Preparation and Pharmacological Evaluation of Novel Glycoprotein (Gp) IIb/IIIa Antagonists. 2. Condensed Heterocyclic Derivatives

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Received May 25, 1999; accepted September 6, 1999

A novel series of platelet receptor glycoprotein (Gp) IIb/IIIa antagonists with condensed heterocycles as their basic core was synthesized. In an *in vitro* assay, *trans*-4-(5-amidinobenzofuran-2-carboxamido)cyclohexyloxyacetic acid 17e and *trans*-3-[4-(5-amidinobenzofuran-2-carboxamido)cyclohexyl]propionic acid 17f produced marked inhibitions with IC_{50} values of 0.018 and 0.006 μM, respectively in a human platelet adenosin-5′-diphospate (ADP)-induced aggregation assay; they also exhibited a wide spectrum of inhibition toward major aggregation agonists (ADP, collagen, thrombin, PMA (tumor promoter) and arachidonic acid). These compounds were >2—3 orders of magnitude more effective in inhibiting platelet aggregation than human umbilical vein endothelial cell (HUVEC) binding. The oral administration of 10 mg/kg of either 17e and 17f to guinea pig, resulted in a 60% inhibition of *ex vivo* platelet aggregation after 5 h. Oral administration of ethyl *trans*-4-(5-amidinobenzofuran-2-carboxamido)cyclohexyloxyacetate 18e (10 mg/kg) resulted in 80% inhibition of platelet aggregation in dogs for 6 h after oral administration with a return to baseline by 24 h. Ethyl *trans*-3-[4-(5-amidinobenzofuran-2-carboxamido)cyclohexyl]propionate 18f (AR0598) produced 80% inhibition for 5 h after oral administration. Prodrug 18e showed a good profile in dogs with a long duration of action. 18e (AR0510) was selected as suitable clinical candidate for development as an orally active antithrombotic agent.

Key words glycoprotein IIb/IIIa; amidinobenzofuran; structure–activity relationship; AR0510

The glycoprotein (Gp) IIb/IIIa is a platelet membrane-bound receptor involved in the final stage of the platelet aggregation process. It plays a central role in the binding of fibrinogen (Fbg) during normal hemostasis and arterial thrombosis.[1—3] Recently, development of receptor antagonists has been an area of active research in many pharmaceutical companies in the quest for a new type of orally active low molecular weight anti-aggregation agent.[4—12]

In the previous report,[13] we described our strategy for the development of naphthalene compounds as GP IIb/IIIa antagonists. Recently, Gp IIb/IIIa antagonists with bicyclic 5-amidinoindoles have been reported by Su and co-workers.[11] The 10-fold difference in potency observed between indole and acyclic compounds suggested a conformational restriction in the indole part or a CH–π interaction effect in that region of the receptor. However, our acyclic compound **2** (Fig. 1) possessed similar inhibitory activity to the naphthalene **1**. This potency suggests that the condensed heterocycle does not provide an additional CH–π interaction effect compared with an acyclic compound within that region of Gp IIb/IIIa. Our previous structure–activity relationship and molecular modeling studies of these naphthalene compounds combined with conformational studies established the importance of geometrical parameters and of the three-point interaction between amide hydrogen bonds, the amidinoaryl moiety, and the carboxylic acid moiety. Carboxylic acid derivative **1** was found to be a potent antagonist of Fbg receptors. In designing our antagonists, we postulated that the affinity of binding to the Fbg receptor is dependent on the geometrical relationship between the carboxylic acid and the amidino group, and we designed the condensed heterocyclic analogues to improve this geometrical relationship. These studies led to the identification of **1** as a lead compound.[13]

In this paper, as shown in Fig. 1, we describe the synthesis, structure–activity relationship, and *in vitro* and *ex vivo* activities of this novel series of condensed heterocyclic compounds with naphthalene compound **1** as lead compound. **17e**, **17f** and a number of other compounds with a cyclohexane unit also have enhanced activity. These compounds are highly selective compared with other RGD-recognizing integrins and demonstrate a wide-ranging potent inhibition toward major aggregation agonists. The corresponding prodrugs, ethyl *trans*-4-(5-amidinobenzofuran-2-carboxamido)-cyclohexyloxyacetate **18e** and ethyl *trans*-3-[4-(5-amidinobenzofuran-2-carboxamido)cyclohexyl]propionate **18f**, are a potent fibrinogen receptor antagonists with a long duration of action after oral administration.

Chemistry Our compounds can be structurally divided into two parts, a basic part (amidinoaryl moiety) and an acidic part (carboxylic acid moiety) (Fig. 1). Preparation of the target compounds used to investigate the effects of the basic part, listed in Table 1, is illustrated in Charts 1 and 2. The cyano acids **3** were prepared from the nitro ester by reduction (*e.g.* hydrogenation), the Sandmeyer reaction and basic hydrolysis. The 6-cyanoindole-2-carboxylic acid **3c** was prepared using Fisher's indolization.[14] Compound **3d** was obtained by N-alkylation from **3c**,[15] and **3h** was prepared from 2-acetyl-4-nitrophenol in four steps according to the procedure for the preparation of **3a**. The amino ester **4** was prepared as reported in the previous paper.

The preparation of **7a**—**h** is shown in Chart 1. Coupling of the aromatic amino ester **4** with each of the cyano acids **3a**, **3c**,[15] **3d**—**g** led to the corresponding intermediates. The amide bonds were prepared using standard coupling

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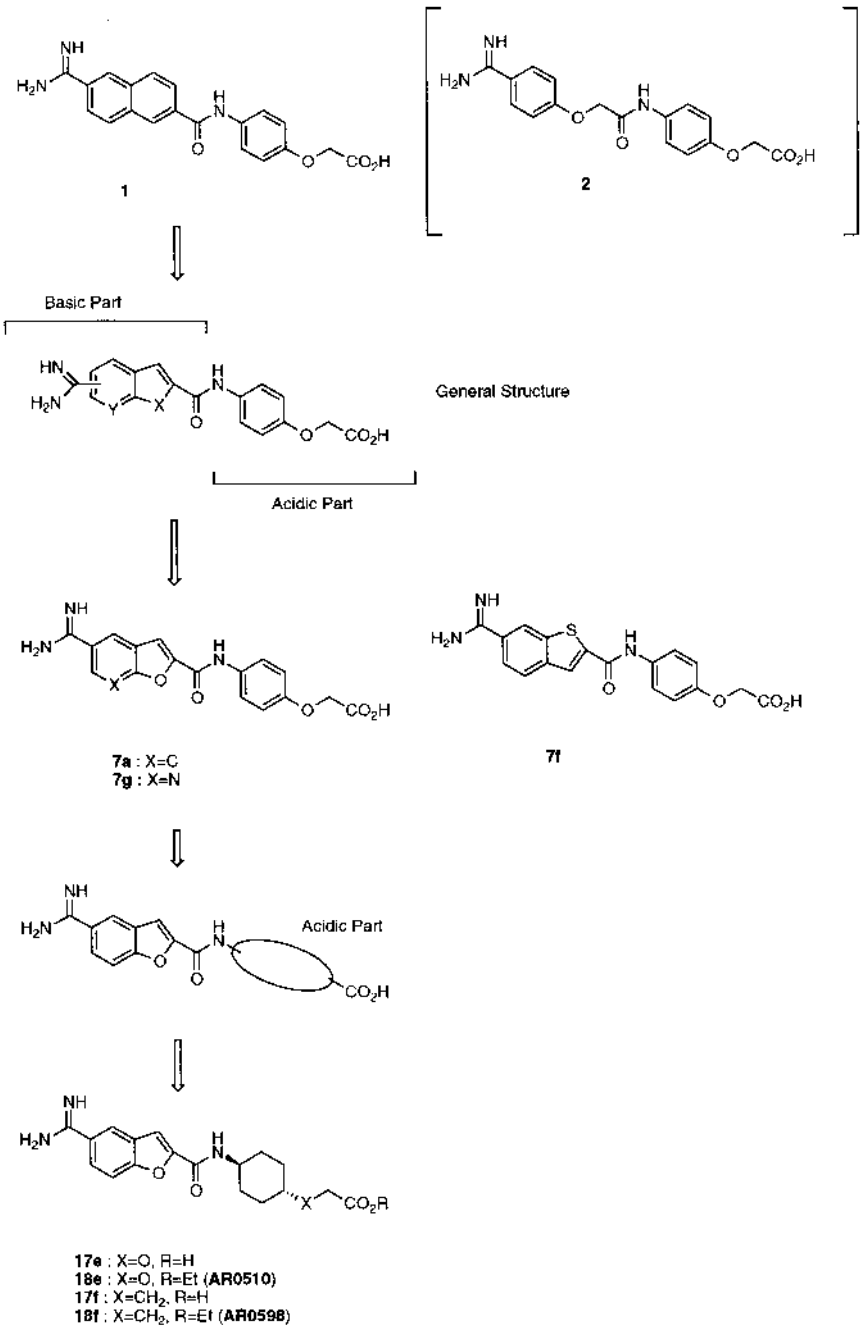
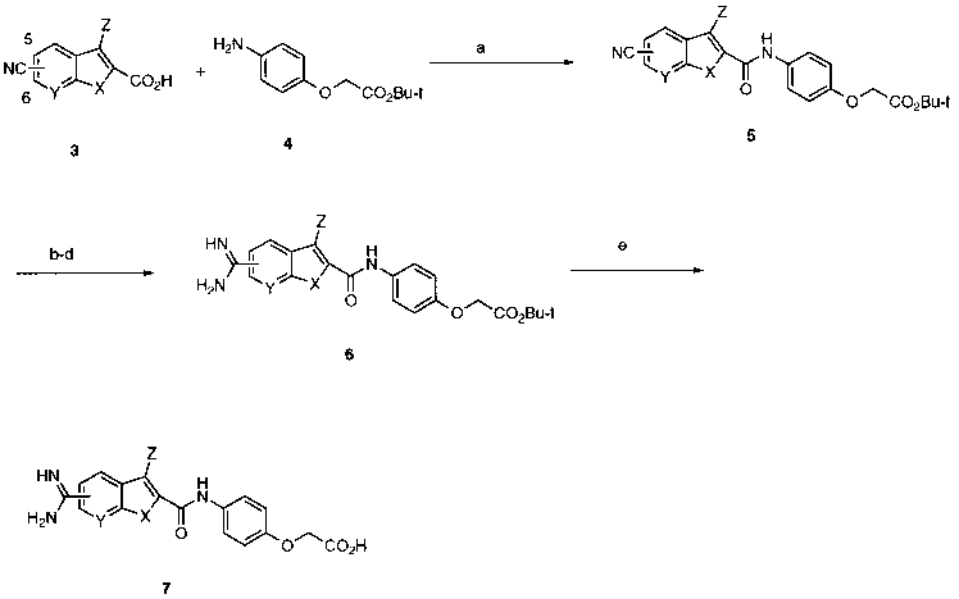


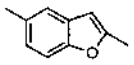
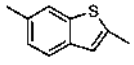
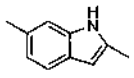
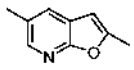
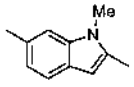
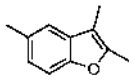
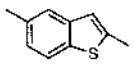
Fig. 1. Progression from Naphthalene compound **1** to Condensed Heterocycles

reagents, *e.g.* 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDI) and diisopropylcarbodiimide.

A known three-step sequence route[16] (thioimidate is formed by first treating the cyano group with hydrogen sulfide followed by alkylation with methyl iodide; next, treatment of the methylthioimidate with ammonium acetate affords the amidine group as the hydriodide salt) was used to convert the cyano group to the amidino group. Deprotection of the *tert*-butyl esters using trifluoroacetic acid (TFA) afforded the target compound in pure form. In **7b**, nitrile **8b**[15] was converted to the corresponding amidine by the Pinner reaction,[17] and basic hydrolysis of ester **9** afforded the carboxylic acid **10**. Condensation with **4** led to the amidino ester **11**. Basic hydrolysis of the ester afforded target compound **7b** (Chart 2). However, during the preparation of 4-(5-amidino-1*H*-indole-2-carboxamide)phenoxyacetic acid, conversion of the nitrile to the corresponding product failed using hydrogen sulfide, and starting nitrile was recovered.

Next, the acidic part was modified. Preparation of the target compounds used to investigate the effect of the acidic part in Tables 4—6 is illustrated in Charts 3—6. The acidic part can be prepared from commercially available starting materials using known methods. Firstly, the acidic part of **7a** was replaced with various carboxylic acids containing an extra ring as shown in Tables 4 and 5. The reactions of **3a**, **3h**, **3i** (**a**: Y=H, **h**: Y=Me, **i**: Y=OMe) with the various acidic derivatives **12a**—**d**, **h**, **l** and **m** offered **13a**—**d**, **h**, **l** and **m** in yields ranging from 38—100%. The cyano group



comp. no.		comp. no.	
7a		7f	
7c		7g	
7d		7h	
7e			

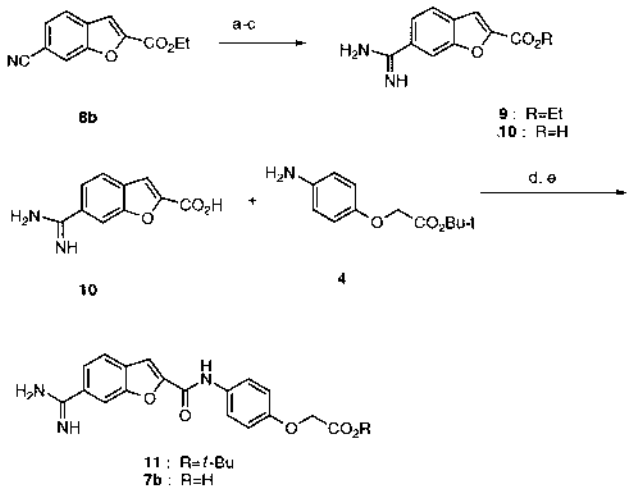
(a) EDI-HCl, HOBt, DMF; (b) H_2S, pyr., TEA; (c) MeI; (d) NH_4OAc; (e) TFA, CH_2Cl_2

Chart 1. Preparation of Condensed Heterocycle Derivatives

of **13** was converted to the corresponding amidino group by a known three-step route with H_2S.[17] In other cases, the Pinner reaction[18] (33%—quantitatively yield) was also used to obtain the amidino derivatives. Cleavage of the *tert*-butyl ester using TFA or saponification of the ethyl ester using sodium hydroxide afforded the acids in 29—91% yield after purification (Chart 3).

Chart 4 illustrates the preparation of **17e**, **f**, **g**, **i**, **j** and **k**. The key intermediate **19** was easily prepared by the procedure described above. The cyano group of **3a** was converted to the imidate by the Pinner reaction using saturated HCl–EtOH. Treatment of the imidate with ammonia in ethanol afforded the amidine. The amidino group was protected as the benzyloxycarbonyl (Z) derivative. The reaction of **19** with the various acidic derivatives **12e**, **f**, **g**, **i**, **j** and **k** gave **20e**, **f**, **g**, **i**, **j** and **k** in 21—94% yield. The amide bonds were prepared using EDI–1-hydroxy-1*H*-benzotriazole (HOBt). Deprotection of Z-protection *via* hydrogenation using 10% palladium on charcoal and cleavage of the *tert*-butyl esters using TFA or saponification of the ethyl ester using sodium hydroxide afforded the desired product **17e**, **f**, **g**, **i**, **j** and **k** in 35—95% yield in analytically pure form. Furopyridines **29e** and **29f** were prepared using the same method (total yield 12—27%) (Chart 5).

Finally, to examine the effects of various condensed heterocycle systems on antagonist activities, a series of derivatives (**27e**, **28d**, **e**, **29d**) was prepared from the acidic derivative **12e** and the corresponding **3c**, **3f** and **3g**, using the



(a) HCl, EtOH; (b) NH_3, EtOh; (c) LiOH, THF-H_2O; (d) Diisopropylcarbodiimide, HOBt hydrate, DMF; (e) TFA, CH_2Cl_2

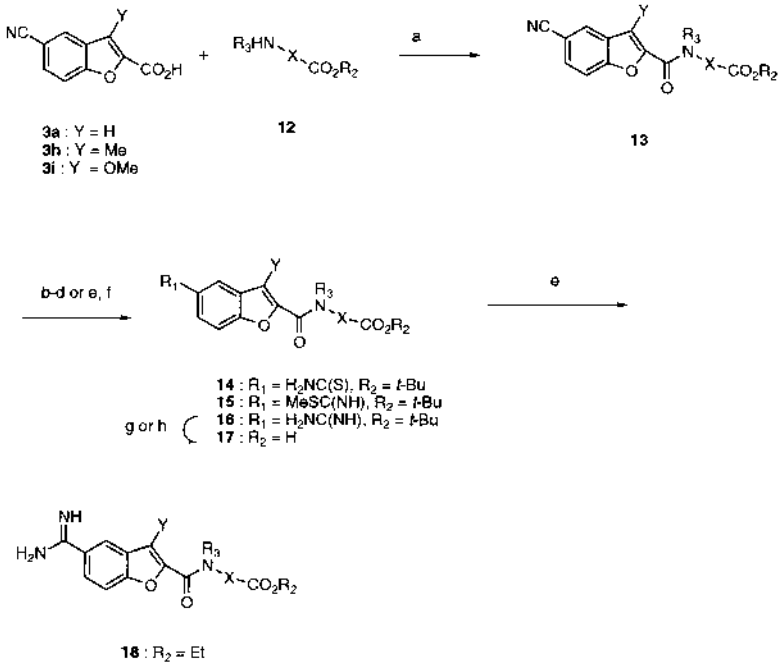
Chart 2. Preparation of **7b**

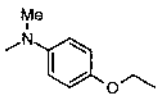
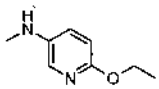
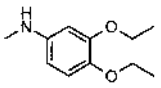
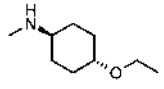
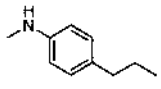
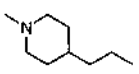
method outlined above, with a total yield of 7—37%. The esters **30e** and **31e** were prepared from acids **27e** and **28e** (Chart 6).

Results and Discussion

In the process of the design of our antagonists, we postulated that the affinity of binding to the Fbg receptor is dependent on the geometrical relationship between the carboxylic acid and the amidino group, and we designed a condensed heterocyclic analogues to improve this geometry. Firstly, modification of **1** as a lead compound involved the basic part.

