New Orally Active Serine Protease Inhibitors: Structural Requirements for Their Good Oral Activity

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Synthesis and structural requirements for good oral activity of a series of *para*-substituted benzoyl esters of 4-hydroxybenzamidine serine protease inhibitors are described. The structure required for good oral activity was found to be general formula **II** whose corresponding ester has to be hydrolyzed in the intestine before absorption through the mucous membranes or in plasma after absorption. Biological evaluation of oral absorption using plasma anti-trypsin activity was useful for rapid evaluation. By measuring their actual plasma concentrations after oral administration, compounds **14** and **16b** were confirmed to show good area under the plasma concentration–time curves (AUC). Their plasma concentrations corresponded to their plasma anti-trypsin activity. Structure–oral activity relationships are discussed.

The serine protease trypsin has long been known to be a key mediator in inflammation. Recent studies have also suggested its role in the pathogenesis of inflammatory diseases such as pancreatitis[1] and reflux esophagitis after gastrectomy.[2] The development of agents to inhibit trypsin could be useful in the treatment of inflammation caused by the hypersecreted trypsin. As a result, inhibition of hypersecreted trypsin has become an attractive therapeutic target. A few examples of benzoguanidine and naphthoamidine derivatives, such as **2a**[3] (FOY-305) and **3**[4] (FUT-187), have been described as orally active, reversible trypsin inhibitors. However, structural requirements for their oral absorption have not yet been determined.

Here, we report the molecular design and structural features of a new series of orally active benzamidine derivatives in which a charged amidinium moiety is contained as a specificity-determining residue. This is the first report of an orally active trypsin inhibitor derived from benzamidine derivatives. The leading candidate in this series is the reversible inhibitor **1a**[5] (Chart 1), which is stable in aqueous medium and also exhibits *in vitro*, *ex vivo*, and *in vivo* anti-trypsin activity.

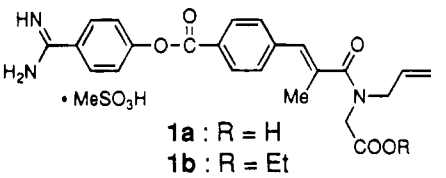
Chemistry

The synthesis of **1a,b** was reported briefly in our previous paper.[5] Full details of the preparation of all the unknown compounds are described in the Experimental Section of this paper. The synthesis of **17a–c** was begun with **19** (Scheme 1).

Selective hydrolysis of **19** afforded **20**, which was esterified with 4-amidinophenol hydrochloride followed by deprotection under acidic conditions to give **21** as a hydrochloride. Compound **21** was used as a key intermediate for the synthesis of **17a-c**. Syntheses of the amine moieties were carried out as follows. Formation of the Schiff base of diethyl ketomalonate (**22**) with *tert*-butyl 4-aminobutyrate or ethyl 4-aminobutyrate followed by catalytic hydrogenation gave **23** or **24**, respectively. Compound **23** was converted to **17b** by condensation with **21** followed by deprotection. Condensation of **24** with **21** gave **17c**.

Esterification of the half-ester **25**, which was obtained by partial hydrolysis of the corresponding diethyl ester, with *tert*-butyl alcohol followed by deprotection gave **27**. *N*-Alkylation of **27** using ethyl 3-bromocrotonate followed by catalytic hydrogenation in the presence of sodium nitrite afforded **28**. Condensation of **28** with **21** followed by acidic deprotection gave **17a**.

Chart 1



1a : R = H
1b : R = Et

Results and Discussion

The molecular design of an orally active inhibitor was carried out through a following stepwise procedure. An orally active inhibitor must be resistant to immediate metabolic inactivation in the intestine. One of the most difficult problems in obtaining an orally active trypsin inhibitor is to block such immediate hydrolysis of its phenol ester moiety in the intestine because most of the potent inhibitors such as **2a** or **3** contain an active phenol ester function which is mechanistically indispensable in these molecules. To obtain information regarding metabolic stability in the small intestine, we carried out a preliminary experiment using rat small intestine homogenate and some known orally active trypsin inhibitors. Treatment of **2a** (FOY-305) with rat small intestine homogenate gave **2b** as an active metabolite which was resistant to further enzymatic degradation.[6] **3** (FUT-187) was also resistant to immediate hydrolysis under the same conditions as described above (Figure 1, supporting information).

On the basis of the information described above, we speculated that introduction of a polar function such as an acidic carboxylic acid on **2b** or a basic cyclic guanidine moiety on **3** into the opposite side of the positively charged guanidine or amidine moiety dramatically

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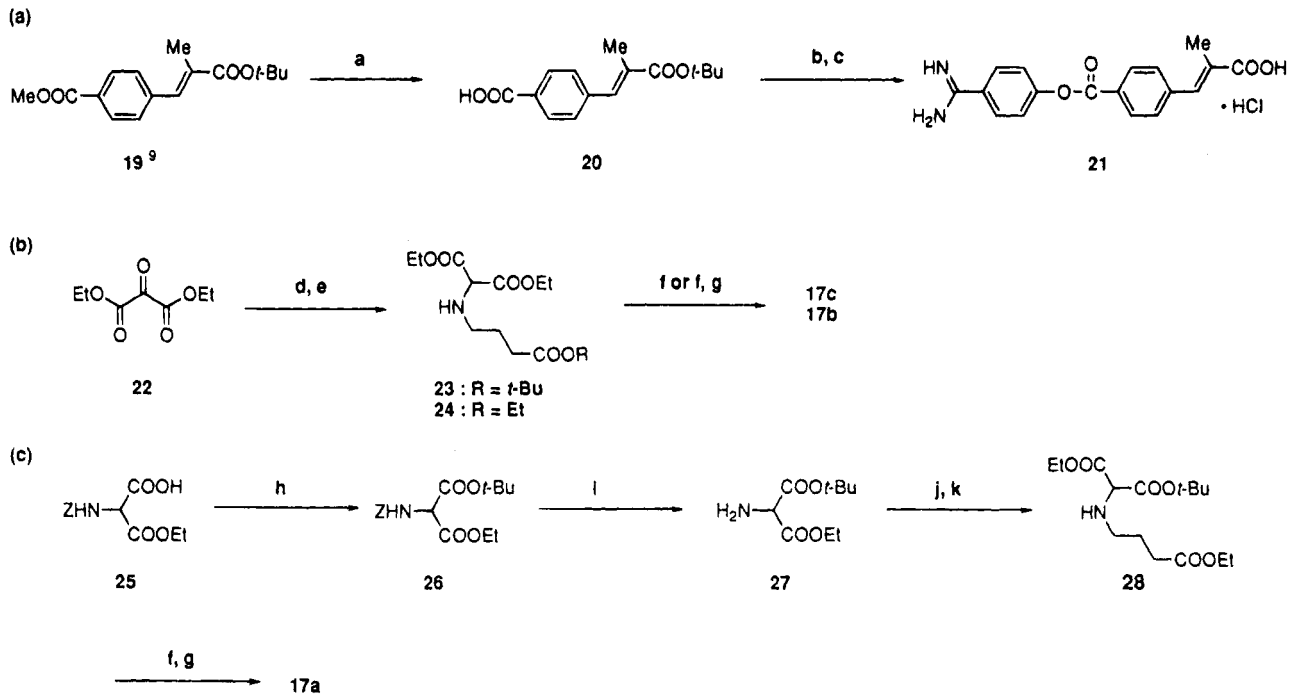
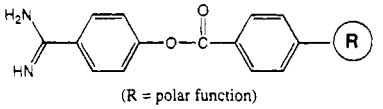
Scheme 1. Preparation of **17a-c**[a]



[a] Reagents: (a) N NaOH, THF, 25 °C, 5 h; (b) 4-amidinophenol hydrochloride, DCC, py, 25 °C, 15 h; (c) 4 N HCl–AcOEt, $CHCl_3$, 0–25 °C, 2 h; (d) alkyl 4-aminobutyrate, benzene, reflux under azeotropic conditions, 2 h; (e) 5% Pd–C, H_2, benzene, 25 °C 18 h; (f) **21**, DCC, py 25 °C, 17 h; (g) TFA, anisole, 25 °C, 2 h; (h) *t*-BuOH, 2-chloro-1-methylpyridinium iodide, diisopropylethylamine, CH_3CN, 25 °C, 2 h; (i) 5% Pd–C, H_2, EtOH, 25 °C, 18 h; (j) ethyl 3-bromocrotonate, diisopropylethylamine, CH_3CN, 25 °C, 12 h; (k) 10% Pd–C, H_2, $NaNO_2$, EtOH, 25 °C, 18 h.

Chart 2. General Formula of the Metabolically Stabilized Molecule



General Formula I

stabilized these molecules to immediate enzymatic inactivation. As expected, **5b** was relatively stabilized by the introduction of a polar carboxylic acid function into the *para*-position of the phenol ester moiety, although compounds **4**[7] and **5a**[7] were extremely unstable in the presence of rat small intestine homogenate (Figure 1, supporting information). Some benzoyl esters of 4-hydroxybenzamidine of general formula **I** (Chart 2) have long been known as potent trypsin inhibitors.[8] Despite its potent *in vitro* inhibitory activity, however, an orally active derivative has never been developed. The information described above for the metabolic stabilization of **2b**, **3**, and **5b** was expected to be applicable to the stabilization of the benzamidine derivative **I**.

Another difficult problem is the molecular design of good permeability through the mucous membrane of the intestine which is unpredictable. Much effort has been made to find orally active molecules among compounds corresponding to the general formula **I**. As reported in our previous paper, **1a,b** exhibited very potent anti-trypsin activity in rat plasma and long duration of action after their oral administration in rats, and oral absorption of **1b** in beagle dogs was confirmed. We felt that this biological method would allow for the rapid evaluation of oral activity in a series of trypsin inhibitors within the following two limitations.

