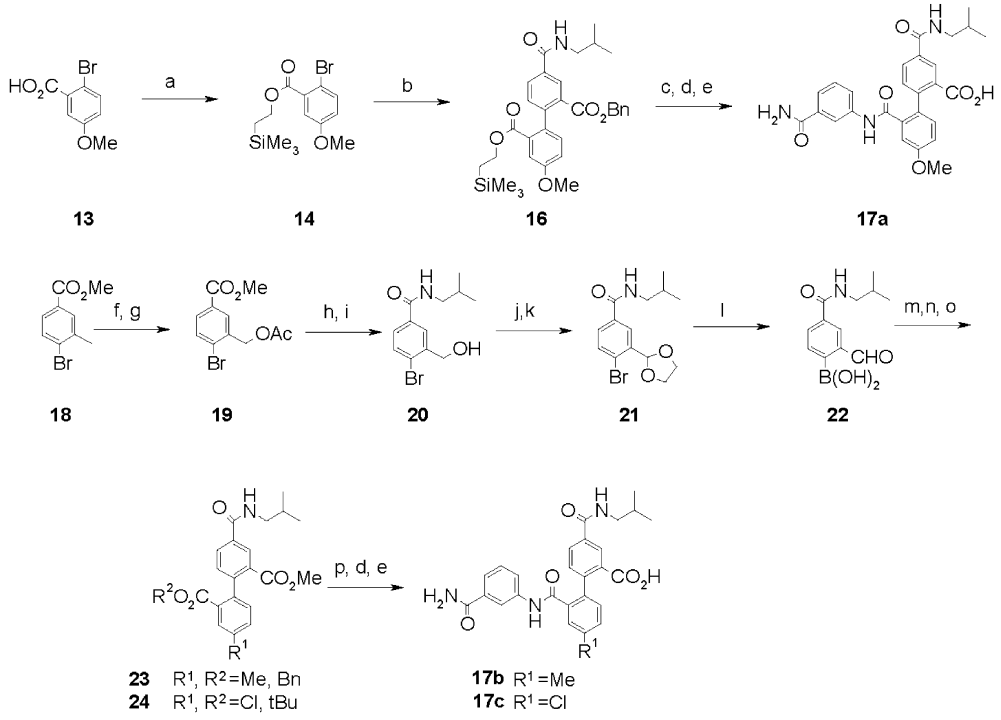
Scheme 1. Reagents and conditions: (a) trifluromethanesulfonic anhydride, pyridine, CH_2Cl_2; (b) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butanol, CH_3CN, H_2O; (c) $(COCl)_2$, isobutylamine, NEt_3, CH_2Cl_2; (d) (2-formylphenyl)boronic acid, $Pd(PPh_3)_4$, K_3PO_4, DMF; (e) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butanol, CH_3CN, H_2O; (f) $ArNH_2$. 1-ethyl-3-(3-dimethylaminopropyl)carbodimide hydrochloride, 1-hydroxybenzotriazole, DMF; (g) NaOH, MeOH; (h) HCl, AcOEt for **7g** and **7h**.

NBS, followed by treatment with AcONa in AcOH, gave acetoxy compound **19**. After hydrolysis of **19**, condensation with amine gave amide **20**. The alcohol group of **20** was converted to an aldehyde with MnO_2, which was protected by acetal, to afford the 1,3-dioxolane analogue **21**. Lithiation of **21** with *n*-BuLi and $B(O^iPr)_3$ followed by quenching with hydrochloric acid gave arylboronic acid **22**. Suzuki cross-coupling reaction with the boronic acid **22** and corresponding 2-bromobenzoate analogues produced biaryls (**23** and **24**), which were converted to the desired products (**17b** and **17c**) by the same method as described in Scheme 1.

The synthesis of compounds **12h**–**k** and **17d**, which have nitrogen atoms at the 4′-position, is shown in Scheme 4. The 4′-nitrobiaryl intermediates (**26** and **27**) were prepared in four steps from methyl 2-bromo-5-nitrobonzoate **25** in the same manner as described in Scheme 2. Hydrolysis of the methyl ester of **27**, followed by condensation of the resulting acid with 3-aminobenzamide, gave amide **28**. Deprotection of the *tert*-butyl group with TFA afforded the desired compound **12h**. Compound **12i** was synthesized by the same method as that for **12h**, from **29**, which was obtained by hydrogenation of the nitro group of **28** in the presence of 10% palladium on carbon. Because of a reaction failure from **29** to **33**,[14] we used a methoxycarbonyl instead of an aminocarbonyl group at the 3-position of the P1 phenyl ring. The intermediate **26** was converted to an amine **30** in the same manner as that for **29**, followed by reductive amination with HCHO, which gave dimethylamino analogue **32**. Hydrolysis of the methyl ester of **32**, followed



Scheme 2. Reagents and conditions: (a) BnBr, $KHCO_3$, DMF for **9a**–**e**, or *tert*-butanol, $MgSO_4$, H_2SO_4 for **9f**; (b) (2-formylphenyl)boronic acid for **10a**–**f** or 2-formyl-4-methoxybezenboronic acid for **10g**, $Pd(PPh_3)_4$, K_3PO_4, DMF; (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butanol, CH_3CN, H_2O; (d) EtI for **11b** or MeI for **11c**–**g**, $KHCO_3$, DMF; (e) H_2, MeOH for **12b**–**e** or TFA, CH_2Cl_2 for **12f**; (f) 3-aminobenzamide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride 1-hydroxybenzotriazole, DMF; (g) NaOH, MeOH.

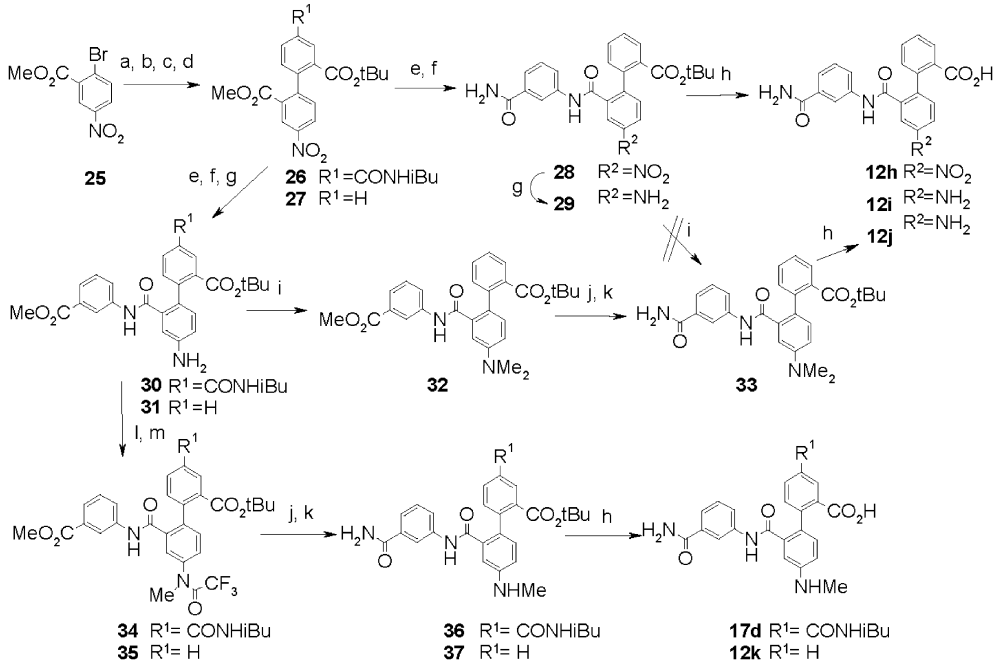


Scheme 3. Reagents and conditions: (a) $TMSCH_2CH_2OH$, DCC, DMF, pyridine, cat. DMAP; (b) benzyl 5-[(isobutylamino)-carbonyl]-2-{[(trifluromethyl)sulfonyl]oxy}benzoate (**15**), bis(pinacolato)diboron, $PdCl_2(dppf)$, K_3PO_4, dioxane; (c) TBAF, THF; (d) 3-aminobezamide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF; (e) NaOH, MeOH; (f) NBS, AIBN, CCl_4; (g) AcONa, AcOH; (h) NaOH, MeOH; (i) isobutylamine, WSC, HOBt, DMF; (j) MnO_2, $CHCl_3$; (k) TsOH, ethyleneglycol, toluene; (l) *n*-BuLi, $B(O\text{-}i\text{-}Pr)_3$, Et_2O, then HCl aq; (m) benzyl 2-bromo-5-methylbenzoate for **23** or *tert*-butyl 2-bromo 5-chlorobenzoate for **24**, $Pd(PPh_3)_4$, K_3PO_4, DMF; (n) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butanol, CH_3CN, H_2O; (o) MeI, $KHCO_3$, DMF; (p) H_2, Pd-C, MeOH for **16b** or TFA, CH_2Cl_2 for **16c.**

by condensation of the resulting acid with ammonia, gave the amide **33**, which converted to the desired product **12j**. Compounds **17d** and **12k** were synthesized similarly as described above via preparation of *N*-methyltrifluoroacetamide analogues (**34** and **35**) in three steps from the aniline analogues (**30** and **31**) under standard conditions, respectively.

3. Results and discussion

To evaluate the synthesized target compounds, the IC_{50} values for the inhibition of TF/FVIIa enzymatic activities were determined using the chromogenic substrate s-2288. We investigated the alternative structures of the amidine moiety for the S1 site by the modification

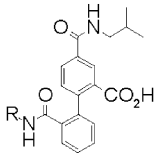


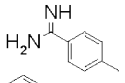
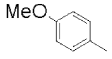
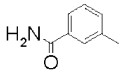
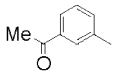
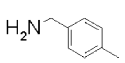
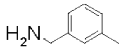
Scheme 4. Reagents and conditions: (a) boronic acid **22** for **26** or (2-formylphenyl)boronic acid for **27**, $Pd(PPh_3)_4$, K_3PO_4, DMF; (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butanol, CH_3CN, H_2O; (d) $MgSO_4$, H_2SO_4, *tert*-butanol, CH_2Cl_2; (e) NaOH, MeOH; (f) 3-aminobenzamide, for **28** or methyl 3-aminobenzoate for **30** and **31**, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF; (g) H_2, Pd–C, MeOH; (h) TFA, CH_2Cl_2; (i) HCHO, $NaBH(OAc)_3$, 1,2-dichloroethane; (j) NaOH, MeOH; (k) NH_4Cl, *i*-Pr_2NEt, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, DMF; (l) TFAA, pyridine, CH_2Cl_2; (m) MeI, K_2CO_3, 2-butanone.

of compound **1** shown in Table 1. Removal of the amidine moiety from compound **1** or introduction of substituents such as MeO and Cl resulted in loss of the activity (**7a**–**d**). In the case of other substituents, though the 3-acetyl and 3-aminomethyl analogues **7f** and **7h**, respectively, did not have potency, the 3-aminocarbonyl and 4-aminomethyl derivatives **7e** and **7g** showed moderate inhibitory activities ($IC_{50} = 19$ and 40 μM, respectively).

In order to understand the SARs for these compounds, the binding conformation of compound **1** to the TF/FVIIa complex was studied.[15] As shown in Figure 2, the molecular modeling suggested that the amidine moiety interacts with Asp 189 in the S1 pocket to form a salt bridge, the central phenyl group lies nearby the S2-pocket, and the carboxylic acid fits optimal binding with His 57. We used the molecular modeling of our compounds **7e** and **7g**. From Figures 3a and b, it is clear that the proposed overall binding mode for each compound is generally similar except for the S1 site. For example, the molecular modeling suggested that the nitrogen of the central amide group might be capable of hydrogen-bonding with the OH of Ser 195, and that the carboxylic acid group also forms a salt bridge with His 57. As for the S1 site, the aminocarbonyl group of compound **7e** is in the S1 specificity pocket, and the carbonyl oxygen of the aminocarbonyl substituent is in close proximity to the OH group of Ser 190 and forms a hydrogen-bond. Moreover, the nitrogen of the aminocarbonyl group forms a hydrogen-bond to the carbonyl oxygen of Trp 215 and Val 227 through a bridging water molecule.[16] This might contribute to the improved potency of compound **7e**. These interactions are different from those of the amidine analogue, compound **1**. Since the donation of a hydrogen to the nearby water and the acceptance of a hydrogen from Ser 190 may

Table 1. In vitro inhibitory activities against TF/FVIIa



Compound	R	IC_{50} (μM)
1		0.089
7a		>200
7b		>200
7c		>200
7d		>200
7e		19
7f		>200
7g		40
7h		>200

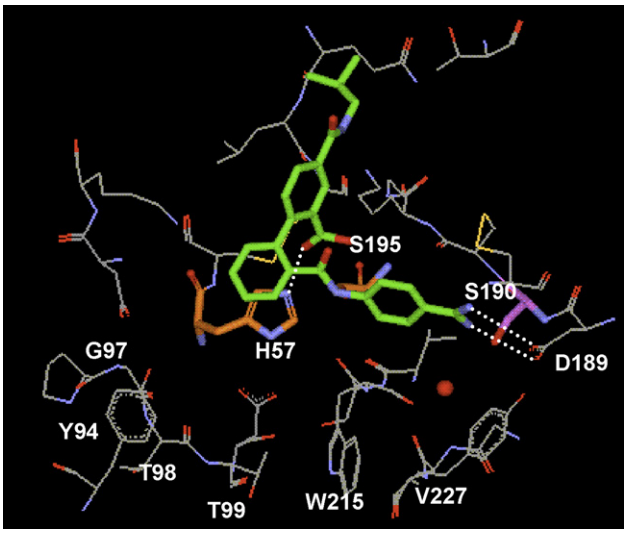
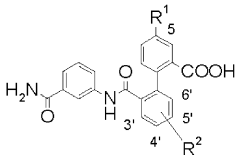


Figure 2. Docking model of compound **1** in TF/FVIIa. Compound is shown in green. H57 and S195 of the catalytic triad are shown in orange. S190 is shown in pink. Residue numbers of some key residues are displayed. Predicted position of water molecule in S1 pocket is indicated by red spheres. The hydrogen-bonds formed by the inhibitor are shown in dotted white line.

both play important roles in the potency, compound **7f**, which is missing the heteroatom that would bind with the water, and compound **7h**, which does not have a carbonyl group that would accept the hydrogen, did not show potency. In the case of compound **7g**, as shown in Figure 2, the aminomethyl group of **7g** forms a salt bridge with Asp 189 similar to that of amidine compound **1**.

