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Bioorganic & Medicinal Chemistry 12 (2004) 2099–2114
Bioorganic & Medicinal Chemistry

Design, synthesis and biological activity of amidinobicyclic compounds (derivatives of DX-9065a) as factor Xa inhibitors: SAR study of S1 and aryl binding sites

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Received 3 December 2003; accepted 24 February 2004

Abstract—Since factor Xa (fXa) plays a pivotal role in the blood coagulation cascade, inhibition of fXa is thought to be an effective treatment for a variety of thrombotic events. (2*S*)-2-[4-[[(3*S*)-1-Acetimidoyl-3-pyrrolidinyl]oxy]phenyl]-3-(7-amidino-2-naphthyl)propanoic acid hydrochloride pentahydrate (DX-9065a) was previously found in our laboratory as a novel orally active factor Xa inhibitor. DX-9065a exhibits a strong inhibitory activity toward fXa by occupying the substrate recognition (called S1) sites and aryl binding sites of fXa. Herein we describe conversions of the amidinonaphthalene and the acetimidoylpyrrolidine moieties of DX-9065a. Some compounds showed remarkably increased in vitro anti-factor Xa and PRCT activities compared with those of DX-9065a. The most promising compound **38** showed four times the prolongation of APTT against DX-9065a after oral administration to rats.

1. Introduction

Thrombotic events are thought to be major causes of fatal diseases, such as myocardial infarction and cerebral infarction. Although several anticoagulants, as well as antiplatelet agents, are clinically prescribed for the treatment and prevention of such events nowadays, warfarin is the only anticoagulant that can be administered orally. Sixma described that an ideal anti-thrombotic should meet six criteria: (a) it should inhibit thrombosis, (b) it should not affect homeostasis to such an extent that problems arise, (c) it should have a long half-life, (d) it should not have serious side effects, (e) it should be absorbed after oral administration, and (f) it should have a large therapeutic range.[1] From these criteria, warfarin still has several defects regarding its use; for example, interaction with other drugs or foods and the necessity of regular monitoring. Therefore, many groups have been expanding massive efforts in the search for new orally active anticoagulants, particularly for inhibitors against thrombin or activated factor Xa (fXa). Thrombin indeed plays a pivotal role, that is, fibrin formation at the final step of the blood coagulation cascade. Thrombin not only converts fibrinogen to fibrin for clot formation, however, but is also directly involved in platelet aggregation, protein C activation, factor XIII activation, and feedback activation of factors V and VIII. These complicated actions of thrombin might imply some difficulties of thrombin inhibitors with its use. Furthermore, fXa, whose major practical role is the generation of thrombin by the limited proteolysis of prothrombin, holds a central position that links the intrinsic and extrinsic activation mechanisms in the final common pathway of coagulation. fXa in combination with fVa and calcium ion on the phospholipid surface forms the prothrombinase complex that generates thrombin via the proteolysis of prothrombin. This activation of thrombin by the complex is a highly amplified process of the blood coagulation cascade. The tenacious role of fXa in the coagulation cascade encouraged us to study fXa inhibitors. We have already reported finding efficacy, and a tendency for less bleeding of highly selective DX-9065a type fXa inhibitors[2] (Fig. 1).

Brandstetter et al. have already reported the structure of the complex of DX-9065a in des-Gla-fXa.[3] Because the

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doi:10.1016/j.bmc.2004.02.032

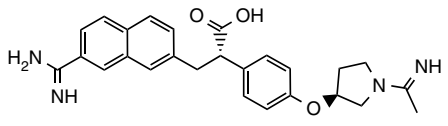


Figure 1. Structure of DX-9065a.

amidinonaphthalene moiety shows a good interaction with the S1 site of fXa on the basis of X-ray analysis, the planar amidinobicyclic aromatic ring moieties were thought to be appropriate structures for the S1 site of fXa. And, the cation hole, which was formed by the carbonyl oxygen atoms of Lys-97 together with the Glu-97 side chain, interacted with the acetimidoylpyrrolidine. Therefore, we synthesized some amidinobicyclic planar aromatic compounds and some basic aliphatic heterocycles to further optimize our fXa inhibitors. As a consequence of this study, we obtained the 6-amidinoindole and the piperidinyloxyphenyl structures as highly interactive moieties for the S1 site and the aryl binding site of fXa, respectively. The structure of **38** was the hybridization of the foregoing structures, and compound **38** showed the best anti-fXa activity and anticoagulant activity with oral administration in the series.