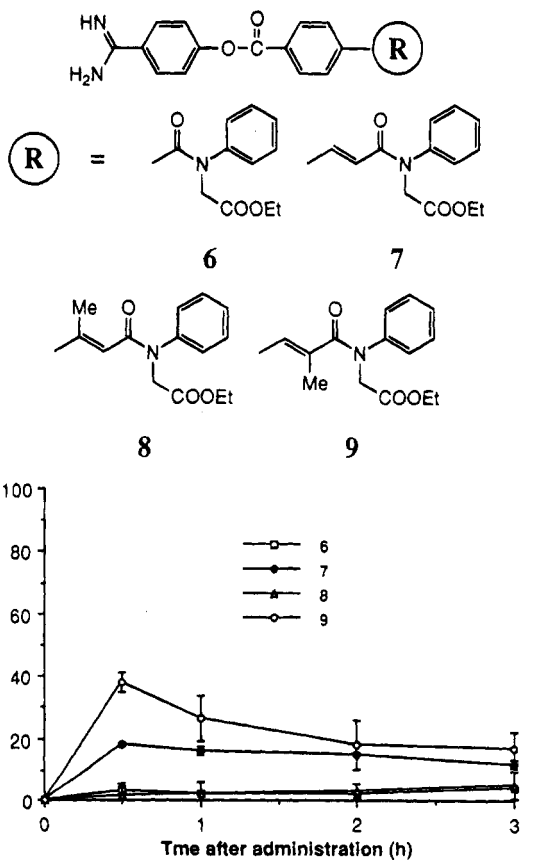


Figure 1. Design of the polar function. Plasma anti-trypsin activity in rats after oral administration of **6–9** (100 mg/kg). Values are means ± SE (n = 3). Metabolic study of all the compounds listed here was not carried out.

(a) All of this series of inhibitors showed nearly the same potency in an *in vitro* assay (IC_{50} 0.004–0.01 μM)

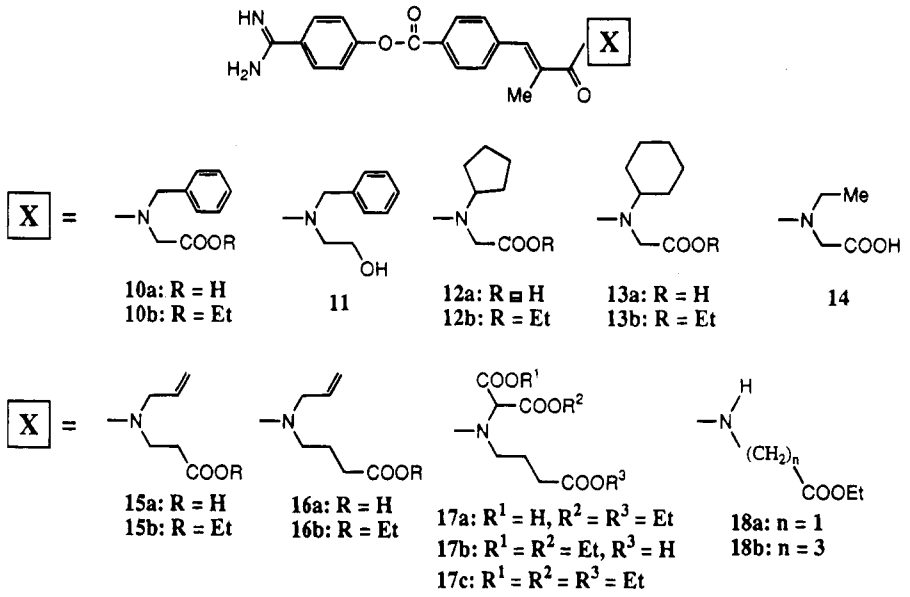


Figure 2. Further modification of the polar function.

because they possess a common 4-amidinophenyl benzoate moiety as a potency-determining function. Additionally, in the presence of plasma, all of the inhibitors listed in this paper also showed proportionally decreased potency (IC_{50} 0.3–0.6 μM) because of their presumed strong interaction with plasma proteins.

(b) Plasma anti-trypsin activity of these compounds in rats showed good dose dependency after their oral administration.[9]

The compounds prepared are listed in Figures 1 and 2. Introduction of an amide moiety as a polar function was useful not only for metabolic stabilization but also for increased solubility in water.[10] Additionally, the amide carbonyl moiety activates the reactivity of the phenol ester, increasing inhibitory activity. Among the compounds synthesized, a series of *N*-phenylglycine amide derivatives listed in Figure 1 showed poor to moderate plasma anti-trypsin activity after oral administration in rats. Insertion of an unsubstituted or substituted *trans*-double bond between the benzoyl group and the amide carbonyl moiety on **6** moderately improved plasma anti-trypsin activity (**7**–**9**). Compound **9** gave quite good results.

Further modification of the polar function (*N*-phenylglycine ethyl ester moiety) on **9** was made (Figure 2). To obtain better solubility in water, *N*-phenylglycine was converted to *N*-benzylglycine (**10a,b**) or *N*-alkylglycine (**12a**–**17c**). Compound **10b** exhibited similar plasma anti-trypsin activity to **9**, while the corresponding carboxylic acid **10a** showed dramatically high plasma anti-trypsin activity (Figure 3). Compound **10b** was presumed to be absorbed as an intact molecule and then metabolized to **10a** because it showed the same extent of metabolic stability ($t_{1/2}$ = 40–50 min) and profile[11] as **10a** in the rat small intestine homogenate (Table 1) and was converted into the active metabolite **10a**, which showed good metabolic stability ($t_{1/2}$ > 60 min), in the rat plasma within 1 min.

Compound **11**, in which the carboxylic acid function of **10a** was reduced to the corresponding alcohol, showed very poor inhibitory activity in plasma. The carboxylic acid function on **10a** was thought to be necessary to obtain high anti-trypsin activity in plasma which corresponds to good oral absorption and good stability in plasma and the liver (Figure 3).[12]

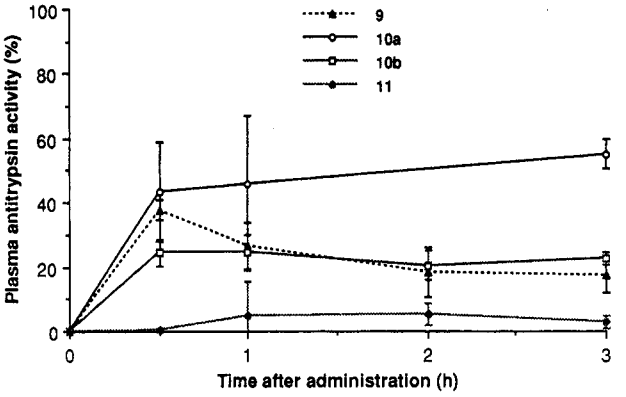


Figure 3. Plasma anti-trypsin activity in rats after oral administration of **9**, **10a,b**, and **11** (100 mg/kg). Values are means ± SE (n = 3).

This result was further confirmed by the following experimental results. Although both compounds **12a** and **13a** exhibited high anti-trypsin activity in plasma, the corresponding ethyl esters **12b** and **13b** showed much lower inhibitory activity in plasma (Figure 4). These results were found to be closely related to the metabolism of the synthesized inhibitors **12b** and **13b** in the small intestine or plasma (Figure 1, supporting information and Table 1).

Clear differences were seen in **12b** and **13b** compared to **12a** and **13a** with regard to their metabolism in the small intestine. Both **12b** and **13b** were inactivated and not converted to **12a** and **13a**, respectively, in the small intestine (Table 1), whereas in plasma, both compounds were rapidly converted to **12a** and **13a**, respectively. As a result, compounds **12b** and **13b** were also considered to be absorbed through the same process as **10b**.

Ethyl ester derivatives **15b** and **16b**, in which the distance between the amide nitrogen and the carboxylic acid function was lengthened, also afforded the corresponding carboxylic acids **15a** and **16a**, upon treatment with rat small intestine homogenate or plasma (Figure 1, supporting information and Table 1). Oral administration of **15b** and **16b** gave high anti-trypsin activity in plasma and long duration of action (Figure 5).

Table 1. *In Vitro* Metabolic Study in the Rat Small Intestine Homogenate and Plasma[a]

	$t_{1/2}$(min)			
compd	small intestine homogenate ($n = 2$)	product	plasma ($n = 2$)	product
10a	50	AP[b] + C[c]	>60	NT[d]
10b	44	AP + C	<1	10a
11	41	AP + C	<5	AP
12a	42	AP + C	23	AP
12b	>60	AP + C	<5	12a
13a	49	AP + C	>60	NT
13b	>60	AP + C	<5	13a
14	>60	AP + C	60	AP
15a	>60	AP + C	47	AP
15b	e	15a	<1	15a
16a	55	AP + C	>60	NT
16b	e	16a	<1	16a
17a	NT	NT	14	AP
17b	NT	NT	29	AP
17c	e	**17a + 17b**	<1	**17a + 17b**

[a] Values are the means from two separate experiments. Details of HPLC analyses are described in the Experimental Section. [b] AP = 4-amidinophenol. [c] C = the corresponding carboxylic acid. [d] NT = not tested. [e] See Figures 7 and 8. Structures of all the metabolites were confirmed on the basis of the t_Rs of the synthesized authentic samples.

Interestingly, the triester derivative **17c** gave a mixture of monocarboxylic acids **17a,b** when treated with rat small intestine homogenate (Figure 1, supporting information). As expected, oral administration of compound **17c** to rats also gave high anti-trypsin activity in plasma (Figure 6).

On the basis of the described evidence above, we concluded that at least the following general formula **II** is required for this series of inhibitors to show high plasma anti-trypsin activity. The carboxylic acid function was absolutely required for rat plasma stability and the presumed better mucous membrane permeability. R should be a less hindered alkyl, alkenyl, or arylalkyl group. Hydrogen was not accepted as an R group to obtain high plasma anti-trypsin activity because **18a,b** showed less plasma anti-trypsin activity (Figure 5).[13] X should be a methylene or branched ester (**17a,b**). Further structural studies related to R and X will be the subject of future reports.

As typical examples, **14** and **16b** were administered orally to rats and their plasma concentrations were studied. Both compound **14** and **16b** showed good area under the plasma concentration–time curves (AUC) and long duration of action (Figures 7 and 8). The only active compound detected in the plasma was **14** or **16a** in each case. These data shown in Figures 7 and 8 corresponded well to the anti-trypsin activities of these compounds in plasma (Figure 5).