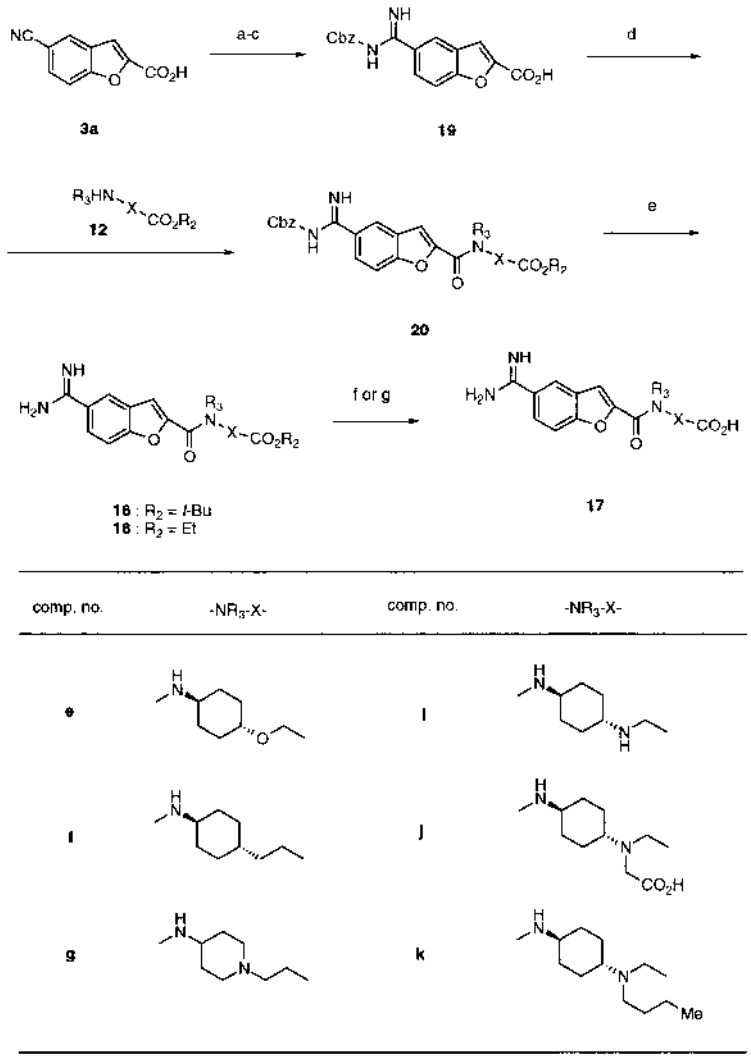
Table 1 shows the synthesized amidino-condensed heterocyclic compounds with their IC_{50} values for inhibition of ADP-induced aggregation of human platelet-rich plasma (PRP).[18] The 5-amidinobenzofuran **7a** and 6-amidinoindole **7c** possessed similar activity to the parent compound **1**, Ro43-8857, and BIBU-52. The 6-amidinobenzothiophene **7f** was approximately four-fold more potent than **1** in the platelet aggregation assay. On the other hand, the 6-amidinobenzofuran **7b** and 5-benzothiophene **7e** were one order



comp. no.	-NR_3-X-	comp. no.	-NR_3-X-
a		d	
b		l, m	
c		h	

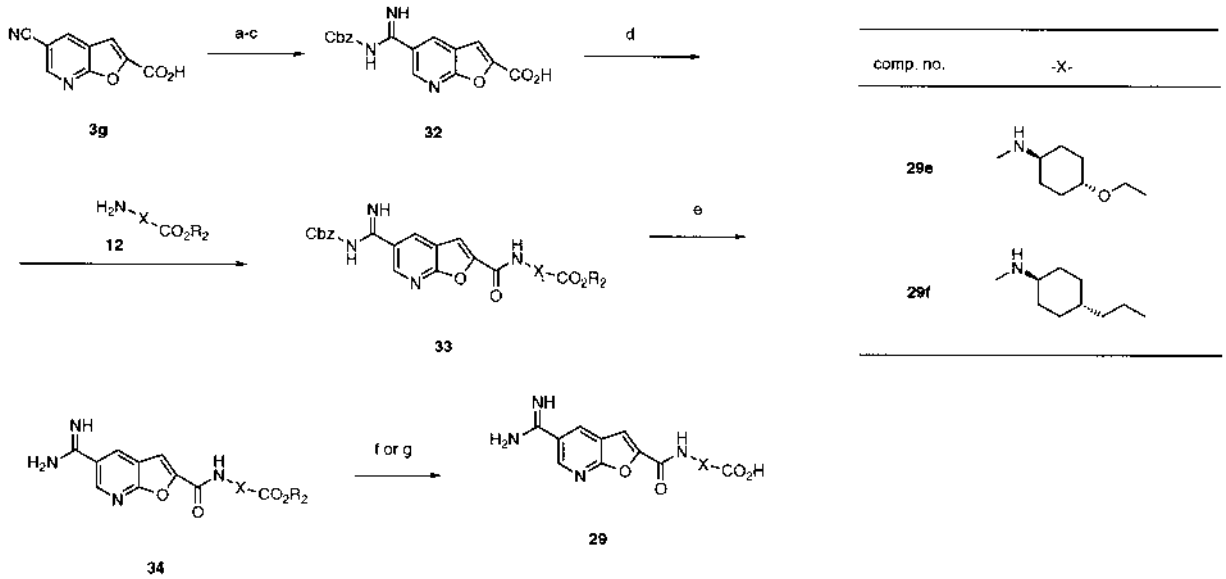
(a) EDI–HOBt, DMF; (b) H_2S, pyr., Et_3N; (c) MeI, acetone; (d) NH_4OAc, MeOH; (e) HCl, EtOH; (f) NH_3, EtOH; (g) TFA, CH_2Cl_2; (h) 1) NaOH, THF–H_2O; 2) HCl

Chart 3. Method A



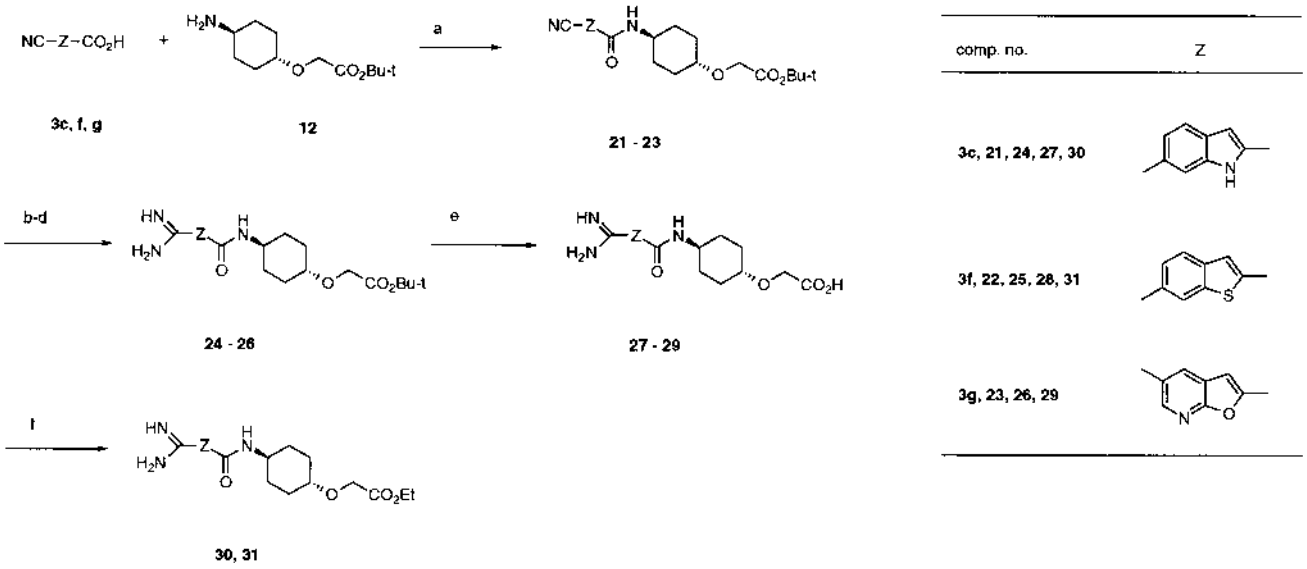
(a) EtOH, HCl; (b) NH_3, EtOH; (c) CbzCl, NaOH, H_2O–THF; (d) R_3NH–X–CO_2R_2, EDI–HOBt, DMF; (e) H_2, 10%Pd–C, $CHCl_3$–MeOH–HCl; (f) 1) NaOH, H_2O–THF; 2) HCl; (g) TFA, CH_2Cl_2

Chart 4. Method B



(a) EtOH, HCl; (b) NH_3, EtOH; (c) CbzCl, NaOH, H_2O–THF; (d) R_3NH–X–CO_2R_2, EDI–HOBt, DMF; (e) H_2, 10%Pd–C, $CHCl_3$–MeOH–HCl; (f) 1) NaOH, H_2O–THF; 2) HCl; (g) TFA, CH_2Cl_2

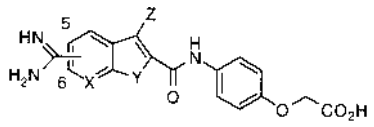
Chart 5



(a) EDI–HOBt, DMF; (b) H_2S, pyr., Et_3N; (c) MeI, acetone; (d) NH_4OAc, MeOH; (e) TFA, CH_2Cl_2; (f) EtOH, methanesuflonic acid

Chart 6

Table 1. The Condensed Heterocyclic Derivatives: *In Vitro* Inhibition of Platelet Aggregation



Comp. no.	Position of amidino moiety	X	Y	Z	Platelet aggregation PRP, IC_{50} (μM)[a]
1					0.07±0.02
2					0.08±0.02
7a	5	CH	O	H	0.033±0.009
7b	6	CH	O	H	0.23±0.03
7c	6	CH	NH	H	0.066±0.012
7d	6	CH	NMe	H	0.23±0.02
7e	5	CH	S	H	0.77±0.033
7f	6	CH	S	H	0.017±0.002
7g	5	N	O	H	0.012±0.002
7h	5	CH	O	Me	0.035±0.003
BIBU-52[9]					0.07±0.006
Ro43-8857[6]					0.085±0.0025

a) Concentration required to inhibit by 50%. Values are the means±S.E.M. of three experiments.

Table 2. Comparison of Apparent Inhibition Constant (Ki_{app})[a] against Various Proteases

Compound no.[a]	Ki_{app} (M) against protease[b]			
	anti-Thrombin	F-Xa	Plasmin	Trypsin
Nafamostat	2.85×10^{-7}	4.75×10^{-6}	1.40×10^{-8}	1.38×10^{-8}
7c	1.26×10^{-4}	6.66×10^{-5}	3.12×10^{-4}	2.14×10^{-5}

a) Substrate concentration; Nafamostat=10 μM, **7c**=100 μM. *b*) Values are expressed as the average of at least two experiments.

Table 3. Inhibitory Effects (10 mg/kg, *p.o.*) of **1**, **7a** and Prodrug of **7a** (Ethyl Ester) on ADP (10 μM)-Induced Platelet Aggregation in Conscious Guinea Pigs

Comp. no.	Inhibition (%)[a]	
	2 h after drug	4 h after drug
1	16.0±1.4	16.7±0.9
7a	22.4±12.2	43.7±1.2
Prodrug of **7a**	6.2±6.2	20.3±20.3

a) Values are the means±S.E.M. of three experiments.

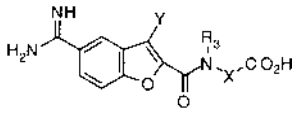
less active than **1**. Replacement of the benzofuran ring in **7a** with a furo[2, 3-*b*]pyridine ring afforded **7g**, which was a potent inhibitor. **7h**, formed by 3-methyl substitution on the benzofuran ring of derivative **7a**, showed similar inhibitory activity to the latter. Addition of an *N*-methyl group to the indole **7d** reduced the activity four-fold compared with the corresponding **7c**. As a result, the 5-amidinobenzofuran, 6-amidinoindole, 6-amidinobenzothiophene, and 5-amidinofuro[2, 3-*b*]pyridine were selected as the basic part.

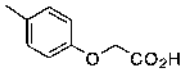
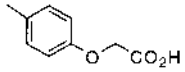
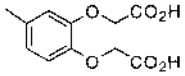
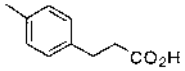
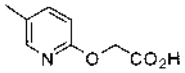
Nafamostat, a serine protease inhibitor with an amidinonaphthol unit, was selected as our lead compound for the development of Gp IIb/IIIa antagonists. These drugs act on blood clotting disorders by suppressing clotting proteases, including thrombin. As a side-effect, they sometimes prolong the bleeding time. The specificity of the condensed heterocyclic compounds for inhibition of a variety of serine proteases was evaluated by measuring the apparent inhibition constant (Ki_{app}).[19] As shown in Table 2, compound **7c** has lost its ability to inhibit these proteases.

7a displayed potent inhibition of human platelet aggregation. However, lead compound **1**, **7a** and its prodrug showed low oral potency in a guinea pig model (Table 3). We attempted to improve the oral potency of these compounds by modifying their physical properties.

Lilly researches have used alkyl chains as the acidic part of Gp IIb/IIIa antagonists.[11] Compound **7a**, which possesses a phenyl ring in the acidic part, was more potent than alkyl chain compounds. We assume that this difference of activity may be due to a steric conformational restriction and to a

Table 4. *In Vitro* Inhibition of Platelet Aggregation by Benzofuran Derivatives



No.	Y	R_3	-X-CO_2H	Preparation method[a)]	Platelet aggregation in PRP, IC_{50} (μM)[b)]
7a	H	H		—	0.033±0.009
17a	H	Me		A (H_2S)	0.11±0.01
17b	H	H		A (H_2S)	0.022±0.002
17c	H	H		A (H_2S)	0.043±0.003
17d	H	H		A (H_2S)	0.067±0.007

a) See Charts 4 and 5. *b*) Concentration required to inhibit by 50%. Values are the means±S.E.M. of three experiments.

more appropriate positioning of the pharmacophore.

Our approach involved modifying the aromatic ring (Table 4). The *N*-methyl amide compound **17a**, in which the *N*-methyl was incorporated into **7a**, showed weak activity. When the phenoxy group was replaced by pyridine and the series of phenyl-substituted analogues, these analogues were not significantly different in potency from the phenoxy analogue **17a**. The next strategy, as shown in Fig. 1, was to design molecules with the acidic part removed to construct a new skeleton, modified to enhance the activity and oral potency (Table 5). These compunds (**17e**—**k**) showed IC_{50} values of 6—70 nM in human PRP with the exception of **17h**. The potency was markedly decreased in **17h** which had an $IC_{50}>5$ μM. The introduction of a methyl or methoxy group in the 3-position of benzofuran led to compounds **17l** and **17m**, respectively. It is interesting to note that the inhibitory potency against the receptors was 0.027 μM for **17l** and 0.13 μM for **17m**. **17m** was, thus, approximately five-fold less potent than **17l** and parent compound **7a**. Bulky substitution may lead to a decrease in antiaggregatory activity, since the methoxy substitution was bulkier than the methyl substitution. This finding indicates, interestingly, that the spatial restriction of this position through introduction of a functional group leads to a change in antiaggregatory activity, and/or that incorporation of a hydrogen bond-accepting group (MeO), as in **17m**, is unsuccessful.

When the benzofuran was replaced by an alternative condensed heterocycle (as shown in Table 6), the resulting analogues (**27e**, **28d**, **28e**, **29d**, **29e**, **29f**) displayed similar inhibitory activity (IC_{50}=0.01—0.03 μM). This result suggested that the basic part was exchangeable with 6-amidinoindole, 6-amidinobenzothiophen, and 5-amidinofuro[2, 3-*b*]pyridine. Since **17e**, **27e**, and **28e** were not soluble in water, esterification of **17e**, **27e**, and **28e** provided the corresponding ethyl esters **18e**, **30e**, and **31e** as prodrugs and these were soluble in water. The effect of **18e**, **30e**, and **31e** at 1 mg/kg i.v. on *ex vivo* ADP (1 μM)-induced whole blood platelet aggregation in anesthetized guinea pigs is shown in Fig. 2. The platelet aggregation response of **30e** and **31e** was 60% or less inhibition within 30 min. These results indicate that benzofuran derivatives produce long lasting of action following i.v. administration and slow metabolism and/or excretion from blood. Furthermore, the *in vitro* and *ex vivo* activities of compounds **17e** and **17f** were evaluated.

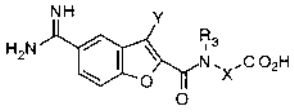
In a study of the antiaggregatory potency of **17e** and **17f**, inhibition of the binding of [^{125}I] Fbg[20] to activated human platelets gave IC_{50} values of 0.011 and 0.003 μM, respectively (Table 7), approximately three orders of magnitude more potent than RGDS (IC_{50}=60 μM). **17e** and **17f** were found to antagonize Gp IIb/IIIa very strongly.

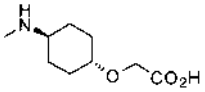
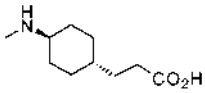
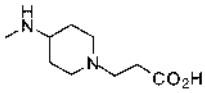
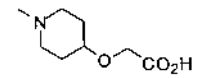
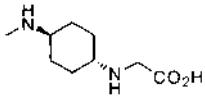
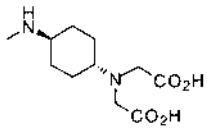
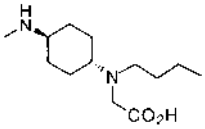
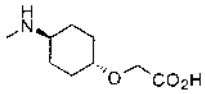
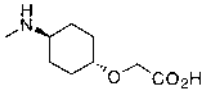
An interesting feature of those compounds was that the inhibition of platelet aggregation in rabbit was similar, in terms of IC_{50} value, to that in human, dog and guinea pig *in vitro* (Table 8). Most non-peptide Gp IIb/IIIa antagonists (SC-54701, Ro43-8857 and BIBU-52 *etc*.) have only a weak effect on rabbit platelets. The distances are summarized in Table 9. The distance between the carbon (C_1) of the amidino or guanidino group and the carbon (C_2) of the carbonyl group was C_1–C_2. The distance between the carbon (C_1) of the amidino or guanidino group and the hetero atom, which can interact with the receptor, was C_1–(hetero atom). The distance between the carbon (C_2) of the carbonyl group and the hetero atom, which can interact with the receptor, was (hetero atom)–C_2. The C_1–(hetero atom) of SK&F 107260 and RGD was 7.7 Å while the C_1–(hetero atom) of **7a** and **17e** was 8.2 Å. Overlay of these compounds showed that the carbonyl group of the 4-amidinophenylcarbonyl unit present in most antagonists does not overlap with the arginine carbonyl group of cyclic peptide SK&F 107260. The distance between the Arg–guanidino group and the Asp–carboxylic group was about 14 Å, according to the superimposed stable conformations of SK&F 107260 and other non-peptide antagonists. The carbonyl oxygen of the 5-amidinobenzofuran-2-ylcarbonyl unit is not capable of accessing the same space as the 4-amidinobiphenylcarbonyl unit in BIBU-52 or the 4-amidinophenylaminocarbonyl unit in SB207448. However it is capable of accessing the same space as the arginine carbonyl oxygen of SK&F 107260 and RGD peptide. This result shows that the interaction of our compounds was a three point interaction of the RGD-type. Although the mechanism underlying this difference of activity is not clear, we assume that this difference in activity for rabbit platelets may be due to this three point interaction of the RGD-type.[13] Recently, Bernat and co-workers reported that SR121787 also potently inhibits platelet aggregation in rabbit.[21] However, our compounds are one order of magnitude more effective (IC_{50}= 0.02—0.07 μM *versus* 0.8 μM for SR121787) and, hence, will be powerful tools for use in rabbit antithrombotic models.

Our compounds inhibited human platelet aggregation induced in PRP by ADP, collagen, thrombin, phorbol 12-myristate 13-acetate (PMA) (tumor promoter) and arachidonic acid and the IC_{50} values are summarized in Table 10. Our compounds demonstrated a wide inhibitory spectrum toward major aggregation agonists.

The Fbg receptor of platelets is a member of the integrin superfamily of receptors. Selectivity between Gp IIb/IIIa and other RGD-recognizing integrins is thought to be an impor-

Table 5. Modification of the Acidic Part of Benzofuran Derivatives and Substitution at the 3-Position of Benzofuran



No.	Y	R_3N-X-CO_2H	Preparation method[a)]	Platelet aggregation in PRP, IC_{50} (μM)[b)]
17e	H		B	0.018±0.004
17f	H		B	0.006±0.0003
17g	H		B	0.017±0.004
17h	H		A (HCl)	>5
17i	H		B	0.017±0.002
17j	H		B	0.009±0.0003
17k	H		B	0.07±0.003
17l	Me		A (H_2S)	0.027±0.009
17m	OMe		A (H_2S)	0.13±0.003

a,*b*) See corresponding footnote to Table 4.

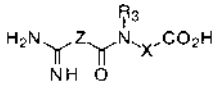
tant requirement in order to avoid side-effects. Our compounds were evaluated for their selectivity in inhibiting human umbilical vein endothelial cell (HUVEC)[22] binding to Fbg, vitronectin (VN), fibronectin (FN) and von Willebrand Factor (vWF). The results are presented in Table 11 and show that **17e** and **17f** was >2—3 orders of magnitude more effective in inhibiting platelet aggregation than HUVEC binding.

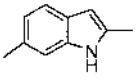
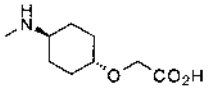
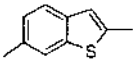
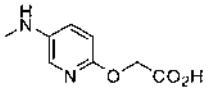
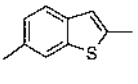
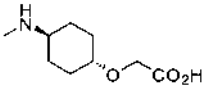
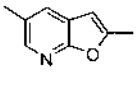
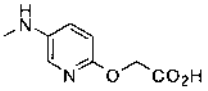
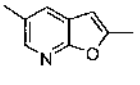
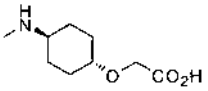
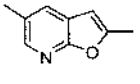
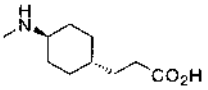
The effects following oral administration of the compounds were evaluated by measuring *ex vivo* inhibition of platelet aggregation.[23] Results for **17e** and **17f** in conscious guinea pigs are shown in Fig. 3. Oral administration of 10 mg/kg of either **17e** and **17f** to guinea pigs, resulted in a 60% inhibition of *ex vivo* platelet aggregation after 5 h. In the guinea pig study, **17f** possessed a good profile with a long duration of action and high potency. The contrasting oral activities of **17e** and **17f** in dogs are shown in Figs. 4 and 5. After oral administration, **17e** produced more than 50% inhibition at 5 h with a return to baseline after 24 h. However, **17f** had no significant *ex vivo* activity. This study demonstrated that **17f** has reduced oral potency in dogs.