2. Chemistry

All compounds reported herein were synthesized as a mixture of two epimers/enantiomers at a carbon of carbonyl group. Carboxylic acid derivatives **5a**–**h**, **6a**, and **6h** were synthesized in a convergent synthesis as outlined in Scheme 1. Wittig reaction of the α-keto ester **2**[2] with the phosphonium salts **1a**–**h** using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base, followed by catalytic hydrogenation, gave the cyanobicyclic derivatives **3a**–**h**. After Pinner reaction of the compounds **3a**–**h** (successive reaction of the compounds with saturated HCl ethanol solution, and then with ammonia in ethanol), esters of the obtained amidine derivatives **4a**–**h** were hydrolyzed to give the carboxylic acid derivatives **5a**–**h**, respectively. Acetimidation of the pyrrolidine moieties was accomplished by treating **5a** or **5h** with ethyl acetimidate hydrochloride in an aqueous solution or ethanol solution. The phosphonium salts **1a**–**h** (except **1c**) were synthesized by treating the alcohol derivatives **7a**–**b**,[4] **12a**–**b**, and **14a**–**c** (**12a**–**b** and **14a**–**c** were prepared by alkylation of esters **11a**[4] or **13a**[4] followed by reduction), with $SOCl_2/PPh_3$ or PBr_3/PPh_3. Synthesis of the phosphonium salt **1c** was accomplished by treating **8**[5] with **9**[6] in ethanol solution, followed by formation of the phosphonium salt using PPh_3 in $ClCH_2CH_2Cl$ solution (Scheme 1).

Syntheses of the benzothiazole derivatives **21a**–**b** and **22a** were shown in Scheme 2. Mitsunobu reaction of **15**[7] with **16**[2] gave the malonate derivative **17**. Reaction of **19a**–**b**, which were prepared subsequent cyanation of **18a**–**b**,[8,9] with **17** gave the cyano derivatives **20a**–**b**. Pinner reaction of **20a**–**b**, followed by hydrolysis in 2 N HCl gave the carboxylic acid derivatives **21a**–**b**, respectively. Acetimidation of **21a** with ethyl acetimidate gave **22a** (Scheme 2).

Syntheses of the naphthalene derivatives **27a**–**c**, **28a**, **29a**, **28d**, **29d**, and **30d** were shown in Scheme 3. Reaction of the phosphonium salt **23**[2] with the α-ketoesters **24a**–**c** using DBU as a base, followed by catalytic hydrogenation, gave the cyano derivatives **25a**–**c**. Pinner reaction of the nitriles **25a**–**c** gave the corresponding amidine derivatives **26**. Reaction of **26** with ethyl acetimidate hydrochloride in an ethanol solution, followed by acid hydrolysis gave the carboxylic acid derivatives **27a**–**c**, respectively. The piperidine derivative **26a** was converted into the other imidoyl derivatives with the corresponding imidate, and then acid hydrolysis gave the carboxylic acid derivatives **28a** and **29a**.

Reaction of the phosphonium salt **23**[2] with the α-ketoesters **2** using DBU as a base, followed by catalytic hydrogenation, gave the nitrile **25d**. Pinner reaction of the nitrile **25d** gave the corresponding amidine derivative **26d**. The pyrrolidine derivative **26d** was converted into some imidoyl derivatives with the corresponding imidate, and then acid hydrolysis gave the carboxylic acid derivatives **28d**, **29d**, and **30d**.