Summary and Conclusions

In conclusion, we have discovered a series of orally active serine protease inhibitors and disclosed some of the structural requirements for good oral activity. Inhibitors possessing general formula **II** were found to show good oral activity, presumably because of good permeability through the mucous membrane and high metabolic stability in both the intestine and plasma. It seems plausible that the major absorption through the mucous membrane of the ethyl ester derivatives **1b**, **15b**, **16b**, and **17c** took place after their enzymatic conversion to the corresponding carboxylic acids **1a**, **15a**, **16a**, and **17a,b**. The poorly absorbed intact molecules,

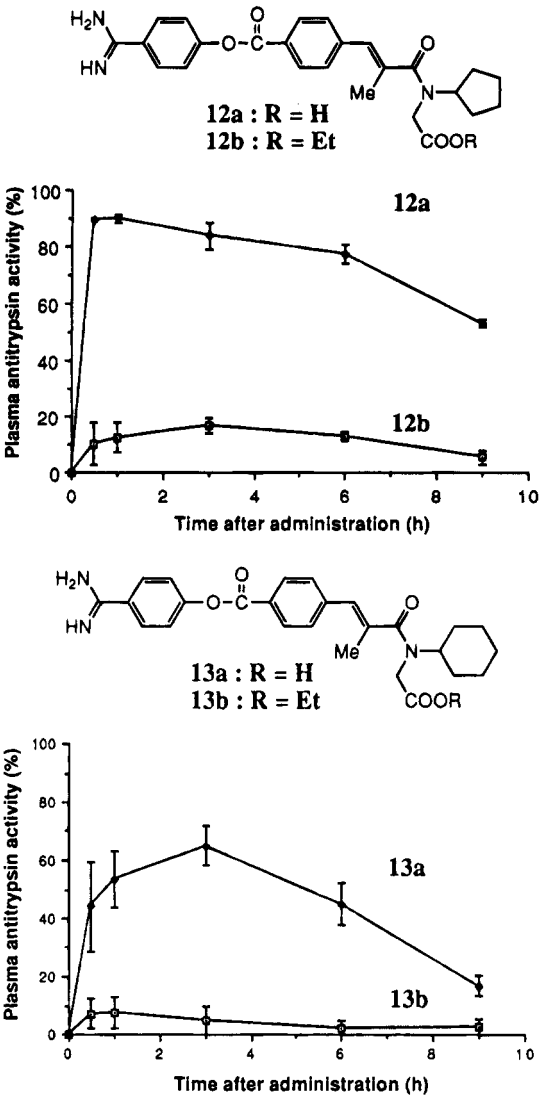


Figure 4. Plasma anti-trypsin activity in rats after oral administration of **12a,b** and **13a,b** (100 mg/kg). Values are means ± SE ($n = 3$).

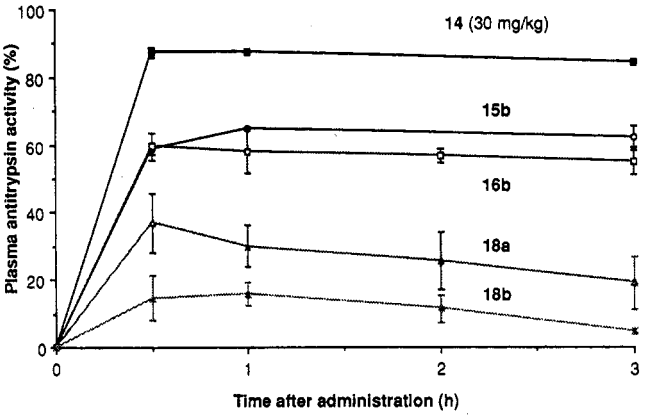


Figure 5. Plasma anti-trypsin activity in rats after oral administration of **14** (30 mg/kg), **15b**, **16b**, and **18a,b** (100 mg/kg). Values are means ± SE ($n = 3$).

whose permeability is presumed to be much less than that of the corresponding carboxylic acid derivatives, were rapidly converted to their respective active metabolites **1a**, **15a**, **16a**, and **17a,c** in plasma.

Compounds **10b**, **12b**, and **13b** showed a unique profile in their metabolism. On the basis of metabolic studies, these compounds seem to be absorbed as intact

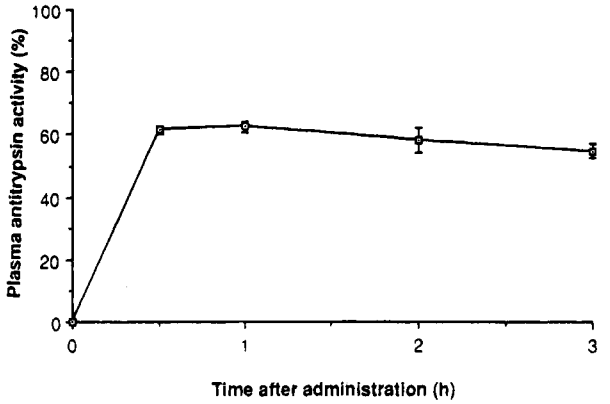
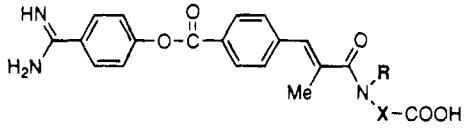


Figure 6. Plasma anti-trypsin activity in rats after oral administration of **17c** (100 mg/kg). Values are means ± SE (n = 3).

Chart 3. Structure Required for Good Oral Activity



General Formula II

molecules and are then converted into **10a**, **12a**, and **13a**, respectively, in plasma. The specificities of the enzymatic hydrolysis of these inhibitors were also disclosed (Table 1). Bulky N-alkyl groups such as N-benzyl in **10b**, N-cyclopentyl in **12b**, and N-cyclohexyl in **13b** were thought to block the hydrolysis of the ethyl ester function in the small intestine to give very low plasma anti-trypsin activity. Biological evaluation of oral activity of the trypsin inhibitors using a synthetic chromogenic substrate was useful for rapid evaluation. Further details of structure–oral activity relationships in this series of inhibitors will be the subject of future reports. The mechanisms of action of these inhibitors using X-ray analysis will also be reported elsewhere.

Experimental Section

Chemistry. General Directions. All ^{1}H and ^{13}C NMR spectra were obtained using a JOEL FX-90Q or Varian VXR-200s or 500s spectrometer. Mass spectra were obtained on a JEOL JMS-DX-303HF spectrometer. IR spectra were measured on Perkin Elmer FT-IR 1760X. Melting points are uncorrected. Column chromatography was carried out on silica gel (E. Merck; particle size 0.063–0.02 mm). Thin layer chromatography was performed on silica gel (Merck Art. No. 5715). HPLC was carried out using a HITACHI L-4000H UV detector and L-6000 pump equipped with a YMC AM-312 ODS column (S-5 μm, 120 Å, 150 × 6 mm i.d.) at 265 nm. All solvents were distilled before use. High-resolution mass spectra of all compounds were within ±3 mu of the theoretical values. 6-Amidino-2-naphthyl 4-[(4,5-dihydro-1H-imidazol-2-yl)amino]benzoate dimethanesulfonate (**3**, FUT-187), 4-guanidinophenyl benzoate (**4**), and 4-amidinophenyl benzoate (**5a**) were prepared according to the reported methods.[4,7]

General Procedure A: Preparation of *N*-Allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]-glycine ethyl ester hydrochloride (1b). Step 1: 4-(Benzyloxycarbonyl)-α-methylcinnamic acid. To a stirred suspension of sodium hydride (183 mmol) in THF (225 mL) was added a solution of *tert*-butyl 2-(diethoxyphosphinyl)-propionate (48.0 g, 180 mmol) in THF (60 mL) dropwise at 5 °C. After stirring for 30 min at 25 °C, a solution of benzyl 4-formylbenzoate (42.9 g, 179 mmol) in THF (165 mL) was added dropwise to the resulting solution at 5 °C. After stirring for an additional 30 min at 25 °C, the reaction mixture was poured into ice-cooled water and extracted with ethyl acetate. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried, and concentrated. Purification by chromatography on silica gel (n-hexane:ethyl acetate = 15:1) gave 56.1 g (89%) of *tert*-butyl 4-(benzyloxycarbonyl)-α-methylcinnamate as a colorless oil: Rf = 0.79 (n-hexane:ethyl acetate = 4:1).

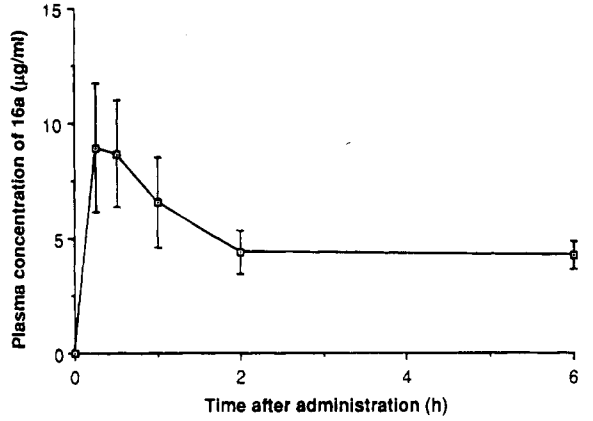


Figure 7. Plasma concentration of **16a** in rats after oral administration of **16b** (100 mg/kg). Values are means ± SD (n = 5).

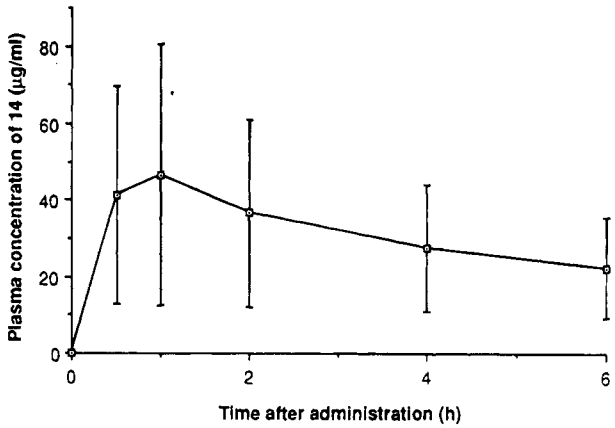


Figure 8. Plasma concentration of **14** in rats after its oral administration (50 mg/kg). Values are means ± SD (n = 4).

The *tert*-butyl ester obtained (56.1 g, 159 mmol) was dissolved in anisole (40 mL) and treated with trifluoroacetic acid (75 mL) at 25 °C. After 2 h, the resulting solution was evaporated, and the residue was triturated with n-hexane/isopropyl ether and washed with isopropyl ether to yield 39.6 g (84%) of the title compound as a white powder: R_f = 0.25 (n-hexane:ethyl acetate:acetic acid = 12:4:1); mp 130.0–131.0 °C; ^{1}H NMR ($CDCl_3$) δ 2.15 (3H, s), 5.40 (2H, s), 7.35–7.55 (7H, m), 7.83 (1H, s), 8.12 (2H, d, J = 8.0 Hz); MS m/e 296 (M^+); IR (KBr) 3449, 1719, 1687 cm^{-1}.