We next examined the effect of ester prodrugs of several compounds in terms of increased absorption and longer duration of action. Oral administration of prodrug **18e** (10 mg/kg) resulted in 80% inhibition of platelet aggregation in dogs for 6 h after oral administration with a return to baseline after 24 h; **18f** produced 80% inhibition for 5 h after oral administration. **18e** showed good profiles in dogs, with a duration of action of over 24 h after *p.o.* administration at 10 mg/kg. The prodrugs exhibited better oral potency (bioavailability=15—18%), suggesting an improved duration of action. From these results, **18e** inhibited platelet aggregation not only in the *in vitro* assay, but also in the *ex vivo* assay.

In conclusion, we have described the design and synthesis of novel Fbg receptor antagonists. The results of structure–activity studies allowed modifications which improved the affinity for Fbg receptors. **17e**, **17f**, and a number of compounds with a cyclohexane moiety have even better *in vitro* activity. These compounds are highly selective compared with other RGD-recognizing integrins and exhibit a wide-

Table 6. Modification of the Basic Part of Benzofuran Derivatives



No.	-Z-	R_3N-X-CO_2H	Platelet aggregation in PRP, IC_{50} (μM)[a]
27e			0.028±0.002
28d			0.022±0.002
28e			0.027±0.009
29d			0.018±0.002
29e			0.012±0.002
29f			0.023±0.007

a) See corresponding footnote to Table 4.

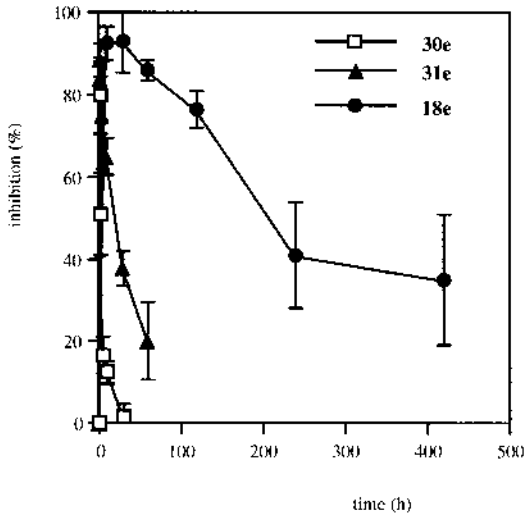


Fig. 2. Effect of Benzofuran **18e** (●), Benzothiophene **31e** (▲) and Indole **30e** (□) Derivatives i.v. (1 mg/kg) on *ex Vivo* Whole Blood Platelet Aggregation in Anesthetized Guinea Pigs ($n=5$)

ranging potent inhibition of major aggregation agonists. The corresponding prodrug **18e** exhibited potent Fbg receptor antagonism and a long duration of action after oral administration. **18e** (AR0510) was selected as a suitable clinical candidate for development as an orally active antithrombotic agent.

Experimental

Chemistry Reagents were purchased from commercial suppliers and used without further purification. Reaction solvents were distilled from an appropriate drying agent before use. Melting points were measured on a Yanaco micromelting point apparatus and are uncorrected. IR and NMR spectra, which were in agreement with the structures cited, were recorded on a Shimadzu IR-420 instrument for IR and a Brucker AM-500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) and a Brucker AC-200 spectrometer (200 MHz for ^{1}H-NMR and 50 MHz for ^{13}C-NMR) for NMR using tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (ppm), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Electron impact (EI)-MS and secondary ion (SI)-MS were taken on a Hitachi M-2000 mass spectrometer.

Purity of selected final compounds was determined by HPLC using a Inertsil ODS-3 column (4.6 i.d.×250 mm, 5 μm, GL Sciences Inc.): flow rate, 1 ml/min; temperature, 40 °C; sample size, 2 μg/ml; injection volume, 1 μl; detection, 270 nm; mobile phase A, 20% MeCN/0.05% TFA in water; mobile phase B, 12% MeCN/0.05% TFA in water (flow rate, 1 ml/min); mobile phase C, 33% MeOH/0.05% TFA in water; mobile phase D, 28% MeOH/0.05% TFA in water (flow rate, 0.75 ml/min). (Although the purity of **2**, **7d**, **17e**, **17m**, **29d**, **30f**, and **31f** was verified by NMR and HPLC analyses, their hygroscopic nature prevented satisfactory elemental analyses being obtained. Compounds were, therefore, judged pure by observation of a single peak (>96%) using two different RP-HPLC methods).

Echistatin was purchased from Bachem (Bubendorf, Switzerland). SC-54701, Ro43-8857 and BIBU-52 were prepared according to a procedure described by Zablocki,[7a] Graul,[7b] Alig[6] and Austel[9].

***tert*-Butyl 4-(5-Cyanobenzofuran-2-carboxamido)phenoxyacetate (5a). (Standard Procedure A)** **3a**[14] (300 mg, 1.60 mmol) and **4** (395 mg,

Table 7. Inhibition of ^{125}I-Fbg Binding to Activated Human Platelets by Gp IIb/IIIa Antagonists

Compound[a]	IC_{50} (μM)[b]
1	0.05
17e	0.011
17f	0.003
SC-54701[7]	0.008
Ro43-8857[6]	0.07
BIBU-52[9]	0.03
Echistatin[17]	0.017
RGDS	60

a) SC-54701, Ro43-5587 and BIBU-52 were prepared according to the methods reported in the literature and patents. *b*) Values are expressed as the average of at least two experiments.

Table 8. Inhibition of ADP-Induced Platelet Aggregation by Gp IIb/IIIa Antagonists from Different Species

Compound	IC_{50} (μM)				
	Human[a]	Dog[b]	Guinea pig[b]	Rabbit[b]	Rat[b]
17e	0.018±0.004	0.057	0.088	0.073	>50
17f	0.006±0.0003	0.023	0.033	0.016	>50
SC-54701[7]	0.037±0.003	0.033	0.033	3	>50
Ro43-8857[6]	0.085±0.0025[c]	0.140	0.275	14	>12.5
BIBU-52[9]	0.067±0.007	0.13	1	5	>50

a) Concentration required to inhibit by 50%. Values are the means±S.E.M. of three experiments. *b*) Values are expressed as the average of at least two experiments. *c*) $n=62$.

Table 9. Pharmacophore Distance of the Three Point Interaction

Comp. no.	Distance (Å)		
	C_1–(hetero atom)	(hetero atom)–C_2	C_1–C_2
RGDF	7.7	6.4	14.0
SK&F107260	7.7	6.9	14.4
1	8.5	7.4	14.0
7a	8.2	5.9	14.2
17e	8.2	6.3	14.3
BIBU-52	10.0	4.6	13.8
SB207448	6.2	8.8	14.9

Table 10. Inhibition of Human Platelet Aggregation by Gp IIb/IIIa Antagonists

Compound	IC_{50} (μM)				
	ADP[a]	Collagen[b]	Thrombin[b]	PMA[b]	Arachidonic acid[b]
17e	0.018±0.004	0.03	0.063	0.125	0.17
17f	0.006±0.0003	0.043	0.038	0.075	0.09
SC-54701[7]	0.037±0.003	0.095	0.065	0.125	0.15
Ro43-8857[6]	0.085±0.003[c]	0.11	0.110	1.15	0.425
BIBU-52[9]	0.067±0.007	0.17	0.04	0.26	1.25

a) Concentration required to inhibit by 50%. Values are the means±S.E.M. of three experiments. *b*) Values are expressed as the average of at least two experiments. *c*) $n=62$.

Table 11. Effect of Gp IIb/IIIa Antagonists on Adhesion of HUVECs to Adhesive Protein-Coated Plates

Compound	IC_{50} (μM)[a]			
	Fbg	VN	FN	vWF
17e	>100	>100	>100	>100
17f	>100	>100	>100	>100
SC 54701[7]	>100	>100	>100	>100
Ro43-8857[6]	>100	>100	>100	>100
BIBU-52[9]	>100	>100	>100	>100

a) Values are expressed as the average of at least two experiments.

1.76 mmol) were dissolved in dimethylformamide (DMF) (40 ml), and HOBt (238 mg, 1.76 mmol) and EDI (342 mg, 1.76 mmol) were added. The mixture was stirred at room temperature for 18 h. Water was added to the reaction mixture, and the mixture was extracted with EtOAc. The extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling matters were distilled from the filtrate under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 512 mg of **5a** as a pale-yellow solid (82%): IR (KBr) cm^{-1}: 2200, 1750, 1680, 1605, 1530, 1505. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 4.53 (2H, s), 6.92—6.96 (2H, m), 7.60—7.75 (5H, m), 8.07 (1H, d, $J=0.8$ Hz).

***tert*-Butyl 4-(5-Amidinobenzofuran-2-carboxamido)phenoxyacetate (6a). (Standard Procedure B)** **5a** (430 mg, 1.10 mmol) was dissolved in a mixed solution of pyridine (30 ml) and Et_3N (TEA) (7 ml), and hydrogen sulfide gas was bubbled through it for 10 min at room temperature, followed by stirring for 18 h.[17] Low boiling material was distilled from the reaction mixture under reduced pressure and the residue was dissolved in EtOAc. The mixture was washed with 2 N aqueous potassium hydrogensulfate solution, water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give the corresponding thioamide as a yellow solid. The thioamide was dissolved in acetone (50 ml, methyl iodide (2 ml) was added and the mixture was refluxed under heating for 40 min. Low boiling material was distilled from the reaction mixture under reduced pressure to give the corresponding thioiminomethyl ester as a yellow solid. Then, MeOH (30 ml) and ammonium acetate (280 mg, 3.64 mmol) were added and the mixture was refluxed under heating for 3 h. Low boiling material was distilled from the reaction mixture under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 596 mg of the hydriodide of **6a** as a yellow solid (quantitatively in 3 steps).: IR (KBr) cm^{-1}: 3700—2900, 1730, 1640. ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, s), 6.93 (2H, s), 7.69 (2H, s), 7.80—8.00 (3H, m), 8.32 (1H, s).

4-(5-Amidinobenzofuran-2-carboxamido)phenoxyacetic Acid (7a). (Standard Procedure C) CH_2Cl_2 (25 ml) was added to the hydriodide (537 mg, 1.00 mmol) of **6a**, followed by TFA (8 ml) then stirring at room temperature for 2 h. Et_2O (100 ml) was added to the reaction mixture and the mixture was stirred for 10 min. The resulting precipitate was collected by filtration and washed with diethyl ether to give 380 mg of the hydriodide of **7a** as a pale-brown solid (79%). mp: >250 °C. IR (KBr) cm^{-1}: 1740, 1690, 1610, 1540, 1505. ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 6.93—6.97 (2H, m), 7.69—7.72 (2H, m), 7.89 (1H, dd, $J=1.7$, 8.8 Hz), 7.91 (1H, s), 7.97 (1H, d, $J=8.8$ Hz), 8.34 (1H, d, $J=1.7$ Hz), 9.11 (2H, br s), 9.38 (2H, br s), 10.57 (1H, s). *Anal*. Calcd for $C_{18}H_{15}N_3O_5\cdot HCl\cdot H_2O$: C, 53.25; H, 4.88; N, 10.88. Found: C, 53.20; H, 4.95; N, 10.36.

Ethyl 6-[(1-Ethoxy)iminomethyl)]benzofuran-2-carboxylate (10) Ethanol (15 ml) was added to ethyl 6-cyano-2-benzofurancarboxylate **8**[14] (135 mg, 0.628 mg) and the mixture was ice-cooled. Hydrogen chloride gas was bubbled through it for 15 min and the mixture was stirred at room temperature for 14 h. Low boiling material was distilled from the reaction mixture under reduced pressure and the residue was dissolved in chloroform. A saturated aqueous sodium hydrogencarbonate solution was added and the mixture was stirred for 10 min. The organic layer was partitioned, washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 137 mg of ethyl 6-[(1-ethoxy)iminoethyl)]benzofuran-2-carboxylate as an orange solid (84%): ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.50 (6H, m), 4.36 (2H, q, $J=7.1$ Hz), 4.45 (2H, q, $J=7.1$ Hz), 7.53 (1H, s), 7.20—7.30 (1H, m), 8.02—8.03 (1H, m).

Ammonium chloride (29.5 mg, 0.551 mmol), a solution (0.4 ml) of ammonia in EtOH, and EtOH (3 ml) were added to ethyl 6-[(1-ethoxy)iminoethyl)]benzofuran-2-carboxylate (137 mg, 0.525 mmol), and the mixture was refluxed under heating for 2 h in a nitrogen atmosphere. Low boiling material was distilled from the reaction mixture under reduced pressure and the residue was washed with Et_2O to give 132 mg of **9** as a yellow solid (94%): ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, $J=7.2$ Hz), 4.38 (2 H, q, $J=7.2$ Hz), 7.76 (1H, dd, $J=1.4$, 8.6 Hz), 7.87 (1H, d, $J=1.4$ Hz), 8.01(1 H, d, $J=8.4$ Hz), 8.26 (1H, s), 9.33 (4H, br s).

9 (129 mg, 0.480 mmol) was hydrolyzed with 1 N aqueous sodium hydroxide to give 65 mg of **10** as a brown solid (56%): IR (KBr) 3700—2700, 1680, 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.74—7.92 (2H, m), 8.02 (1H, d, $J=8.3$ Hz), 8.24 (1H, s), 9.18 (2H, br s), 9.26 (2H, br s).

***tert*-Butyl 4-(6-Amidinobenzofuran-2-carboxamido)phenoxyacetate (11)** In the same manner as in standard procedure A, 6-amidino-2-benzofurancarboxylic acid hydrochloride **10** (63 mg, 0.26 mmol) and **4** (66 mg, 0.30 mmol) were condensed and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 109 mg of the hydrochloride of **11** as a colorless solid (91%): IR (KBr) cm^{-1}: 1750, 1710, 1630. ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, m), 6.90—6.97 (2H, m), 7.60—7.80 (3H, m), 7.90 (1H, s), 8.05 (1H, d, $J=7.8$ Hz), 8.20 (1H, s), 10.65 (1H, br s).

4-(6-Amidinobenzofuran-2-carboxamido)phenoxyacetic Acid (7b) In the same manner as in standard procedure C, the hydrochloride (109 mg, 2.45 mmol) of **11** was treated with TFA (2.5 ml) to give 96 mg (quantitative) of the hydrochloride of **7b** as a pale-yellow solid: mp: >250 °C. IR (KBr) cm^{-1}: 3700—2800, 1730, 1690, 1520, 1505. ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 6.93—6.97 (2H, m), 7.69—7.72 (2H, m), 7.79 (1H, dd, $J=1.6$, 8.3 Hz), 7.89 (1H, d, $J=1.6$ Hz), 8.06 (1H, d, $J=8.3$ Hz), 8.20 (1H, s), 9.32 (2H, br s), 9.45 (2H, br s), 10.63 (1H, br s). *Anal*. Calcd for $C_{18}H_{15}N_3O_5\cdot 2.2CF_3CO_2H\cdot 1.8H_2O$: C, 42.44; H, 3.08; N, 7.68. Found: C, 42.47; H, 3.19; N, 7.40.

6-Cyano-1*H*-indole-2-carboxylic Acid (3c) Compound **3c** was prepared from **8c**[15] in a single step according to the procedure for the preparation of **3g** (59%): IR (KBr) cm^{-1}: 3700—2700, 2250, 1700, 1520. ^{1}H-NMR (DMSO-d_6) δ: 7.20 (1H, d, $J=1.9$ Hz), 7.39 (1H, d, $J=9.1$ Hz), 7.80—7.87 (2H, m). MS *m/z*: 186 (M^+).

4-(6-Amidino-1*H*-indole-2-carboxamido)phenoxyacetic Acid (7c) In the same manner as in standard procedure A, 6-cyano-2-indolcarboxylic acid **3c** (295 mg, 1.59 mmol) and **4** (386 mg, 1.74 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 622 mg (quantitative) of **5c** as a pale-brown solid: IR (KBr) cm^{-1}: 3700—2900, 2200, 1730, 1650, 1540. ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.64 (2H, s), 6.89—6.99 (2H, m), 7.35—7.55 (2H, m), 7.61—7.71 (2H, m),

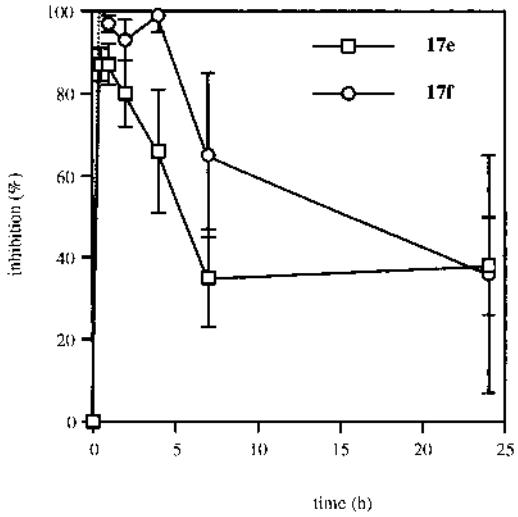


Fig. 3. Effect of **17e** (□) and **17f** (○) *p.o.* (10 mg/kg) on *ex Vivo* ADP-(10 μM) Induced Whole Blood Platelet Aggregation in Conscious Guinea Pigs ($n=5$)

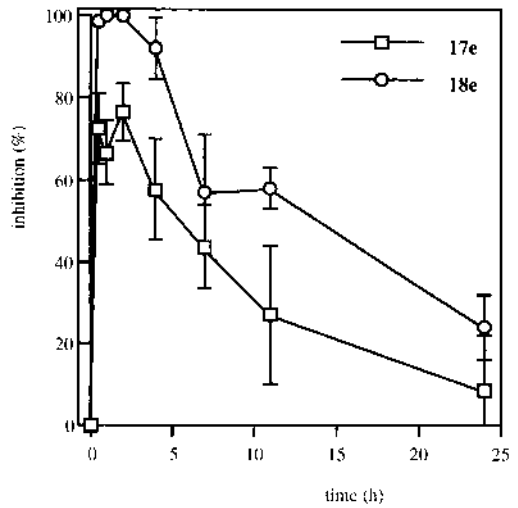


Fig. 4. Effect of **17e** (□) and **18e** (○) *p.o.* (10 mg/kg) on *ex Vivo* ADP-(10 μM) Induced Whole Blood Platelet Aggregation in Dogs ($n=5$)

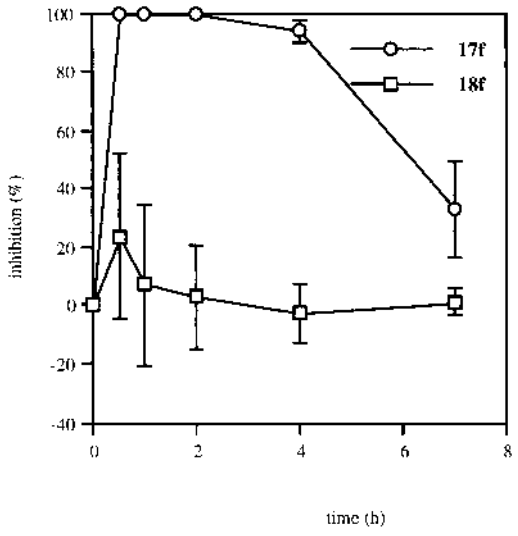


Fig. 5 Effect of **17f** (○) and **18f** (□) *p.o.* (10 mg/kg) on *ex Vivo* ADP-(10 μM) Induced Whole Blood Platelet Aggregation in Dogs ($n=5$)

7.85—8.00 (2H, m), 10.32 (1H, br s), 12.23 (1H, br s).

In the same manner as in standard procedure B, the cyano group of **5c** (630 mg, 1.61 mmol) was converted to an amidino group to give 891 mg of the hydriodide of **6c** as a viscous brown oil (quantitatively, in 3 steps): ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, s), 6.89—6.99 (2H, m), 7.43 (1H, d, J=9.2 Hz), 7.51 (1H, s), 7.64—7.74 (2H, m), 7.86—7.95 (2H, m), 10.33 (1H, br s).