From the above modeling study, we considered that there is a possibility to modify the central phenyl ring since there is no interaction between its moiety and a specific pocket formed by Gly 97, Tyr 94, Thr 98, and Thr 99, which is known as the S2 site. In order to regain the potency lost upon replacing the amidine moiety, our synthetic efforts were focused on the additional substitutions on the phenyl ring (Table 2). From the SAR studies shown in Table 1, the 3-aminocarbonyl analogue **7e** was identified as the lead structure for further optimization. In regard to the synthetic efficiency, we undertook to understand the SARs by the use of 5-unsubstituted compounds (**12a**–**k**). Substitutions on the central phenyl ring yielded interesting results. Compared to the unsubstituted compound **12a**, the substitution of the methyl group at the 4′-position increased potency, although the substitutions at other positions did not show the enhancement of the potency (**12b**–**e**). Among 4′-substituted compounds (**12f**–**k**), though NO_2 and NH_2 analogues did not have enhanced activity, Cl, MeO, and the dimethylamino groups contributed to the binding affinity (**12f**, **12g**, and **12i**) and the methylamino analogue **12k** also showed relatively potent activity. From the result of the SAR of the 5-unsubstituted series of compounds, selected compounds having 5-isobutylaminocarbonyl moiety were prepared (**17a**–**d**). Comparing the series of the 5-unsubstituted compounds (**12a**–**k**) with those containing the 5-isobutylaminocarbonyl (**17a**–**d**), similar results were observed. That is, introduction of methyl, Cl, and MeO groups at the 4′-position(**17a**–**c**) resulted in increased potency compared to the 4′-unsubstituted compound**7e**. In addition, the methylamino analogue **17d** was about 20-fold more potent than the corresponding unsubstituted analogue **7e**.

Table 2. In vitro inhibitory activities against TF/FVIIa



Compound	R^1	R^2	IC_{50} (μM)
7e	CONH-*i*-Bu	H	19
12a	H	H	>200
12b	H	3′-Me	>200
12c	H	4′-Me	89
12d	H	5′-Me	>200
12e	H	6′-Me	>200
12f	H	4′-Cl	102
12g	H	4′-MeO	137
12h	H	4′-NO_2	>200
12i	H	4′-NH_2	>200
12j	H	4′-NMe_2	94
12k	H	4′-NHMe	23
17a	CONH-*i*-Bu	4′-MeO	7.6
17b	CONH-*i*-Bu	4′-Me	3.3
17c	CONH-*i*-Bu	4′-Cl	3.7
17d	CONH-*i*-Bu	4′-NHMe	0.69

In order to understand the enhancement of the potency by the modification of the central phenyl ring, we used a molecular modeling of compound **17d** (Fig. 4). The molecular modeling suggested that the aminocarbonyl

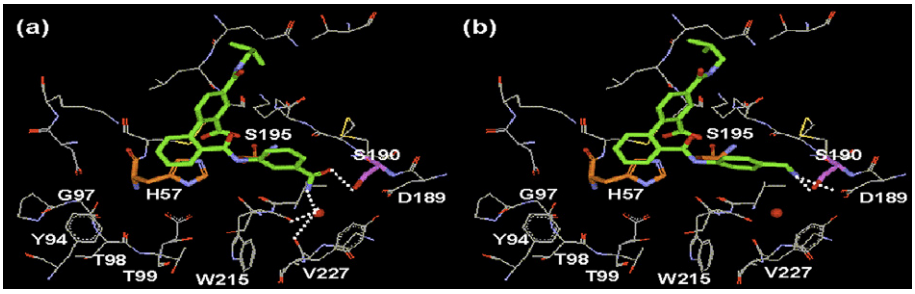


Figure 3. Docking model of (a) **7e** and (b) **7g** in human IF/FVIIa. Color schemes are the same as those of Figure 2.

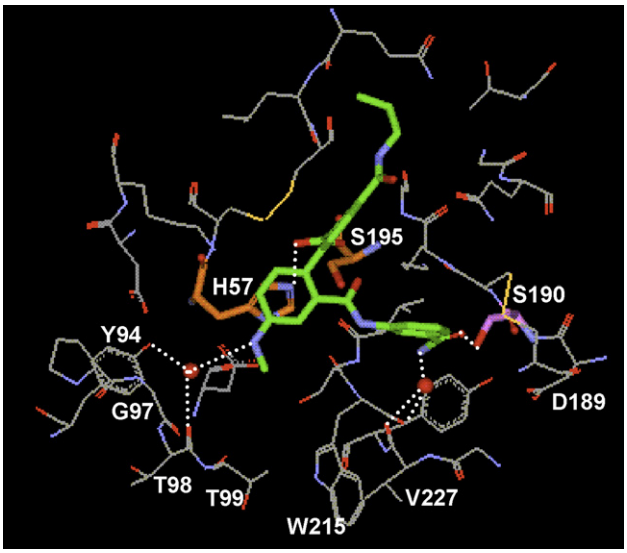


Figure 4. Docking model of **17d** in human TF/FVIIa. Color schemes are the same as those of Figure 2. Predicted positions of water molecules in S1 and S2 pockets are displayed.

P1 group of **17d** sits in the S1 site in a manner similar to that portion of **7e** when bound to the enzyme. As for the S2 site, the molecular modeling suggested that the nitrogen of the methylamino substituent formed a hydrogen-bonding network among the phenol oxygen of Tyr 94 and the carbonyl oxygen of Thr 98 through a structurally conserved water molecule.[16] Furthermore, the methyl group of the methylamino moiety contributes to the binding affinity through the hydrophobic interaction in the S2 site. This is supported by the fact that the absence of a hydrogen-bonding network between the enzyme and the substituents such as MeO and NMe_2 results in loss of potency (**12g** and **12j**) and the 4′-NH_2 analogue **12i**, which lacks the methyl group of **12k**, also decreases the potency. Although the reasons why the other 4′-substituted analogues (**12c**, **12f**, **12g**, and **17a–c**) increase the potency compared with the unsubstituted analogues (**12a** and **7e**) are not well understood at this point, this result might be caused by the van der Waals interaction or hydrophobic interaction between these substituents and the S2 site.

The non-amidine compound **17d** and the amidine analogue, compound **1**, were assayed for their inhibitory potencies against a larger series of trypsin-like proteinases, and the resulting IC_{50} values are reported in Table 3. The inhibitors were also tested in standard clotting assays including prothrombin time (PT) and activated partial thromboplastin time (APTT) determinations, which were used as qualitative in vitro indicators of the potential antithrombotic activity.

From this it can be seen that amidine compound **1**, which was used as the starting compound, is found to inhibit against FXa and trypsin. On the other hand, the compound **17d** has better selectivity for TF/FVIIa over other serine proteases in comparison to the amidine analogue, compound **1**. As described above, in the S1 site the aminocarbonyl group of **17d** is bound to Ser 190, a specific residue at FVIIa compared to FXa that may exhibit the higher degree of specificity against FXa than that of amidine compound **1**. In the case of the selectivity against trypsin, we suspect that the difference in the residues at site S2 between FVIIa and trypsin plays an important role. The residues at site S2 are Gly 97, Tyr 94, Thr 98, and Thr 99 for FVIIa, as described above, and the corresponding residues are Lys 97, Tyr 94, Thr 98, and Leu 99 for trypsin, which causes the different forms of the S2 pockets: that is, the S2 pocket of trypsin is smaller in size than that of FVIIa (Fig. 5). The molecular modeling suggested that

Table 3. Selectivity profiles of TF/VIIa inhibitors

Compound	IC_{50} (μM)				CT_2 (μM)	
	TF/VIIa	FXa	Thrombin	Trypsin	PT	APTT
1	0.090	0.88	>200	4.8	4.2	4.3
17d	0.69	>200	>200	>200	150	>300 (1.0-fold)

PT/CT_2, concentration of inhibitor required to double the prothrombin time in human plasma.
$APTT/CT_2$, concentration of inhibitor required to double the activated partial thromboplastin time in human plasma.
In bracket, ploidy of the elongation at concentrations of 300 μM.

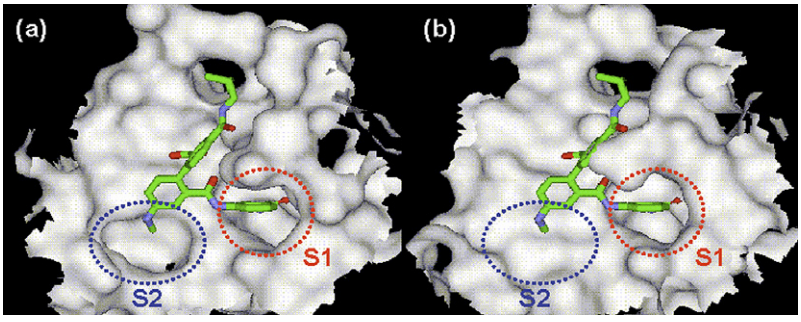


Figure 5. The active site regions of (a) human TF/FVIIa (PDBcode: 1DAN) and (b) human trypsin (PDB code: ITRN). The proteins are displayed with Connoly surface. S1/S2 pockets are indicated as dotted circles. Compound **17d** is shown in green. Docking pose of **17d** with TF/FVIIa is transported to trypsin for the comparison.

the interaction of **17d** with trypsin was less favorable than that with TF/FVIIa, due to the lack of hydrophobic interaction between methylamino substituent of **17d** and the S2 pocket of trypsin.

The PT/CT_2 value for compound **17d** is 150 μM. Furthermore, as expected for the selective TF/FVIIa inhibitors, it should be noted that compound **17d** does not prolong the APTT at all, even at concentrations of 300 μM. On the other hand, the amidine analogue, compound **1**, doubled the APTT at concentrations of 4.3 μM, which may be caused by the inhibition of FXa. Though the IC_{50} and $PTCT_2$ values of **17d** were reduced compared to that of compound **1**, the relative selectivity was significantly more desirable. This property of high selectivity would be expected to escape the bleeding risk.

4. Conclusion

Optimization of the substituents on the P1 phenyl portion of compound **1** led to a neutral or less basic alternative for amidine moieties such as the aminocarbonyl and aminomethyl moieties. By further optimization of the substituents on the central phenyl ring, a highly potent and selective TF/VIIa inhibitor, **17d**, was discovered. This non-amidine compound is positioned to be a valuable and novel lead in the exploration of selective TF/FVIIa inhibitors.

5. Experimental

5.1. Chemistry

In general, reagents and solvents were used as purchased, without further purification. Melting points were determined with a Yanaco MP-500D melting point apparatus and left uncorrected. ^{1}H NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer. Chemical shifts were expressed in δ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). Mass spectra were recorded on a JEOL JMS-LX2000 spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, and N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of theoretical values.

5.2. Methyl 5-formyl-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate (3)

To a solution of methyl 5-formyl-2-hydroxybenzoate (8.89 g, 49.3 mmol) and pyridine (16.0 mL, 197 mmol) in CH_2Cl_2 (89 mL) was added trifluoromethanesulfonic anhydride (14.9 mL, 88.8 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was partitioned between CH_2Cl_2 and H_2O, extracted with CH_2Cl_2, and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to give **3** (7.47 g, 48%) as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.94 (3H, s), 7.84 (1H, d, J = 8.5 Hz), 8.32 (1H, dd, J = 2.1 Hz, 8.5 Hz), 8.55 (1H, d, J = 2.1 Hz), 10.11 (1H, s); FAB-MS (m/z): 313 $(M+H)^+$.

5.3. Methyl 5-[(isobutylamino)carbonyl]-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate (4)

To a solution of **3** (6.50 g, 20.8 mmol), $NaH_2PO_4{\cdot}H_2O$ (3.25 g, 20.8 mmol), and 2-methyl-2-butene (11.0 mL, 104 mmol) in t-BuOH/H_2O/CH_3CN mixture (98 mL, 12:2:1 v/v) was added sodium chlorite (9.41 g, 80 wt %, 104 mmol), and the mixture was stirred at room temperature for 1 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a colorless solid (6.83 g). To a solution of the compound obtained above (6.50 g, 19.8 mmol) in CH_2Cl_2 (65 mL) were added oxalyl chloride (2.07 mL, 23.8 mmol) and DMF (0.10 mL), and the mixture was stirred at room temperature for 2 h. The mixture was concentrated in vacuo. To a solution of the compound obtained above and NEt_3 (2.76 mL, 19.8 mmol) in CH_2Cl_2 (65 mL) at 0 °C was added 2-methyl-1-propanamine (1.45 g, 19.8 mmol), and the mixture was stirred at 0 °C for 1 h. The mixture was partitioned between $CHCl_3$ and H_2O and extracted with $CHCl_3$, and the organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 4:1) to give **4** (4.09 g, 54%) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.90 (6H, d, J = 6.7 Hz), 1.77–1.93 (1H, m), 3.08-3.14 (2H, m), 3.92 (3H, s), 7.70 (1H, d, J = 8.6 Hz), 8.24 (1H, dd, J = 2.3 Hz, 8.6 Hz), 8.50 (1H, d, J = 2.3 Hz), 8.83 (1H, t, J = 5.7 Hz); FAB-MS (m/z): 384 $(M+H)^+$.