Step 2: *N*-Allyl-*N*-[4-(benzyloxycarbonyl)-α-methylcinnamoyl]glycine Ethyl Ester. A mixture of 4-(benzyloxycarbonyl)-α-methylcinnamic acid (30.0 g, 100 mmol) and thionyl chloride (200 mL) was heated at reflux for 1 h and evaporated. To a stirred solution of ethyl N-allylglycinate (14.1 g, 100 mmol) in pyridine–CH_2Cl_2 (100 mL–100 mL) was added dropwise a solution of the acid chloride in CH_2Cl_2 (100 mL) at 5 °C. After 2 h at 25 °C, the mixture was poured into cold water and extracted with ethyl acetate. The organic layer was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated. The crude residue was chromatographed on silica gel (n-hexane:ethyl acetate = 4:1) to yield 39.0 g (90%) of the title compound as a pale yellow oil: R_f = 0.35 (n-hexane:ethyl acetate = 2:1); ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.5 Hz), 2.10 (3H, s), 4.13 (4H, s), 4.21 (2H, q, J = 7.5 Hz), 5.10–5.10 (2H, m), 5.38 (2H, s), 5.81 (1H, t of dd, J = 5.0, 12 Hz), 6.83 (1H, brs), 7.23–7.50 (7H, m), 8.07 (2H, d, J = 8.0 Hz); MS m/e 421 (M^+).

Step 3: *N*-Allyl-*N*-(4-carboxy-α-methylcinnamoyl)glycine Ethyl Ester. To a stirred solution of the benzyl ester obtained (3.0 g, 13.0 mmol) in anisole (28 mL) was added methanesulfonic acid (13.9 mL, 214 mmol) at 25 °C. After 2 h, the reaction mixture was poured into ice–water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated. The crude residue was purified by silica gel chromatography (*n*-hexane:ethyl acetate = 4:1) to yield 2.93 g (68%) of the carboxylic acid as a brown oil: R_f = 0.19 (*n*-hexane:ethyl acetate = 3:2); ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, *J* = 7.5 Hz), 2.18 (3H, s), 4.15 (4H, s), 4.22 (2H, q, *J* = 7.5 Hz), 5.15–5.40 (2H, m), 5.82 (1H, t of dd, *J* = 5.0, 12 Hz), 6.69 (1H, brs), 7.40 (2H, d, *J* = 8.0 Hz), 8.10 (2H, d, *J* = 8.0 Hz); MS *m/e* 331 (M^+); IR (neat) 2984, 1756, 1689, 1650, 1294 cm^{-1}.

Step 4: *N*-Allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]glycine Ethyl Ester. To a stirred solution of 4-amidinophenol hydrochloride (31.5 g, 183 mmol) and the carboxylic acid obtained in pyridine (200 mL) was added 1,3-dicyclohexylcarbodiimide (44.5 g, 216 mmol) at 25 °C. After 15 h, the resulting urea was removed by filtration, and the filtrate was evaporated. The residue was chromatographed on silica gel (chloroform:methanol:acetic acid = 50:2:1) to yield 47.7 g (64%) of hydrochloride salt of **1b** as a white amorphous solid.

To a stirred solution of the hydrochloride salt (47.7 g, 98 mmol) in chloroform (500 mL) was added methanesulfonic acid (6.35 mL, 98 mmol) at 5 °C. After 1 h at 25 °C, evaporation of the resulting solution was repeated several times to remove volatile hydrochloric acid. The residue was solidified with diethyl ether to afford **1b** quantitatively as a white amorphous solid: R_f = 0.36 (chloroform:methanol:acetic acid = 10:2:1); mp 135–136 °C; ^{1}H NMR (CD_3OD) δ 1.30 (3H, brt), 2.15 (3H, brs), 2.70 (3H, s, CH_3SO_3H), 4.05–4.30 (6H, m), 5.20–5.40 (2H, m), 5.90 (1H, m), 6.70 (1H, m), 7.50–7.60 (4H, m),7.90 (2H, d, *J* = 8.0 Hz), 8.20 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 176.92, 171.38, 168.69, 166.40, 157.72, 143.78, 137.30, 135.25, 132.28, 131.64, 131.42, 130.70, 129.97, 128.11, 124.98, 119.65, 63.31, 54.98, 40.40, 17.22, 15.40; IR (KBr) 3279, 1741, 1679, 1266 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{27}N_3O_5$ + H^+ 450.5031, found 450.2018.

Ethyl Esters of General Formula II. The title compounds **6**–9, **10b**, **11**, **12b**, **13b**, **15b**, **16b**, and **18a,b** were prepared according to general procedure A described in the preparation of **1b**.

***N*-[4-[(4-Amidinophenoxy)carbonyl]benzoyl]-*N*-phenylglycine ethyl ester acetate (6):** R_f = 0.49 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 160–163 °C; ^{1}H NMR (CD_3OD) δ 1.35 (3H, t, *J* = 7.0 Hz), 1.95 (3H, s, CH_3CO_2H), 4.25 (2H, q, *J* = 7.0 Hz), 4.65 (2H, s), 7.10–7.35 (5H, m), 7.45–7.60 (4H, m), 7.90 (2H, d, *J* = 9.0 Hz), 8.05 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 170.34, 164.95, 156.58, 144.29, 131.14, 130.75, 130.67, 130.43, 130.36, 129.95, 129.87, 129.58, 129.31, 128.92, 128.76, 128.54, 127.27, 123.98, 123.02, 117.12, 62.57, 53.28, 14.51; IR (KBr) 3270, 1746, 1681, 1635,1608, 1598, 1570 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{23}N_3O_5$ + H^+ 446.1716, found 446.1712.

***N*-[4-[(4-Amidinophenoxy)carbonyl]cinnamoyl]-*N*-phenylglycine ethyl ester hydrochloride (7):** R_f = 0.59 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 201–205 °C; ^{1}H NMR (CD_3OD) δ 1.25 (3H, t, *J* = 7.5 Hz), 4.20 (2H, q, *J* = 7.5 Hz), 4.52 (2H, s), 6.58 (1H, d, *J* = 15.0 Hz), 7.40–7.60 (9H, m), 7.69 (1H, d, *J* = 15.0 Hz), 7.90 (2H, d, *J* = 8.0 Hz), 8.15 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 170.33, 167.63, 165.09, 156.65, 143.33, 142.02, 141.52, 131.73, 131.61, 130.95, 130.84, 130.64, 129.71, 129.20, 129.05, 127.07, 124.01, 123.89, 122.18, 122.12, 62.44, 52.87, 14.56; IR (KBr) 3099, 1744, 1660, 1607, 1494, 1418, 1382 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{25}N_3O_5$ + H^+ 472.1873, found 472.1861.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-β-methylcinnamoyl]-*N*-phenylglycine ethyl ester hydrochloride (8):** R_f = 0.44 ($CHCl_3$:MeOH:AcOH = 15:2:1); mp 209–210 °C; ^{1}H NMR (CD_3OD) δ 1.30 (3H, t, *J* = 7.5 Hz), 2.42 (3H, s), 4.22 (2H, q, *J* = 7.5 Hz), 4.50 (2H, s), 6.10 (1H, brs), 7.40 (2H, d, *J* = 8.0 Hz), 7.41 (5H, s), 7.50 (2H, d, *J* = 7.5 Hz), 7.92 (2H, d, *J* = 8.0 Hz), 8.10 (2H, d, *J* = 7.5 Hz); ^{13}C NMR (CD_3OD) δ 170.54, 169.20, 167.66, 165.23, 156.69, 148.85, 148.61, 143.81, 131.39, 131.33, 130.63, 129.78, 129.30, 128.74, 127.39, 127.03, 123.96, 122.53, 62.44, 52.38, 18.05, 14.53; IR (KBr) 3259, 1746, 1737, 1674, 1633, 1606, 1492, 1373 cm^{-1}; HRMS (FAB) calcd for $C_{28}H_{27}N_3O_5$ + H^+ 486.2029, found 486.2018.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-phenylglycine ethyl ester methanesulfonate (9):** R_f = 0.47 ($CHCl_3$:MeOH:AcOH = 15:2:1); mp 178–180 °C; ^{1}H NMR (CD_3OD) δ 1.30 (3H, t, *J* = 7.5 Hz), 1.90 (3H, brs), 2.71 (3H, s, CH_3SO_3H), 4.22 (2H, q, *J* = 7.5 Hz), 4.55 (2H, s), 6.68 (1H, brs), 7.23 (2H, d, *J* = 8.0 Hz), 7.30–7.41 (5H, m), 7.50 (2H, d, *J* = 8.0 Hz), 7.91 (2H, d, *J* = 8.0 Hz), 8.10 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 174.77, 170.46, 167.67, 165.31, 156.72, 144.68, 142.84, 136.48, 133.77, 131.15, 130.66, 130.46, 130.13, 128.91, 128.75, 128.27, 39.50 (CH_3SO_3H), 16.42, 144.54; IR (KBr) 3387, 1743, 1651, 1607, 1493, 1418 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{29}N_2O_8$ + H^+ 486.5094, found 486.2008.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-benzylglycine ethyl ester hydrochloride (10b):** R_f = 0.43 ($CHCl_3$:MeOH:AcOH = 15:2:1); mp 104–106 °C; ^{1}H NMR (CD_3OD) δ 1.20–1.35 (3H, m), 2.15 (3H, s), 4.00–4.21 (4H, m), 4.70–4.82 (2H, m), 6.75 (1H, brs), 7.40 (5H, brs), 7.55 (4H, brd, *J* = 7.5 Hz), 7.95 (2H, d, *J* = 8.0 Hz), 8.10 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 176.15, 170.23, 167.70, 165.38, 156.75, 142.76, 137.60, 136.43, 131.29, 131.09, 130.76, 130.68, 130.42, 130.08, 129.97, 129.77, 129.41, 129.39, 128.98, 128.92, 128.86, 128.84, 128.44, 127.08, 124.01, 123.07, 117.06, 62.40, 54.91, 51.02, 50.46, 48.12, 16.38, 14.50; IR (KBr) 3352, 1741, 1678, 1606, 1480, 1411 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{29}N_3O_5$ + H^+ 500.5625, found 500.2193.