In the same manner as in standard procedure C, the hydriodide (891 mg, 1.61 mmol) of **6c** was treated with TFA (10 ml) to give 489 mg of the hydriodide of **7c** as a brown solid (63%): mp: 205—245 °C (dec.). IR (KBr) cm^{-1}: 3700—3100, 1660, 1520, 1400. ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 6.90—7.00 (2H, m), 7.45 (1H, dd, J=1.3, 8.4 Hz), 7.51 (1H, s), 7.65—7.75 (2H, m), 7.91 (1H, d, J=8.4 Hz), 7.95 (1H, s), 8.82 (2H, br s), 9.27 (2H, br s), 10.33 (1H, br s). *Anal.* Calcd for $C_{18}H_{16}N_4O_4\cdot 2HI\cdot 1.5H_2O$: C, 34.04; H, 3.33; N, 8.82. Found: C, 33.97; H, 3.13; N, 9.12.

6-Cyano-1-methylindole-2-carboxylic Acid (3d) To a stirred suspension of NaH (60% in oil, 41.0 mg, 1.03 mmol) in dry DMF (5 ml) was added **8d** (200 mg, 0.93 mmol) portionwise at 0 °C over 20 min, and then methyliodide (0.062 ml, 1.00 mmol) was added. The mixture was stirred at room temperature for 2 h, and poured into aqueous NH_4Cl. The precipitate was collected by filtration and washed with water and EtOH to give 85 mg (40%) of **3d** as a yellow solid. Compound **3d** was prepared from **8d** in a single step according to the procedure for the preparation of **3e** (70%): ^{1}H-NMR (DMSO-d_6) δ: 4.08 (3H, s), 7.30 (1H, d, J=0.6 Hz), 7.43 (1H, dd, J=0.6, 8.3 Hz), 7.85 (1H, d, J=8.3 Hz), 8.26 (1H, s).

4-(6-Amidino-1-methylindole-2-carboxamido)phenoxyacetic Acid (7d) In the same manner as in standard procedure A, 6-cyano-1-methyl-2-indolcarboxylic acid **3d** (52 mg, 0.26 mmol) and **4** (63 mg, 0.29 mmol) were condensed to give 109 mg (quantitative) of **5d** as a colorless solid: IR (KBr) cm^{-1}: 3700—3000, 2200, 1740, 1505. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 4.11 (3H, s), 4.52 (2H, s), 6.89—6.93 (2H, m), 7.26 (1H, s), 7.39 (1H, d, J=9.0 Hz), 7.49—7.56 (2H, m), 7.70—7.85 (2H, m).

In the same manner as in standard procedure B, the cyano group of **5d** (105 mg, 0.259 mmol) was converted to an amidino group to give 240 mg of the hydriodide of **6d** as a brown solid (quantitatively, in 3 steps): ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.09 (3H, s), 4.64 (2H, s), 6.87—6.94 (2H, m), 7.36 (1H, s), 7.54 (1H, d, J=8.8 Hz), 7.62—7.71 (2H, m), 7.91 (1H, d, J=8.8 Hz), 8.18 (1H, s), 10.23 (1H, br s).

In the same manner as in standard procedure C, the hydriodide (240 mg, 0.26 mmol) of **6d** was treated with TFA (2 ml) to give 128 mg (quantitative) of the hydriodide of **7d** as a yellow solid: mp: >250 °C. IR (KBr) cm^{-1}: 3700—2800, 1640, 1505, 1390. ^{1}H-NMR (DMSO-d_6) δ: 4.09 (3H, s), 4.64 (2H, s), 6.94 (2H, s), 7.37 (1H, s), 7.54 (1H, dd, J=1.5, 8.4 Hz), 7.62—7.71 (2H, m), 7.92 (1H, d, J=8.4 Hz), 8.19 (1H, s), 8.90 (2H, br s), 9.28 (2H, br s). *Anal.* Calcd for $C_{19}H_{18}N_4O_4\cdot HI$: C, 46.17; H, 3.87; N, 11.33. Found: C, 20.01; H, 3.34; N, 7.12. HPLC t_{RA} 9.7 min (96.7%), t_{RC} 17.5 min (96.0%).

Ethyl 5-Cyanobenzo[*b*]thiophen-2-carboxylate (8e) Compound **8e** was prepared from ethyl 5-nitrobenzothiophen-2-carboxylate[24] by two steps according to the procedure for the preparation of **8g** (5%): ^{1}H-NMR (DMSO-d_6) δ: 1.45 (3H, t, J=7.1 Hz), 4.42 (2H, q, J=7.1 Hz), 7.65 (1H, dd, J=1.5, 8.5 Hz), 7.96 (1H, d, J=8.5 Hz), 8.09 (1H, s), 8.21 (1H, s). MS *m/z*: 231 (MH^+).

5-Cyanobenzo[*b*]thiophen-2-carboxylic Acid (3e) A mixture of **8e** (600 mg, 2.60 mmol), LiOH–H_2O (109 mg, 2.60 mmol), H_2O (20 ml) and tetrahydrofuran (THF) (10 ml) was stirred at room temperature for 1 h. The solution was acidified with 1 N HCl to pH 2—3 and precipitated solids were collected by filtration to give 274 mg (quantitative) of **3e** as an orange solid: IR (KBr) cm^{-1}: 2200, 1660, 1520, 1300. ^{1}H-NMR (DMSO-d_6) δ: 7.85 (1H, dd, J=1.5, 8.6 Hz), 8.19 (1H, s), 8.30 (1H, d, J=8.6 Hz), 8.55 (1H, s).

4-(5-Amidinobenzo[*b*]thiophen-2-carboxamido)phenoxyacetic Acid (7e) In the same manner as in standard procedure A, **3e** (520 mg, 2.56 mmol) and **4** (632 mg, 2.82 mmol) were condensed to give 888 mg of **5e** as an orange solid (85%). IR (KBr) cm^{-1}: 2200, 1740, 1635, 1600, 1500. ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, s), 6.94 (2H, s), 7.70—7.61 (2H, m), 7.83 (1H, dd, J=1.5, 8.4 Hz), 8.30 (1H, d, J=8.4 Hz), 8.40 (1H, s), 8.61 (1H, d, J=1.5 Hz), 10.59 (1H, br s).

In the same manner as in standard procedure B, the cyano group of **5e** (850 mg, 2.08 mmol) was converted to an amidino group to give 469 mg of the hydriodide of **6e** as a yellow solid (41% in 3 steps): IR (KBr) cm^{-1}: 1755, 1640, 1510, 1230, 1160. ^{1}H-NMR (DMSO-d_6) δ: 1.45 (9H, s), 4.63 (2H, s), 6.87—6.96 (2H, m), 7.62—7.70 (2H, m), 7.84 (1H, d, J=8.4 Hz), 8.26—8.35 (1H, m), 8.45 (1H, s).

In the same manner as in standard procedure C, the hydriodide (459 mg, 0.830 mmol) of **6e** was treated with TFA (7 ml) to give 338 mg of the hydrio-

dide of **7e** as a brown solid (84%): mp: >210 °C (dec.). IR (KBr) cm^{-1}: 3700—2700, 1680, 1640, 1510. ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 6.93—6.97 (2H, m), 7.65—7.68 (2H, m), 7.84 (1H, dd, J=1.8, 8.6 Hz), 8.32 (1H, d, J=8.6 Hz), 8.42 (2H, m), 9.26 (2H, br s), 9.42 (2H, br s), 10.58 (1H, br s). *Anal*. Calcd for $C_{18}H_{17}N_3O_4S \cdot HI \cdot 0.5CF_3CO_2H$: C, 41.17; H, 3.00; N, 7.58. Found: C, 42.52; H, 3.33; N, 7.33.

6-Cyanobenzo[*b*]thiophen-2-carboxylic Acid (3f) Compound **3f** was prepared from ethyl 6-nitrobenzothiophen-2-carboxylate[25] by three steps according to the procedure for the preparation of **3g** (36%): IR (KBr) cm^{-1}: 3600—3000, 2150, 1660. ^{1}H-NMR (DMSO-d_6) δ: 7.82 (1H, dd, J=1.4, 8.2 Hz), 8.10—8.25 (1H, m), 8.70 (1H, s).

4-(6-Amidinobenzo[*b*]thiophen-2-carboxamido)phenoxyacetic Acid (7f) In the same manner as in standard procedure A, **3f** (280 mg, 1.38 mmol) and **4** (341 mg, 1.52 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 485 mg of **5f** as a yellow solid (90%): IR (KBr) cm^{-1}: 200, 1740, 1635, 1500. ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, s), 6.97—6.91 (2H, m), 7.62—7.67 (2H, m), 7.82 (1H, dd, J=1.4, 8.4 Hz), 8.19 (1H, d, J=8.4 Hz), 8.41 (1H, s), 8.70 (1H, s), 10.60 (1H, br s).

In the same manner as in standard procedure B, the cyano group of **5f** (475 mg, 1.22 mmol) was converted to an amidino group to give 462 mg of the hydriodide of **6f** as a yellow solid (68% in 3 steps).: IR (KBr) cm^{-1}: 3700—2700, 1730, 1635, 1500. ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, s), 6.94—7.01 (2H, m), 7.63—7.68 (2H, m), 7.81 (1H, d, J=8.5 Hz), 8.24 (1H, d, J=8.5 Hz), 8.43 (1H, s), 8.57 (1H, s), 8.50—10.00 (4H, br s), 10.58 (1H, s).

In the same manner as in standard procedure C, the hydriodide (407 mg, 0.736 mmol) of **6f** was treated with TFA (7 ml) to give 345 mg of the hydriodide of **7f** as a red-brown solid (94%): mp: >250 °C. IR (KBr) cm^{-1}: 3700—2800, 1740, 1680, 1635, 1500. ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 6.93—6.97 (2H, m), 7.65—7.70 (2H, m), 7.81 (1H, dd, J=1.5, 8.5 Hz), 8.23 (1H, d, J=8.5 Hz), 8.44 (1H, s), 8.58 (1H, s), 9.21 (2H, br s), 9.41 (2H, br s), 10.59 (1H, br s). *Anal*. Calcd for $C_{18}H_{17}N_3O_4S \cdot HI \cdot 0.7CF_3CO_2H$: C, 40.3; H, 3.09; N, 7.27. Found: C, 40.53; H, 3.38; N, 6.96.

5-Cyanofuro[2,3-*b*]pyridine-2-carboxylic Acid (3g) Ethyl 5-nitrofuro[2,3-*b*]pyridine-2-carboxylate[26] (850 mg, 3.60 mmol) in EtOH (30 ml) was hydrogenated over 10% palladium on carbon (80 mg) under atmospheric pressure at room temperature for 15 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue (711 mg, 96%) was used in the next step. To a cooled (3 °C) mixture of ethyl 5-aminofuro[2,3-*b*]pyridine-2-carboxylate (700 mg, 3.40 mmol) in water (21 ml) and THF (10 ml), 12 N HCl (3.1 ml) was added over 5 min. After stirring the mixture for 10 min, sodium nitrite (265 mg, 3.85 mmol) in water (10 ml) was added dropwise for 15 min to maintain the temperature of the solution at 2—3 °C. Stirring was continued for another 20 min, then sodium carbonate in water was added to adjust the pH of the solution to about 6. The solution obtained was added dropwise over 10 min at 3 °C to a solution of potassium dicyanocuprate prepared from a mixture of cuprous cyanide (623 mg, 6.97 mmol) and potassium cyanide (1.31 g, 13.9 mmol) in water (20 ml). The mixture was left for 1.5 h at 3 °C and then heated to 50 °C for 2 h. After being cooled to room temperature, the mixture was poured into water and extracted with EtOAc. The extract was washed with water and brine and dried over anhydrous $MgSO_4$. After evaporation of the solvent, the residue was purified by silica gel column chromatography (hexane/EtOAc) to give 163 mg of **8g** as a yellow solid (20%): IR (KBr) cm^{-1}: 2200, 1720, 1290. ^{1}H-NMR (DMSO-d_6) δ: 1.36 (3H, t, J=7.0 Hz), 4.40 (2H, q, J=7.0 Hz), 7.87 (1H, s), 8.88 (1H, d, J=2.1 Hz), 8.99 (1H, d, J=2.1 Hz).

Compound **3g** was prepared from **8g** in a single step according to the procedure for the preparation of **3e**: ^{1}H-NMR (DMSO-d_6) δ: 7.75 (1H, s), 8.85 (1H, d, J=2.0 Hz), 8.96 (1H, d, J=2.0 Hz).

4-(5-Amidinofuro[2,3-*b*]pyridinebenzofuran-2-carboxamido)phenoxyacetic Acid (7g) **7g** was prepared like compound **7a** with the following changes. 5-cyanofuro[2,3-*b*]pyridine-2-carboxylic acid **3g** (108 mg, 0.574 mmol) and **4** (141 mg, 0.632 mmol) were condensed to give 232 mg of **5g** as an orange solid (quantitatively): ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.65 (2H, s), 6.85—6.95 (2H, m), 7.65—7.74 (2H, m), 7.84 (1H, s), 8.90 (1H, d, J=2.0 Hz), 8.96 (1H, d, J=2.0 Hz), 10.69 (1H, br s).

In the same manner as in standard procedure B, the cyano group of **5g** (430 mg, 1.10 mmol) was converted to an amidino group to give 35 mg of the hydriodide of **6g** as a yellow solid (11% in 3 steps): ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 4.66 (2H, s), 6.85—6.96 (2H, m), 7.64—7.75 (2H, m), 7.93 (1H, s), 8.75 (1H, d, J=2.2 Hz), 8.87 (1H, d, J=2.2 Hz).

In the same manner as in standard procedure C, the hydriodide (28 mg, 0.068 mmol) of **6g** was treated with TFA (0.5 ml) to give 22 mg of the hydriodide of **7g** as a yellow solid (56%): mp: >250 °C (dec.). IR (KBr) cm^{-1}: 3350, 1660, 1600, 1500. ^{1}H-NMR (DMSO-d_6) δ: 4.67 (2H, s), 6.90—6.97 (2H, m), 7.67—7.75 (2H, m), 7.92 (1H, s), 8.75 (1H, d, J=2.1 Hz), 8.87 (1H, d, J=2.1 Hz), 9.38 (2H, br s), 9.53 (2H, br s), 10.69 (1H, br s). *Anal*. Calcd for $C_{17}H_{14}N_4O_5 \cdot 1.4CF_3CO_2H$: C, 46.27; H, 3.02; N, 10.90. Found: C, 45.91; H, 3.42; N, 11.48.

***tert*-Butyl 4-(5-Amidino-3-methylbenzofuran-2-carboxamido)phenoxyacetate (6h)** In the same manner as in standard procedure A, 5-cyano-3-methyl-2-benzofurancarboxylic acid **3h** (427 mg, 1.6 mmol) and **4** (500 mg, 2.24 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 562 mg of **5h** as a colorless solid (74%): IR (KBr) cm^{-1}: 2450, 1715, 1660, 1610, 1535, 1505. ^{1}H-NMR (DMSO-d_6) δ: 1.50 (9H, s), 2.69 (3H, s), 4.52 (2H, s), 6.91—6.95 (2H, m), 7.59—7.63 (2H, m), 7.72 (1H, dd, J=1.6, 8.6 Hz), 7.99—8.00 (1H, m), 8.23 (1H, s).

In the same manner as in standard procedure B, the cyano group of **5h** (550 mg, 1.35 mmol) was converted to an amidino group, followed by purification by silica gel column chromatography ($CHCl_3$/MeOH) to give 520 mg of the hydriodide of **6h** as a pale-brown solid (70% in 3 steps): ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 2.64 (3H, s), 4.65 (2H, s), 6.81 (2H, d, J=9.1 Hz), 7.71 (2H, d, J=9.1 Hz), 7.89—7.91 (2H, m), 8.32—8.33 (1H, m), 9.32 (4H, br s).

4-(5-Amidino-3-methylbenzofuran-2-carboxamido)phenoxyacetatic Acid (7h) In the same manner as in standard procedure C, the hydriodide (510 mg, 0.925 mmol) of **6h** was treated with TFA (6 ml) to give 382 mg of hydroiodide of **7h** as a brown solid (83%): mp: 220—228 °C (dec.). IR (KBr) cm^{-1}: 3300, 3100, 1730, 1670, 1610, 1535, 1510. ^{1}H-NMR (DMSO-d_6) δ: 2.64 (3H, s), 4.67 (2H, s), 6.91—6.94 (2H, m), 7.70—7.73 (2H, m), 7.90 (1H, d, J=8.6 Hz), 7.92 (1H, dd, J=1.8, 8.6 Hz), 8.34 (1H, d, J=1.8 Hz), 9.14 (2H, br s), 9.38 (2H, br s), 10.42 (1H, s). *Anal*. Calcd for $C_{19}H_{17}N_3O_5 \cdot HI \cdot 0.8CF_3CO_2H$: C, 42.19; H, 3.21; N, 7.17. Found: C, 42.00; H, 3.31; N, 6.89.

***tert*-Butyl 4-(Methylamino)phenoxyacetate (12a)** *tert*-Butyl 4-aminophenoxyacetate (7.00 g, 31.4 mmol) and succinimide (3.11 g, 31.4 mmol) were added to EtOH (40 ml), followed by a 37% aqueous formaldehyde solution (2.55 g, 31.4 mmol). The mixture was refluxed under heating for 4 h. Low boiling material was distilled from the reaction mixture under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc) to give 8.37 g of *tert*-butyl 4-(succinimidomethylamino)phenoxyacetate as a yellow solid (84%). This solid (8.30 g, 26.0 mmol) was dissolved in DMSO (50 ml) and sodium borohydride (989 mg, 26.0 mmol) was added, followed by stirring at 100 °C for 30 min. After cooling, water was poured into the reaction mixture which was then extracted with Et_2O. The extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 3.62 g of **3a** as a yellow oil (59%): ^{1}H-NMR (DMSO-d_6) δ: 1.42 (9H, s), 2.61 (3H, d, J=5.2 Hz), 4.51 (2H, s), 6.43—6.46 (2H, m), 6.67—6.71 (2H, m).

***tert*-Butyl 4-(5-Amidinobenzofuran-2-*N*-methylcarboxamido)phenoxyacetate (16a)** In the same manner as standard procedure A, **3a** (300 mg, 1.60 mmol) and **12a** (417 mg, 1.76 mmol) were condensed and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 652 mg (quantitative) of **13a** as a yellow solid: ^{1}H-NMR (DMSO-d_6) δ: 1.40 (9H, m), 3.36 (3H, s), 4.69 (2H, s), 6.93—6.96 (2H, m), 7.25—7.35 (2H, m), 7.65—7.82 (2H, m), 7.96 (1H, s), 8.15 (1H, s).

In the same manner as standard procedure B, the cyano group of **13a** (652 mg, 1.60 mmol) was converted to an amidino group and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 392 mg of **16a** as a yellow solid (44% in 3 steps): ^{1}H-NMR (DMSO-d_6) δ: 1.42 (9H, s), 3.37 (3H, s), 4.69 (2H, s), 6.95—7.00 (2H, m), 7.25—7.35 (2H, m), 7.46 (1H, br s), 7.76 (2H, s), 8.08 (1H, s).

4-(5-Amidinobenzofuran-2-*N*-methylcarboxamido)phenoxyacetic Acid (17a) In the same manner as standard procedure C, **16a** (390 mg, 0.708 mmol) was treated with TFA (5 ml) to give 281 mg of **17a** as a yellow solid (80%): mp: 185—195 °C (dec.). IR (KBr) cm^{-1}: 3700—2800, 1750, 1690, 1640, 1505. ^{1}H-NMR (DMSO-d_6) δ: 3.37 (3H, s), 4.71 (2H, s), 6.43 (1H, br s), 6.95—7.05 (2H, m), 7.25—7.35 (2H, m), 7.76 (2H, s), 8.10 (1H, s), 9.16 (2H, br s), 9.26 (2H, br s), 13.11 (1H, br s). *Anal*. Calcd for $C_{19}H_{17}N_3O_5 \cdot 1.6C_2HO_2F_3$: C, 48.50; H, 3.41; N, 7.64. Found: C, 48.49; H, 3.44; N, 7.93.

Di-*tert*-butyl [[4-(5-Amidinobenzofuran-2-carboxamido)-*o*-phenylene]-dioxy]diacetate (13b) In the same manner as standard procedure A, **3a** (200 mg, 1.07 mmol) and **12b** (415 mg, 1.17 mmol) were condensed and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 473 mg

of **13b** as a colorles solid (84%): IR (KBr) cm^{-1}: 2220, 1740, 1735, 1640. ^{1}H-NMR (DMSO-d_6) δ: 1.48 (9H, s), 1.50 (9H, s), 4.61 (2H, s), 4.65 (2H, s), 6.90 (1H, d, J=8.7 Hz), 7.15 (1H, dd, J=8.7, 2.4 Hz), 7.52 (1H, d, J=2.4 Hz), 7.66—7.78 (2H, m), 8.07 (1H, s).