5.4. Methyl 2′-formyl-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylate (5)

To a solution of **4** (1.00 g, 2.61 mmol), (2-formylphenyl)boronic acid (391 mg, 2.61 mmol), and K_3PO_4 (831 mg, 3.91 mmol) in DMF (10 mL) was added $Pd(PPh_3)_4$ (90 mg, 0.078 mmol), and the mixture was stirred at 100 °C for 5 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to give **5** (822 mg, 93%) as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.92 (6H, d, J = 6.8 Hz), 1.86–1.96 (1H, m), 3.10–3.17 (2H, m), 3.57 (3H, s), 7.26–7.31 (1H, m), 7.47 (1H, d, J = 7.9 Hz), 7.57–7.66 (1H, m), 7.68–7.75 (1H, m), 7.94 (1H, dd, J = 1.3 Hz, 7.7 Hz), 8.12 (1H, dd, J = 1.8 Hz, 7.9 Hz), 8.42 (1H, d, J = 1.8 Hz), 8.74 (1H, t, J = 5.7 Hz), 9.74 (1H, s); FAB-MS (m/z): 340 $(M+H)^+$.

5.5. 4′-[(Isobutylamino)carbonyl]-2′-(methoxycarbonyl)biphenyl-2-carboxylic acid (6)

To a solution of **5** (3.00 g, 8.84 mmol), $NaH_2PO_4{\cdot}2H_2O$ (1.38 g, 8.85 mmol), and 2-methyl-2-butene (4.7 mL,

44.4 mmol) in *t*-BuOH/H_2O/CH_3CN mixture (30 mL, 6:2:1 v/v) was added sodium chlorite (4.00 g, 80 wt %, 35.4 mmol), and the mixture was stirred at room temperature for 6 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **6** (3.14 g, 100%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.91 (6H, d, J = 6.6 Hz), 1.80–1.95 (1H, m), 3.08–3.17 (2H, m), 3.57 (3H, s), 7.16–7.21 (1H, m), 7.31 (1H, d, J = 7.9 Hz), 7.44–7.53 (1H, m), 7.55–7.64 (1H, m), 7.88–7.96 (1H, m), 8.04 (1H, dd, J = 1.8 Hz, 8.1 Hz), 8.35 (1H, d, J = 1.8 Hz), 8.69 (1H, t, J = 5.7 Hz), 12.55 (1H, br s); FAB-MS (m/z): 356 $(M+H)^+$.

5.6. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (7e)

To a solution of **6** (355 mg, 0.999 mmol) and 3-aminobenzamide (185 mg, 1.20 mmol) in DMF (7.0 mL) were added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC·HCl) (230 mg, 1.20 mmol) and 1-hydroxybenzotriazole (HOBt) (162 mg, 1.20 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 99:1) to give a colorless solid (458 mg). To a solution of the compound obtained above in MeOH (8.0 mL) was added 1 M NaOH/H_2O (1.8 mL, 1.8 mmol), and the mixture was refluxed for 2 h. After cooling, the reaction mixture was acidified with 1 M HCl/H_2O (1.8 mL, 1.8 mmol). The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give **7e** (124 mg, 29%) as a colorless solid: mp 137–138 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.8 Hz), 1.77–1.92 (1H, m), 3.04–3.13 (2H, m), 7.21–7.26 (1H, m), 7.26–7.35 (3H, m), 7.47–7.57 (3H, m), 7.61 (1H, d, J = 7.8 Hz), 7.66–7.72 (1H, m), 7.86 (1H, s), 7.95 (1H, dd, J = 1.9 Hz, 8.3 Hz), 8.01 (1H, s), 8.30 (1H, d, J = 1.9 Hz), 8.63 (1H, t, J = 5.8 Hz), 10.20 (1H, s), 12.88 (1H, br s); FAB-MS (m/z): 458 $(M+H)^+$; Anal. Calcd for $C_{26}H_{25}N_3O_5 \cdot 0.7H_2O \cdot 0.1$AcOEt: C, 65.93; H, 5.70; N, 8.74. Found: C, 66.13; H, 5.53; N, 8.54.

5.7. 2′-(Anilinocarbonyl)-4-[(isobutylamino)carbonyl]-biphenyl-2-carboxylic acid (7a)

Compound **7a** was synthesized from **6** and aniline following the same procedure as that for **7e**. Compound **7a** was obtained as a colorless solid (49%, 121 mg): mp 115–117 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (6H, d, J = 6.3 Hz), 1.76–1.92 (1H, m), 3.08 (2H, t, J = 5.8 Hz), 7.00 (1H, t, J = 7.3 Hz), 7.15-7.30 (4H, m), 7.40–7.55 (4H, m), 7.61 (1H, dd, J = 1.9 Hz, 6.8 Hz), 7.92 (1H, dd, J = 1.9 Hz, 8.0 Hz), 8.28 (1H, d, J = 1.9 Hz), 8.63 (1H, t, J = 5.8 Hz), 10.32 (1H, br s), 12.92 (1H, br s); FAB-MS (m/z): 417 $(M+H)^+$; Anal. Calcd for $C_{25}H_{24}N_2O_4 \cdot 0.6H_2O$: C, 70.28; H, 5.94; N, 6.56. Found: C, 70.15; H, 5.92; N, 6.48.

5.8. 4-[(Isobutylamino)carbonyl]-2′-{[(4-methoxyphenyl)amino]carbonyl}biphenyl-2-carboxylic acid (7b)

Compound **7b** was synthesized from **6** and 4-methoxyaniline following the same procedure as that for **7e**. Compound **7b** was obtained as a colorless solid (60%, 157 mg): mp 149–151 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.3 Hz), 1.77–1.92 (1H, m), 3.09 (2H, t, J = 5.8 Hz), 3.68 (3H, s), 6.80 (2H, d, J = 9.8 Hz), 7.17–7.23 (1H, m), 7.27 (1H, d, J = 7.8 Hz), 7.36 (2H, d, J = 9.8 Hz), 7.44–7.54(1H, m), 7.64 (1H, dd, J = 1.9 Hz, 6.8 Hz), 7.92 (1H, dd, J = 1.9 Hz, 7.8 Hz), 8.27 (1H, d, J = 1.9 Hz), 8.63 (1H, t, J = 5.8 Hz), 10.12 (1H, br s), 12.94 (1H, br s); FAB-MS (m/z): 447 $(M+H)^+$; Anal. Calcd for $C_{26}H_{26}N_2O_5 \cdot 0.3H_2O$: C, 69.10; H, 5.93; N, 6.20. Found: C, 68.96; H, 6.01; N, 6.02.

5.9. 2′-{[(4-Chlorophenyl)amino]carbonyl}-4- [(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (7c)

Compound **7c** was synthesized from **6** and 4-chloroaniline following the same procedure as that for **7e**. Compound **7c** was obtained as a colorless solid (26%, 84 mg): mp 117–118 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.4 Hz), 1.79–1.93 (1H, m), 3.09 (2H, t, J = 5.8 Hz), 7.22–7.27 (1H, m), 7.27–7.34 (3H, m), 7.47–7.58 (4H, m), 7.68 (1H, dd, J = 1.5 Hz, 7.3 Hz), 7.95 (1H, dd, J = 1.9 Hz, 7.8 Hz), 8.30 (1H, d, J = 1.5 Hz), 8.64 (1H, t, J = 5.8 Hz), 10.22 (1H, s), 12.81 (1H, br s); FAB-MS (m/z): 451 $(M+H)^+$; HRMS (ESI). Calcd for $C_{25}H_{23}ClN_2O_4$: 451.1424. Found: 451.1420.

5.10. 2′-{[(3-Chlorophenyl)amino]carbonyl}-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (7d)

Compound **7d** was synthesized from **6** and 3-chloroaniline following the same procedure as that for **7e**. Compound **7d** was obtained as a colorless solid (36%, 114 mg): mp 107–109 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.4 Hz), 1.79–1.92 (1H, m), 3.09 (2H, t, J = 5.8 Hz), 7.05–7.10 (1H, m), 7.20–7.33 (3H, m), 7.40 (1H, d, J = 8.8 Hz), 7.46–7.58 (3H, m), 7.67 (1H, dd, J = 2.0 Hz, 7.3 Hz), 7.96 (1H, dd, J = 2.0 Hz, 7.8 Hz), 8.30 (1H, d, J = 2.0 Hz), 8.64 (1H, t, J = 5.8 Hz), 10.30 (1H, s), 12.78 (1H, br s); FAB-MS (m/z): 451 $(M+H)^+$; HRMS (ESI) Calcd for $C_{25}H_{23}ClN_2O_4$: 451.1424. Found: 451.1416.

5.11. 2′-{[(3-Acetylphenyl)amino]carbonyl}-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (7f)

Compound **7f** was synthesized from **6** and 1-(3-aminophenyl)ethanol following the same procedure as that for **7e**. Compound **7f** was obtained as a colorless solid (65%, 203 mg): mp 107–108 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.4 Hz), 1.77–1.92 (1H, m), 2.51 (3H, s), 3.09 (2H,

t, J = 6.0 Hz), 7.25 (1H, dd, J = 0.8 Hz, 7.2 Hz), 7.31 (1H, d, J = 8.0 Hz), 7.40 (1H, t, J = 8.0 Hz), 7.48–7.58 (2H, m), 7.63 (1H, d, J = 8.0 Hz), 7.67–7.78 (2H, m), 7.95 (1H, dd, J = 2.0 Hz, 8.0 Hz), 8.09 (1H, s), 8.30 (1H, d, J = 2.0 Hz), 8.63 (1H, t, J = 6.0 Hz), 10.31 (1H, s), 12.85 (1H, br s); FAB-MS (m/z): 459 $(M+H)^+$; HRMS (FAB) Calcd for $C_{27}H_{27}N_2O_5$: 459.1920. Found: 459.1922.

5.12. 2′-({[4-(Aminomethyl)phenyl]amino}carbonyl)-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (7g)

To a solution of **6** (320 mg, 0.900 mmol) and *tert*-butyl (4-aminobenzyl)carbamate (200 mg, 0.900 mmol) in DMF (9.0 mL) were added WSC·HCl (345 mg, 1.80 mmol) and HOBt (276 mg, 1.80 mmol), and the mixture was stirred at 50 °C for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 97:3) to give a yellow amorphous (350 mg). To a solution of the compound obtained above in MeOH (6.0 mL) was added 1 M NaOH/H_2O (1.3 mL, 1.3 mmol), and the mixture was refluxed for 2 h. After cooling, the reaction mixture was acidified with 1 M HCl/H_2O (2.6 mL, 2.6 mmol) and concentrated in vacuo. To a solution of the compound obtained above in 1,4-dioxane (20 mL) was added 1 M HCl/H_2O (4.0 mL, 4.0 mmol), and the mixture was stirred at 50 °C for 12 h. The reaction mixture was concentrated in vacuo. The residue was purified by ODS-gel column chromatography (CH_3CN/0.001 HCl aq = 23:77) to give **7g** (150 mg, 95%) as a colorless amorphous powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.8 Hz), 1.81–1.88 (1H, m), 3.08 (2H, t, J = 6.0 Hz), 3.92 (2H, br s), 7.24 (1H, dd, J = 1.6 Hz, 7.2 Hz), 7.29–7.34 (3H, m), 7.49–7.54 (4H, m), 7.66 (1H, dd, J = 1.6 Hz, 7.2 Hz), 7.95 (1H, dd, J = 1.6 Hz, 7.6 Hz), 8.19 (3H, br s), 8.29 (1H, d, J = 1.6 Hz), 8.66 (1H, t, J = 6.0 Hz), 10.19 (1H, br s); FAB-MS (m/z): 446 $(M+H)^+$ Anal. Calcd for $C_{26}H_{27}N_3O_4 \cdot 1.1HCl \cdot 1.5H_2O$: C, 60.92; H, 6.11; N, 8.20; Cl, 7.61. Found: C, 60.69; H, 6.11; N, 8.31; Cl, 7.33.