2-[*N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-benzylamino]ethanol acetate (11): R_f = 0.55 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 164–168 °C; ^{1}H NMR (CD_3OD) δ 1.95 (3H, s, CH_3CO_2H), 2.10–2.15 (3H, m), 3:54 (2H, t, *J* = 5.0 Hz), 3,73 (2H, brs), 4.80 (2H, brs), 6.69 (1H, s), 7.31–7.38 (5H, m), 7.52 (4H, d, *J* = 9.0 Hz), 7.93 (2H, d, *J* = 9.0 Hz), 8.18 (2H, d, *J* = 9.0 Hz); ^{13}C NMR (CD_3OD) δ 176.01, 167.62, 165.35, 156.69, 143.06, 138.35, 137.11, 131.24, 130.63, 130.38, 129.80, 129.36, 128.78, 128.59, 128.10, 127.02, 123.95, 60.44, 59.85, 54.96, 51.27, 16.81; IR (KBr) 3412, 1737, 1678, 1607, 1475, 1452 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{27}N_3O_4$ + H^+ 458.2080, found 458.2092.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-cyclopentylglycine ethyl ester acetate (12b):** R_f = 0.31 ($CHCl_3$:MeOH:AcOH = 20:2:1); mp 138–141 °C; ^{1}H NMR (CD_3OD) δ 1.10–1.20 (3H, m), 1.45–1.80 (6H, m), 1.80–2.05 (ca. 7H, m), 2.05–2.25 (3H, m), 3.95–4.30 (5H, m), 4.30–4.60 (2H, m), 6.60–6.70 (1H, brs), 7.40–7.65 (4H, m), 7.83–7.96 (2H, m), 8.13–8.28 (2H, m); ^{13}C NMR (CD_3OD) δ 175.64, 171.01, 167.87, 165.54, 156.86, 143.07, 137.31, 131.41, 130.73, 130.68, 130.52, 128.99, 127.22, 124.08, 62.38, 62.64, 44.44, 30.74, 24.84, 16.48, 14.51; IR (KBr) 3500, 1742, 1682, 1605, 1494, 1454, 1407 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{31}N_3O_5$ + H^+ 478.5550, found 478.2351.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-cyclopentylglycine ethyl ester acetate (13b):** R_f = 0.43 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 140–142 °C; ^{1}H NMR (CD_3OD) δ 1.05–1.75 (9H, m), 1.80–2.00 (4H, m), 1.95 (ca. 4H, s, CH_3CO_2H), 2.15 (3H, brs), 3.80 (1H, m), 4.05–4.25 (4H, m), 6.65 (1H, brs), 7.50–7.60 (4H, m), 7.90 (2H, d, *J* = 8.0 Hz), 8.25 (2H, d, *J* = 9.5 Hz); ^{13}C NMR (CD_3OD) δ 175.39, 170.91, 167.86, 165.53, 156.83, 143.07, 137.28, 131.45, 131.32, 131.26, 131.08, 131.03, 130.72, 130.45, 129.00, 128.77, 127.28, 124.08, 123.15, 62.28, 60.19, 44.15, 32.18, 26.80, 26.48, 26.11, 22.95, 16.42, 14.50; IR (KBr) 3415, 1737, 1607, 1411 cm^{-1}; HRMS (FAB) calcd for $C_{28}H_{33}N_3O_5$ + H^+ 492.5809, found 492.2492.

Ethyl 3-[*N*-allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]amino]propionate acetate (15b): R_f = 0.56 ($CHCl_3$:MeOH:AcOH = 10:2:1); ^{1}H NMR (CD_3OD) δ 1.25 (3H, m), 1.95 (3H, s, CH_3CO_2H), 2.10 (3H, s), 2.70 (2H, m), 3.75 (2H, m), 4.05–4.20 (4H, m), 5.20–5.40 (2H, m), 5.90 (1H, m), 6.65 (1H, brs), 7.50–7.60 (4H, m), 7.90 (2H, d, *J* = 7.5 Hz), 8.20 (2H, d, *J* = 7.5 Hz); ^{13}C NMR (CD_3OD) δ 180.48, 175.76, 167.88, 166.41, 165.55, 164.10, 163.04, 156.82, 143.07,

137.03, 133.24, 133.21, 131.50, 131.48, 131.37, 131.32, 131.13, 131.04, 130.72, 130.67, 130.52, 129.28, 129.00, 127.33, 124.08, 120.80, 118.99, 118.41, 106.07, 105.86, 105.61, 62.15, 62.05, 611.85, 60.48, 60.42, 53.99, 52.47, 53.24, 52.47, 45.79, 42.56, 42.45, 33.70, 32.53, 23.45, 16.48, 14.50; IR (KBr) 3402, 1735, 1608, 1553, 1447, 1417 cm^{-1}; HRMS (FAB) calcd for $C_{26}H_{29}N_3O_5$ + H^+ 464.2186, found 464.2203.

Ethyl 4-[*N*-allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]amino]butyrate methanesulfonate (16b): R_f = 0.43 ($CHCl_3$:MeOH:AcOH = 15:2:1); mp 112–116 °C; ^1H NMR (CD_3OD) δ 1.24 (3H, brt), 1.94 (3H, s, CH_3CO_2H), 1.85–2.00 (2H, m), 2.13 (3H, s), 2.38 (2H, brt), 3.48 (2H, t, *J* = 7.0 Hz), 4.10 (4H, m), 5.20–5.35 (2H, m), 5.80–6.00 (1H, m), 6.64 (1H, s), 7.52 (2H, d, *J* = 8.5 Hz), 7.56 (2H, d, *J* = 9.0 Hz), 7.92 (2H, d, *J* = 9.0 Hz), 8.22 (2H, d, *J* = 8.5 Hz); ^{13}C NMR (CD_3OD) δ 179.82, 175.50, 167.69, 165.45, 156.67, 143.00, 137.08, 134.63, 133.80, 131.29, 131.00, 130.60, 130.42, 128.83, 127.17, 123.95, 118.40, 117.09, 61.45, 52.60, 45.16, 32.31, 24.68, 23.36, 16.59, 14.51; IR (KBr) 3256, 1743, 1677, 1606, 1485, 1410 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{31}N_3O_5$ + H^+ 478.5550, found 478.2359.

***N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]glycine ethyl ester methanesulfonate (18a):** R_f = 0.35 ($CHCl_3$:MeOH:AcOH = 15:2:1); mp 162–167 °C; ^1H NMR (CD_3OD) δ 1.30 (3H, t, *J* = 7.0 Hz), 2.15 (3H, s), 2.69 (ca. 5H, s, CH_3SO_3H), 4.03 (2H, s), 4.21 (2H, q, *J* = 7.0 Hz), 7.41 (1H, s), 7.54 (2H, d, *J* = 8.5 Hz), 7.60 (2H, d, *J* = 8.5 Hz), 7.93 (2H, d, *J* = 8.5 Hz), 8.23 (2H, d, *J* = 8.5 Hz); ^{13}C NMR (CD_3OD) δ 171.92, 171.20, 167.47, 165.25, 156.62, 143.27, 135.44, 133.83, 131.22, 131.03, 130.72, 130.64, 129.08, 127.02, 123.95, 117.05, 62.19, 42.49, 39.57, 14.67, 14.59; IR (KBr) 3402, 1738, 1683, 1652, 1609, 1536, 1498, 1412 cm^{-1}; HRMS (FAB) calcd for $C_{22}H_{23}N_3O_5$ + H^+ 410.1716, found 410.1740.

Ethyl 4-[*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]amino]butyrate acetate (18b): R_f = 0.31 ($CHCl_3$:MeOH:AcOH = 15:2:1); mp 175–177 °C; ^1H NMR (CD_3OD) δ 1.22 (3H, t, *J* = 7.5 Hz), 1.90 (2H, tt, *J* = 8.0 Hz), 2.00 (6H, s, CH_3CO_3H), 2.1 (3H, s), 2.40 (2H, t, *J* = 7.5 Hz), 3.35 (2H, t, *J* = 8.0 Hz), 4.12 (2H, q, *J* = 7.5 Hz), 7.31 (1H, brs), 7.53 (2H, d, *J* = 8.0 Hz), 7.59 (2H, d, *J* = 8.0 Hz), 7.95 (2H, d, *J* = 8.0 Hz), 8.21 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 175.00, 174.79, 171.88, 167.57, 165.28, 156.69, 143.47, 136.05, 133.08, 131.19, 131.13, 130.66, 130.61, 130.54, 128.98, 126.96, 123.94, 61.48, 40.24, 32.53, 25.70, 20.94, 14.76, 14.58; IR (KBr) 3304, 1735, 1682, 1649, 1605, 1529, 1490, 1412 cm^{-1}; HRMS (FAB) calcd for $C_{24}H_{27}N_3O_5$ + H^+ 438.2029, found 438.2029.

General Procedure B: Preparation of *N*-Allyl-*N*-[4-[(2-amidinophenoxy)carbonyl]-α-methylcinnamoyl]glycine Methanesulfonate (1a). The solution of *N*-allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]glycine *tert*-butyl ester acetate prepared according to general procedure A (166 g, 310 mmol) in trifluoroacetic acid (1000 g) was stirred at 25 °C for 1.5 h. After evaporation, the residue was solidified with diethyl ether to afford 144 g (87%) of trifluoroacetic acid salt of **1a** as a white amorphous solid. The resulting trifluoroacetic acid salt was converted to the methanesulfonic acid salt as described for **1b** to afford **1a** quantitatively as a white amorphous solid: R_f = 0.18 (chloroform:methanol:acetic acid = 20:2:1); mp 134–136 °C; ^1H NMR (CD_3OD) δ 2.20 (3H, brt), 2.69 (3H, s, CH_3SO_3H), 4.08–4.22 (4H, m), 5.20–5.36 (2H, m), 6.06–6.55 (1H, m), 7.48–7.60 (4H, m), 7.95 (2H, d, *J* = 8.0 Hz), 8.20 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 13.32, 39.49 ($MeSO_3H$), 47.32, 53.98, 118.72, 124.10, 127.22, 129.05, 129.74, 130.54, 130.76, 131.31, 131.38, 134.38, 136.53, 143.01, 156.88, 165.55, 167.86, 172.07, 176.03; IR (KBr) 3101, 1736, 1687, 1266 cm^{-1}; HRMS (FAB) calcd for $C_{23}H_{23}N_3O_5$ + H^+ 422.4512, found 422.1736.

Compounds of General Formula II. The title compounds **10a**, **12a**, **13a**, **14**, **15a**, and **16a** were prepared according to general procedure B described in the preparation of **1a**.