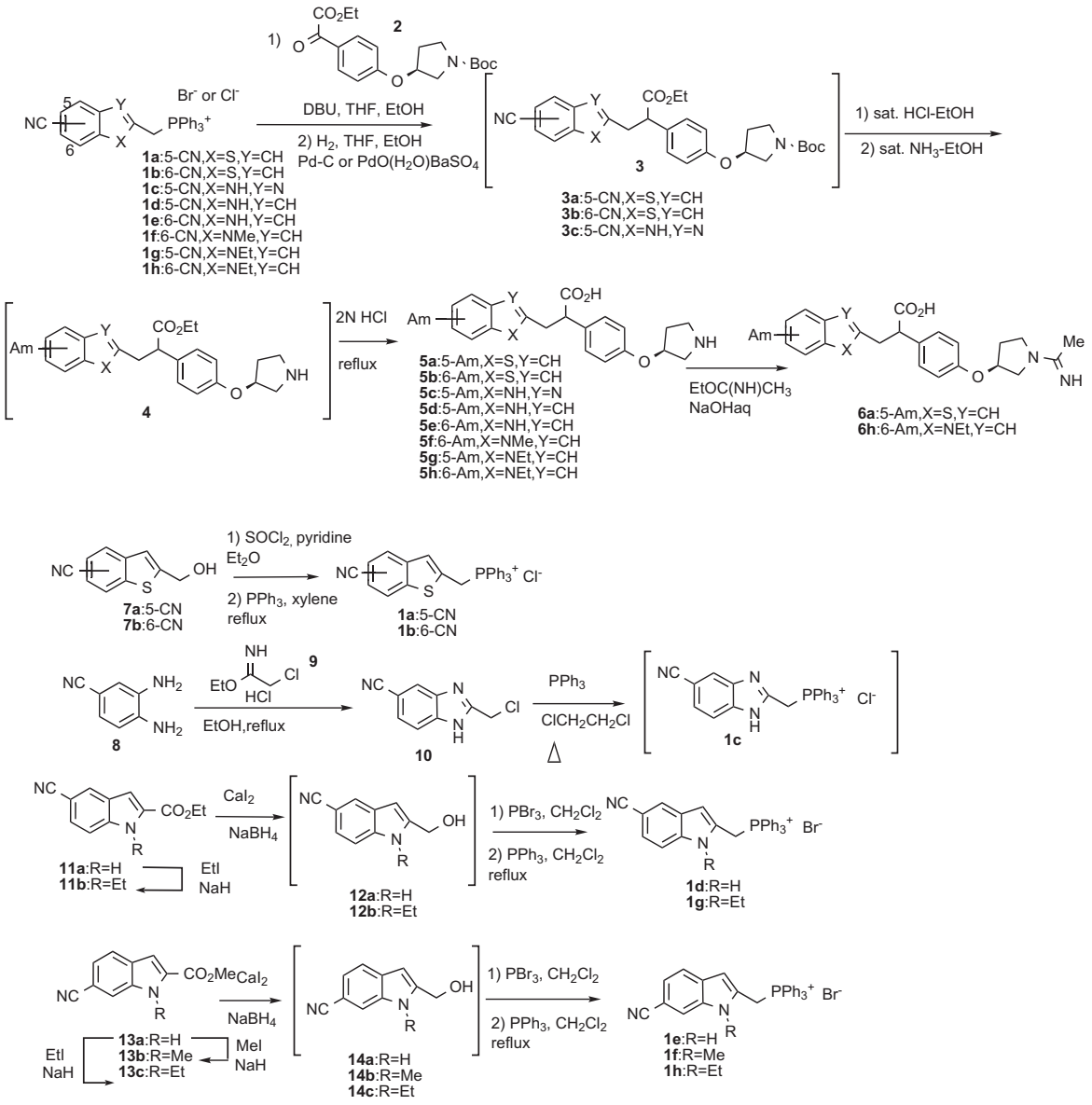
The α-ketoesters **24a**–**c** were prepared by Mitsunobu reaction of ethyl 4-hydroxyphenylglyoxalate **31**[2] and the commercially available alcohols **32a**–**c** (Scheme 3).

Syntheses of the benzothiophene derivatives **34** and **36**, and the indole derivative **38** were shown in Scheme 4. These compounds were prepared by six steps from the phosphonium salt **1a** or **1h** in the same manner as described for **27a**–**c** (Scheme 4).