5.13. 2′-({[4-(Aminomethyl)phenyl]amino}carbonyl)-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (7h)

Compound **7h** was synthesized from **6** and *tert*-butyl (3-aminobenzyl)carbamate following the same procedure as that for **7g**. Compound **7h** was obtained as a colorless amorphous powder (39%, 143 mg): ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.0 Hz), 1.79–1.92 (1H, m), 3.09 (2H, t, J = 4.8 Hz), 3.92 (2H, br s), 7.15 (1H, d, J = 7.6 Hz), 7.24 (1H, dd, J = 2.0 Hz, 6.8 Hz), 7.26–7.38 (3H, m), 7.47–7.57 (2H, m), 7.66 (1H, dd, J = 2.0 Hz, 7.2 Hz), 7.76 (1H, s), 7.96 (1H, dd, J = 2.0 Hz, 8.4 Hz), 8.25 (3H, br s), 8.31 (1H, d, J = 2.0 Hz), 8.67 (1H, t, J = 6.0 Hz), 10.22 (1H, s), 12.88 (1H, br s); FAB-MS (m/z): 446 $(M+H)^+$; Anal. Calcd for $C_{26}H_{27}N_3O_4 \cdot 1.0HCl \cdot 1.5H_2O$: C, 61.35; H, 6.14; N, 8.26; Cl, 6.97. Found: C, 61.30; H, 6.10; N, 8.18; Cl, 6.87.

5.14. Benzyl 2′-formyl-4-methylbiphenyl-2-carboxylate (10c)

To a solution of **8c** (3.00 g, 14.0 mmol) in DMF (30 mL) were added K_2CO_3 (2.90 g, 21.0 mmol) and benzylbromide (1.7 mL, 14.3 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **9c** (4.42 g, 100%) as a colorless oil. To a solution of **9c** (2.04 g, 6.68 mmol) and (2-formylphenyl)boronic acid (1.00 g, 6.67 mmol) in DMF (15 mL) were added $Pd(PPh_3)_4$ (231 mg, 0.200 mmol) and K_3PO_4 (2.12 g, 9.99 mmol), and the mixture was stirred at 100 °C for 3 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to give **10c** (1.95 g, 88%) as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.43 (3H, s), 5.00 (2H, s), 7.05–7.12 (2H,m), 7.21–7.33 (5H, m), 7.46–7.56 (1H, m), 7.59–7.67 (1H, m), 7.77–7.80 (1H, m), 7.82 (1H, dd, J = 1.5 Hz, 7.5 Hz), 9.69 (1H, s); FAB-MS (m/z): 331 $(M+H)^+$.

5.15. Benzyl 2′-formylbiphenyl-2-carboxylate (10a)

Compound **10a** was synthesized from **8a** following the same procedure as that for **10c**. Compound **10a** was obtained as a colorless oil (81%, 1.71 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.02 (2H, s), 7.06–7.14 (2H, m), 7.24–7.33 (4H, m), 7.82 (1H, dd, J = 1.3 Hz, 7.5 Hz), 7.50–7.72 (4H, m), 7.84 (1H, dd, J = 1.5 Hz, 7.5 Hz), 7.99 (1H, dd, J = 1.5 Hz, 7.5 Hz), 9.69 (1H, s); FAB-MS (m/z): 317 $(M+H)^+$.

5.16. Benzyl 2′-formyl-3-methylbiphenyl-2-carboxylate (10b)

Compound **10b** was synthesized from **8b** following the same procedure as that for **10c**. Compound **10b** was obtained as a colorless oil (72%, 2.94 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.34 (3H, s), 4.87 (1H, d, J = 12.2 Hz), 4.95 (1H, d, J = 12.2 Hz), 6.95–7.03 (2H,m), 7.20–7.31 (4H, m), 7.38–7.51 (2H, m), 7.53–7.60 (1H, m), 7.65 (1H, dt, J = 1.7 Hz, 7.5 Hz), 7.86 (1H, dd, J = 1.3 Hz, 7.5 Hz), 9.65 (1H, s); FAB-MS (m/z): 331 $(M+H)^+$.

5.17. Benzyl 2′-formyl-5-methylbiphenyl-2-carboxylate (10d)

Compound **10d** was synthesized from **8d** following the same procedure as that for **10c**. Compound **10d** was obtained as a colorless oil (83%, 1.84 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: δ 2.40 (3H, s), 5.00 (2H, s), 7.07–7.13 (2H,m), 7.18 (1H, br s), 7.23–7.32 (4H, m), 7.38–7.43 (1H, m), 7.49–7.56 (1H, m), 7.64 (1H, dt, J = 1.5 Hz, 7.5 Hz), 7.83 (1H, dd, J = 1.3 Hz, 7.7 Hz), 7.91 (1H, d, J = 8.0 Hz), 9.69 (1H, s); FAB-MS (m/z): 331 $(M+H)^+$.

5.18. Benzyl 2′-formyl-6-methylbiphenyl-2-carboxylate (10e)

Compound **10e** was synthesized from **8b** following the same procedure as that for **10c**. Compound **10e** was obtained as a colorless oil (31%, 675 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.91 (3H, s), 4.95 (2H, s), 7.08–7.13 (2H, m), 7.16 (1H, dd, J = 0.8 Hz, 7.5 Hz), 7.27–7.33 (3H, m), 7.44–7.60 (3H, m), 7.66 (1H, dt, J = 1.5 Hz, 7.5 Hz), 7.78 (1H, d, J = 7.7 Hz), 7.85 (1H, dd, J = 1.1 Hz, 7.7 Hz), 9.60 (1H, s); FAB-MS (m/z): 331 $(M+H)^+$.

5.19. *tert*-Butyl 4-chloro-2′-formylbiphenyl-2-carboxylate (10f)

Compound **10f** was synthesized from **8f** following the same procedure as that for **10c** except for the protection with the acid group by *tert*-butyl group.[17] Compound **10f** was obtained as a colorless oil (97%, 3.25 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.10 (9H, s), 7.30 (1H, d, J = 7.8 Hz), 7.39 (1H, d, J = 8.0 Hz), 7.60-7.78 (3H, m), 7.84 (1H, d, J = 2.2 Hz), 7.95 (1H, dd, J = 1.3 Hz, 7.7 Hz), 9.75 (1H, s); FAB-MS (m/z): 317 $(M+H)^+$.

5.20. Methyl 2′-formyl-4′-methoxybiphenyl-2-carboxylate (10g)

Compound **10g** was synthesized from **8g** following the same method of that for **10c** except for cross-coupling between **9g** and 2-formyl-4-methoxyboronic acid. Compound **10g** was obtained as a colorless oil (55%, 410 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.55 (3H, s), 3.87 (3H, s), 7.21 (1H, d, J = 8.4 Hz), 7.27 (1H, dd, J = 2.7 Hz, 8.4 Hz), 7.36 (1H, dd, J = 1.3 Hz, 7.5 Hz), 7.39 (1H, d, J = 2.7 Hz), 7.57 (1H, dt, J = 1.3 Hz, 7.7 Hz), 7.66 (1H, dt, J = 1.5 Hz, 7.7 Hz), 7.60–7.78 (3H, m), 7.84 (1H, d, J = 2.2 Hz), 7.95 (1H, dd, J = 1.3 Hz, 7.5 Hz), 7.93 (1H, dd, J = 1.5 Hz, 7.7 Hz), 9.65 (1H, s); FAB-MS (m/z): 271 $(M+H)^+$.

5.21. 2-Benzyl 2′-methyl 4-methylbiphenyl-2,2′-dicarboxylate (11c)

To a solution of **10c** (1.95 g, 5.90 mmol), $NaH_2PO_4{\cdot}2H_2O$ (920 mg, 5.90 mmol), and 2-methyl-2-butene (3.2 mL, 30.2 mmol) in *t*-BuOH/H_2O/CH_3CN mixture (27 mL, 6:2:1 v/v) was added sodium chlorite (2.67 g, 80 wt %, 23.6 mmol), and the mixture was stirred at room temperature for 9 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give as a colorless solid (2.04 g). To a solution of compound obtained above (1.00 g, 2.89 mmol) in DMF (10 mL) were added K_2CO_3 (798 mg, 5.77 mmol) and MeI (0.54 mL, 8.67 mmol), and the mixture was stirred at room temperature for 3 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **11c** (1.04 g, 100%) as a colorless oil: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.40 (3H, s), 3.50 (3H, s), 5.00 (2H, br s), 7.06–7.12 (3H,m), 7.18 (1H, dd, J = 1.5 Hz, 7.7 Hz), 7.26–7.32 (3H, m), 7.38–7.46 (2H, m), 7.55 (1H, dt, J = 1.5 Hz, 7.5 Hz), 7.72 (1H, d, J = 1.5 Hz), 7.81 (1H, dd, J = 1.5 Hz, 7.7 Hz); FAB-MS (m/z): 361 $(M+H)^+$.

5.22. 2′-[(Benzyloxy)carbonyl]biphenyl-2-carboxylic acid (11a)

Compound **11a** was synthesized from **10a** following the same procedure as that for **11c** without protection of the acid by methyl group. Compound **11a** was obtained as a colorless amorphous powder (100%, 1.80 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.01 (2H, s), 7.08–7.26 (5H, m), 7.26–7.32 (3H, m), 7.38–7.56 (2H, m), 7.58 (1H, dt, J = 1.5 Hz, 7.5 Hz), 7.85 (1H, dd, J = 1.2 Hz, 7.7 Hz), 7.92 (1H, dd, J = 1.2 Hz, 7.7 Hz), 12.45 (1H, br s); FAB-MS (m/z): 333 $(M+H)^+$.

5.23. 2-Benzyl 2′-ethyl 3-methylbiphenyl-2,2′-dicarboxylate (11b)

Compound **11b** was synthesized from **10b** following the same procedure as that for **11c** except for the protection of the acid by the ethyl group. Compound **11b** was obtained as a colorless oil (89%, 2.95 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.86 (3H, t, J = 7.1 Hz), 2.29 (3H, s), 3.84–4.00 (2H, m), 4.81–4.98 (2H, m), 6.99–7.07 (3H,m), 7.21 (1H, dd, J = 1.1 Hz, 7.3 Hz), 7.24–7.32 (4H, m), 7.34–7.41 (1H, m), 7.44–7.58 (2H, m), 7.84 (1H, dt, J = 1.7 Hz, 7.5 Hz); FAB-MS (m/z): 375 $(M+H)^+$.

5.24. 2-Benzyl 2′-methyl 5-methylbiphenyl-2,2′-dicarboxylate (11d)

Compound **11d** was synthesized from **10d** following the same procedure as that for **11c**. Compound **11d** was obtained as a colorless oil (100%, 1.04 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.37 (3H, s), 3.49 (3H, s), 4.97–5.02 (2H, m), 7.02 (1H, br s), 7.06–7.13 (2H, m), 7.20 (1H, dd, J = 1.1 Hz, 7.7 Hz), 7.26–7.32 (4H, m), 7.40–7.47 (1H, m), 7.56 (1H, dt, J = 1.5 Hz, 7.5 Hz), 7.82 (1H, dd, J = 1.3 Hz, 7.7 Hz), 7.84 (1H, d, J = 7.7 Hz); FAB-MS (m/z): 361 $(M+H)^+$.

5.25. 2-Benzyl 2′-methyl 6-methylbiphenyl-2,2′-dicarboxylate (11e)

Compound **11e** was synthesized from **10e** following the same procedure as that for **11c**. Compound **11e** was obtained as a colorless oil (100%, 468 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.87 (3H, s), 3.50 (3H, s), 4.92 (1H, d, J = 16.3 Hz), 4.96 (1H, d, J = 16.3 Hz), 7.07 (1H, d, J = 7.7 Hz), 7.09–7.15 (3H, m), 7.27–7.51 (3H, m), 7.57 (1H, dt, J = 1.3 Hz, 7.5 Hz), 7.73 (1H, d, J = 6.8 Hz), 7.89 (1H, dd, J = 1.3 Hz, 7.7 Hz); FAB-MS (m/z): 361 $(M+H)^+$.

5.26. 2-*tert*-Butyl 2′-methyl 4-chlorobiphenyl-2,2′-dicarboxylate (11f)

Compound **11f** was synthesized from **10f** following the same procedure as that for **11c**. Compound **11f** was

obtained as a colorless oil (94%, 3.32 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.12 (9H, s), 3.56 (3H, s), 7.19–7.26 (2H, m), 7.49–7.56 (1H, m), 7.60–7.68 (2H, m), 7.78 (1H, d, J = 2.4 Hz), 7.95 (1H, d, J = 7.7 Hz); FAB-MS (m/z): 347 $(M+H)^+$.

5.27. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4′-methylbiphenyl-2-carboxylic acid (12c)

To a solution of **11c** (1.03 g, 2.86 mmol) in MeOH (20 mL) was added 10% Pd/C powder (100 mg), and the mixture was stirred in a hydrogen atmosphere at room temperature for 12 h. The catalyst was filtered on Celite and the filtrate was concentrated in vacuo to give a solid (773 mg). To a solution of the compound obtained above and *tert*-butyl (4-aminobenzyl)carbamate (879 mg, 5.70 mmol) in DMF (10 mL) were added WSC·HCl (820 mg, 4.28 mmol) and HOBt (578 mg, 4.28 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 97:3) to give a colorless solid (892 mg). To a solution of the residual solid (840 mg, 2.16 mmol) in MeOH (10 mL) was added 1 M NaOH/H_2O (3.4 mL, 3.4 mmol), and the mixture was refluxed for 12 h. After cooling, the reaction mixture was acidified with 1 M HCl/H_2O (1.8 mL, 1.8 mmol). The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give **12c** (812 mg, 100%) as a colorless solid: mp 200–201 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.42 (3H, s), 3.34 (3H, br s), 4.08 (1H, br s), 7.10 (1H, d, J = 7.8 Hz), 7.20 (1H, d, J = 7.3 Hz), 7.25–7.35 (3H, m), 7.35–7.43 (1H, m), 7.43–7.53 (3H, m), 7.57 (1H, d, J = 7.8 Hz), 7.80 (1H, dd, J = 1.5 Hz, 7.3 Hz), 7.88 (1H, br s), 7.94 (1H, br s), 10.02 (1H, s), 12.80 (1H, br s); FAB-MS (m/z): 375 $(M+H)^+$. Anal. Calcd for $C_{22}H_{18}N_2O_4$·1.0H_2O·1.0-MeOH: C, 65.08; H, 5.70; N, 6.60. Found: C, 65.23; H, 5.71; N, 6.65.