***N*-[4-[(4-Amidinophenoxy)carbonyl)-α-methylcinnamoyl]-*N*-benzylglycine methanesulfonate (10a):** R_f = 0.36 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 140–141 °C; ^1H NMR (CD_3OD) δ 2.15 (3H, s), 2.70 (ca. 5H, s, CH_3SO_3H), 4.10 (2H, brs), 4.75 (2H, m), 6.75 (1H, m), 7.45–7.60 (4H, m), 7.95 (2H, d, *J* = 8.5 Hz), 8.20 (2H, d, *J* = 8.5 Hz); ^{13}C NMR (CD_3OD) δ 176.29, 171.91, 167.86, 165.54, 156.88, 142.97, 137.79, 136.72, 131.36, 130.76, 130.49, 130.06, 130.01, 129.99, 129.94, 129.91, 129.89, 129.36, 129.06, 128.98, 128.95, 128.94, 128.48, 127.20, 124.08, 54.83, 50.62, 50.32, 47.67, 39.52, 16.33; IR (KBr) 3449, 1736, 1686, 1655, 1639, 1475, 1459 cm^{-1}; HRMS (FAB) calcd for $C_{27}H_{25}N_3O_5$ + H^+ 472.5106, found 472.1902.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-cyclopentylglycine methanesulfonate (12a):** R_f = 0.16 ($CHCl_3$:MeOH:AcOH = 20:2:1); mp 190–192 °C; ^1H NMR (CD_3OD) δ 1.50–1.80 (6H, m), 1.80–2.00 (2H, m), 2.17 (3H, s), 2.70 (ca. 6H, s, CH_3SO_3H), 4.05 (2H, s), 4.30–4.50 (1H, m), 6.69 (1H, s), 7.50–7.70 (4H, m), 7.92 (2H, d, *J* = 8.0 Hz), 8.22 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 175.64, 172.58, 167.85, 165.57, 156.87, 143.15, 137.43, 131.38, 130.75, 130.51, 129.19, 127.20, 124.08, 61.66, 43.99, 39.49, 30.74, 24.87, 16.48; IR (KBr) 3372, 1737, 1686, 1606, 1484, 1459, 1411 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{27}N_3O_5$ + H^+ 450.5031, found 450.2041.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-cyclohexylglycine methanesulfonate (13a):** R_f = 0.12 ($CHCl_3$:MeOH:AcOH = 20:2:1); mp 205–206 °C; ^1H NMR (CD_3OD) δ 1.05–1.95 (10H, m), 2.10–2.20 (3H, m), 2.70 (ca. 5H, s, CH_3SO_3H), 3.70–3.90 (1H, m), 4.00–4.20 (2H, m), 6.67 (1H, s), 7.53 (2H, d, *J* = 8.0 Hz), 7.57 (2H, d, *J* = 8.0 Hz), 7.93 (2H, d, *J* = 8.0 Hz), 8.23 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 175.39, 171.35, 167.86, 165.56, 165.50, 156.88, 156.85, 143.24, 143.15, 138.30, 131.44, 131.30, 131.00, 130.75, 130.44, 127.23, 127.20, 124.08, 60.24, 43.79, 39.49, 32.20, 26.83, 26.16, 16.41, 14.35; IR (KBr) 3500–2700, 1737, 1688, 1607, 1587, 1489, 1457, 1411 cm^{-1}; HRMS (FAB) calcd for $C_{26}H_{29}N_3O_5$ + H^+ 464.5290, found 464.2164.

***N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-ethylglycine methanesulfonate (14):** R_f = 0.35 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 215–217 °C; ^1H NMR (CD_3OD) δ 1.22 (3H, br), 2.10 and 2.15 (3H, brs, each), 2.72 (3H, s, CH_3SO_3H), 3.57 (2H, br), 4.17 and 4.24 (2H, brs, each), 6.67 and 6.72 (1H, brs, each), 7.47–7.70 (4H, m), 7.93 (2H, d, *J* = 8.0 Hz), 8.22 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 175.67, 172.20, 167.78, 165.50, 156.84, 143.07, 136.97, 131.36, 131.29, 130.98, 130.73, 130.48, 129.52, 129.24, 128.96, 127.13, 124.05, 97.25, 51.30, 47.09, 46.15, 43.04, 39.49, 20.73, 16.38, 14.16, 12.45; IR (KBr) 3436, 1734, 1676, 1649, 1604, 1482, 1411 cm^{-1}; HRMS (FAB) calcd for $C_{22}H_{23}N_3O_5$ + H^+ 410.4477, found 410.1693.

3-[*N*-Allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl] amino]propionic acid methanesulfonate (15a): R_f = 0.54 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 173–174 °C; ^1H NMR (CD_3OD) δ 2.13 (3H, s), 2.67 (2H, t, *J* = 8.0 Hz), 2.70 (ca. 4H, s, CH_3SO_3H), 3.60–3.80 (2H, m), 4.15–4.20 (2H, m), 5.20–5.35 (2H, m), 5.80–6.02 (1H, m), 6.65 (1H, s), 7.53 (2H, d, *J* = 8.0 Hz), 7.56 (2H, d, *J* = 8.0 Hz), 7.92 (2H, d, *J* = 8.0 Hz), 8.21 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 175.70, 167.88, 165.57, 156.89, 143.13, 137.09, 134.82, 131.35, 130.74, 130.51, 129.28, 128.97, 127.20, 124.09, 118.38, 118.35, 53.27, 42.61, 39.49, 33.04, 33.02, 16.46; IR (KBr) 3600–2700, 1737, 1687, 1606, 1482, 1414 cm^{-1}; HRMS (FAB) calcd for $C_{24}H_{25}N_3O_5$ + H^+ 436.1873, found 436.1880.

4-[*N*-Allyl-*N*-[4-[(4-amidinophenoxy)carbonyl]-α-methylcinnamoyl]amino]butyric acid methanesulfonate (16a): R_f = 0.48 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 159–162 °C; ^1H NMR (CD_3OD) δ 1.98 (2H, tt, *J* = 7.5, 5.0 Hz), 2.30–2.48 (2H, m), 2.75 (3H, s, CH_3SO_3H), 3.50 (2H, t, *J* = 7.5 Hz), 4.10 (2H, d, *J* = 5.0 Hz), 5.20–5.38 (2H, m), 5.79-6.02 (1H, m), 6.62 (1H, s), 7.55 (2H, d, *J* = 8.0 Hz), 7.58 (2H, d, *J* = 8.0 Hz), 7.90 (2H, d, *J* = 8.0 Hz), 8.21 (2H, d, *J* = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 176.29, 175.32, 167.51, 165.40, 156.70, 143.09, 137.21, 134.80, 134.06, 131.32, 130.77, 130.50, 128.85, 127.08, 124.03, 118.47, 52.59, 40.17, 32.12, 23.52, 16.68; IR (KBr) 3091, 1728, 1707, 1606, 1479 1415 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{27}N_3O_5$ + H^+ 450.2029, found 450.2036.

4-[(4-Amidinophenoxy)carbonyl]benzoic Acid Trifluoroacetate (5b). To a stirred solution of 4-amidinophenol hydrochloride (963 mg, 5.60 mmol) and mono-*tert*-butyl terephthalate (1.24 g, 5.60 mmol) in pyridine (20 mL) was added

1,3-dicyclohexylcarbodiimide (DCC; 1.38 g, 6.72 mmol) at 25 °C. After 15 h, the resulting urea was removed by filtration and the filtrate was evaporated. The residue was chromatographed on silica gel (chloroform:methanol:acetic acid = 50:1:1) to yield 1.40 g (63%) of *tert*-butyl 4-[(4-amidinophenoxy)-carbonyl]benzoate acetate as a white amorphous solid: R_f = 0.68 (chloroform:methanol:acetic acid = 10:2:1).

The *tert*-butyl ester obtained (1.40 g, 3.50 mmol) was treated with trifluoroacetic acid (14 mL) at 25 °C. After 0.5 h, the resulting solution was evaporated and the residue was triturated and washed with diethyl ether to yield 1.11 g (80%) of **5b** as a white powder: R_f = 0.49 (chloroform:methanol:acetic acid = 10:2:1); mp 290–295 °C; ^{1}H NMR (DMSO-d_6) δ 7.60 (2H, d, J = 8.5 Hz), 7.95 (2H, d, J = 8.5 Hz), 8.20 (2H, d, J = 9.0 Hz), 8.25 (2H, d, J = 9.0 Hz), 9.10 (2H, m), 9.40 (2H, m); ^{13}C NMR (DMSO-d_6) δ 166.49, 165.11, 163.65, 158.34, 154.45, 135.70, 132.06, 130.18, 129.97, 129.77, 126.17, 122.68; IR (KBr) 3328, 1748, 1698, 1670 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{12}N_2O_4$ + H$^+$ 285.2745, found 285.0858.

Preparation of Compounds 17a–c. Step 1: 4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamic Acid Hydrochloride (21). To a stirred solution of *tert*-butyl 4-[4-(methoxycarbonyl)phenyl]-α-methylcinnamate (**19**) (16.6 g, 60 mmol) in THF (300 mL) was added 4 N NaOH (18.0 mL, 72 mmol) dropwise at 25 °C. After 5 h, the reaction mixture was concentrated and the aqueous layer was washed with diethyl ether. The aqueous layer was neutralized with 2 N HCl (36.0 mL) dropwise at 5 °C and extracted with ethyl acetate. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated. The crude carboxylic acid **20** was used for the next step.

To a stirred solution of the crude carboxylic acid **20** (10.7 g, 40.7 mmol) and 4-amidinophenol hydrochloride (7.0 g, 40.7 mmol) in pyridine (150 mL) was added DCC (12.6 g, 61.1 mmol) at 25 °C. After 15 h, the resulting urea was removed by filtration and the filtrate was evaporated. The residue was chromatographed on silica gel (chloroform:methanol:acetic acid = 20:2:1) to yield an oily product.

To a stirred solution of the resulting oil in chloroform (100 mL) was added 4 N HCl in ethyl acetate (150 mL) at 0 °C. After 2 h at 25 °C, the reaction mixture was evaporated. The residue was triturated and washed with ethyl acetate to yield 11.3 g (52% for three steps) of **21** as a white powder: R_f = 0.30 ($CHCl_3$:MeOH:AcOH = 10:2:1); ^{1}H NMR (CD_3OD) δ 2.12 (3H, s), 7.54 (2H, d, J = 8.0 Hz), 7.60 (2H, d, J = 8.0 Hz), 7.75 (1H, s), 7.95 (2H, d, J = 8.0 Hz), 8.21 (2H, d, J = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 171.22, 167.66, 165.34, 156.76, 143.16, 138.27, 132.61, 131.28, 130.95, 130.70, 129.45, 127.04, 124.01, 14.41; IR (KBr) 3362, 1737, 1686, 1606, 1486, 1414 cm^{-1}; FAB MS m/e 325 (M$^+$ + 1 − HCl).