3. Result and discussion

Anti-fXa activities, anti-fIIa activities, and the plasma recalcification times (PRCT) of the synthesized compounds are shown in Tables 1–3. Further, the anticoagulant activities (activated partial thromboplastin time; APTT) of compounds **6h**, **27a**, **36**, and **38** orally administered to rats are shown in Table 4.

These compounds were a mixture of two epimers at the carbon where the carboxylic acid was substituted. Compound **44** is a mixture of DX-9065a and the epimer. Anti-fXa activity of DX-9065a is two times higher than that of **44**. The epimer of DX-9065a at carbon with carboxyl moiety showed about 10 times less active against fXa. Anti-fXa activity was influenced by the stereo of the carbon where the carboxylic acid was substituted.[2] According to the above observation, a single stereoisomer would show two times higher anti-fXa activity than the mixture of two stereoisomers.

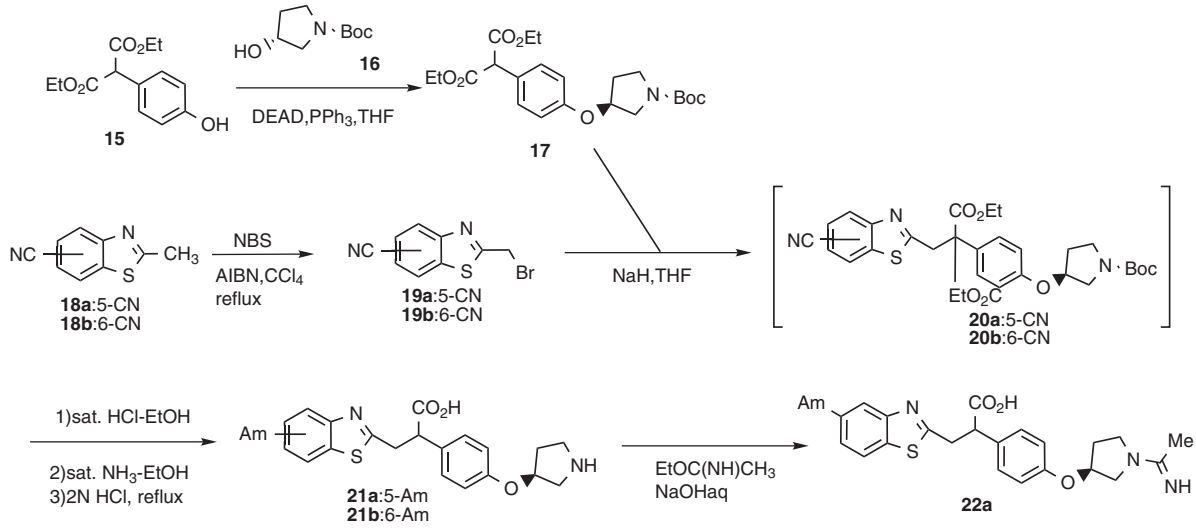


Scheme 1.