5.28. 2′-({[3- (Aminocarbonyl)phenyl]amino}carbonyl)-biphenyl-2-carboxylic acid (12a)

Compound **12a** was synthesized from **11a** following the same procedure as that for **12c** without deprotection of the benzyl group. Compound **12a** was obtained as a colorless solid (72%, 702 mg): mp 135–136 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.20–7.25 (2H, m), 7.27–7.35 (2H,m), 7.37–7.43 (1H, m), 7.45–7.58 (5H, m), 7.63–7.68 (1H, m), 7.82 (1H, dd, J = 1.5 Hz, 7.8 Hz), 7.86 (1H, br s), 7.94 (1H, br s), 10.04 (1H, s), 12.81 (1H, br s); FAB-MS (m/z): 361 $(M+H)^+$. Anal. Calcd for $C_{21}H_{16}N_2O_4$·0.5AcOEt: C, 68.31; H, 4.98; N, 6.93. Found: C, 68.14; H, 4.88; N, 6.88.

5.29. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-3′-methylbiphenyl-2-carboxylic acid (12b)

Compound **12b** was synthesized from **11b** following the same procedure as that for **12c**. Compound **12b** was obtained as a colorless solid (8.0%, 53 mg): mp 130–132 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.38 (3H, s), 7.02 (1H, d, J = 6.8 Hz), 7.24–7.40 (6H, m), 7.42–7.51 (3H, m), 7.76 (1H, dd, J = 1.0 Hz, 6.9 Hz), 7.85 (1H, br s), 7.87 (1H, br s), 9.99 (1H, s), 12.87 (1H, br s); FAB-MS (m/z): 375 $(M+H)^+$. Anal. Calcd for $C_{22}H_{18}N_2O_4$·0.7H_2O: C, 68.28; H, 5.05; N, 7.24. Found: C, 68.31; H, 5.14; N, 7.21.

5.30. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-5′-methylbiphenyl-2-carboxylic acid (12d)

Compound **12d** was synthesized from **11d** following the same procedure as that for **12c**. Compound **12d** was obtained as a colorless solid (84%, 929 mg): mp 212–214 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.37 (3H, s), 7.02 (1H, s), 7.20 (1H, dd, J = 0.9 Hz, 7.8 Hz), 7.26–7.32 (3H, m), 7.37–7.43 (1H, m), 7.46–7.60 (2H, m), 7.81 (1H, dd, J = 1.5 Hz, 7.8 Hz), 7.86 (1H, br s), 7.93 (1H, br s), 9.94 (1H, s), 12.84 (1H, br s); FAB-MS (m/z): 375 $(M+H)^+$; HRMS (ESI). Calcd. for $C_{22}H_{18}N_2O_4$: 375.1345. Found: 375.1349.

5.31. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-6′-methylbiphenyl-2-carboxylic acid (12e)

Compound **12e** was synthesized from **11e** following the same procedure as that for **12c**. Compound **12e** was obtained as a colorless solid (80%, 361 mg): mp 236–238 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.93 (3H, s), 7.12 (1H, d, J = 7.3 Hz), 7.24–7.32 (2H, m), 7.39–7.46 (4H, m), 7.46–7.55 (3H, m), 7.83–7.91 (3H, m), 10.03 (1H, s), 12.86 (1H, br s); FAB-MS (m/z): 375 $(M+H)^+$. Anal. Calcd for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.53; H, 4.84; N, 7.39.

5.32. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4′-chlorobiphenyl-2-carboxylic acid (12f)

Compound **12f** was synthesized from **11f** following the same procedure as that for **12c**. Compound **12f** was obtained as a colorless solid (25%, 367 mg): mp 132–135 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.20–7.36 (4H, m), 7.39–7.45 (1H,m), 7.48–7.55 (2H, m), 7.55–7.62 (2H, m), 7.69 (1H, d, J = 2.0 Hz), 7.82–7.89 (2H, m), 7.96 (1H, br s), 10.27 (1H, s), 12.72 (1H, br s); FAB-MS (m/z): 395 $(M+H)^+$. Anal. Calcd for $C_{21}H_{15}ClN_2O_4$·0.5H_2O: C, 62.46; H, 3.99; N, 6.94; Cl, 8.78. Found: C, 62.13; H, 3.86; N, 6.91; Cl, 8.84.

5.33. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4′-methoxybiphenyl-2-carboxylic acid (12g)

Compound **12g** was synthesized from **10g** following the same procedure as that for **12c**. Compound **12g** was obtained as a colorless solid (81%, 432 mg): mp 125–127 °C (MeOH–H_2O); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.87 (3H, s), 7.07 (1H, dd, J = 2.4 Hz, 8.4 Hz), 7.13 (1H, d, J = 8.4 Hz), 7.17–7.23 (2H, m), 7.26–7.34 (2H, m), 7.38 (1H, dt, J = 1.3 Hz, 7.5 Hz), 7.44–7.53 (2H, m), 7.57 (1H, d, J = 8.4 Hz), 7.78 (1H, dd, J = 1.3 Hz, 7.5 Hz), 7.87 (1H, br s), 7.93 (1H, br s), 10.05 (1H, s), 12.77 (1H, s); ESI-MS

(*m*/*z*): 389 $(M-H)^-$; HRMS (ESI) Calcd for $C_{22}H_{18}N_2O_5$: 391.1294. Found: 391.1296.

5.34. 2-(Trimethylsilyl)ethyl 2-bromo-5-methoxybenzoate (14)

To a solution of **13** (2.50 g, 10.8 mmol) and 2-trimethylsilylethanol (1.53 g, 12.9 mmol) in DMF (5.0 mL) and pyridine (2.5 mL) were added DCC (2.45 g, 11.9 mmol) and DMAP (2 mg), and the mixture was stirred at room temperature for 12 h. The resulting precipitate was filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 9:1) to give **14** (2.00 g, 56%) as a colorless oil: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.06 (9H, s), 0.99–1.09 (2H, m), 3.73 (3H, s), 4.26–4.35 (2H, m), 7.00 (1H, dd, J = 3.1 Hz, 8.8 Hz), 7.18 (1H, d, J = 3.1 Hz), 7.55 (1H, d, J = 8.8 Hz).

5.35. Benzyl 5-[(isobutylamino)carbonyl]-2-{[(trifluoromethyl)sulfonyl]oxy}benzoate (15)

Compound **15** was synthesized from benzyl 5-formyl-2-hydroxybenzoate following the same procedure as that for **4**. Compound **15** was obtained as a colorless solid (85%, 2.89 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.6 Hz), 1.75–1.92 (1H, m), 3.06–3.13 (2H, m), 5.42 (2H, s), 7.33–7.45 (3H, m), 7.45–7.52 (2H, m), 7.71 (1H, d, J = 8.6 Hz), 8.23 (1H, dd, J = 2.4 Hz, 8.6 Hz), 8.48 (1H, d, J = 2.4 Hz), 8.82 (1H, t, J = 5.9 Hz); FAB-MS (*m*/*z*): 460 $(M+H)^+$.

5.36. 2-Benzyl 2′-[2-(trimethylsilyl)ethyl] 4-[(isobutylamino)carbonyl]-4′-methoxybiphenyl-2,2′-dicarboxylate (16)

To a solution of **14** (1.40 g, 4.23 mmol) and **15** (971 mg, 2.11 mmol) in DMF (10 mL) were added bis(pinacolato)diboron (1.07 g, 4.53 mmol), $PdCl_2(dppf)\cdot CH_2Cl_2$ (86 mg, 0.105 mmol), and K_3PO_4 (1.35 g, 6.35 mmol), the mixture was stirred at 120 °C for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 4:1) to give **16** (401 mg, 34%) as a colorless amorphous powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ: −0.07 (9H, s), 0.63–0.73 (2H, m), 0.97 (6H, d, J = 6.9 Hz), 1.88–2.00 (1H, m), 3.15–3.23 (2H, m), 3.89 (3H, s), 3.98–4.07 (2H, m), 5.08–5.13 (2H, m), 7.15–7.23 (4H, m), 7.33–7.39 (5H, m), 8.11 (1H, dd, J = 1.9 Hz, 7.9 Hz), 8.43 (1H, d, J = 1.9 Hz), 8.74 (1H, t, J = 5.9 Hz); FAB-MS (*m*/*z*): 562 $(M+H)^+$.

5.37. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4-[(isobutylamino)carbonyl]-4′-methoxybiphenyl-2-carboxylic acid (17a)

To a solution of **16** (480 mg, 0.850 mmol) in DMF (5.0 mL) was added TBAF (1.0 M solution in THF, 1.0 mL, 1.0 mmol). The mixture was stirred at room temperature for 8 days, then partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2) to give a pale yellow amorphous (219 mg). To a solution of the compound obtained above and *tert*-butyl (4-aminobenzyl)carbamate (110 mg, 0.713 mmol) in DMF (4.0 mL) were added WSC·HCl (137 mg, 0.715 mmol) and HOBt (96 mg, 0.710 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and H_2O and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2) to give a colorless solid (128 mg). To a solution of the residual solid in MeOH (2.0 mL) was added 1 M NaOH/H_2O (0.50 mL, 0.50 mmol), and the mixture was refluxed for 2 h. After cooling, the reaction mixture was acidified with 1 M HCl/H_2O (0.60 mL, 0.60 mmol). The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give **17a** (60 mg, 57%) as a colorless solid: mp 135–137 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (6H, d, J = 6.3 Hz), 1.78–1.92 (1H, m), 3.04-3.12 (2H, m), 3.88 (3H, s), 7.10 (1H, dd, J = 2.5 Hz, 8.3 Hz), 7.18 (1H, d, J = 8.3 Hz), 7.23 (1H, d, J = 2.5 Hz), 7.26–7.34 (3H, m), 7.51 (1H, d, J = 7.8 Hz), 7.63 (1H, d, J = 8.3 Hz), 7.88 (1H, s), 7.92 (1H, dd, J = 1.9 Hz, 7.8 Hz), 8.00 (1H, s), 8.26 (1H, d, J = 1.9 Hz), 8.61 (1H, t, J = 5.8 Hz), 10.19 (1H, s), 12.84 (1H, br s); FAB-MS (*m*/*z*): 490 $(M+H)^+$; Anal. Calcd for $C_{27}H_{27}N_3O_6\cdot 1.0H_2O$: C, 63.90; H, 5.76; N, 8.28. Found: C, 63.74; H, 5.64; N, 8.08.

5.38. Methyl 3-[(acetyloxy)methyl]-4-bromobenzoate (19)

To a solution of methyl 4-bromo-3-methylbenzoate (**18**) (13.5 g, 58.9 mmol) in CCl_4 (300 mL) were added NBS (12.5 g, 70.3 mmol) and AIBN (5 mg). The mixture was stirred at 70 °C for 2 h, then partitioned between $CHCl_3$ and H_2O, and the organic layer was washed with 5% $NaHCO_3$ in H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a colorless solid. To a solution of the compound obtained above in AcOH (60 mL) was added AcONa (4.81 g, 117 mmol), and the mixture was stirred at 100 °C for 12 h. After concentrated in vacuo, the mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with 5% $NaHCO_3$ in H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 5:1) to give **19** (10.8 g, 64%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (3H, s), 3.93 (3H, s), 5.22 (2H, s), 7.66 (1H, d, J = 8.2 Hz), 7.85 (1H, dd, J = 2.0 Hz, 8.2 Hz), 8.06 (1H, d, J = 2.0 Hz).

5.39. 4-Bromo-3-(hydroxymethyl)-*N*-isobutylbenzamide (20)

To a solution of **19** (10.7 g, 37.3 mmol) in MeOH (140 mL) was added 1 M NaOH/H_2O (112 mL, 112 mmol), and the mixture was stirred at room

temperature for 2 h. After cooling, the reaction mixture was acidified with 1 M HCl/H_2O (112 mL, 112 mmol). The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give a colorless solid (8.29 g). To a solution of the compound obtained above (4.30 g, 18.6 mmol) and 2-methylpropan-1-amine (4.08 g, 55.8 mmol) in DMF (40 mL) were added WSC·HCl (5.33 g, 27.9 mmol) and HOBt (4.18 g, 27.9 mmol), and the mixture was stirred at room temperature for 12 h. After adding H_2O to the reaction mixture, the resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give **20** (4.27 g, 80%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.88 (6H, d, J = 6.6 Hz), 1.76–1.92 (1H, m), 3.04-3.12 (2H, m), 4.54 (1H, d, J = 4.2 Hz), 5.53 (1H, br s), 7.64–7.67 (2H, m), 8.02 (1H, s), 8.55 (1H, t, J = 5.5 Hz).