Step 2: Preparation of Secondary Amines. Compound 23. A solution of diethyl ketomalonate (**22**; 2.56 mL, 16.8 mmol) and *tert*-butyl 4-aminobutyrate (2.54 g, 16.0 mmol) in benzene (100 mL) was heated to reflux under azeotropic conditions for 2 h. After cooling to 25° C, the reaction mixture was hydrogenated in the presence of 500 mg of 5% Pd–C under an atmosphere of hydrogen. The catalyst was removed by filtration of the reaction mixture through Celite. The filtrate was evaporated to give an oily residue, which was then purified by column chromatography on silica gel (ethyl acetate:*n*-hexane = 1:4) to yield 3.45 g (68%) of amine **23** as a colorless oil: R_f = 0.35 (ethyl acetate:*n*-hexane = 1:2); ^{1}H NMR ($CDCl_3$) δ 1.30 (6H, t, J = 7.0 Hz), 1.43 (9H, s), 1.65 (1H, brs), 1.82 (2H, m), 2.30 (2H, t, J = 7.0 Hz), 2.63 (2H, t, J = 7.0 Hz), 4.02 (1H, s), 4.22 (4H, q, J = 7.0 Hz).

The following compound **24** was obtained according to the procedure described for the preparation of **23**.

Compound 24: R_f = 0.28 (ethyl acetate:*n*-hexane = 1:2); ^{1}H NMR ($CDCl_3$) δ 1.21 (3H, t, J = 7.5 Hz), 1.23 (6H, t, J = 7.5 Hz), 1.40–2.10 (4H, m), 2.28 (1H, t, J = 8.5 Hz), 2.62 (1H, t, J = 8.0 Hz), 4.00 (1H, s), 4.05 (2H, q, J = 7.5 Hz), 4.10 (4H, q, J = 7.5 Hz).

Compound 28. To a stirred solution of compound **25** (8.5 g, 30 mmol), which was prepared by a partial hydrolysis of diethyl [*N*-(benzyloxycarbonyl)amino]malonate, and *tert*-butyl alcohol (14.1 mL, 150 mmol) in acetonitrile (100 mL) were added diisopropylethylamine (20.9 mL, 120 mmol) and 2-chloro-1-methylpyridium iodide (15.3 g, 60 mmol) at 25 °C. After 2 h, the reaction mixture was poured into ice–water and extracted with ethyl acetate. The organic layer was washed with 1 N HCl and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (ethyl acetate:*n*-hexane = 1:4) to yield 5.10 g (51%) of **26** as a pale yellow oil: R_f = 0.67 (ethyl acetate:*n*-hexane = 1:1); ^{1}H NMR ($CDCl_3$) δ 1.25 (3H, t, J = 7.0 Hz), 1.45 (9H, s), 4.70 (2H, q, J = 7.0 Hz), 5.85 (1H, d, J = 9.0 Hz), 5.50–5.80 (1H, m), 7.20–7.40 (5H, m).

The reaction mixture of **26** (5.5 g, 16.3 mmol) and 5% Pd–C (500 mg) in ethanol (50 mL) was stirred vigorously under an atmosphere of hydrogen at 25° C for 18 h. Removal of the catalyst was carried out by the filtration of the reaction mixture through Celite. The filtrate was evaporated to give an oily residue, which was chromatographed on silica gel (ethyl acetate:*n*-hexane = 1:1) to yield 2.87 g (87%) of amine **27** as a pale brown oil: R_f = 0.26 (ethyl acetate:*n*-hexane = 1:1); ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 7.0 Hz), 1.45 (9H, s), 4.03 (1H, s), 4.20 (2H, q, J = 7.0 Hz).

To a stirred solution of amine **27** (5.34 g, 26 mmol) and ethyl 3-bromocrotonate (6.7 g, 34.8 mmol) in acetonitrile (40 mL) was added diisopropylethylamine (4.55 mL, 26 mmol) at 25 °C. After 12 h, the reaction mixture was poured into ice–water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (ethyl acetate:*n*-hexane = 1:4) to yield 6.70 g (75%) of *tert*-butyl ethyl 2-[[3-(ethoxycarbonyl)-2-propenyl]amino]malonate as a pale yellow oil: R_f = 0.54 (ethyl acetate:*n*-hexane = 1:1); ^{1}H NMR ($CDCl_3$) δ 1.29 (6H, t, J = 7.0 Hz), 1.43 (9H, s), 3.42 (2H, d, J = 6.0 Hz), 3.91 (1H, s), 4.10–4.30 (5H, m), 6.03 (1H, d, J = 16.0 Hz), 6.92 1H, dt, J = 16.0, 6.0 Hz).

A reaction mixture of the oily product (6.2 g, 19.6 mmol), $NaNO_2$ (1.35 g, 19.6 mmol), and 5% Pd–C (500 mg) in ethanol (50 mL) was stirred vigorously under an atmosphere of hydrogen at 25 °C for 18 h. The catalyst was removed by filtration, and the filtrate was evaporated to afford an oily residue, which was chromatographed on silica gel (ethyl acetate:*n*-hexane = 1:3) to yield 2.87 g (87%) of amine **28** as a pale brown oil: R_f = 0.42 (ethyl acetate:*n*-hexane = 1:1); ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.0 Hz), 1.28 (3H, t, J = 7.0 Hz), 1.43 (9H, s), 1.60–2.00 (2H, m), 2.38 (2H, t, J = 7.0 Hz), 2.62 (2H, t, J = 7.0 Hz), 3.90 (1H, s), 4.10 (2H, q, J = 7.0 Hz), 4.20 (2H, q, J = 7.0 Hz).

Step 3: Coupling Reaction of 21 with Secondary Amines. Compound 17c. Thionyl chloride (1.03 mL, 14.1 mmol) was slowly added to a stirred suspension of **21** (5.07 g, 14.1 mmol) in DMF (20 mL) at 0 °C under an argon atmosphere. The suspension was stirred at 0 °C for 4 h. The resulting solution was added to the solution of **24** (3.16 g, 10.9 mmol) in pyridine (30 mL) dropwise at 0 °C. After 17 h, the solvent was evaporated below 40 °C and the residue was chromatographed on silica gel (chloroform:methanol:acetic acid = 50:1:1) to yield 2.21 g (32%) of the hydrochloric acid salt of **17c**. The conversion to its methanesulfonate was carried out according to the method described in our previous paper.[5]

Diethyl 2-[*N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-[3-(ethoxycarbonyl)propyl]amino]-malonate methanesulfonate (17c): R_f = 0.59 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 110 ~ 113 °C; ^{1}H NMR (CD_3OD) δ 1.12–1.19 (3H, m), 1.31 (6H, t, J = 7.0 Hz), 1.90–2.00 (2H, m), 2.15 (3H, s), 2.58 (2H, t, J = 7.0 Hz), 2.70 (ca. 4H, s, CH_3SO_3H), 3.42–3.57 (2H, m), 3.98–4.19 (3H, m), 4.26 (4H, q, J = 7.0 Hz), 6.68 (1H, s), 7.52 (2H, d, J = 9.0 Hz), 7.58 (2H, d, J = 9.0 Hz), 7.91 (2H, d, J = 9.0 Hz), 8.20 (2H, d, J = 9.0 Hz); ^{13}C NMR (CD_3OD) δ 175.46, 174.42, 167.76, 167.05, 165.46, 156.81, 142.77, 136.25, 131.36, 130.74, 130.31, 130.07, 129.12, 127.16, 124.06, 63.16, 61.57, 50.69, 39.49, 31.51, 24.97, 16.29, 14.50; IR (KBr) 3398, 1737, 1687, 1638, 1607, 1484 cm^{-1}; HRMS (FAB) calcd for $C_{31}H_{37}N_3O_9$ + H$^+$ 596.6416, found 596.2582.

The corresponding *tert*-butyl esters of **17a,b** were obtained according to the procedure described for preparation of **17c**.

***tert*-butyl ester of 17a**: yield 6%; R_f = 0.33 (chloroform:methanol:acetic acid = 10:2:1).

***tert*-butyl ester of 17b**: yield 34%; R_f = 0.61 (chloroform:methanol:acetic acid = 10:2:1).

Step 4: Deprotection of the *tert*-Butyl Ester. Ethyl Hydrogen 2-[*N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-[3-(ethoxycarbonyl)propyl]amino]-malonate Acetate (17a). To a stirred solution of the *tert*-butyl ester of **17a** (370 mg, 0.58 mmol) in anisole (5 mL) was added trifluoroacetic acid (5 mL, 65 mmol) at 25 °C. After 2 h, the reaction mixture was evaporated and the residue was chromatographed on silica gel (chloroform:methanol:acetic acid = 30:2:1) to yield 300 mg (81%) of **17a** as a white solid: R_f = 0.23 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 167–169 °C; ^1H NMR (CD_3OD) δ 1.10–1.40 (6H, m), 1.90 (3H, s, CH_3CO_2H), 1.90–2.20 (5H, m), 2.35 (2H, t, J = 7.0 Hz), 3.50–3.70 (2H, m), 4.0–4.3 (4H, m), 4.95 (1H, brs), 6.61 and 6.76 (1H, s, each), 7.50–7.60 (4H, m), 7.92 (2H, d, J = 8.0 Hz), 8.21 (2H, d, J = 8.0 Hz); ^{13}C NMR (CD_3OD) δ 181.55, 176.11, 175.03, 174.62, 167.86, 165.52, 163.45, 156.79, 132.14, 131.43, 131.34, 131.11, 131.03, 130.83, 130.71, 130.60, 130.54, 130.22, 129.89, 129.79, 129.76, 129.71, 129.69, 128.99, 127.32, 124.32, 124.15, 124.07, 117.39, 115.30, 62.78, 62.75, 62.69, 62.61, 61.61, 61.51, 33.07, 33.02, 23.93, 23.83, 16.42, 16.28, 16.25, 16.22, 14.51, 14.43 (rotamers); IR (KBr) 3387, 1737, 1688, 1607, 1571, 1543, 1432 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{33}N_3O_9 + H^+ - CO_2$ 524.2397, found 524.2394.

The following compound **17b** was obtained according to the procedure described for preparation of **17a**.

4-[*N*-[4-[(4-Amidinophenoxy)carbonyl]-α-methylcinnamoyl]-*N*-[1,1-bis(ethoxycarbonyl)methyl]amino]-butyric acid acetate (17b): R_f = 0.28 ($CHCl_3$:MeOH:AcOH = 10:2:1); mp 240–244 °C; ^1H NMR (CD_3OD) δ 1.10–1.44 (6H, m), 1.80–2.40 (10H, m), 3.40–3.70 (3H, m), 4.00–4.40 (4H, m), 6.61 and 6.72 (1H, s, each), 7.30–8.30 (8H, m); IR (KBr) 3402, 1737, 1687, 1608, 1562, 1414 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{33}N_3O_9 + H^+$ 568.2295, found 568.2280.