In the same manner as standard procedure B, the cyano group of **13b** (463 mg, 0.881 mmol) was converted to an amidino group and 244 mg of **16b** was obtained as a yellow solid (42% in 3 steps): ^{1}H-NMR (DMSO-d_6) δ: 1.44 (9H, s), 1.45 (9H, s), 4.66 (4H, s), 6.93 (1H, d, J=8.9 Hz), 7.35 (1H, dd, J=8.9, 2.1 Hz), 7.52 (1H, d, J=2.1 Hz), 7.85—8.00 (3H, m), 8.33 (1H, s), 9.34 (4H, br s), 10.52 (1H, br s).

[[4-(5-Amidinobenzofuran-2-carboxamido)-*o*-phenylene]dioxy]diacetic Acid (17b) In the same manner as standard procedure C, **16b** (187 mg, 0.280 mmol) was treated with TFA (3 ml) to give 127 mg of **17b** as a pale-brown solid (82%): mp >250 °C. IR (KBr) cm^{-1}: 3300, 1660, 1200, 1135. ^{1}H-NMR (DMSO-d_6) δ: 4.68 (2H, s), 4.69 (2H, s), 6.93 (1H, d, J=9.5 Hz), 7.41—7.43 (2H, m), 7.89 (1H, s), 7.89 (1H, dd, J=8.8, 1.7 Hz), 7.96 (1H, d, J=8.8 Hz), 8.33 (1H, d, J=1.7 Hz), 9.24 (2H, br s), 9.38 (2H, br s), 13.00 (1H, br s), 13.01 (1H, br s). *Anal*. Calcd for $C_{20}H_{17}N_3O_8\cdot HCl\cdot H_2O$: C, 49.85; H, 4.10; N, 8.72. Found: C, 49.58; H, 4.19; N, 8.78.

Methyl 3-(4-Aminophenyl)propionate (12c) 4-Aminocinnamic acid (15.0 g, 77.6 mmol) was added to a mixed solvent of MeOH (250 ml) and $CHCl_3$ (150 ml); sulfuric acid (3 ml) was then added, followed by refluxing under heating for 47 h. The reaction mixture was concentrated under reduced pressure, the residue was made weakly alkaline with saturated aqueous sodium hydrogencarbonate, and the mixture was extracted with EtOAc. The extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 7.84 g of methyl 4-aminocinnamate as a yellow solid (72%). This solid (7.80 g, 44.1 mmol) was dissolved in MeOH (250 ml) and 10% palladium-carbon (780 mg) was added. The mixture was stirred at room temperature for 19 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 5.98 g of **12c** as a colorless solid (76%): mp: 47—49.5 °C. IR (KBr) cm^{-1}: 3700—2500, 1710, 1600, 1500, 1425. ^{1}H-NMR ($CDCl_3$) δ: 2.57 (2H, t, J=6.8 Hz), 2.83 (2H, t, J=6.8 Hz), 3.66 (3H, s), 6.55—6.65 (2H, m), 6.95—7.10 (2H, m).

Methyl 3-[4-(5-Amidinobenzofuran-2-carboxamido)phenyl]propionate In the same manner as standard procedure A, **3a** (200 mg, 1.07 mmol) and **12c** (211 mg, 1.18 mmol) were condensed to give 371 mg (quantitative) of methyl 3-[4-(5-cyanobenzofuran-2-carboxamido)phenyl]propionate as a yellow solid: IR (KBr) cm^{-1}: 2200, 1735, 1685, 1600, 1520. ^{1}H-NMR (DMSO-d_6) δ: 2.63 (2H, t, J=7.0 Hz), 2.84 (2H, t, J=7.0 Hz), 3.59 (3H, s), 7.15—7.25 (2H, m), 7.65—7.75 (2H, m), 7.86 (1H, s), 7.94 (2H, s), 8.44 (1H, s), 10.59 (1H, br s).

In the same manner as standard procedure B, the cyano group of the above compound (350 mg, 1.01 mmol) was converted to an amidino group and purified by silica gel column chromatography (CH_2Cl_2/MeOH) to give 206 mg of the title compound as a yellow solid (41% in 3 steps): IR (KBr) cm^{-1}: 1700, 1645, 1600, 1535. ^{1}H-NMR (DMSO-d_6) δ: 2.64 (2H, t, J=7.4 Hz), 2.85 (2H, t, J=7.4 Hz), 3.59 (3H, s), 7.20—7.30 (2H, m), 7.65—7.75 (2H, m), 7.85—8.01 (3H, m), 8.33 (1H, s), 8.92 (2H, br s), 9.36 (2H, br s), 10.59 (1H, br s).

3-[4-(5-Amidinobenzofuran-2-carboxamido)phenyl]propionic Acid (17c). (Standard Procedure D) The above compound (162 mg, 0.329 mmol) was suspended in THF (6 ml) and 1 N NaOH (3.0 ml, 3.0 mmol) was added, followed by stirring at room temperature for 1 h. The reaction mixture was adjusted to pH 2 with 1 N HCl and concentrated under reduced pressure. The resulting precipitate was collected by filtration and washed with water to give 82 mg of **17c** as a yellow solid (64%): mp: >250 °C. IR (KBr) cm^{-1}: 3600—2700, 1700, 1650, 1600, 1535. ^{1}H-NMR (DMSO-d_6) δ: 2.57 (2H, t, J=7.7 Hz), 2.82 (2H, t, J=7.7 Hz), 7.20—7.30 (2H, m), 7.70—7.80 (2H, m), 7.91 (1H, dd, J=1.8, 8.8 Hz), 7.97 (1H, d, J=8.8 Hz), 8.02 (1H, s), 8.37 (1H, d, J=1.8 Hz), 9.26 (2H, br s), 9.47 (2H, br s), 10.67 (1H, s), 12.11 (1H, br s). *Anal*. Calcd for $C_{19}H_{16}N_3O_4\cdot HCl\cdot 1.3H_2O$: C, 55.63; H, 4.82; N, 10.24. Found: C, 55.45; H, 4.86; N, 10.60.

Ethyl 5-[5-(Benzyloxycarbonylamidino)furo[2,3-*b*]pyridine-2-carbonylamino]-2-pyridyloxyacetate (20d) In the same manner as standard procedure A, **19** (2.00 g, 6.18 mmol) and ethyl 5-aminopyridyl-2-oxyacetate **12d** (500 mg, 2.60 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 264 mg of **20d** as a yellow solid (21%): ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J=7.1 Hz), 4.14 (2H, q, J=7.1 Hz), 4.89 (2H, s), 5.15 (2H, s), 6.98 (1H, d, J=8.9 Hz), 7.30—7.50 (5H, m), 7.75—7.88 (2H, m), 8.12 (1H, d, J=8.9 Hz), 8.49 (1H, s), 10.72 (1H, br s).

5-(5-Amidinofuro[2,3-*b*]pyridine-2-carbonylamino)-2-pyridyloxyacetate Acid (17d). (Standard Procedure E) $CHCl_3$ (30 ml), EtOH (50 ml), conc. HCl (0.5 ml, 6.0 mmol) and 10% palladium–carbon (40 mg) were added to **20d** (254 mg, 0.491 mmol), and the mixture was stirred at room temperature for 14 h under a hydrogen atmosphere. The reaction mixture was filtered and low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 230 mg (quantitative) of **16d** as a brown solid.

In the same manner as standard procedure D, **16d** (220 mg, 0.484 mmol) was hydrolyzed with 1 N NaOH (20 ml, 20 mmol) to give 72 mg of **17d** as a colorless solid (35%): mp: >250 °C. IR (KBr) cm^{-1}: 3400, 1740, 1620. ^{1}H-NMR (DMSO-d_6) δ: 4.81 (1H, s), 6.95 (1H, d, J=8.9 Hz), 7.85—8.05 (1H, m), 8.14 (1H, dd, J=2.6, 8.9 Hz), 8.37 (1H, s), 8.51 (1H, d, J=2.6 Hz), 9.28 (2H, br s), 9.46 (2H, br s), 10.90 (1H, br s). *Anal*. Calcd for $C_{17}H_{14}N_4O_5\cdot HCl\cdot 1.2H_2O$: C, 49.51; H, 4.25; N, 13.59. Found: C, 49.48; H, 4.56; N, 12.32.

***tert*-Butyl *trans*-(4-Aminocyclohexyloxy)acetate (12e)** Toluene (200 ml) was added to a mixture of *tert*-4-aminocyclohexanol (5.00 g, 43.4 mmol), *N*,*N*-dimethylurea (3.82 g, 43.4 mmol), 37% formalin (50 ml), *N*-methylmorpholine (9.54 ml, 86.8 mmol) and dioxane (10 ml), and the mixture was heated for about 5 h while removing water by azeotropic distillation. Low boiling material was distilled from the reaction mixture under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to give 7.40 g of *trans*-5-(4-hydroxycyclohexyl)-1,3-dimethylhexahydro-2-oxo-1,3,5-triazine as a colorless solid (75%): ^{1}H-NMR ($CDCl_3$) δ: 1.23—1.46 (4H, m), 1.88—2.13 (4H, m), 2.74—2.90 (1H, m), 2.85 (6H, s), 3.46 (1H, d, J=4.6 Hz), 3.55—3.68 (1H, m), 4.21 (4H, s).

tert-5-(4-Hydroxycyclohexyl)-1,3-dimethylhexahydro-2-oxo-1,3,5-triazine (1.00 g, 4.40 mmol) and *tert*-butyl bromoacetate (1.29 g, 6.60 mmol) were dissolved in toluene (13 ml), and tetra-*n*-butylammonium hydrogensulfate (45 mg, 0.13 mmol) was added to the mixture. A solution of NaOH (13.2 g, 330 mmol) dissolved in water (13.2 ml) was added dropwise, and the mixture was stirred at room temperature for 15 h. The organic layer was partitioned, washed with water and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to give 680 mg of *trans*-5-[4-[(*tert*-butoxycarbonyl)methyloxy]cyclohexyl]-1,3-dimethylhexahydro-2-oxo-1,3,5-triazine as a colorless solid (45%): ^{1}H-NMR ($CDCl_3$) δ: 1.16—1.44 (4H, m), 1.47 (9H, s), 1.93—2.06 (2H, m), 2.06—2.18 (2H, m), 2.75—2.88 (1H, m), 2.84 (6H, s), 3.23—3.49 (1H, m), 3.98 (2H, s), 4.20 (4H, s).

trans-5-[4-[(*tert*-Butoxycarbonyl)methyloxy]cyclohexyl]-1,3-dimethylhexahydro-2-oxo-1,3,5-triazine (300 mg, 0.879 mmol) was dissolved in *tert*-butanol (5 ml) and a saturated aqueous ammonium chloride solution (5 ml) was added, followed by refluxing under heating for 2 h. The reaction mixture was adjusted to pH 10 with 1 N NaOH and extracted with benzene. The extract was washed with water and saturated brine, and dried over anhydrous Na_2SO_4. After filtration, low boiling material was distilled from the filtrate under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH) to give 130 mg of **12e** as a colorless solid (57%): ^{1}H-NMR ($CDCl_3$) δ: 1.07—1.50 (4H, m), 1.47 (9H, s), 1.82—2.45 (6H, m), 2.69—2.88 (1H, m), 3.42—4.24 (1H, m), 3.98 (2H, s).

***tert*-Butyl *trans*-[4-[5-(Benzyloxycarbonylamidino)benzofuran-2-carboxamido]cyclohexyloxy]acetate (20e)** In the same manner as standard procedure A, **19** (183 mg, 0.541 mmol) and **12e** (124 mg, 0.541 mmol) were condensed, and purified by silica gel column chromatography (CH_2Cl_2/MeOH) to give 251 mg of **20e** as a colorles solid (84%): ^{1}H-NMR ($CDCl_3$) δ: 1.24—1.60 (4H, m), 1.48 (9H, m), 2.17—2.25 (4H, m), 3.30—3.48 (1H, m), 3.92—4.05 (1H, m), 4.00 (2H, s), 5.22 (2H, s), 6.45 (1H, d, J=8.0 Hz), 7.27—7.60 (7H, m), 7.95 (1H, dd, J=1.3, 8.8 Hz), 8.21 (1H, d, J=1.3 Hz).

***trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyloxy]acetic Acid (17e)** *tert*-Butanol (13 ml) and 10% palladium–carbon (20 mg) were added to **20e** (135 mg, 0.246 mmol) and the mixture was refluxed with heating for 7 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. TFA (1 ml) was added to the residue and the mixture was stirred at room temperature for 1 h. Et_2O was then added to the reaction mixture and the resulting precipitate was collected by filtration and washed with Et_2O to give 103 mg of **17e** as a colorless solid (88%): ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.57 (4H, m), 1.81—1.96 (2H, m), 1.96—2.13 (2H, m), 3.25—3.55 (1H, m), 3.65—3.90 (1H, m), 4.03 (2H, s), 7.71 (1H, s), 7.82—7.97 (2H, m), 8.28 (1H, s), 8.64 (1H, d, J=8.0 Hz), 9.18 (2H, br s), 9.36 (2H,

br s), 12.00—12.90 (1H, m).

Ethyl *trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyloxy]-acetate (18e) EtOH (8 ml) and methanesulfonic acid (360 mg, 3.74 mmol) were added to **17e** (300 mg, 0.758 mmol) and the mixture was refluxed under heating for 1 h. The reaction mixture was concentrated to about 1/4 under reduced pressure and Et_2O (8 ml) was added. The precipitated sediment was washed with Et_2O to give 350 mg of **18e** as a colorless solid (95%): mp: 242—245 °C. IR (KBr) cm^{-1}: 3600—2700, 1745, 1673, 1635, 1590, 1520. ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.60 (4H, m), 1.21 (3H, t, J=7.1 Hz), 1.78—2.15 (4H, m), 2.38 (3H, s), 3.20—3.50 (1H, m), 3.68—3.93 (1H, m), 4.12 (2H, q, J=7.1 Hz), 4.13 (2H, s), 7.72 (1H, s), 7.80—7.95 (2H, m), 8.29 (1H, d, J=1.0 Hz), 8.65 (1H, d, J=8.0 Hz), 9.06 (2H, br s), 9.36 (2H, br s). *Anal*. Calcd for $C_{19}H_{17}N_3O_5 \cdot C_1H_4O_3S_1$: C, 52.16; H, 6.04; N, 8.69. Found; C, 52.08; H, 6.01; N, 8.62.

***trans*-[4-[(5-Amidinobenzofuran-2-carboxamido]cyclohexyloxy]acetic Acid (17e)** In the same manner as standard procedure D, **18e** (1.00 g, 2.07 mmol) was hydrolyzed with 1 N NaOH to give 761 mg of the hydrochloride of **17e** as a colorless solid (93%): mp: >250 °C. IR (KBr) cm^{-1}: 1660, 1530, 1450, 1430. ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.50 (4H, m), 1.87—1.92 (2H, m), 1.98—2.10 (2H, m), 3.25—3.55 (1H m), 3.70—3.85 (1H, m), 4.04 (2H, s), 7.75 (1H, s), 7.87 (1H, dd, J=1.5, 8.8 Hz), 7.91 (1H, d, J=8.8 Hz), 8.30 (1H, d, J=1.5 Hz), 8.69 (1H, d, J=8.0 Hz), 9.20 (2H, br s), 9.43 (2H, br s), 12.00—12.90 (1H, m). *Anal*. Calcd for $C_{18}H_{21}N_3O_5 \cdot HCl \cdot 2H_2O$: C, 50.06; H, 6.07; N, 9.73. Found: C, 49.67; H, 6.52; N, 9.92. HPLC t_{RA} 13.6 min (98.1%), t_{RC} 13.0 min (98.9%).

Ethyl *trans*-3-[4-[5-(Benzyloxycarbonylamidino)benzofuran-2-carboxamido]cyclohexyl]propionate (20f) In the same manner as standard procedure A, **19** (3.98 g, 11.8 mmol) and **12f** (3.00 g, 14.1 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 4.66 g of **20f** as a colorless solid (76%): IR (KBr) cm^{-1}: 1730, 1640, 1600, 1530. ^{1}H-NMR ($CDCl_3$) δ: 1.00—1.15 (2H, m), 1.22—1.32 (5H, m), 1.40—1.48 (2H, m), 1.80—1.86 (2H, m), 2.05—2.15 (2H, m), 2.30—2.38 (2H, m), 3.90—3.93 (1H, m), 4.13 (2H, q, J=7.1 Hz), 5.22 (2H, s), 6.47 (1H, d, J=7.0 Hz), 7.25—7.50 (6H, m), 7.53 (1H, d, J=8.8 Hz), 7.95 (1H, dd, J=1.7, 8.8 Hz), 8.23 (1H, d, J=1.7 Hz).

Ethyl *trans*-3-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyl]-propionate (18f) In the same manner as standard procedure E, **20f** (2.85 g, 5.48 mmol) was subjected to hydrogen reduction and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 2.18 g of **18f** as a colorless solid (94%): mp: >250 °C. IR (KBr) cm^{-1}: 3700—2600, 1720, 1640, 1520. ^{1}H-NMR (DMSO-d_6) δ: 0.97—1.07 (2H, m), 1.13—1.24 (4H, m), 1.35—1.48 (4H, m), 1.71—1.88 (4H, m), 2.26—2.35 (2H m), 3.71—3.78 (1H, m), 4.05 (2H, q, J=7.1 Hz), 7.74 (1H, s), 7.87 (1H, dd, J=8.7, 1.8 Hz), 7.90 (1H, d, J=8.7 Hz), 8.30 (1H, d, J=1.8 Hz), 8.67 (1H, d, J=8.1 Hz), 9.31 (4H, br s). *Anal*. Calcd for $C_{17}H_{27}N_3O_4 \cdot HCl \cdot 0.25H_2O$: C, 59.15; H, 6.74; N, 9.85. Found: C, 59.06; H, 6.77; N, 9.90.

***trans*-3-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyl]propionic Acid (17f)** In the same manner as standard procedure D, **18f** (1.59 g, 3.77 mmol) was hydrolyzed to give 1.45 g of **17f** as a pale-brown solid (67%): mp: >250 °C. IR (KBr) cm^{-1}: 3600—2500, 1700, 1630, 1500. ^{1}H-NMR (DMSO-d_6) δ: 0.95—1.70 (2H, m), 1.10—1.20 (1H, m), 1.20—1.49 (4H, m), 1.71—1.89 (4H, m), 2.19—2.28 (2H, m), 3.69—3.80 (1H, m), 7.63 (1H, s), 7.71—7.94 (2H, m), 8.30 (1H, d, J=1.4 Hz), 8.67 (1H, d, J=8.2 Hz), 9.20 (2H, br s), 9.47 (2H, br s). *Anal*. Calcd for $C_{19}H_{23}N_3O_4 \cdot HCl \cdot 0.8H_2O$: C, 55.90; H, 6.32; N, 10.29. Found: C, 55.89; H, 6.36; N, 10.07.

Ethyl 3-(4-Aminopiperidino)propionate (12g) 4-Piperodinone (10.0 g, 73.7 mmol) and potassium carbonate (30.6 g, 221 mmol) were added to DMF (100 ml), and then ethyl 3-bromopropionate (10.0 ml, 78.0 mmol) was added. The mixture was filtered and the filtrate was added to saturated aqueous sodium hydrogencarbonate (150 ml). The mixture was extracted with EtOAc. The mixture was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 9.70 g of ethyl 3-(4-oxopiperidino)propionate as a pale-yellow oil (66%).