5.40. 4-Bromo-3-(1,3-dioxolan-2-yl)-*N*-isobutylbenzamide (21)

To a solution of **20** (3.21 g, 11.2 mmol) in $CHCl_3$ (55 mL) was added MnO_2 (9.74 g, 112 mmol), and the mixture was stirred at 70 °C for 1 h. After cooling, the reaction mixture was filtered through a pad of Celite and concentrated in vacuo to give a colorless solid. To a solution of the solid obtained above and ethylene glycol (1.39 g, 22.4 mmol) in toluene (110 mL) was added 4-methylbenzenesulfonic acid monohydrate (20 mg, 0.116 mmol), and the mixture was refluxed for 12 h. After cooling, to the reaction mixture was added 5% $NaHCO_3$ in H_2O. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was washed with diisopropyl ether to give **21** (3.53 g, 96%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.88 (6H, d, J = 6.6 Hz), 1.76–1.92 (1H, m), 3.04–3.11 (2H, m), 3.97–4.06 (2H, m), 4.06–4.16 (2H, m), 5.97 (1H, s), 7.71–7.82 (2H, m), 8.00 (1H, s), 8.62 (1H, t, J = 5.9 Hz).

5.41. {2-Formyl-4-[(isobutylamino)carbonyl]phenyl}boronic acid (22)

To the solution of **21** (630 mg, 1.92 mmol) in THF (19 mL) was added at -78 °C 1.59 M *n*-butyllithium/hexane (2.66 mL, 4.23 mmol), and the mixture was stirred at −78 °C for 2 h. To the reaction mixture was added triisopropyl borate (0.89 mL, 3.84 mmol), allowed to warm to room temperature over 12 h. To the reaction mixture was added 1 M HCl/H_2O (9.0 mL, 9.0 mmol) and the mixture was stirred at room temperature for 3 h. After adding 1 M NaOH/H_2O (18 mL, 18 mmol), the mixture was partitioned between Et_2O and H_2O, and after the water layer was acidified with 1 M HCl/H_2O (10 mL, 10 mmol), the water layer was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **22** (280 mg, 59%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.90 (6H, d, J = 6.8 Hz), 1.81–1.92 (1H, m), 3.11 (2H, t, J = 6.4 Hz), 7.66 (1H, d, J = 7.7 Hz), 8.07 (1H, dd, J = 2.6 Hz, 7.7 Hz), 8.31–8.35 (3H, br), 8.65 (1H, t, J = 6.0 Hz), 10.17 (1H, s).

5.42. 2′-Benzyl 2-methyl 4-[(isobutylamino)carbonyl]-4′-methylbiphenyl-2,2′-dicarboxylate (23)

To a solution of **22** (280 mg, 1.08 mmol) and **9c** (395 mg, 1.3 mmol) in DMF (11 mL) were added K_3PO_4 (344 mg, 1.62 mmol) and $Pd(PPh_3)_4$ (62 mg, 0.054 mmol), and the mixture was stirred at 120 °C for 24 h. After cooling, the mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 2:1) to give a colorless oil. To a solution of the compound obtained above, $NaH_2PO_4{\cdot}2H_2O$ (115 mg, 0.955 mmol), and 2-methyl-2-butene (0.50 mL, 4.78 mmol) in *t*-BuOH/H_2O/CH_3CN mixture (18 mL, 6:2:1 v/v) was added sodium chlorite (432 mg, 80 wt %, 3.82 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a colorless amorphous. To a solution of the acid and MeI (0.089 mL, 1.43 mmol) in DMF (10 mL) was added K_2CO_3(197 mg, 1.43 mmol), and the mixture was stirred at room temperature for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **23** (0.44 g, 52%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.92 (6H, d, J = 6.8 Hz), 1.82–1.97 (1H, m), 3.09–3.17 (2H, m), 3.53 (3H, s), 4.98–5.04 (2H, m), 7.03–7.08 (2H, m), 7.10 (1H, d, J = 7.9 Hz), 7.23–7.31 (4H, m), 7.44 (1H, d, J = 7.9 Hz), 7.75 (1H, s), 8.01 (1H, dd, J = 2.0 Hz, 7.9 Hz), 8.29 (1H, d, J = 2.0 Hz), 8.65 (1H, t, J = 6.0 Hz); FAB-MS(m/z): 474 $(M+H)^+$.

5.43. 2-*tert*-Butyl 2′-methyl 4-chloro-4′-[(isobutylamino)carbonyl]biphenyl-2,2′-dicarboxylate (24)

Compound **24** was synthesized from **22** and **9f** following the same procedure as that for **23**. Compound **24** was obtained as a colorless solid (21%, 185 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.90 (6H, d, J = 6.0 Hz), 1.14 (9H, s), 1.80–1.95 (1H, m), 3.08–3.16 (2H, m), 3.60 (3H, s), 7.24 (1H, d, J = 8.3 Hz), 7.34 (1H, d, J = 7.9 Hz), 7.67 (1H, dd, J = 2.2 Hz, 8.3 Hz), 7.82 (1H, d, J = 2.2 Hz), 8.10 (1H, dd, J = 1.5 Hz, 7.9 Hz), 8.43 (1H, d, J = 1.5 Hz), 8.72 (1H, t, J = 5.9 Hz); FAB-MS (m/z): 446 $(M+H)^+$.

5.44. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4-[(isobutylamino)carbonyl]-4′-methylbiphenyl-2-carboxylic acid (17b)

To a solution of **23** (440 mg, 0.955 mmol) in MeOH (10 mL) was added 10% Pd/C powder (44 mg), and the mixture was stirred under hydrogen pressure (3.0 kg/cm^2) at room temperature for 12 h. The catalyst was filered on Celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30:1) to give a

colorless oil (350 mg). To a solution of the compound obtained above and 3-aminobenzamide (339 mg, 1.49 mmol) in DMF (11 mL) were added WSC·HCl (316 mg, 1.65 mmol) and HOBt (282 mg, 1.65 mmol), and the mixture was stirred at 60 °C for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 97:3) to give a colorless solid (150 mg). To a solution of the residual solid in MeOH (6.0 mL) was added 1 M NaOH/H_2O (0.46 mL, 0.46 mmol), and the mixture was stirred at room temperature for 12 h. After cooling, the reaction mixture was acidified with 1 M HCl/H_2O (0.46 mL, 0.46 mmol). The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give **17b** (71 mg, 49%) as a colorless solid: mp 143–144 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (6H, d, J = 6.8 Hz), 1.79–1.89 (1H, m), 2.43 (3H, s), 3.08 (2H, t, J = 6.4 Hz), 7.12 (1H, d, J = 7.6 Hz), 7.27–7.35 (4H, m), 7.49–7.51 (2H, m), 7.62 (1H, d, J = 8.4 Hz), 7.86 (1H, br s), 7.93 (1H, dd, J = 2.0 Hz, 8.0 Hz), 8.00 (1H, br s), 8.28 (1H, d, J = 2.0 Hz), 8.62 (1H, t, J = 6.0 Hz), 10.18 (1H, br s), 12.85 (1H, br s); FAB-MS(m/z): 474 $(M+H)^+$; Anal. Calcd for $C_{27}H_{27}N_3O_5 \cdot 1.3H_2O$: C, 65.26; H, 6.00; N, 8.46. Found: C, 65.30; H, 5.73; N, 8.19.

5.45. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)- 4′-chloro-4-[(isobutylamino)carbonyl]biphenyl-2-carboxylic acid (17c)

Compound **17c** was synthesized from **24** following the same procedure as that for **17b** except for deprotection of the *tert*-butyl group under acidic conditions. Compound **17c** was obtained as a colorless solid (49%, 80 mg): mp 155–158 °C (MeOH–H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (6H, d, J = 6.3 Hz), 1.80–1.90 (1H, m), 3.09 (2H, t, J = 6.3 Hz), 7.27–7.33 (4H, m), 7.52 (1H, d, J = 7.9 Hz), 7.59–7.64 (2H, m), 7.75 (1H, d, J = 2.5 Hz), 7.87 (1H, br s), 7.96 (1H, dd, J = 1.9 Hz, 7.8 Hz), 8.00 (1H, br s), 8.32 (1H, d, J = 2.0 Hz), 8.65 (1H, t, J = 5.3 Hz), 10.36 (1H, br s), 12.85 (1H, br s); FAB-MS (m/z): 494 $(M+H)^+$; Anal. Calcd for $C_{26}H_{24}ClN_3O_5 \cdot 1.0H_2O$: C, 61.00; H, 5.12; N, 8.21; Cl, 6.92. Found: C, 61.24; H, 4.98; N, 8.23; Cl, 7.08.

5.46. 2-*tert*-Butyl 2′-methyl 4-[(isobutylamino)carbonyl]-4′-nitrobiphenyl-2,2′-dicarboxylate (26)

Compound **26** was synthesized from **21** and methyl 2-bromo-5-nitrobenzoate (**25**) following the same procedure as that for **22**. Compound **26** was obtained as a yellow solid (40%, 2.15 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.92 (6H, d, J = 6.8 Hz), 1.16 (9H, s), 1.83–1.94 (1H, m), 3.13 (2H, t, J = 6.4 Hz), 3.64 (3H, s), 7.33 (1H, d, J = 8.0 Hz), 7.58 (1H, d, J = 8.4 Hz), 8.07 (1H, dd, J = 1.8 Hz, 7.9 Hz), 8.37 (1H, d, J = 1.7 Hz), 8.49 (1H, dd, J = 2.4 Hz, 8.4 Hz), 8.69 (1H, d, J = 2.4 Hz), 8.74 (1H, t, J = 6.2 Hz); FAB-MS (m/z): 446 $(M+H)^+$.

5.47. 2′-*tert*-Butyl 2-methyl 4-nitrobiphenyl-2,2′-dicarboxylate (27)

Compound **27** was synthesized from **25** following the same procedure as that for **22**. Compound **27** was obtained as a yellow solid (30%, 4.32 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.17 (9H, s), 3.61 (3H, s), 7.22 (1H, d, J = 7.3 Hz), 7.51–7.60 (2H, m), 7.61–7.68 (1H, m), 7.93 (1H, d, J = 7.7 Hz), 8.46 (1H, dd, J = 2.5 Hz, 8.4 Hz), 8.66 (1H, d, J = 2.5 Hz); FAB-MS (m/z): 358 $(M+H)^+$.

5.48. *tert*-Butyl 4′-amino-4-[(isobutylamino)carbonyl]-2′-({[3-(methoxycarbonyl)phenyl]amino}carbonyl)biphenyl-2-carboxylate (30)

To a solution of **27** (1.30 g, 2.85 mmol) in MeOH (20 mL) was added 1 M NaOH/H_2O (3.0 mL, 3.0 mmol), and the mixture was refluxed for 2 h. After cooling and concentrating, the reaction mixture was acidified with 1 M HCl/H_2O (1.8 mL, 1.8 mmol). The mixture was partitioned between AcOEt and H_2O and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a pale yellow solid (1.23 g). To a solution of the compound obtained above (960 mg) and methyl 3-aminobenzoate (656 mg, 4.34 mmol) in DMF (20 mL) was added WSC·HCl (623 mg, 3.25 mmol) and HOBt (439 mg, 3.25 mmol), and the mixture was stirred at 80 °C for 12 h. The mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2) to give a yellow solid (1.28 g). To a solution of the residual solid in MeOH (20 mL) were added 10% Pd/C powder (130 mg), and the mixture was stirred in a hydrogen atmosphere at room temperature for 12 h. The catalyst was filtrated on Celite and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 30:1) to give **30** (1.00 g, 85%) as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.87 (6H, d, J = 6.6 Hz), 1.27 (9H, s), 1.78–1.89 (1H, m), 3.07 (2H, t, J = 6.2 Hz), 3.82 (3H, s), 5.46 (2H, br s), 6.72 (1H, dd, J = 2.0 Hz, 8.3 Hz), 6.84 (1H, s), 6.86–6.89 (1H, m), 7.26 (1H, d, J = 8.1 Hz), 7.39 (1H, t, J = 7.9 Hz), 7.60 (1H, d, J = 7.7 Hz), 7.69 (1H, d, J = 7.9 Hz), 7.86 (1H, dd, J = 1.8 Hz, 8.0 Hz), 8.12 (1H, d, J = 2.0 Hz), 8.22 (1H, br s), 8.57 (1H, t, J = 5.7 Hz), 10.09 (1H, br s); FAB-MS (m/z): 546 $(M+H)^+$.

5.49. *tert*-Butyl 2′-({[3-(aminocarbonyl)phenyl]amino}carbonyl)-4′- nitrobiphenyl-2-carboxylate (28)

Compound **28** was synthesized from **26** and 3-aminobenzamide following the same procedure as that for **30** without reduction of the nitro group. Compound **28** was obtained as a yellow solid (87%, 3.84 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.21 (9H, s), 7.25–7.38 (3H, m), 7.45–7.66 (4H, m), 7.85–8.02 (4H, m), 8.40 (1H, dd, J = 2.4 Hz, 8.4 Hz), 8.53 (1H, d, J = 2.4 Hz), 10.43 (1H, s).

5.50. *tert*-Butyl 4′-amino-2′-({[3- (aminocarbonyl)phenyl]amino}carbonyl)biphenyl-2-carboxylate (29)

Compound **29** was synthesized from **28** with reduction of the nitro group following the same procedure of as that for **30**. Compound **29** was obtained as a yellow solid (69%, 2.13 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.27 (9H, s), 5.39 (2H, br s), 6.69 (1H, dd, J = 2.4 Hz, 8.4 Hz), 6.82 (1H, d, J = 8.2 Hz), 6.85 (1H, d, J = 2.4 Hz), 7.19 (1H, dd, J = 1.1 Hz, 7.7 Hz), 7.24–7.35 (3H, m), 7.38–7.45 (1H, m), 7.45–7.52 (2H, m), 7.66 (1H, dd, J = 1.3 Hz, 7.7 Hz), 7.85 (1H, br s), 7.95 (1H, br s), 9.65 (1H, s); FAB-MS (m/z): 432 $(M+H)^+$.