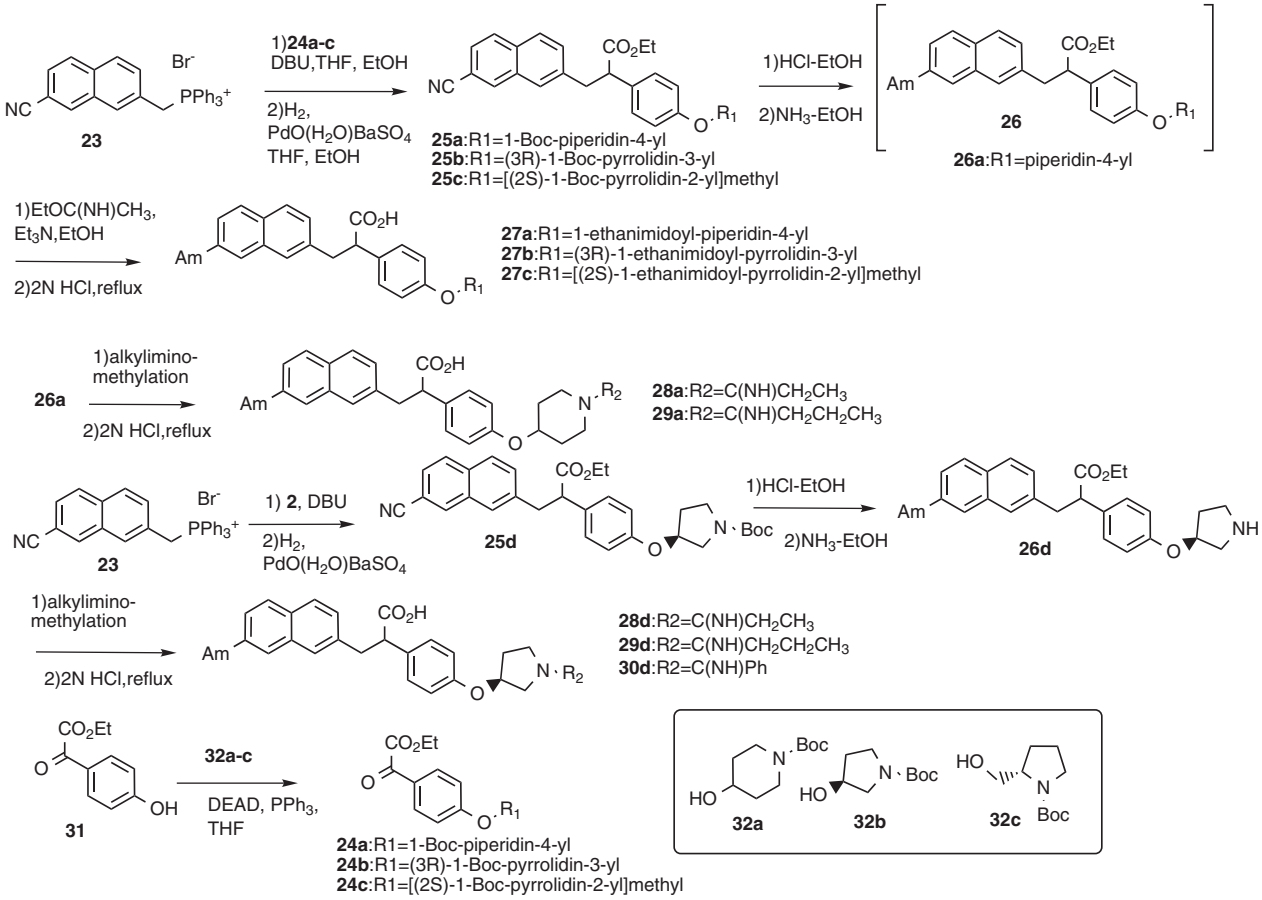
The result of optimization of the bicyclic aromatic ring is shown in Table 1. Indole and benzothiophene rings, five–six membered bicyclic aromatic rings, were considered to be favorable for the anti-fXa activities and anticoagulant activities. Especially, 5-amidinobenzothiophene derivative **5a** and 6-amidino-1-ethylindole derivative **5h** exhibited good activities comparable to that of 7-amidinonaphthalene derivative **42**. Their acetimidoyl derivatives **6a** and **6h** had high activities, and compound **6h** showed two times higher anti-fXa and anticoagulant activity in comparison with those of compound **44** (Table 1).

The results of the optimization of aryl binding site fitting moiety are shown in Table 2. Compounds **28d** and **29d**, which have more hydrophobic moieties, showed much higher activities than those of compound **44**. Compound **27a**, having an acetimidoylpiperidine moiety showed the strongest activity among these naphthalene derivatives **27** to **30** and **44**.

Since the 5-amidinobenzothiophene derivative and 6-amidino-1-ethylindole derivative showed good activities in vitro, compounds **34**, **36**, and **38** were synthesized. Compound **38** showed anti-fXa and anticoagulant activities similar to those of compound **27a**. In spite of being a mixture of epimers, the anti-fXa activity and anticoagulant activity of compounds **27a** and **38** were higher than those of DX-9065a (anti-fXa; 0.07 μM, PRCT; 0.5 μM) (Table 3). In our previous report, we separated two epimers of **25d** with recrystallization.[2] HPLC study revealed that **25d** and **44** were 1:1 mixture of epimers.[2] All diastereomeric mixtures (include **25d**, **26d**, and **44**) in this report did not separate