Then, ethyl 3-(4-oxopiperidino)propionate (1.29 g, 6.47 mmol) and benzylamine (0.85 ml, 7.78 mmol) were dissolved in EtOH (40 ml). A solution of sodium cyanoborohydride (256 mg, 4.22 mmol) dissolved in EtOH (20 ml) was added at room temperature and then acetic acid (1 ml) was added to adjust the pH to 6—7. The mixture was stirred at room temperature for 18 h and conc. HCl (3 ml) added to adjust the pH to 1—2. The resulting precipitate was collected by filtration and saturated aqueous sodium hydrogencarbonate (150 ml) was added to adjust the mixture pH to 8—9. The mixture was extracted with EtOAc, and the extract was washed with saturated brine and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 1.39 g of ethyl 3-(4-benzylaminopiperidino)propionate as a colorless oil (74%). This oil (1.11 g, 3.82 mmol) was dissolved in EtOH (120 ml) and 10% palladium–carbon (320 mg) was added. The mixture was refluxed with heating for 5 h under a hydrogen atmosphere. The reaction mixture was filtered and low boiling material was distilled from the filtrate under reduced pressure to give 617 mg of **12g** as a colorless oil (81%): IR (neat) cm^{-1}: 3300, 2900, 1720, 1600. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.1 Hz), 1.29—1.45 (2H, m), 1.77—1.83 (2H, m), 2.05 (2H, td, J=2.4, 11.6 Hz), 2.44—2.52 (2H, m), 2.63—2.72 (3H, m), 2.79—2.87 (2H, m), 4.14 (2H, q, J=7.1 Hz).

Ethyl 3-[4-[5-(Benzyloxycarbonylamidino)benzofuran-2-carboxamido]-piperidino]propionate (20g) In the same manner as standard procedure A, **19** (1.08 g, 3.19 mmol) and **12g** (610 mg, 3.05 mmol) were condensed to give 1.10 g of **20g** as a colorless solid (69%): IR (KBr) cm^{-1}: 3300, 2930, 1725, 1660, 1635, 1520, 1250. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.2 Hz), 1.53—1.72 (2H, m), 2.02—2.07 (2H, m), 2.23 (2H, td, J=11.5, 2.1 Hz), 2.51 (2H, q, J=7.2 Hz), 2.73 (2H, t, J=7.0 Hz), 2.87—2.93 (2H, m), 3.99—4.03 (1H, m), 4.15 (3H, q, J=7.2 Hz), 5.23 (2H, s), 6.48 (1H, d, J=8.3 Hz), 7.30—7.48 (6H, m), 7.55 (1H, d, J=8.8 Hz), 7.96 (1H, dd, J=8.8, 1.9 Hz), 8.24 (1H, d, J=1.9 Hz).

Ethyl 3-[4-(5-Amidinobenzofuran-2-carboxamido)piperidino]propionate (18g) The **20g** (400 mg, 0.768 mmol) was dissolved in EtOH (100 ml) and 10% palladium–carbon (80 mg) was added. The mixture was stirred at room temperature for 4 h under a hydrogen atmosphere. The reaction mixture was filtered and low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel (NH type)[27] column chromatography ($CHCl_3$/MeOH) to give 246 mg of **18g** as a colorless solid (83%): ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J=7.2 Hz), 1.51—1.70 (2H, m), 2.02—2.07 (2H, m), 2.16—2.28 (2H, m), 2.50 (2H, t, J=6.7 Hz), 2.73 (2H, t, J=6.7 Hz), 2.86—2.92 (2H, m), 3.94—4.06 (1H, m), 4.15 (2H, q, J=7.2 Hz), 4.31 (3H, br s), 6.52 (1H, br s), 7.47 (1H, d, J=0.6 Hz), 7.53 (1H, d, J=8.7 Hz), 7.70 (1H, dd, J=8.7, 1.7 Hz), 7.93 (1H, d, J=1.7 Hz).

3-[4-(5-Amidinobenzofuran-2-carboxamido)piperidino]propionic Acid (17g) In the same manner as standard procedure D, **18g** (142 mg, 0.367 mmol) was hydrolyzed to give 150 mg of **17g** as a colorless solid (95%): mp: >250 °C. IR (KBr) cm^{-1}: 3250, 1715, 1660, 1195. ^{1}H-NMR (DMSO-d_6) δ: 2.01—2.06 (4H, m), 2.86 (2H, t, J=7.5 Hz), 3.06—3.13 (2H, m), 3.23—3.48 (4H, m), 4.07—4.10 (1H, m), 7.85 (1H, m), 7.89 (1H, d, J=8.8 Hz), 7.92 (1H, d, J=8.8 Hz), 8.33 (1H, s), 9.06 (1H, d, J=7.5 Hz), 9.25 (2H, br s), 9.47 (2H, br s), 11.10 (1H, br s). *Anal*. Calcd for $C_{18}H_{22}N_4O_4 \cdot 2HCl \cdot 1.4H_2O$: C, 47.36; H, 5.92; N, 12.27. Found: C, 47.08; H, 5.55; N, 12.07.

Methyl *N*-(5-Amidinobenzofuran-2-carbonyl)-4-piperidinyloxyacetate In the same manner as standard procedure A, **3a** (185 mg, 0.988 mmol) and *tert*-butyl 4-piperidinyloxyacetate **12h** (255 mg, 1.18 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 289 mg of **13h** as a colorless solid (76%).

In the same manner as standard procedure F, the cyano group of **13h** (280 mg, 0.728 mmol) was converted to an amidino group and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 62 mg of the title compound as a colorless solid (22%): ^{1}H-NMR ($CDCl_3$) δ: 1.55—1.57 (2H, m), 1.92—1.96 (2H, m), 3.38—3.50 (2H, m), 3.69—3.73 (1H, m), 3.66 (3H, m), 3.92—3.94 (2H, m), 4.20 (2H, m), 7.55 (1H, m), 7.87 (1H, dd, J=2.0, 8.8 Hz), 7.92 (1H, d, J=8.8 Hz), 8.27 (1H, d, J=2.0 Hz,), 9.34 (4H, br s).

***N*-(5-Amidinobenzofuran-2-carbonyl)-4-piperidinyloxyacetic Acid (17h)** In the same manner as standard procedure D, the above compound (60 mg, 0.16 mmol) was hydrolyzed with 1 N NaOH solution (1.0 ml, 1.0 mmol) to give 22 mg of **17h** as a colorless solid (37%): mp: 217—228 °C (dec.). IR (KBr) cm^{-1}: 3300, 3100, 1730, 1640, 1120. ^{1}H-NMR (DMSO-d_6) δ: 1.55—1.57 (2H, m), 1.91—1.95 (2H, m), 3.37—3.48 (2H, m), 3.69—3.72 (1H, m), 3.91—3.94 (2H, m), 4.09 (2H, s), 7.55 (1H, s), 7.87 (1H, dd, J=2.0, 8.8 Hz), 7.93 (1H, d, J=8.8 Hz), 8.27 (1H, d, J=2.0 Hz), 9.24 (2H, s), 9.43 (2H, s). *Anal*. Calcd for $C_{17}H_{19}N_3O_5 \cdot HCl \cdot 1.5H_2O$: C, 49.94; H, 5.67; N, 10.20. Found: C, 49.83; H, 5.32; N, 10.00.

***tert*-Butyl *trans*-[4-Aminocyclohexyl-*N*-(*tert*-butoxycarbonyl)amino]-acetate (12i)** *trans*-4-(Triphenylmethylamino)cyclohexylamine (2.73 g, 7.66 mmol) and potassium carbonate (2.22 g, 16.08 mmol) were added to DMF (100 ml) and *tert*-butyl bromoacetate (1.57 g, 8.04 mmol) was then added dropwise under ice-cooling. The mixture was stirred for 30 min at room temperature for 30 min. The reaction mixture was filtered and water (200 ml) was added to the filtrate. The mixture was extracted with EtOAc. The extract was washed with water and saturated with brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from

the filtrate under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 2.98 g of *tert*-butyl *trans*-[4-(triphenylmethylamino)cyclohexylamino]acetate as a colorless solid (83%). This solid (2.40 g, 5.10 mmol), 4-dimethylaminopyridine (0.31 g, 2.55 mmol) and pyridine (0.81 g, 10.20 mmol) were dissolved in CH_2Cl_2 (40 ml) and a solution of di-*tert*-butyl dicarbonate (1.17 g, 5.35 mmol) dissolved in CH_2Cl_2 (10 ml) was added dropwise under ice-cooling, which was followed by stirring for 2 h. Water (100 ml) and 1 N HCl (20 ml) were then added and the mixture was extracted with $CHCl_3$. The extract was dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 1.82 g of *tert*-butyl *trans*-[4-(triphenylmethylamino)cyclohexyl-*N*-(*tert*-butoxycarbonyl)amino]acetate as a colorless solid (63%). This solid (1.82 g, 3.19 mmol) was dissolved in EtOH (60 ml) and 10% palladium–carbon (0.40 g) was added. The mixture was refluxed with heating for 16 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. Water (20 ml) and 1 N HCl (10 ml) were added to the residue and the mixture was extracted with Et_2O. Saturated aqueous sodium hydrogencarbonate was added to the aqueous layer to make it alkaline, and the mixture was extracted with $CHCl_3$. The extract was dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 0.67 g of **12i** as a pale-yellow solid (64%): IR (KBr) cm^{-1}: 3400, 2900, 1738, 1690, 1435. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.55 (4H, m), 1.44 (9H, s), 1.46 (9H, s), 1.70—2.00 (4H, m), 2.45—2.70 (1H, m), 3.65 (2H, s), 3.90—4.15 (1H, m).

***tert*-Butyl *trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyl-*N*-(*tert*-butoxycarbonyl)amino]acetate (16i)** In the same manner as standard procedure A, **19** (690 mg, 2.04 mmol) and **12i** (670 mg, 2.04 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 493 mg of **20i** as a colorless solid (37%): IR (KBr) cm^{-1}: 3600—3100, 2910, 1740, 1650, 1620, 1585, 1510, 1438. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.60 (4H, m), 1.45 (9H, s), 1.48 (9H, s), 1.75—2.25 (4H, m), 3.68 (2H, s), 3.75—4.02 (1H, m), 4.02—4.25 (1H, m), 5.22 (2H, m), 6.54 (1H, d, J=8.3 Hz), 7.25—7.55 (7H, m), 7.95 (1H, dd, J=1.6, 8.8 Hz), 8.21 (1H, d, J=1.6 Hz), 9.70—10.20 (2H, br s).

20i (482 mg, 0.743 mmol) was dissolved in EtOH (20 ml) and 10% palladium–carbon (150 mg) was added, followed by refluxing with heating for 16 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel (NH type) column chromatography ($CHCl_3$/MeOH) to give 328 mg of **16i** as a pale-yellow solid (86%): IR (KBr) cm^{-1}: 3600—3000, 2920, 1735, 1635, 1590, 1520, 1432. ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.70 (4H, m), 1.45 (9H, s), 1.48 (9H, s), 1.70—2.30 (4H m), 3.68 (2H, s), 3.80—4.03 (1H, m), 4.03—4.30 (1H, m), 4.30—5.20 (3H, m), 6.30—6.75 (1H, m), 7.45 (1H, s), 7.52 (1H, m), 7.70 (1H, d, J=8.9 Hz), 7.94 (1H, s).

***trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexylamino]-acetic Acid (17i)** **16i** (216 mg, 0.420 mmol) was dissolved in CH_2Cl_2 (8 ml) and TFA (4 ml) was added. The mixture was stirred at room temperature for 23 h. The reaction mixture was concentrated to about 1/3 under reduced pressure and Et_2O (50 ml) was added. The precipitated sediment was washed with Et_2O and collected by filtration to give 219 mg of **17i** as a colorless solid (89%): mp: >250 °C. IR (KBr) cm^{-1}: 3600—2600, 1665, 1530, 1450. ^{1}H-NMR (DMSO-d_6) δ: 1.37—1.56 (4H, m), 1.88—2.00 (2H, m), 2.00—2.20 (2H, m), 3.00—3.12 (1H, m), 3.70—3.83 (1H, m), 3.94 (2H, s), 7.72 (1H, s), 7.86 (1H, dd, J=1.8, 8.8 Hz), 7.91 (1H, d, J=8.8Hz), 8.29 (1H, d, J=1.8 Hz), 8.74 (1H, d, J=8.0 Hz), 8.96 (2H, br s), 9.21 (2H, br s), 9.37 (2H, br s). *Anal*. Calcd for $C_{22}H_{22}N_4O_4 \cdot 1.5C_2HO_2F_3 \cdot 3H_2O$: C, 43.23; H, 5.10; N, 9.60. Found: C, 42.99; H, 5.40; N, 9.28.

Di-*tert*-butyl *trans*-(4-Aminocyclohexylamino)diacetate (12j) 1,4-Diaminocyclohexane (*cis*, *trans* mixture) (24.0 g, 210 mmol) and TEA (16.3 ml, 117 mmol) were dissolved in CH_2Cl_2 (400 ml). A solution of triphenylmethyl chloride (32.5 g, 117 mmol) dissolved in CH_2Cl_2 (100 ml) was added dropwise with ice-cooling and the mixture was stirred for 15 min at room temperature for 45 min. The reaction mixture was filtered and low boiling material was distilled from the filtrate under reduced pressure. Water (100 ml) was added to the residue and extracted with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4. After filtration, low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel (NH type) column chromatography ($CHCl_3$/MeOH) to give 18.4 g of 1-amino-4-(triphenylmethylamino)cyclohexane as a pale-yellow oil (25%).

1-Amino-4-(triphenylmethylamino)cyclohexane (18.4 g, 51.7 mmol) and potassium carbonate (15.0 g, 108.6 mmol) were added to DMF (250 ml), and *tert*-butyl bromoacetate (20.7 g, 106.0 mmol) was added dropwise under ice-cooling, followed by stirring at room temperature for 2.5 h. Thereafter, triethylamine (14.8 ml, 106 mmol) was added and the mixture was stirred for 1.5 h. The reaction mixture was filtered and water (500 ml) was added to the filtrate. The mixture was extracted with EtOAc and the extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 3.56 g of di-*tert*-butyl *trans*-[4-(triphenylmethylamino)cyclohexylamino]-diacetate as a colorless solid (12%) and 16.23 g of di-*tert*-butyl *cis*-[4-(triphenylmethylamino)cyclohexylamino]diacetate as a viscous pale-yellow oil (54%).

Di-*tert*-butyl *trans*-[4-(triphenylmethylamino)cyclohexylamino]-diacetate (3.46 g, 5.92 mmol) was dissolved in MeOH (100 ml) and mixed with 10% palladium–carbon (0.75 g). The mixture was refluxed with heating for 7 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. 1 N HCl (30 ml) and water (50 ml) were added to the residue, the mixture was extracted with $CHCl_3$, and the extract was dried over anhydrous Na_2SO_4. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 2.13 g (quantitative) of **12j** as a pale-yellow oil: IR ($CHCl_3$) cm^{-1}: 2910, 1725, 1590, 1480, 1442. ^{1}H-NMR ($CDCl_3$) δ: 0.95—1.65 (4H, m), 1.45 (18H, s), 1.80—2.00 (4H, m), 2.50—2.78 (2H, m), 3.45 (4H, m).

Di-*tert*-butyl *trans*-[4-[5-(Benzyloxycarbonylamidino)benzofuran-2-carboxamido]cyclohexylamino]diacetate (20j) In the same manner as standard procedure A, **19** (2.04 g, 6.04 mmol) and **12j** (2.07 g, 6.04 mmol) were condensed and purified by silica gel column chromatography (EtOAc/*n*-hexane) to give 2.04 g of **20j** as a colorless solid (51%): IR(KBr) cm^{-1}: 3700—3000, 2910, 1722, 1657, 1618, 1587, 1510. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.60 (4H, m), 1.46 (18H, s), 1.90—2.25 (4H, m), 2.60—2.95 (1H, m), 3.47 (4H, s), 3.80—4.05 (1H, m), 5.23 (2H, s), 6.41 (1H, d, J=8.3 Hz), 7.25—7.60 (7H, m), 7.95 (1H, dd, J=1.9, 8.8 Hz), 8.23 (1H, d, J=1.5 Hz).

Di-*tert*-butyl *trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexylamino]diacetate (16j) **20j** (1.60 g, 2.41 mmol) was dissolved in *tert*-butanol (100 ml) and 10% palladium-carbon (0.56 g) was added, followed by refluxing with heating for 14 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel (NH type) column chromatography ($CHCl_3$/MeOH) to give 1.20 g of **16j** as a pale-brown solid (94%): mp: 78—81 °C. IR (KBr) cm^{-1}: 3700—3000, 2960, 2910, 1725, 1638, 1590, 1570, 1520. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.60 (4H, m), 1.46 (18H, s), 1.95—2.28 (4H, m), 2.65—2.88 (1H, m), 3.47 (4H, s), 3.85—4.07 (1H, m), 6.44 (1H, d, J=6.2 Hz), 7.47 (1H, s), 7.52 (1H, d, J=8.8 Hz), 7.69 (1H, d, J=8.3 Hz), 7.93 (1H, s).

***trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexylamino]diacetic Acid (17j)** In the same manner as standard procedure C, **16j** (1.17 g, 2.22 mmol) was treated with TFA (14 ml) to give 1.11 g of **17j** as a colorless solid (77%): mp: 210 °C (dec.). IR (KBr) cm^{-1}: 3700—2500, 1658, 1592, 1528, 1450. ^{1}H-NMR (DMSO-d_6) δ: 1.30—1.65 (4H, m), 1.72—2.10 (4H, m), 2.90—3.10 (1H, m), 3.81 (4H, s), 7.71 (1H, s), 7.85 (1H, dd, J=1.7, 8.8 Hz), 7.92 (1H, d, J=8.7 Hz), 8.28 (1H, s), 8.69 (1H, d, J=8.1 Hz), 9.17 (2H, br s), 9.37 (2H, br s). *Anal*. Calcd for $C_{20}H_{24}N_4O_6 \cdot 1.8C_2HO_2F_3$: C, 45.60; H, 4.18; N, 9.01. Found: C, 45.37; H, 4.43; N, 9.28.

***tert*-Butyl *trans*-(4-Aminocyclohexyl-*N*-*n*-butylamino)acetate (12k)** *trans*-1,4-Diaminocyclohexane (12.0 g, 105 mmol) was reacted with triphenylmethyl chloride (14.7 g, 52.5 mmol) to give 11.1 g of *trans*-1-amino-4-(triphenylmethylamino)cyclohexane as a colorless solid (30%). This solid (2.00 g, 5.61 mmol) and *n*-butylaldehyde (400 mg, 5.61 mmol) were dissolved in EtOH (70 ml) and a suspension of sodium cyanoborohydride (220 mg, 3.64 mmol) suspended in EtOH (30 ml) was added dropwise. Then, acetic acid (0.6 ml) was added to adjust the pH to 6—7 and the mixture was stirred at room temperature for 14 h. Water (200 ml) was added to the reaction mixture and the mixture was concentrated to 1/3 under reduced pressure. The condensate was extracted with $CHCl_3$ and the extract was dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure and the residue was purified by silica gel (NH type) column chromatography (*n*-hexane/$CHCl_3$) to give 1.72 g *trans*-1-(*n*-butylamino)-4-(triphenylmethylamino)cyclohexane as a colorless oil (74%). This oil (1.72 g, 4.17 mmol) and potassium carbonate (1.21 g, 8.75 mmol) were added to DMF (80 ml) and *tert*-butyl bromoacetate (850 mg, 4.38 mmol) was added dropwise at room temperature, followed by stirring for 4.5 h. The reaction mixture was filtered and water (100 ml) was

added to the filtrate. The mixture was extracted with EtOAc and the extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure and the residue was purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 1.88 g of *tert*-butyl *trans*-[4-(triphenylmethylamino)cyclohexyl-*N*-*n*-butylamino]acetate as a colorless oil (86%). This oil (1.74 g, 3.31 mmol) was dissolved in EtOH (60 ml) and 10% palladium–carbon (520 mg) was added. The mixture was refluxed under heating for 4 h under a hydrogen atmosphere. The reaction mixture was filtered through Celite and low boiling material was distilled from the filtrate under reduced pressure. Water (30 ml) and 1 N HCl (10 ml) were added to the residue and the mixture was extracted with Et_2O. Sodium hydrogencarbonate was added to the aqueous layer to make it alkaline and the mixture was extracted with $CHCl_3$. The extract was dried over anhydrous Na_2SO_4. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 680 mg of **12k** as a yellow oil (72%): IR (KBr) cm^{-1}: 3500—3000, 2900, 1730, 1590, 1450. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=7.0 Hz), 1.00—1.60 (8H, m), 1.45 (9H, m), 1.70—2.05 (4H, m), 2.45—2.75 (4H, m), 3.20 (2H, s).