5.51. *tert*-Butyl 4′-amino-2′-({[3- (methoxycarbonyl)phenyl]amino}carbonyl)biphenyl-2-carboxylate (31)

Compound **31** was synthesized from **27** following the same procedure as that for **30**. Compound **31** was obtained as a colorless solid (73%, 783 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.27 (9H, s), 3.82 (3H, s), 5.39 (2H, br s), 6.70 (1H, d, J = 8.2 Hz), 6.81–6.87 (2H, m), 7.21 (1H, d, J = 7.5 Hz), 7.27–7.46 (3H, m), 7.56–7.69 (3H, m), 8.17 (1H, s), 9.87 (1H, s); FAB-MS (m/z): 447 $(M+H)^+$.

5.52. *tert*-Butyl 4′-(dimethylamino)-2′-({[3-(methoxycarbonyl)phenyl]amino}carbonyl)biphenyl-2-carboxylate (32)

To a solution of **30** (243 mg, 0.544 mmol) and HCHO (37% solution in H_2O, 1.3 mL, 1.63 mmol) in 1,2-dichloroethane (5.0 mL) and AcOH (326 mg, 5.44 mmol) was added $NaBH(OAc)_3$ (346 mg, 1.63 mmol), and the mixture was stirred at room temperature for 10 h. The mixture was partitioned between AcOEt and 5% $NaHCO_3$ in H_2O, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 4:1) to give **32** (155 mg, 60%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.26 (9H, s), 3.00 (6H, s), 3.83 (3H, s), 6.88 (1H, d, J = 8.0 Hz), 6.95–7.04 (2H, m), 7.22 (1H, d, J = 7.5 Hz), 7.30–7.49 (3H, m), 7.58–7.72 (3H, m), 8.18 (1H, s), 9.96 (1H, s); FAB-MS (m/z): 475 $(M+H)^+$.

5.53. *tert*-Butyl 4-[(isobutylamino)carbonyl]-2′-({[3-(methoxycarbonyl)phenyl]amino}carbonyl)-4′-[methyl(trifluoroacetyl)amino]biphenyl-2-carboxylate (34)

To a solution of **30** (300 mg, 0.550 mmol) and pyridine (0.06 mL, 0.715 mmol) in CH_2Cl_2 (6.0 mL) was added TFAA (0.09 mL, 0.660 mmol), and the mixture was stirred at room temperature for 3 days. The mixture was partitioned between AcOEt and 5% $NaHCO_3$ in H_2O, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/AcOEt = 4:1) to give a colorless solid. To a solution of the compound obtained above and K_2CO_3 (53 mg, 0.383 mmol) in 2-butanone (5.0 mL) was added MeI (136 mg, 0.958 mmol), and the mixture was stirred at 40 °C for 4 h. After cooling, the mixture was partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **34** (190 mg, 53%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.89 (6H, d, J = 6.6 Hz), 1.22 (9H, s), 1.78–1.92 (1H, m), 3.09 (2H, t, J = 6.6 Hz), 3.40 (3H, s), 3.83 (3H, s), 7.33–7.46 (3H, m), 7.60–7.70 (2H, m), 7.75 (1H, d, J = 7.3 Hz), 7.88 (1H, br s), 7.95–8.01 (1H, m), 8.15 (1H, br s), 8.24 (1H, br s), 8.67 (1H, t, J = 5.5 Hz), 10.21 (1H, br s); FAB-MS (m/z): 656 $(M+H)^+$.

5.54. *tert*-Butyl 2′-({[3-(methoxycarbonyl)phenyl]amino}carbonyl)-4′-[methyl(trifluoroacetyl)amino]biphenyl-2-carboxylate (35)

Compound **35** was synthesized from **31** following the same procedure as that for **34**. Compound **35** was obtained as a colorless solid (83%, 254 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.22 (9H, s), 3.83 (3H, s), 3.83 (3H, s), 7.25–7.38 (2H, m), 7.38–7.48 (2H, m), 7.50–7.58 (1H, m), 7.58–7.66 (2H, m), 7.70 (1H, d, J = 7.9 Hz), 7.74–7.84 (2H, m), 8.12 (1H, s), 10.05 (1H, s); FAB-MS (m/z): 557 $(M+H)^+$.

5.55. *tert*-Butyl 2′-({[3-(aminocarbonyl)phenyl]amino}carbonyl)-4- [(isobutylamino)carbonyl]-4′-(methylamino)biphenyl-2-carboxylate (36)

To a solution of **35** (175 mg, 0.267 mmol) obtained above in MeOH (7.0 mL) was added 1 M NaOH/H_2O (0.8 mL, 0.8 mmol), and the mixture was refluxed for 2 h. After cooling, the reaction mixture was acidified with 5% citric acid in H_2O. The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give a pale yellow solid (115 mg). To a solution of the compound obtained above and NH_4Cl (41 mg, 0.766 mmol) in DMF (11 mL) were added WSC·HCl (55 mg, 0.288 mmol), HOBt (39 mg, 0.289 mmol), and *N*-ethyl-*N*-isopropylpropan-2-amine (*i*-Pr_2NEt) (99 mg, 0.766 mmol). The mixture was stirred at room temperature for 12 h, then partitioned between AcOEt and H_2O, and extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50:1) to give **36** (78 mg, 75%) as a colorless amorphous powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.96 (6H, d, J = 6.6 Hz), 1.83–1.96 (1H, m), 2.77 (3H, s), 3.25 (2H, t, J = 6.6 Hz), 5.68 (1H, br), 6.28 (1H, br), 6.54 (1H, t, J = 5.7 Hz), 6.68 (1H, dd, J = 2.6 Hz, 8.4 Hz), 6.93 (1H, d, J = 8.3 Hz), 7.00 (1H, d, J = 2.6 Hz), 7.22–7.26 (2H, m), 7.32–7.35 (1H, m), 7.42–7.45 (1H, m), 7.58 (1H, t, J = 1.8 Hz), 7.69 (1H, dd, J = 2.0 Hz, 8.0 Hz), 8.12 (1H, d, J = 1.8 Hz), 8.56 (1H, s); FAB-MS (m/z): 545 $(M+H)^+$.

5.56. *tert*-Butyl 2′-({[3-(aminocarbonyl)phenyl]amino}carbonyl)-4′- (dimethylamino)biphenyl-2-carboxylate (33)

Compound **33** was synthesized from **32** following the same procedure as that for **36**. Compound **33** was obtained as a colorless solid (50%, 125 mg): ^{1}H NMR

(300 MHz, DMSO-d_6) δ: 1.25 (9H, s), 2.99 (3H, s), 6.88 (1H, dd, J = 2.6 Hz, 8.4 Hz), 6.96 (1H, d, J = 2.6 Hz), 6.99 (1H, d, J = 8.4 Hz), 7.21 (1H, d, J = 7.7 Hz), 7.26–7.37 (3H, m), 7.43 (1H, dt, J = 1.4 Hz, 7.5 Hz), 7.46–7.58 (3H, m), 7.69 (1H, dd, J = 1.3 Hz, 7.7 Hz), 7.87 (1H, br s), 9.77 (1H, s); FAB-MS (*m*/*z*): 458 $(M-H)^-$.

5.57. *tert*-Butyl 2′-({[3-(aminocarbonyl)phenyl]amino}carbonyl)-4′-(methylamino)biphenyl-2-carboxylate (37)

Compound **37** was synthesized from **35** following the same procedure as that for **36**. Compound **37** was obtained as a colorless solid (58%, 119 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.27 (9H, s), 2.76 (3H, d, J = 4.6 Hz), 5.92–6.00 (1H, m), 6.68 (1H, dd, J = 2.4 Hz, 8.2 Hz), 6.79 (1H, d, J = 2.4 Hz), 6.89 (1H, d, J = 8.2 Hz), 7.21 (1H, d, J = 7.5 Hz), 7.24–7.36 (3H, m), 7.42 (1H, dt, J = 1.3 Hz, 7.5 Hz), 7.46–7.54 (2H, m), 7.67 (1H, dd, J = 1.3 Hz, 7.7 Hz), 7.86 (1H, br s), 7.95 (1H, br s), 9.69 (1H, s); FAB-MS (*m*/*z*): 444 $(M-H)^-$.

5.58. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4-[(isobutylamino)carbonyl]-4′-(methylamino)biphenyl-2-carboxylic acid (17d)

To a solution of **36** (75 mg, 0.143 mmol) in CH_2Cl_2 (5 mL) was added trifluoroacetic acid (2 mL), and the mixture was stirred at room temperature for 12 h. The mixture was concentrated in vacuo. To the residue was added 1 M NaOH in H_2O, and the solution was then acidified with 5% citric acid in H_2O. The resulting precipitate was filtered, washed with H_2O, and dried in vacuo to give **17d** (36 mg, 52%) as a colorless solid: mp 156–159 °C (H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.87 (6H, d, J = 6.8 Hz), 1.78–1.91 (1H, m), 2.43 (3H, s), 3.67 (2H, t, J = 6.4 Hz), 6.03 (1H, br s), 6.67 (1H, dd, J = 2.4 Hz, 8.3 Hz), 6.79 (1H, d, J = 2.4 Hz), 6.94 (1H, d, J = 8.3 Hz), 7.18–7.32 (3H, m), 7.49 (1H, d, J = 7.8 Hz), 7.59 (1H, d, J = 9.3 Hz), 7.85–7.88 (2H, m), 7.99 (1H, br s), 8.19 (1H, d, J = 1.9 Hz), 8.90 (1H, d, J = 1.9 Hz), 10.09 (1H, s), 12.87 (1H, br s); FAB-MS (*m*/*z*): 489 $(M+H)^+$; Anal. Calcd for $C_{27}H_{28}N_4O_5 \cdot 1.5H_2O$: C, 62.90, H, 6.06; N, 10.87. Found: C, 63.00; H, 5.82; N, 10.66.

5.59. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4′-nitrobiphenyl-2-carboxylic acid (12h)

Compound **12h** was synthesized from **28** following the same procedure as that for **17d**. Compound **12h** was obtained as a colorless solid (56%, 112 mg): mp 230–233 °C (H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.24–7.36 (3H, m), 7.45–7.51 (1H, m), 7.52–7.65 (4H, m), 7.88 (1H, br s), 7.92 (1H, dd, J = 1.0 Hz, 7.8 Hz), 7.97 (1H, br s), 8.36 (1H, dd, J = 2.4 Hz, 8.3 Hz), 8.48 (1H, d, J = 2.4 Hz), 10.45 (1H, s), 12.77 (1H, br s); FAB-MS (*m*/*z*): 404 $(M-H)^-$; Anal. Calcd for $C_{21}H_{15}N_3O_6 \cdot 0.8H_2O$: C, 60.09; H, 3.99; N, 10.01. Found: C, 60.33; H, 3.82; N, 9.66.

5.60. 4′-Amino-2′-({[3- (aminocarbonyl)phenyl]amino}carbonyl)biphenyl-2-carboxylic acid (12i)

Compound **12i** was synthesized from **29** following the same procedure as that for **17d**. Compound **12i** was obtained as a colorless amorphous powder (88%, 150 mg): ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.64 (2H, br s), 6.93 (1H, dd, J = 1.5 Hz, 8.3 Hz), 7.01 (1H, d, J = 8.3 Hz), 7.07 (1H, d, J = 1.5 Hz), 7.10–7.22 (1H, m), 7.22–7.34 (2H, m), 7.34–7.40 (1H, m), 7.42–7.55 (3H, m), 7.75 (1H, dd, J = 1.5 Hz, 7.9 Hz), 7.86 (1H, br s), 7.91 (1H, br s), 9.97 (1H, s); FAB-MS (*m*/*z*): 376 $(M+H)^+$; HRMS (ESI) Calcd for $C_{21}H_{17}N_3O_4$: 376.1297. Found: 376.1290.

5.61. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4′-(dimethylamino)biphenyl-2-carboxylic acid (12j)

Compound **12j** was synthesized from **33** following the same procedure as that for **17d**. Compound **12j** was obtained as a colorless solid (40%, 40 mg): mp 141–144 °C (H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.00 (6H, s), 6.85 (1H, dd, J = 2.9 Hz, 8.3 Hz), 6.93 (1H, d, J = 2.9 Hz), 7.01 (1H, d, J = 8.3 Hz), 7.18 (1H, d, J = 7.9 Hz), 7.24–7.37 (3H, m), 7.44 (1H, dt, J = 1.5 Hz, 7.4 Hz), 7.49 (1H, d, J = 7.9 Hz), 7.56 (1H, d, J = 8.3 Hz), 7.72 (1H, dd, J = 1.5 Hz, 7.9 Hz), 7.86 (1H, br s), 7.92 (1H, br s), 9.96 (1H, s), 12.81 (1H, br s); FAB-MS (*m*/*z*): 404 $(M+H)^+$; Anal. Calcd for $C_{23}H_{21}N_3O_4 \cdot 0.1H_2O \cdot 0.5AcOEt$: C, 66.83; H, 5.65; N, 9.35. Found: C, 66.94; H, 5.25; N, 9.48.