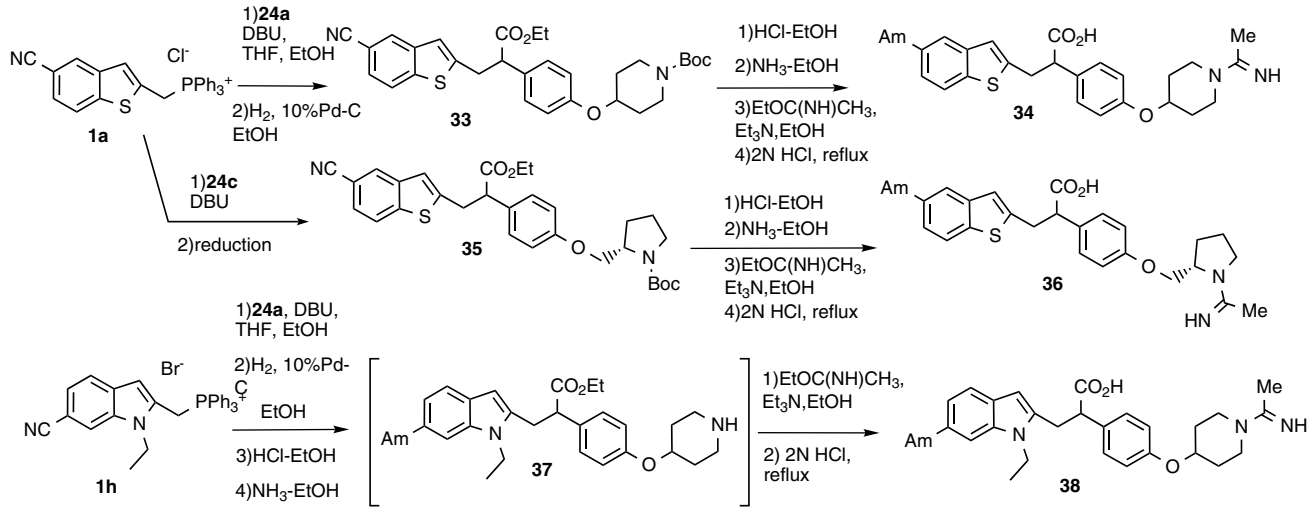
Scheme 2.



Scheme 3.

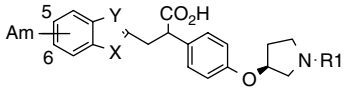
on the TLC. It can therefore presumed that all compounds in Table 1–3 are 1:1 mixture of the epimers.

Some compounds were tested for their ex vivo anticoagulant activity following their oral administration to rats at the dose of 100 mg/kg. The results are shown in Table 4. All compounds showed prolongation of thromboplastin time (APPT) to over 4 h after oral administration. Compound **38** showed four times prolongation of APPT at 30 min after oral administration, and twofold pro-



Scheme 4.

Table 1. In vitro anticoagulant and enzyme inhibitory activity (bicyclic aromatic ring optimization)



Compound		X	Y	R1	fXa (IC_{50}: μM)	fIIa (IC_{50}: μM)	PRCT (CT2: μM)
39[a]	5-Am	O	CH	H	1.5	230	14
40[a]	6-Am	O	CH	H	1.7	>1000	16
41[a]	6-Am	CH=CH	CH	H	4.1	100	22
42[a]	7-Am	CH=CH	CH	H	0.2	>1600	3.2
5a	5-Am	S	CH	H	0.35	100	3.1
5b	6-Am	S	CH	H	0.8	>1000	8.4
5c	5-Am	NH	N	H	0.7	380	9.5
5d	5-Am	NH	CH	H	0.17	190	1.45
5e	6-Am	NH	CH	H	0.27	>1000	3.5
5f	6-Am	NMe	CH	H	0.3	110	2
5g	5-Am	NEt	CH	H	1.6	>800	8
5h	6-Am	NEt	CH	H	0.16	8.4	1.15
21a	5-Am	S	N	H	1.1	140	8
21b	6-Am	S	N	H	8.4	>1600	72
43[a]	5-Am	O	CH	$C(NH)CH_3$	0.5	15	6
44[a]	7-Am	CH=CH	CH	$C(NH)CH_3$	0.112	>1200	0.96
6a	5-Am	S	CH	$C(NH)CH_3$	0.37	>1000	2.4
6h	6-Am	NEt	CH	$C(NH)CH_3$	0.054	6.8	0.35
22a	5-Am	S	N	$C(NH)CH_3$	0.6	>600	5

[a] Compounds **39–44** have already been reported.[2]

longation of APPT even after 4 h against the control (Table 4).