Evaluation of Plasma Anti-trypsin Activity in Rats.[14] Male Wistar rats (210–260 g; Japan Charles River) were fasted for 24 h prior to experiments, but water was available ad libitum. After oral administration of drugs (100 or 30 mg/10 mL/kg of body weight), blood was taken from the jugular vein at regular intervals. Trypsin (2 μM/mL, 0.1 mL) and then the chromogenic substrate Boc-Phe-Ser-Arg-AMC (0.1 mM, 0.8 mL) were added successively to the plasma (0.1 mL) prepared from the blood. The reaction mixture was incubated at 37 °C for 15 min and the reaction quenched by addition of 30% acetic acid (1 mL). Fluorescence of the released 7-amino-4-methylcoumarin (AMC) was measured (excitation, 380 nm; emission, 460 nm) using a HITACHI 850 fluorescence spectrometer. Plasma anti-trypsin activity was calculated according to the following equation.

$$\text{inhibition (\%)} = \frac{\text{pre(F)} - \text{test(F)}}{\text{pre(F)}} \times 100$$

where pre(F) is the fluorescence of the control group (drugs were not administered) and test(F) is the fluorescence of the tested group.

Evaluation of Oral Absorption of 14 and 16b. Analytical Method. Ion-paired high-performance liquid chromatography (HPLC) using an internal standard (IS) capable of simultaneous determination of **14** and **16a** was developed. HPLC was carried out using a Uvidec 100 III (JASCO) apparatus equipped with a Capcell pak C18, S 5 μm SG120 column (100 × 6 mm i.d.) at 275 nm. Blood samples were centrifuged for 1 min at 10 000 rpm, and then 0.2 mL aliquots of separated plasma samples were immediately added to 3 mL of acetonitrile containing 1% of methanesulfonic acid at 5 °C and centrifuged for 10 min at 3 000 rpm at 5 °C. The supernatant, to which 50 μL of IS solution (18 μg/mL) was added, was evaporated to dryness. The residue was dissolved in 0.2 mL of the mobile phase, and 50–100 μL of this solution was loaded on the column. The limit of detection was 50 ng/mL with an interassay coefficient of variation of <10%.

Absorption Study in Rats. Male Sprague–Dawley (SD) rats weighing 280 ± 20 g were fasted for 20 h prior to and during the expriment. The drugs dissolved in physiological saline were administered orally at doses of 50 mg/10 mL/kg for **14** and 100 mg/5 mL/kg for **16b**. Blood samples were taken from the jugular vein with heparinized syringes at 0.25, 0.5, 1.0, 2.0, 4.0, and 6.0 h after oral administration.

Pharmacokinetic Analysis. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from individual plasma concentration curves. The area under the plasma concentration–time curves (AUC) was calculated by the trapezoidal method.

Evaluation of Metabolic Stability in Rat Plasma and Small Intestine Homogenate. Freshly prepared small intestine from rats fasted for 20 h were washed with saline and homogenized with 8 vol of phosphate buffer (1/10 M $NaHPO_4/NaH_2PO_4$, pH 7.4). The homogenates were centrifuged at 600*g* for 15 min, and the supernatants were used in the experiments. The metabolic stabilities of drugs were evaluated as their half-lives (min).

1. Stability Study in Plasma. Drugs were dissolved in 80% aqueous acetonitrile to a final concentration of 2 mg/mL. To the plasma (1.0 mL) was added the solution of drug (5 μL), and the mixture was incubated at 37 °C for 60 min. Aliquots of 0.1 mL each were taken at regular intervals and the reaction quenched by addition of 1.5 mL of acetonitrile containing an internal standard (IS). After centrifugation at 3000 rpm for 10 min, the supernatant was evaporated to dryness. The residue was dissolved in 0.1 mL of the mobile phase, and 10–20 μL of this solution was loaded onto the HPLC column.

2. Stability Study in Small Intestine Homogenate. Drugs were dissolved in 80% aqueous acetonitrile to a final concentration of 5 mg/mL. To the homogenate (1.0 mL) was added the drug solution (10 μL), and the mixture was incubated at 37 °C for 60 min. Aliquots of 0.1 mL each were taken at regular intervals and the reaction quenched by addition of 0.5 mL of acetonitrile containing IS. After centrifugation at 3000 rpm for 10 min, 10–20 μL of the supernatant was loaded onto the HPLC column.

HPLC Conditions. 2a: t_R 15.7 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% sodium n-dodecylsulfate (SDS) in H_2O–MeOH (1:1):5% sodium *n*-heptylsulfate (SHS) in H_2O–MeOH = 250:170:1:3:8; IS *n*-propyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **2b**: t_R 17.9 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 250:170:1:3:8; IS *n*-propyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **3**: t_R 10.9 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 400:180:1:3:8; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.2 mL/min. **4**: t_R 10.2 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **5a**: t_R 14.4 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *n*-propyl 4-hydroxybenzoate; flow rate 0.7 mL/min. **5b**: t_R 13.7 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *i*-propyl 4-hydroxybenzoate; flow rate 0.8 mL/min. **10a**: t_R 15.0 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *n*-heptyl 4-hydroxybenzoate; flow rate 1.7 mL/min. **10b**: t_R 12.3 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *n*-amyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **11**: t_R 10.6 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **12a**: t_R 11.0 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **12b**: t_R 11.0 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 500:150:2:5:12; IS *n*-hexyl 4-hydroxybenzoate; flow rate 0.8 mL/min. **13a**: t_R 16.2 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *n*-hexyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **13b**: t_R

10.4 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 500:150:2:5:12; IS *n*-hexyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **14**: t_R 12.5 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 400:250:1:3:8; IS *i*-propyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **15a**: t_R 9.7 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *i*-butyl 4-hydroxybenzoate; flow rate 0.8 mL/min. **15b**: t_R 16.8 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *n*-hexyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **16a**: t_R 16.1 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *n*-propyl 4-hydroxybenzoate; flow rate 0.7 mL/min. **16b** :t_R 16.5 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **17a**: t_R 14.6 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 400:250:1:3:8; IS *i*-butyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **17b**: t_R 11.0 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *i*-butyl 4-hydroxybenzoate; flow rate 0.8 mL/min. **17c**: t_R 17.6 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.0 mL/min. **18a**: t_R 8.6 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 400:250:1:3:8; IS *iso*-amyl 4-hydroxybenzoate; flow rate 1.5 mL/min. **18b**: t_R 11.1 min; mobile phase MeOH:H_2O:CH_3CO_2H:5% SDS in H_2O–MeOH (1:1):5% SHS in H_2O–MeOH = 450:200:2:5:12; IS *iso*-amyl 4-hydroxybenzoate; flow rate 0.8 mL/min.

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Supporting Information Available: Detailed data for *ex vivo* studies and oral absorption studies (7 pages). Ordering information is given on any current masthead page.

References

(1) Sobajima, H.; Hayakawa, T.; Kondo, T.; Shibata, T.; Kitagawa, M.; Sakai, Y.; Ishiguro, H.; Tanikawa, M.; Nakae, Y. Effect of a New Synthetic Trypsin Inhibitor on Taurocholate-Induced Acute Pancreatitis in Rats. *Pancreas* **1993**, *8*, 240–247.

(2) Sasaki, I.; Suzuki, Y.; Naito, H.; Funayama, Y.; Kamiyama, Y.; Takahashi, M.; Matsuo, T.; Fukushima, K.; Matsuno, S.; Sato, T. Effect of Camostat in the Treatment of Reflux Esophagitis after Gastrectomy: an Experimental Study in Rats and a Pilot Clinical Study. *Biomed. Res.* **1989**, *10* (Suppl. 1), 167–173.

(3) Otsuki, M.; Tani, S.; Okabayashi, Y.; Nakamura, T.; Fujii, M.; Fujisawa, T.; Itho, H. Therapeutic Effects of Camostat on Caerulein-Induced Acute Pancreatitis in Rats. *Biomed. Res.* **1989**, *10* (Suppl. 1), 25–31.

(4) Nakayama, T.; Taira, S.; Ikeda, M.; Ashizawa, H.; Oda, M.; Arakawa, K.; Fujii, S. Synthesis and Structure-Activity Study of Protease Inhibitors. V. Chemical Modification of 6-Amidino-2-naphthyl 4-Guanidinobenzoate. *Chem. Pharm. Bull.* **1993**, *41*, 117–125.

(5) Senokuchi, K.; Nakai, H.; Nakayama, Y.; Odagaki, Y.; Sakaki, K.; Kato, M.; Maruyama, T.; Miyazaki, T.; Ito, H. New Orally Active Serine Protease Inhibitors. J. Med. Chem. **1995**, *38*, 2521–2523.

(6) Half-life of **2b** in the rat small intestine homogenate was ca. 20 min.

(7) Yaegashi, T.; Nunomura, S.; Okutome, T.; Nakayama, T.; Kurumi, M.; Sakurai, Y.; Aoyama, T.; Fujii, S. Synthesis and Structure-Activity Study of Protease Inhibitors. III. Amidinophenols and Their Benzoyl Esters. *Chem. Pharm. Bull.* **1984**, *32*, 4466–4477.

(8) Tanizawa, K.; Kasaba, Y.; Kanaoka, Y. "Inverse Substrates" for Trypsin. Efficient Enzymatic Hydrolysis of Certain Esters with a Cationic Center in the Leaving Group. *J. Am. Chem. Soc.* **1977**, *99*, 4485–4488.

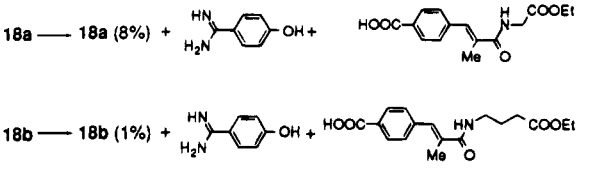
(9) See ref 5.

(10) Better solubility in water gave better reproducibility in absorption experiment.

(11) Both **10a,b** were slowly hydrolyzed at the phenol ester moiety in the rat small intestine homogenate.

(12) On the basis of the good plasma anti-trypsin activity, **10a** was presumed to possess sufficient stability against metabolism in the liver.

(13) Treatment of **18a,b** with the rat small intestine homogenate for 30 min gave *p*-amidinophenol, the inactivated corresponding carboxylic acids, and the unaffected starting materials **18a** (8%) and **18b** (1%), respectively.



(14) Biological evaluation of oral absorption using plasma anti-trypsin activity was useful for rapid evaluation because all of the inhibitors possessing 4-(benzoyloxy)benzamidine as a common partial structure showed almost the same *in vitro* inhibitory activity (4–10 nM) against the isolated enzyme within standard errors.

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