***tert*-Butyl *trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyl-*N*-*n*-butylamino]acetate (16k)** In the same manner as standard procedure A, **19** (785 mg, 2.32 mmol) and **12k** (680 mg, 2.32 mmol) were condensed and purified by silica gel column chromatography (*n*-hexane/EtOAc) to give 1.06 g of **20k** as a colorless solid (75%): IR (KBr) cm^{-1}: 3600—3000, 2900, 1720, 1635, 1510, 1440. ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.05 (3H, m), 1.15—1.60 (8H, m), 1.46 (9H, m), 1.80—2.30 (4H, m), 2.45—2.80 (3H, m), 3.23 (2H, m), 3.80—4.05 (1H, m), 5.23 (2H, s), 6.43 (1H, d, J=8.3 Hz), 7.20—7.60 (7H, m), 7.95 (1H, dd, J=8.7, 1.9 Hz), 8.23 (1H, d, J=1.9 Hz).

In the same manner as standard procedure E, **20k** (941 mg, 1.56 mmol) was subjected to hydrogen reduction and purified by silica gel (NH type) column chromatography ($CHCl_3$/MeOH) to give 625 mg of **16k** as a pale-yellow solid (85%): IR (KBr) cm^{-1}: 3600—3000, 2900, 1720, 1635, 1520, 1450. ^{1}H-NMR ($CDCl_3$) δ: 0.85—1.00 (3H, m), 1.10—1.65 (8H, m), 1.46 (9H, m), 1.80—2.30 (4H, m), 2.40—2.80 (3H, m), 3.23 (2H, s), 3.80—4.10 (1H, m), 6.30—6.60 (1H, m), 7.35—7.60 (2H, m), 7.60—7.80 (1H, m), 7.94 (1H. s).

***trans*-[4-(5-Amidinobenzofuran-2-carboxamido)cyclohexyl-*N*-*n*-butylamino]acetic Acid (17k)** In the same manner as standard procedure C, **16k** (100 mg, 0.212 mmol) was treated with TFA (10 ml) to give 120 mg of **17k** as a colorless solid (88%): mp: 136—138 °C. IR (KBr) cm^{-1}: 3600—2800, 1665, 1530, 1450. ^{1}H-NMR (DMSO-d_6) δ: 0.92 (3H, t, J=7.4 Hz), 1.25—1.40 (2H, m), 1.40—1.55 (2H, m), 1.55—1.78 (4H, m), 1.90—2.10 (4H, m), 3.05—3.40 (3H, m), 3.80—3.95 (1H, m), 4.10 (2H, s), 7.72 (1H, s), 7.86 (1H, dd, J=8.8, 1.9 Hz), 7.91 (1H, d, J=8.8 Hz), 8.29 (1H, d, J=1.9 Hz), 8.74 (1H, d, J=8.1 Hz), 9.29 (2H, br s), 9.37 (2H, br s). *Anal*. Calcd for $C_{22}H_{30}N_4O_4 \cdot 1.9C_2HO_2F_3$: C, 49.10; H, 5.09; N, 8.88. Found: C, 49.28; H, 5.49; N, 9.20.

***tert*-Butyl *trans*-[4-(5-Amidino-3-methylbenzofuran-2-carboxamido)-cyclohexyloxy]acetate (16l)** In the same manner as standard procedure A, **3b** (1.71 g, 8.52 mmol) and **12e** (380 mg, 1.66 mmol) were condensed to give 600 mg of **13l** as a colorless solid (18%): IR (KBr) cm^{-1}: 3250, 2900, 2200, 1750, 1630, 1120. ^{1}H-NMR ($CDCl_3$) δ: 1.26—1.55 (4H, m), 1.49 (9H, s), 2.12—2.17 (4H, m), 2.63 (3H, s), 3.34—3.45 (1H, m), 3.92—4.07 (1H, m), 4.01 (2H, s), 6.42 (1H, d, J=7.9 Hz), 7.53 (1H, d, J=8.6 Hz), 7.68 (1H, dd, J=8.6, 1.5 Hz), 7.96 (1H, d, J=1.5 Hz).

***tert*-Butyl *trans*-[4-[(5-Amidino-3-methylbenzofuran-2-carboxamido)-cyclohexyloxy]acetate (16l). (Standard Procedure F) 13l** (3.30 g, 8.59 mmol) was dissolved in EtOH (100 ml) and the solution was ice-cooled. HCl gas was bubbled through it for 20 min, followed by stirring for 18 h. Low boiling material was distilled from the reaction mixture under reduced pressure, and the residue obtained and EtOH (100 ml) were ice-cooled. NH_3 gas was bubbled through it for 20 min, and the mixture was refluxed with heating for 3 h. Low boiling material was distilled from the reaction mixture under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 3.10 g of **16l** as a pale-brown solid (82% in 2 steps): IR (KBr) cm^{-1}: 3250, 2900, 1720, 1640, 1600, 1110. ^{1}H-NMR (DMSO-d_6) δ: 1.25—1.31 (2H, m), 1.39—1.56 (2H, m), 1.43 (9H, s), 1.82—1.85 (2H, s), 2.02—2.04 (2H, m), 2.54—2.62 (1H, m), 2.58 (3H, s), 3.78—3.80 (1H, m), 4.00 (2H, s), 7.83 (1H, d, J=8.7 Hz), 7.88 (1H, dd, J=8.7, 1.9 Hz), 8.29 (1H, d, J=1.9 Hz), 8.45 (1H, d, J=8.1 Hz), 9.33 (4H, br s).

***trans*-[4-(5-Amidino-3-methylbenzofuran-2-carboxamido)cyclohexyl-oxy]acetic Acid (17l)** In the same manner as standard procedure D, **16l** (2.85 g, 6.51 mmol) was treated with LiOH–H_2O (821 mg, 19.5 mmol) to give 2.39 g of **17l** as a yellow solid (90%): mp: >250 °C. IR (KBr) cm^{-1}: 1720, 1635, 1580, 1540, 1455, 1230, 1115. ^{1}H-NMR (DMSO-d_6) δ: 1.25—1.30 (2H, m), 1.41—1.49 (2H, m), 1.83—1.85 (2H, m), 2.03—2.05 (2H, m), 2.57 (3 H s), 3.29—3.34 (1H, m), 3.77—3.80 (1H, m), 4.04 (2H, s), 7.83 (1H, d, J=8.7 Hz), 7.87 (1H, dd, J=8.7, 1.9 Hz), 8.29 (1H, d, J=1.9 Hz), 8.46 (1H, d, J=8.1 Hz), 9.17 (2H, s), 9.36 (2H, s), 12.52 (1H, br s). *Anal*. Calcd for $C_{19}H_{23}N_3O_5 \cdot HCl \cdot 0.5H_2O$: C, 54.48; H, 6.02; N, 10.03. Found: C, 54.50; H, 6.18; N, 9.96.

5-Cyano-3-methoxybenzofuran-2-carboxylic Acid (3i) Methyl 5-bromo-2-hydroxybenzoate (25.6 g, 129 mmol), copper(I) cyanide (20.8 g, 257 mmol) and copper sulfate (200 mg) were added to *N*-methyl-2-pyrrolidone (250 ml) and the mixture was refluxed under heating for 2 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature and poured into a mixture of water (500 ml) and ethylenediamine (10 ml). After filtration, the filtrate was extracted with EtOAc. The extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/*n*-hexane) to give 3.35 g of methyl 5-cyano-2-hydroxybenzoate as a colorless solid (15%). This solid (3.35 g, 18.9 mmol) and potassium carbonate (5.23 g, 37.0 mmol) were added to DMF (45 ml), and ethyl bromoacetate (2.21 ml, 19.9 mmol) was gradually added, followed by stirring at room temperature for 18 h. Water was added to the reaction mixture and the mixture was extracted with an equal mixture of EtOAc and *n*-hexane. The extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give a crude product, ethyl 4-cyano-2-methoxycarbonylphenoxyacetate. This product (3.39 g, 19.3 mmol) was dissolved in EtOH (20 ml) and the obtained solution was gradually added to a solution of metallic sodium (622 mg, 27.1 mmol) dissolved in ethanol (50 ml). The mixture was stirred at room temperature for 45 min. Low boiling material was distilled from the reaction mixture under reduced pressure and water (200 ml) was added to the residue. Dilute HCl was added to adjust the pH to 2—3. The resulting precipitate was collected by filtration to give 2.98 g (quantitative) of ethyl 5-cyano-3-hydroxybenzofuran-2-carboxylate as a colorless solid: IR (KBr) cm^{-1}: 2200, 1680, 1620, 1590. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.1 Hz), 4.33 (2H, q, J=7.1 Hz), 7.80 (1H, d, J=8.7 Hz), 7.91 (1H, d, J=8.7 Hz), 8.38 (1H, s).

Ethyl 5-cyano-3-hydroxybenzofuran-2-carboxylate (200 mg, 0.866 mmol), DMSO (131 mg, 1.04 mmol) and potassium carbonate (132 mg, 0.953 mmol) were added to acetone (140 ml), and the mixture was refluxed under heating for 1.5 h. Low boiling material was distilled from the reaction mixture under reduced pressure and water (200 ml) was added to the residue, which was followed by extraction with EtOAc. The extract was washed with water and saturated brine, and dried over anhydrous $MgSO_4$. After filtration, low boiling material was distilled from the filtrate under reduced pressure to give 193 mg of ethyl 5-cyano-3-methoxybenzofuran-2-carboxylate (91%). The compound (170 mg, 0.694 mmol) obtained was dissolved in MeOH (5 ml) and potassium hydroxide (160 mg, 2.86 mmol) was added, followed by refluxing under heating for 45 min. Low boiling material was distilled from the reaction mixture under reduced pressure and 1 N HCl was added to the residue to adjust the pH to 2—3. The resulting precipitate was collected by filtration to give 123 mg of **3i** as a colorless solid (82%): IR (KBr) cm^{-1}: 2300, 1690, 1585, 1490. ^{1}H-NMR (DMSO-d_6) δ: 4.22 (3H, s), 7.85 (1H, d, J=8.8 Hz), 7.94 (1H, dd, J=1.7, 8.8 Hz), 8.56 (1H, s).

***tert*-Butyl *trans*-[4-(5-Amidino-3-methoxybenzofuran-2-carboxamido)-cyclohexyloxy]acetate (16m)** In the same manner as standard procedure A, **3i** (220 mg, 1.01 mmol) and **12e** (244 mg, 1.06 mmol) were condensed to give 316 mg of **13m** as a colorless solid (73%): IR (KBr) cm^{-1}: 2200, 1740, 1640, 1540. ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.50 (4H, m), 1.43 (9H, s), 1.80—1.90 (2H, m), 1.95—2.05 (2H, m), 3.25—3.35 (1H, m), 3.73—3.82 (1H, m), 4.20 (3H, s), 7.80 (1H, d, J=8.6 Hz), 7.90 (1H, dd, J=1.3, 8.6 Hz), 8.48 (1H, d, J=1.3 Hz).

In the same manner as standard procedure B, the cyano group of **13m** (310 mg, 0.724 mmol) was converted to an amidino group and purified by silica gel (NH type) column chromatography ($CHCl_3$/MeOH) to give 185 mg of **16m** as a colorless solid (57%): IR (KBr) cm^{-1}: 1740, 1640, 1520. ^{1}H-NMR ($CDCl_3$) δ: 1.30—1.70 (4H, m), 1.49 (9H, s), 2.00—2.20 (4H, m), 3.35—3.45 (1H, m), 4.01 (2H, s), 4.00—4.10 (1H, m), 4.29 (3H, s), 7.60—7.79 (1H, m), 7.51 (1H, d, J=8.7 Hz), 7.67 (1H, dd, J=1.5, 8.7 Hz), 8.05 (1H, s).

***trans*-[4-(5-Amidino-3-methoxybenzofuran-2-carboxamido)cyclohexyloxy]acetic Acid (17m)** In the same manner as standard procedure C, **16m** (180 mg, 0.404 mmol) was treated with TFA (2 ml) to give 179 mg of **17m** as a colorless solid (86%): mp: 130—131 °C. IR (KBr) cm^{-1}: 1720, 1640,

1530. ^{1}H-NMR (DMSO-d_6) δ: 1.20—1.43 (2H, m), 1.43—1.50 (2H, m), 1.80—1.90 (2H, m), 2.00—2.10 (2H, m), 3.30—3.40 (1H, m), 3.70—3.85 (1H, m), 4.03 (2H, s), 4.27 (3H, s), 7.84 (1H, d, J=8.8 Hz), 7.88 (1H, dd, J=8.8, 1.8 Hz), 7.98 (1H, d, J=8.0 Hz), 8.35 (1H, d, J=1.8 Hz), 9.15 (2H, br s), 9.38 (2H, br s), 12.50 (1H, br s). *Anal*. Calcd for $C_{19}H_{23}N_3O_6 \cdot C_2HO_2F_3 \cdot 1.1H_2O$: C, 48.20; H, 5.05; N, 8.03. Found: C, 48.08; H, 4.61; N, 8.01. HPLC t_{RA} 21.4 min (97.3%), t_{RD} 15.4 min (97.7%).

***tert*-Butyl *trans*-[4-(6-Amidinoindole-2-carboxamido)cyclohexyloxy]-acetate (24e)** In the same manner as standard procedure A, 6-cyanoindole-2-carboxylic acid **3c** (244 mg, 1.31 mmol) and **12l** (300 mg, 1.31 mmol) were condensed. The reaction mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 465 mg of **21e** as a brown solid (89%): IR (KBr) cm^{-1}: 3600—3000, 2900, 2200, 1730, 1630, 1540. ^{1}H-NMR ($CDCl_3$) δ: 1.15—1.75 (4H, m), 1.49 (9H, s), 2.05—2.27 (4H, m), 3.30—3.50 (1H, m), 3.90—4.12 (1H, m), 4.02 (2H, s), 6.09 (1H, d, J=7.9 Hz), 6.86 (1H, d, J=1.3 Hz), 7.35 (1H, dd, J=1.4, 8.3 Hz), 7.71 (1H, d, J=8.3 Hz), 7.79 (1H, s), 9.96 (1H, br s).

In the same manner as standard procedure B, the cyano group of **21e** (465 mg, 1.17 mmol) was converted to an amidino group to give 343 mg of **24e** as a brown solid (54%): IR (KBr) cm^{-1}: 3600—2800, 1725, 1660, 1630, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.55 (4H, m), 1.43 (9H, s), 1.80—2.10 (4H, m), 3.67—3.92 (1H, m), 4.01 (2H, s), 7.28 (1H, s), 7.41 (1H, dd, J=1.5, 8.4 Hz), 7.84 (1H, d, J=8.5 Hz), 7.89 (1H, s), 8.45—9.50 (4H, br s), 12.23 (1H, br s).

***trans*-[4-(6-Amidinoindole-2-carboxamido)cyclohexyloxy]acetic Acid (27e)** In the same manner as standard procedure C, **24e** (824 mg, 1.52 mmol) was treated with TFA (6 ml) to give 641 mg of **27e** as a brown solid (87%): mp: >250 °C. IR (KBr) cm^{-1}: 3600—2800, 1720, 1670, 1630, 1520. ^{1}H-NMR (DMSO-d_6) δ: 1.10—1.55 (4H, m), 1.78—2.20 (4H, m), 3.65—3.90 (1H, m), 4.04 (2H, s), 7.29 (1H, s), 7.41 (1H, dd, J=8.4, 1.3 Hz), 7.84 (1H, d, J=8.5 Hz), 7.90 (1H, s), 8.45 (1H, d, J=7.6 Hz), 8.89 (2H, br s), 9.25 (2H, br s), 12.24 (1H, s). *Anal*. Calcd for $C_{18}H_{21}N_3O_4 \cdot 0.9C_2HO_2F_3 \cdot 2.4H_2O$: C, 47.16; H, 5.54; N, 11.11. Found: C, 46.76; H, 5.20; N, 11.49.

Ethyl *trans*-[4-(6-Amidinoindole-2-carboxamido)cyclohexyloxy]acetate (30e) In the same manner of **18e**, **27e** (641 mg, 1.32 mmol) was reacted with EtOH to give 495 mg of methanesulfonate of **30e** as a pale-yellow solid (78%): mp: 263—266 °C. IR (KBr) cm^{-1}: 3600—2800, 1745, 1670, 1630, 1560. ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.55 (4H, m), 1.21 (3H, t, J=7.1 Hz), 1.82—2.15 (4H, m), 2.35 (3H, s), 3.70—3.90 (1H, m), 4.13 (2H, q, J=7.1 Hz), 4.13 (2H, s), 7.29 (1H, d, J=1.5 Hz), 7.41 (1H, dd, J=1.5, 8.5 Hz), 7.84 (1H, d, J=8.4 Hz), 7.90 (1H, s), 8.42 (1H, d, J=7.8 Hz), 8.83 (2H, br s), 9.24 (2H, br s), 12.20 (1H, br s). *Anal*. Calcd for $C_{20}H_{26}N_4O_4 \cdot 1.2CH_4SO_3 \cdot 1.4H_2O$: C, 48.21; H, 5.88; N, 10.91. Found: C, 48.32; H, 6.43; N, 10.63. HPLC t_{RB} 22.5 min (>99.5%), t_{RC} 10.6 min (97.5%).

***trans*-[4-(6-Amidinobenzo[*b*]thiophen-2-carboxamido)cyclohexyloxy]-acetic Acid (28e)** In the same manner as standard procedure A, 6-cyanobenzo[*b*]thiophen-2-carboxylic acid **3f** (400 mg, 1.97 mmol) and **12e** (474 mg, 2.07 mmol) were condensed to give 471 mg of *tert*-butyl *trans*-[4-(6-cyanobenzo[*b*]thiophen-2-carboxamido)cyclohexyloxy]acetate **22e** as an orange solid (58%): IR (KBr) cm^{-1}: 3700—3000, 2200, 1730. ^{1}H-NMR (DMSO-d_6) δ: 1.23—1.33 (2H, m), 1.35—1.45 (2H, m), 1.43 (9H, s), 1.85—1.95 (2H, m), 1.98—2.08 (3H, m), 3.70—3.80 (1H, m), 7.79 (1H, dd, J=8.3, 1.5 Hz), 8.12 (1H, d, J=8.3 Hz), 8.20 (1H, s), 8.64 (1H, d, J=1.5 Hz).

In the same manner as standard procedure B, the cyano group of **22e** (450 mg, 1.09 mmol) was converted to an amidino group and the reaction mixture was purified by silica gel (NH type) column chromatography ($CHCl_3$/MeOH) to give 147 mg of *tert*-butyl *trans*-[4-(6-amidinobenzo[*b*]-thiophen-2-carboxamido)cyclohexyloxy]acetate **25e** as a yellow solid. In the same manner as standard procedure C, this solid was treated with TFA (2 ml) to give 104 mg of **28e** as a yellow solid (20%): mp: >250 °C. IR (KBr) cm^{-1}: 3700—2700, 1740, 1670, 1600. ^{1}H-NMR (DMSO-d_6) δ: 1.20—1.33 (2H, m), 1.35—1.45 (2H, m), 1.85—1.97 (2H, m), 2.00—2.13 (2H, m), 3.43 (1H, m), 3.70—3.80 (1H, m), 4.04 (2H, s), 7.78 (1H, dd, J=1.5, 8.5 Hz), 8.15 (1H, d, J=8.5 Hz), 8.23 (1H, s), 8.53 (1H, s), 8.69 (1H, d, J=7.8 Hz), 9.18 (2H, br s), 9.40 (2H, br s). *Anal*. Calcd for $C_{18}H_{21}N_3O_4S_1 \cdot 1.2\ C_2HO_2F_3$: C, 45.02; H, 4.11; N, 7.72. Found: C, 45.24; H, 4.27; N, 7.85.

Ethyl *trans*-[4-[(6-Amidinobenzo[*b*]thien-2-yl)carbonylamino]cyclohexyloxy]acetate (31e) In the same manner as **18e**, **28e** (85 mg, 0.174 mmol) was reacted with EtOH to give 89 mg (quantitative) of the methanesulfonate of **31e** as a colorless solid: ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.50 (4H, m), 1.21 (3H, t, J=7.1 Hz), 1.85—2.15 (4H, m), 2.35 (3H, s), 3.65—3.85 (1H, m), 4.12 (2H, q, J=7.1 Hz), 4.13 (2H, s), 7.78 (1H, d, J=8.5 Hz), 8.16 (1H, d, J=8.5 Hz), 8.23 (1H, s), 8.52 (1H, s), 8.70 (1H, d, J=8.0 Hz), 9.01 (2H, br s), 9.38 (2 H, br s). *Anal*. Calcd for $C_{20}H_{25}N_3O_4 \cdot 2CH_4SO_3 \cdot 6.5H_2O$: C, 38.79; H, 5.35; N, 6.65. Found: C, 38.85; H, 6.17; N, 6.32. HPLC t_{RB} 24.0 min (>99.5%), t_{RC} 11.2 min (>99.5%).