5.62. 2′-({[3-(Aminocarbonyl)phenyl]amino}carbonyl)-4′-(methylamino)biphenyl-2-carboxylic acid (12k)

Compound **12k** was synthesized from **37** following the same procedure as that for **17d**. Compound **12k** was obtained as a colorless solid (49%, 48 mg): mp 135–137 °C (H_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.75 (3H, s), 5.98 (1H, br s), 6.65 (1H, dd, J = 2.5 Hz, 8.3 Hz), 6.77 (1H, d, J = 2.5 Hz), 6.91 (1H, d, J = 8.3 Hz), 7.16 (1H, d, J = 7.3 Hz), 7.25–7.36 (3H, m), 7.42 (1H, dt, J = 1.4 Hz, 7.4 Hz), 7.46–7.55 (2H, m), 7.70 (1H, dd, J = 1.0 Hz, 7.8 Hz), 7.86 (1H, br s), 7.92 (1H, br s), 9.95 (1H, s); FAB-MS (*m*/*z*): 390 $(M+H)^+$; HRMS (ESI) Calcd for $C_{22}H_{19}N_3O_4$: 390.1454. Found: 390.1464.

5.63. Docking study

Docking simulation was carried out using GOLD (CCDC, Cambridge, UK) software at the active site of TF/FVIIa complex (PDB code: 1DAN). After docking, energy minimization was performed based on the MMFF94s force field using MOE 2004.03 (Chemical Computing Group Inc., Montreal, CA).

5.64. Biology

5.64.1. Chromogenic assay. The hydrolysis rates of synthetic substrates were assayed by continuously measuring absorbance at 405 nm at 37 °C with a microplate spectrophotometer (Spectramax 340PC, Molecular

Devices Co. California, USA). Reaction mixtures (40 μL) were prepared in 96-well plates containing chromogenic substrates and an inhibitor in either 20 mM HEPES, 0.01% BSA, 5 mM $CaCl_2$, pH 7.4, or 0.15 M NaCl. Reactions were initiated with 10 μL portions of the enzyme solution. Enzymes and substrates were used as follows: TF/FVIIa and S-2288; factor Xa and s-2222; thrombin and S-2238; and trypsin and S-2222. The concentration of an inhibitor required to inhibit enzyme activity by 50% (IC_{50}) was calculated from concentration-response curves in which the logit transformation of residual activity was plotted against the logarithm of inhibitor concentration.

5.64.2. Plasma clotting time assay. Citrated blood samples from human were collected in accordance with Astellas Research Ethics Committee. Platelet-poor plasma was centrifuged at 3000 rpm for 10 min and stored at −40 °C until use. Plasma clotting times were recorded using a KC10A coagulometer (Amelung Co., Lehbringsweg, Germany) at 37 °C. Prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using Hemos Recombiplastin and Hemoliance (Instrumentation Laboratory Company Lexington, MA, USA), respectively. Coagulation times for each test sample were compared with coagulation times measured using 4% DMSO in water as a control. The concentration required to double clotting time (CT_2) was estimated from each individual concentration–response curve. Each measurement was performed three times and represented as the mean value.

Acknowledgments

The authors deeply thank Dr. Toshio Okazaki for his helpful support in preparing this manuscript, and are also grateful to the staff of the Division of Analytical Science Laboratories for the elemental analysis and spectral measurements.

References and notes

1. (a) Vlasuk, G. P. Direct Factor Xa Inhibitors. In *New Therapeutic Agents in Thrombosis and Thrombolysis*; Sasahara, A. A., Loscalzo, J., Eds.; Mercel Dekker: New York, 1997; p 261; (b) Bick, R. L.; Kalpan, H. *Clin. North Am.* **1998**, *82*, 409; (c) Vacca, J. P. *Curr. Opin. Chem. Biol.* **2000**, *4*, 394; (d) Braunwald, E.; Califf, R. M.; Cannon, C. P.; Fox, K. A.; Fuster, V.; Gibler, W. B.; Harrington, R. A.; King, S. B.; Kleiman, N. S.; Theroux, P.; Topol, E. J.; Van de Werf, F.; White, H. D.; Willerson, J. T. *Am. J. Med.* **2000**, *108*, 41.
2. Samama, M.; Bara, L.; Gouin-Thibaut, I. *Drugs.* **1996**, *52*, 8.
3. Hirsh, J.; Fuster, V. *Circulation* **1994**, *89*, 1469.
4. (a) Gage, B. F.; Fihn, S. D.; White, R. H. *Am. J. Med.* **2000**, *109*, 481; (b) Stein, P. D.; Grandison, D.; Hua, T. A. *Postgrad. Med. J.* **1994**, *70*, S72; (c) Hirsh, J.; Potter, L. *Arch. Int. Med.* **1994**, *154*, 282.
5. (a) Kimball, S. D. *Handbook Exp. Pharmacol.* **1999**, *132*, 367; (b) Manear, K. *Curr. Med. Chem.* **1998**, *5*, 457.
6. (a) Betz, A. *Exp. Opin. Ther. Patents* **2001**, *11*, 1007; (b) Rai, R.; Sprengeler, P. A.; Elrod, K. C.; Young, W. B. *Curr. Med. Chem.* **2001**, *8*, 101.
7. (a) Mann, K. G.; Nesheim, M. E.; Church, W. R.; Haley, P.; Krishnaswamy, S. *Blood* **1990**, *76*, 1; (b) Darvem, E. W.; Fujikawa, K.; Kisiel, W. *Biochemistry* **1991**, *30*, 10363.
8. (a) Sitco, G. R.; Ramjit, D. R.; Stabilito, I. I.; Lehman, D.; Lynch, J. J.; Vlasuk, G. P. *Circulation* **1992**, *85*, 805; (b) Harker, L. A.; Hanson, S. R.; Kelly, A. B. *Thromb. Haemost.* **1995**, *74*, 464; (c) Nicolini, F. A.; Lee, P.; Malcky, J. L.; Lefkovits, J.; Kottke-Marchant, K.; Plow, E. F.; Topol, E. J. *Blood Coagulation Fibrinolysis* **1996**, *7*, 39; (d) Harker, L. A.; Hanson, S. R.; Kelly, A. B. *Thromb. Haemost.* **1997**, *78*, 736.
9. (a) Ruef, J.; Katus, H. A. H. *Expert. Opin. Investig. Drugs* **2003**, *12*, 669; (b) Morrisey, J. H. *Thromb. Haemost.* **2001**, *86*, 66; (c) Bouma, B. N. M.; Joost, C. M. *Thromb. Haemost.* **1999**, *82*, 243.
10. (a) Harker, L. A.; Hanson, S. R.; Wilcox, J. N.; Kelly, A. B. *Haemostasis* **1996**, *26*, 76; (b) Girard, T. J.; Nicholson, N. S. *Curr. Opin. Pharmacol.* **2001**, *1*, 159; (c) Szalony, J. A.; Taite, B. B.; Giard, T. J.; Nicholson, N. S.; LaChance *J. Thromb. Thrombolysis* **2002**, *14*, 113.
11. (a) Senokuchi, K.; Ogawa, K. WO9941231, 1999; (b) Butenas, S.; Ribarik, N.; Mann, K. G. *Biochemistry* **1993**, *32*, 6531; (c) Uchiba, M.; Okajima, K.; Abe, H.; Okabe, H.; Takatsuki, K. *Thromb. Res.* **1994**, *74*, 155; (d) Semple, J. E.; Rowley, D. C.; Brunck, T. K.; Ripka, W. C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 315; (e) Roussel, P.; Bradley, M.; Kane, P.; Bailey, C.; Arnold, R.; Cross, A. *Tetrahedron* **1999**, *55*, 6219; (f) Jokobsen, P.; Ritsmar, P. B.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2095; (g) Jokobsen, P.; Horneman, A. M.; Persson, E. *Bioorg. Med. Chem.* **2000**, *8*, 2803; (h) Kohrt, J. T.; Filipski, K. J.; Rapundalo, S. T.; Cody, W. L.; Edmunds, J. J. *Tetrahedron Lett.* **2000**, *41*, 6041; (i) Young, W. B.; Kolesnikov, A.; Rai, R.; Sprengeler, P. A.; Leahy, E. M.; Shrader, W. D.; Sangalang, J.; Burgess-Henry, J.; Spender, J.; Elrod, K.; Creagar, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2253; (j) Bisacchi, G. S.; Sutton, J. C., Jr.; Slusarchyk, W. A.; Treuner, U. D.; Zhao, G.; Cheney, D. L.; Wu, S. C.;Shi, Y. WO0242273, 2002; (k) Hanessian, S.; Therrien, E.; Granberg, K.; Nilsson, I. *Bioorg. Med, Chem. Lett.* **2002**, *12*, 2907; (l) Robinson, L. A.; Saiah, E. M. K. *Annu. Rep. Med. Chem.* **2002**, *37*, 85; (m) Parlow, J. J.; Stevens, A. M.; Stegeman, R. A.; Stallings, W. C.; Kurumbail, R. G.; South, M. S. *J. Med. Chem.* **2003**, *46*, 4297; (n) Klingler, O.; Matter, H.; Schudok, M.; Bajaj, S. P.; Czech, J.; Lorenz, M.; Nestler, H. P.; Shreuder, H.; Wildgoose, P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1463; (o) Parlow, J. J.; Kurumbail, R. G.; Stegeman, R. A.; Stevens, A. M.; Stallings, W. C.; South, M. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3721; (p) Klingler, O.; Matter, H.; Schudok, M.; Donghi, M.; Czech, J.; Lorenz, M.; Nestler, H. P.; Szillat, H.; Shreuder, H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3715; (q) Buckman, B. O.; Chou, Yuo-Ling; McCarrick, M.; Liang, A.; Lents, D.; Mohan, R.; Morrissey, M. M.; Shaw, K. J.; Trinh, L.; Light, D. R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2249; (r) Sagi, K.; Fujita, K.; Sugiki, M.; Takahashi, M.; Takehana, S.; Tashiro, K.; Kayahara, T.; Yamanashi, M.; Fukuda, Y.; Oono, S.; Okajima, A.; Iwata, S.; Shoji, M.; Sakurai, K. *Bioorg. Med. Chem.* **2005**, *13*, 1487; (s) Kohrt, J. T.; Filipski, K. J.; Cody, W. L.; Cai, C.; Dudley, D. A.; Huis, C. V.; Willardsen, A.; Narasimhan, L. S.; Zhang, E.; Rapundalo, S. T.; Saiya-Cork, K.; Leadley, R. J.; Edmunds, J. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1060; (t) Rai, R.; Kolesnikov, A.; Sprengeler, P. A.; Torkelson, S.; Ton, T.; Katz, B. A.; Yu, C.; Hendrix, J.; Shrader, W. D.; Stephens, R.; Cabuslay, R.; Sanford, E.; Young, W. B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2270.

12. (a) Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwoddddod, A. M.; Luettgen, J. M.; Liang, L.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, *44*, 566; (b) Pauls, H. W.; Ewing, W. R. *Curr. Top. Med. Chem.* **2001**, *1*, 83; (c) Schumacher, W. A.; Balasubramanian, N.; St. Laurent, D. R.; Seiler, S. M. *Eur. J. Pharmacol.* **1994**, *259*, 165; (d) Hauptmann, J.; Markwardt, F. *Semin. Thromb. Hemost.* **1992**, 200.
13. Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508.
14. A substantial amount of *tert*-butyl 4′-(dimethylamino)-2′-{[(3-{[(hydroxymethyl)amino]carbonyl}phenyl)amino]carbonyl}biphenyl-2- carboxylate, which was obtained from reaction between HCHO and the carboxamide group of **29**, was obtained in 60% yield: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.25 (9H, s), 2.99 (6H, s), 4.67 (2H, t, J = 6.2 Hz), 5.59–5.67 (1H, m), 6.87 (1H, d, J = 8.4 Hz), 6.96 (1H, s), 6.99 (1H, d, J = 8.4 Hz), 7.21 (1H, d, J = 7.8 Hz), 7.26–7.38 (2H, m), 7.40–7.51 (2H, m), 7.57 (1H, d, J = 8.0 Hz), 7.69 (1H, d, J = 7.8 Hz), 7.97 (1H, br s), 8.98–9.06 (1H, m), 9.80 (1H, s); FAB-MS (m/z): 488 $(M-H)^-$.
15. In the course of our research, the X-ray crystal structure of compound **1** bound to the Des-Gla-FVIIa/sTF complex was reported by Granberg and co-workers: Granberg, K. L; Petersen, J. F. W.; Anderson, M.; Nardi, F.; Darby, N.; Lindskog, P.; Slater, T.; Zetterberg, F. J.; Stocker, A.; Caulkett, P.; Preston, J.; Walker, R.; Gordon, C.; Fahlander, U. E. *The 226th ACS National Meeting*, Poster 85, New York, September 7–11, 2003. The binding mode of compound **1** is generally similar between the X-ray crystal structure and our molecular modeling.
16. Before docking simulation, we have checked the conservation of water molecules in S1 and S2 sites referred in this text for 24 structures of FVIIa deposited in Protein Data Bank. As for S1 site, the water molecule is conserved among 19 out of 24 structures. (Note that this water molecule is also well conserved in trypsin with similar hydrogen-bonding networks as FVIIa). As for S2 site, the corresponding position is occupied with hydrophilic groups of ligand molecule in 5 entries and the water molecule is conserved among 10 out of remaining 19 structures.
17. Wright, S. W.; Hageman, D. L.; Wright, A. S.; McClure, L. D. *Tetrahedron Lett.* **1997**, *38*, 7345.