6-Amidinobenzo[*b*]thiophen-2-carboxylic Acid A method similar to standard procedure F was used. For this ethyl 6-cyanobenzothiophen-2-carboxylate (170 mg, 0.736 mmol) was allowed to react with ethanol in the presence of hydrogen chloride to give ethyl 6-[(1-ethoxy)iminomethyl)]-2-benzofurancarboxylate. This compound was allowed to react with ammonia to give 71 mg of ethyl 6-amidinobenzo[*b*]thiophen-2-carboxylate hydrochloride as a colorless solid (34%): ^{1}H-NMR (DMSO-d_6) δ: 1.37 (3H, t, J=7.1 Hz), 4.39 (2H, q, J=7.1 Hz), 7.82 (1H, dd, J=1.7, 8.5 Hz), 8.24 (1H, d, J=8.5 Hz), 8.33 (1H, s), 8.58 (1H, s), 9.21 (3H, br s).

Ethyl 6-amidinobenzo[*b*]thiophen-2-carboxylate hydrochloride (69 mg, 0.24 mmol) was hydrolyzed with 1 N NaOH to give 47 mg of 6-amidinobenzo[*b*]thiophen-2-carboxylic acid hydrochloride as a brown solid (72%): ^{1}H-NMR (DMSO-d_6) δ: 7.81 (1H, dd, J=1.7, 8.4 Hz), 8.14 (1H, s), 8.20 (1H, d, J=8.4 Hz), 8.56 (1H, s), 9.29 (2H, br s), 9.44 (2H, br s).

Ethyl 5-(6-Amidinobenzo[*b*]thiophen-2-carboxamido)pyridyl-2-oxyacetate In the same manner as standard procedure A, 6-amidinobenzo[*b*]thiophen-2-carboxylic acid hydrochloride (45 mg, 0.18 mmol) and ethyl 5-aminopyridyl-2-oxyacetate (38 mg, 0.19 mmol) were condensed and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give the title compound as a colorless solid: ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J=7.1 Hz), 4.13 (2H, q, J=7.1 Hz), 4.90 (2H, s), 6.95—7.00 (1H, m), 7.82 (1H, dd, J=1.7, 8.4 Hz), 8.08—8.11 (1H, m), 8.23 (1H, d, J=8.4 Hz), 8.48 (2H, s), 8.58 (1H, s), 9.30 (4H, br s).

5-(6-Amidinobenzo[*b*]thiophen-2-carboxamido)pyridyl-2-oxyacetic Acid (28d) In the same manner as standard procedure D, the above compound was treated with 1 N NaOH (0.6 ml, 0.6 mmol) to give 38 mg of **28d** as a colorless solid (47% in 2 steps): mp: >250 °C. IR (KBr) cm^{-1}: 3700—2700, 1690, 1630, 1580. ^{1}H-NMR (DMSO-d_6) δ: 4.83 (2H, s), 6.98 (1H, d, J=8.9 Hz), 7.83 (1H, dd, J=1.5, 8.4 Hz), 8.08 (1H, dd, J=2.6, 8.9 Hz), 8.25 (1H, d, J=8.4 Hz), 8.45 (1H, s), 8.48 (1H, d, J=2.6 Hz), 8.59 (1H, s), 9.15 (2H, br s), 9.43 (2H, br s). *Anal*. Calcd for $C_{17}H_{14}N_3O_4S \cdot HCl$: C, 50.19; H, 3.72; N, 13.77. Found: C, 50.52; H, 4.10; N, 13.46.

***tert*-Butyl *trans*-[4-(5-Amidinofuro[2,3-*b*]pyridine-2-carboxamido)cyclohexyloxy]acetate (26e)** In the same manner as standard procedure A, 5-cyanofuro[2,3-*b*]pyridine-2-carboxylic acid **3g** (288 mg, 1.53 mmol) and **12e** (433 mg, 1.89 mmol) were condensed to give 280 mg of *tert*-butyl *trans*-4-(cyanofuro[2,3-*b*]pyridine-2-carbonylamino)cyclohexyloxyacetate **23e** as a colorless solid (46%): IR (KBr) cm^{-1}: 2200, 1740, 1650, 1460. ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.90 (4H, m), 1.48 (9H, s), 2.10—2.40 (1H, m), 3.30—3.50 (1H, m), 3.90—4.10 (1H, m), 4.01 (2H, s), 6.57 (1H, d, J=6.3 Hz), 7.53 (1H, s), 8.36 (1H, d, J=2.0 Hz), 8.72 (1H, d, J=2.0 Hz).

In the same manner as standard procedure B, the cyano group of **23e** (275 mg, 0.69 mmol) was converted to an amidino group to give 66 mg of **26e** as a colorless solid (23% in 3 steps): IR (KBr) cm^{-1}: 1750, 1650. ^{1}H-NMR ($CDCl_3$) δ: 1.25—1.70 (4H, m), 1.49 (9H, s), 2.00—2.40 (4H, m), 3.30—3.55 (1H, m), 3.90—4.10 (1H, m), 4.01 (2H, s), 4.00—5.50 (3H, br s), 6.50—7.00 (1H, br s), 7.47 (1H, s), 8.35 (1H, br s), 8.75 (1H, br s).

***trans*-[4-(5-Amidinofuro[2,3-*b*]pyridine-2-carboxamido)cyclohexyloxy]acetic Acid (29e)** In the same manner as standard procedure C, **26e** (65 mg, 0.16 mmol) was treated with TFA (1.5 ml) to give 60 mg of **29e** as a yellow solid (65%): mp: 135—160 °C (dec.). IR (KBr) cm^{-1}: 1660, 1530, 1380. ^{1}H-NMR (DMSO-d_6) δ: 1.05—1.70 (4H, m), 1.50—1.60 (2H, m), 2.00—2.10 (2H, m), 3.65—3.95 (1H, m), 4.03 (2H, s), 7.74 (1H, s), 8.69 (1H, d, J=2.0 Hz), 8.83 (1H, d, J=2.0 Hz), 9.22 (1H, br s), 9.54 (1H, br s). *Anal*. Calcd for $C_{17}H_{20}N_4O_5 \cdot 1.7C_2HO_2F_3$: C, 44.21; H, 3.95; N, 10.11. Found: C, 44.21; H, 4.34; N, 10.06.

Ethyl *trans*-3-[4-[5-(Benzyloxycarbonylamidino)furo[2,3-*b*]pyridine-2-carboxamido]cyclohexyl]propionate (33f) In the same manner as standard procedure A, **32** (215 mg, 0.634 mmol) and **12f** (149 mg, 0.634 mmol) were condensed to give 193 mg of **33f** as a colorless solid (59%): IR (KBr) cm^{-1}: 1745, 1710, 1620, 1500. ^{1}H-NMR ($CDCl_3$) δ: 1.05—1.18 (2H, m), 1.20—1.49 (6H, m), 1.50—1.67 (2H, m), 1.75—1.90 (2H, m), 2.05—2.18 (2H, m), 2.25—2.45 (2H, m), 3.85—3.95 (1H, m), 4.13 (2H, q, J=7.1 Hz), 5.22, 5.23 (2H, each s), 6.62—6.70 (1H, m), 7.25—7.50 (6H, m), 8.50, 8.58 (1H, each s), 8.89—8.94 (1H, m).

Ethyl *trans*-3-[4-(5-Amidinofuro[2,3-*b*]pyridine-2-carboxamido)cyclohexyl]propionate (34f) In the same manner as standard procedure E, **33f** (183 mg, 0.352 mmol) was subjected to hydrogen reduction in the presence of 10% palladium–carbon (30 mg) to give 77 mg of **34f** as a yellow solid (48%). Then, 70 mg of **33f** was recovered: IR (KBr) cm^{-1}: 3700—3000, 1710, 1620. ^{1}H-NMR (DMSO-d_6) δ: 0.95—1.13 (2H, m), 1.15—1.30 (4H,

m), 1.35—1.58 (4H, m), 1.70—1.90 (4H, m), 2.27—2.34 (2H, m), 3.70—3.90 (1H, m), 4.06 (2H, q, J=7.2 Hz), 7.75 (1H, s), 8.71 (1H, s), 8.80—8.84 (1H, m), 8.84 (1H, s), 9.20—9.60 (4H, br s).

***trans*-3-[4-(5-Amidinofuro[2,3-*b*]pyridine-2-carboxamido)cyclohexyl]-propionic Acid (29f)** In the same manner as standard procedure D, **34f** (67 mg, 0.15 mmol) was hydrolyzed with 1 N NaOH (0.58 ml, 0.58 mmol) to give 28 mg of **29f** as a colorless solid (43%): mp: >250 °C. IR (KBr) cm^{-1}: 3600—2600, 1680, 1610, 1570. ^{1}H-NMR (DMSO-d_6) δ: 0.95—1.08 (2H, m), 1.35—1.50 (4H m), 1.70—1.95 (4H, m), 2.20—2.35 (2H, m), 3.71—3.83 (1H, m), 7.77 (1H, s), 8.73 (1H, d, J=2.2 Hz), 8.83 (1H, br s), 8.85 (1H, d, J=2.2 Hz), 9.43 (2H, br s), 9.64 (2H, br s). *Anal*. Calcd for $C_{18}H_{22}N_4O_4\cdot HCl\cdot 1.2H_2O$: C, 51.91; H, 6.15; N, 13.45. Found: C, 52.19; H, 5.99; N, 13.06.

Ethyl 5-[5-(Benzyloxycarbonylamidino)furo[2,3-*b*]pyridine-2- carboxamido]-2-pyridyloxyacetate (33d) In the same manner as standard procedure A, **32** (100 mg, 0.295 mmol) and **12d** (62.3 mg, 0.324 mmol) were condensed to give 68 mg of **33d** as a colorless solid (45%): ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7.1 Hz), 4.24 (2H, q, J=7.1 Hz), 4.89 (2H, s), 5.25, 5.28 (2H, each s), 6.90—6.94 (1H, m), 7.30—7.45 (5H, m), 7.62, 7.63 (1H, each s), 8.03—8.10 (1H, m), 8.32—8.41 (1H, m), 8.53, 8.67 (1H, each s), 8.92, 8.97 (1H, each s).

Ethyl 5-(5-Amidinofuro[2,3-*b*]pyridine-2-carboxamido)-2-pyridyloxyacetate (34d) $CHCl_3$ (5 ml), EtOH (5 ml), 1 N HCl (0.6 ml, 0.6 mmol) and 10% palladium–carbon (20 mg) were added to **33d** (68 mg, 0.13 mmol), and the mixture was stirred at room temperature for 1.5 h under a hydrogen atmosphere. The reaction mixture was filtered and low boiling material was distilled from the filtrate under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH) to give 42 mg of **34d** as a yellow solid (66%): ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, J=7.1 Hz), 4.14 (2H, q, J=7.1 Hz), 4.90 (2H, s), 6.99 (1H, d, J=9.0 Hz), 7.99 (1H, s), 8.15 (1H, dd, J=2.6, 9.0 Hz), 8.53 (1H, d, J=2.6 Hz), 8.76 (1H, d, J=2.2 Hz), 8.89 (1H, d, J=2.2 Hz).

5-(5-Amidinofuro[2,3-*b*]pyridine-2-carboxamido)-2-pyridyloxyacetic Acid (29d) THF (0.5 ml) and 1 N NaOH (0.43 ml, 0.43 mmol) were added to **34d** (42 mg, 0.085 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was adjusted to pH 2—3 with 1 N HCl and concentrated under reduced pressure. The resulting precipitate was filtered to give 36 mg of **29d** as a colorless solid (92%): mp: >250 °C. IR (KBr) cm^{-1}: 3600—2700, 1660, 1590, 1520. ^{1}H-NMR (DMSO-d_6) δ: 4.79 (2H, s), 6.96 (1H, d, J=8.9 Hz), 7.99 (1H, s), 8.14 (1H, dd, J=2.6, 8.9 Hz), 8.53 (1H, d, J=2.6 Hz), 8.77 (1H, d, J=2.2 Hz), 8.89 (1H, d, J=2.2 Hz), 9.37 (2H, br s), 9.60 (2H, br s). *Anal*. Calcd for $C_{16}H_{13}N_5O_5\cdot HCl\cdot 1.2H_2O$: C, 46.49; H, 4.00; N, 16.94. Found: C, 46.87; H, 3.87; N, 16.50. HPLC t_{RA} 14.5 min (99.1%), t_{RC} 11.8 min (97.2%).

Determination of Suppression of ADP-Induced Aggregation of Human Platelets[19] PRP was prepared from the blood taken from healthy volunteers by centrifugation in the presence of 0.38% sodium citrate, and used for the determination.

Two minutes after the test compounds were added to the above PRP, ADP (1—5 μM) was added, at a concentration such that primary aggregation alone was observed. The suppression of ADP aggregation by the compounds was evaluated. The percentage suppression was determined by varying the concentration of the compounds and the concentration of the compound at which the aggregation was suppressed by 50% (IC_{50}) was calculated; this was taken as the activity of the compound. Throughout the platelet aggregation assay, Ro43-8857 was used as a reference compound, and we confirmed that the IC_{50} values of this compound varied significantly with PRPs from different blood donors (85 nM±33; mean±S.E.M. from three different donors/each assay, n=62). Then 1 μM to 10 μM ADP was added to induce platelet aggregation in order to maintain 85 nM±13 for the IC_{50} value of this compound. We confirmed that the number of platelets in PRP was within the normal range (2—4×10^5/μl) before the start of each platelet aggregation assay. We used the PRP without matching the platelet numbers. The concentration of ADP (1.25—2.5 μM) was chosen each time to give a similar range to the first aggregation (30—50% aggregation in the aggregometer used). For each compound, at least three assays were performed with PRPs from different donors to calculate the IC_{50} value. We used Ro43-8857 as a reference compound in each assay in order to check the reproducibility, and believe that the assay is reliable enough (85 nM±2.5; mean±S.E.M. from three different donors/each assay, n=62).

In Table 8, we used PRPs or WPSs (washed platelet suspension) from beagle dogs (Keari, Osaka, Japan), male Hartley guinea-pigs (Keari, Osaka, Japan), Japanese white rabbits (Keari, Osaka, Japan), and Wistar rats (Keari, Osaka, Japan) instead of humans.

In Table 10, we used collagen (1 μg/ml, Sigma Chemical., U.S.A.), thrombin (0.1 u/ml, from human plasma, Sigma Chemical., U.S.A.), PMA (0.5 μM, Funakoshi, Japan), and arachidonic acid (5 mM, free type, Sigma Chemical., U.S.A.) instead of the above ADP.

Anti-protease Activity[22] The serine protease-inhibition actions of GP IIb/IIIa antagonists were assessed using human α-thrombin, human plasmin, human blood coagulation factor Xa and bovine β-trypsin. Briefly, 10 nM protease was mixed with 10^{-4} M GP IIb/IIIa antagonist or DMSO in Tris–HCl buffered saline for 10 min at room temperature. Protease activity was measured following the addition of 2 mM at the respective synthetic substrate by monitoring the absorption at 405 nm at 25 °C on a DV-7400 Spectrophotometer (Beckman). The synthetic substrates used were S-2303 for thrombin, S-2238 for FXa and S-2251 for plasmin and β-trypsin. The Ki_{app} value was obtained by plotting the percentage inhibition against the inhibitor concentrations. Values are the average of at least two determinations. Nafamostat mesylate (Futhan) was used as a respective protease inhibitor.

^{125}I-Fbg Binding to Washed Platelets Human Fbg was labeled with ^{125}I using enzymobeads (Bio Rad, Laboratories Hercules, CA) and the labeled protein was separated from unbound ^{125}I by passage through a PD-10 column. The specific activity was approximately 0.13 mCi/mg. WPS activated by 1 μM A23187 in the presence of 1 μg/ml ^{125}I-Fbg with or without antagonist for 5 min, was layered onto 20% w/v sucrose+20 mg/ml BSA (albumin, bovine) in saline and centrifuged at 7000×***g*** for 5 min. The radioactivity associated with the platelet pellet was counted in a ***g*** counter (Packard Instruments, Downer Grove, IL). Non-specific binding was determined in the presence of 200 μg unlabeled Fbg. Values are the average of at least two determinations.

Inhibition of *ex Vivo* ADP-Induced Aggregation in Guinea Pig Male Hartley guinea-pigs (Keari, Osaka, Japan) weighing 350—470 g were anesthetized with a combination of 120 mg/kg i.p. phenobarbital Na and 60 mg/kg i.p. pentobarbital Na. The trachea was exposed and cannulated with a tube(C0). Ventilation was started immediately using a small animal respirator (model683, Harvard). The left carotid artery and the right jugular vein were cannulated for blood sampling (into 1 : 10 3.8% sodium citrate) and i.v. compound administration (1 ml/kg in distilled water) respectively. One hundred and eighty μl of blood was collected at 0, 10, 30, 60, 120, 240 and 420 min after administration of the compounds used for the analysis of ADP (1 μM)-induced whole blood platelet aggregation. Briefly, the number of platelets in whole blood was counted before, and 1 min after, the addition of 1 μM ADP. Values are the means±S.E.M. of five experiments (n=5).

Male Hartley guinea pigs (Keari, Osaka, Japan) weighing 350—470 g, fasted for 24 h, were used for this *ex vivo* study. Compounds were suspended in 0.5% methyl cellulose and administered orally to conscious animals at a dose of 10 mg/kg. Blood samples were collected from the abdominal artery 2 and 4 h after administration, and PRP was prepared by centrifugation by a similar method to that used in the *in vitro* study. Inhibition of ADP (10 μM)-induced platelet aggregation was determined by comparing the responses in the samples from drug-administered guinea pigs with those from the vehicle control group at each time point. Values are the means±S.E.M. of five experiments (n=5).

Inhibition of *ex Vivo* ADP-Induced Aggregation in Dog Conscious beagle dogs (Keari, Osaka, Japan) weighing between 8.3—10.2 kg were given the test compound orally. 5 ml of venous blood was drawn at predetermined intervals from a foreleg with 1/10 vol. of 3.8% citrate and centrifuged to obtain PRP. Aggregation of the PRP was measured by addition of 10 μM ADP and was monitored in an aggregometer (NBS Heme Tracer 601, MC Medical, Osaka, Japan). Inhibition of aggregation by the test compounds was determined by comparing the aggregation response (%Tmax) after compound administration to that before. Values are the means±S.E.M. of five experiments (n=5).

Inhibition of HUVEC Attachment to Fbg, vWF, FN and VN HUVEC were purchased from Kurabo (Osaka, Japan). Cells were maintained in MCDB-107 medium (Kyokuto Pharm., Japan) supplemented with 10% Fetal calf serum (FCS) (Filtron), 10 ng/ml endothelial cell growth factor (ECGF), 10 ng/ml epidermal growth factor (EGF), 10 μg/ml transferrin (Intergen Co., NY, U.S.A.), and 1 μg/ml insulin (Intergen Co.). Confluent HUVEC were detached with 0.05% trypsin–0.53 mM EDTA solution. After being washed twice with phosphate-buffered saline, the cells (1×10^6/ml) were suspended in culture medium containing 0.2% BSA. The adhesion of HUVEC to Fbg, vWF, FN or VN was measured by a modification of the known method. Briefly, microtiter plates were coated with an adhesive protein (15 μg/ml Fbg (Green Cross, Osaka, Japan), 3 μg/ml vWF (Green Cross), 5.0 μg/ml FN (Funakoshi, Tokyo, Japan) or 0.23 μg/ml VN (Funakoshi)) and blocked with 1% BSA. Cells (96-well) were added to each

well and incubated for 2 h at 37 °C in the presence of various concentrations of the test compound. After being washed with PBS, the adherent cells were stained with 0.2% crystal violet solution (10% EtOH+2.8% formalin). The dye was solubilized by the addition of 50% EtOH (in 10 mM Tris–HCl, pH7.4) and the number of adherent cells was quantified by reading the absorbance at 590 nm. Nonspecific adhesion was assessed by the adhesion of cells to BSA-coated wells. Values are the average of at least two determinations.

Acknowledgments The authors thank Dr. Fumio Mori, Dr. Feng Wang, Dr. Atsuyuki Ashimori, Mr. Hajime Ebisu, Ms. Ruriko Ikegawa and Mr. Takehiro Okada for their encouragement and helpful discussions throughout this study.

Supporting Information Available: HPLC analyses of compounds **7d**, **17e**, **17m**, **29d**, **30e**, and **31e** (1 page) and pharmacokinetic data for **17e** and **18e** (1 page). Ordering information is given on any current masthead page.

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