4. Conclusion

Compound **38** exhibited potent and highly selective anti-fXa activity and anticoagulant activity in vitro as well as ex vivo test in rats.

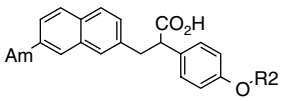
The optimization of the amidinonaphthalene moiety and pyrrolidine moiety of DX-9065a showed that a conversion of these two moieties could increase both in vitro as well as ex vivo activities. One of the combinations, conversion of the 6-amidino-1-ethylindole moiety to the S1 site and the piperidine moiety to the aryl binding site, would be favorable for fXa inhibitors.

5. Experimental section

5.1. General

Melting points were determined on a Büchi 520 apparatus in glass capillary tubes and are uncorrected.

Table 2. In vitro anticoagulant and enzyme inhibitory activity (aliphatic heterocyclic ring optimization)



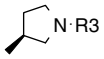
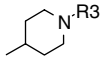
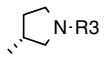
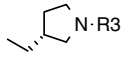
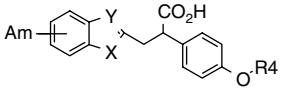
	R2	R3	fXa (IC_{50}: μM)	fIIa (IC_{50}: μM)	PRCT (CT2: μM)
44		$C(NH)CH_3$	0.112	>1200	0.96
28d		$C(NH)CH_2CH_3$	0.062	330	0.7
29d		$C(NH)CH_2CH_2CH_3$	0.056	200	0.72
30d		C(NH)CPh	0.088	220	5.2
27a		$C(NH)CH_3$	0.011	>1200	0.34
28a		$C(NH)CH_2CH_3$	0.021	300	0.54
29a		$C(NH)CH_2CH_2CH_3$	0.054	>1200	1.1
27b		$C(NH)CH_3$	0.29	68	2.5
27c		$C(NH)CH_3$	0.2	140	1.6

Table 3. In vitro anticoagulant and enzyme inhibitory activity (hybrid optimization)



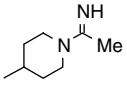
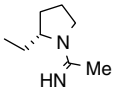
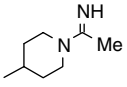
		X	Y	R4	fXa (IC_{50}: μM)	fIIa (IC_{50}: μM)	PRCT (CT2: μM)
34	5-Am	S	CH		0.045	180	0.44
36	5-Am	S	CH		0.044	180	0.54
38	6-Am	NEt	CH		0.011	2.5	0.3

Table 4. Ex vivo anticoagulant activity on oral administration

Compound	Test/control APTT[a] ratio (dose 100 mg/kg $n = 4$)			
	0.5 h	1 h	2 h	4 h
DX-9065a	1.63 ± 0.09	1.51 ± 0.11	1.48 ± 0.04	1.28 ± 0.04
6h	2.69 ± 0.20	3.60 ± 0.20	2.41 ± 0.06	1.66 ± 0.06
27a	2.12 ± 0.08	2.18 ± 0.07	1.69 ± 0.04	1.39 ± 0.09
36	1.68 ± 0.08	1.64 ± 0.08	1.59 ± 0.04	1.47 ± 0.03
38	4.07 ± 0.66	3.96 ± 0.44	3.37 ± 0.17	2.19 ± 0.16

Values are means ± SE.

[a] Activated partial thromboplastin time. Methods are described in Section 5.

Column chromatography was performed on Merck silica gel 60 (particle size 0.060–0.200 or 0.040–0.063 mm) and on Daiaion HP-20 (highly porous polymer type synthetic adsorbent; Mitsubishi Chemical Industries). Preparative HPLC was performed with a reverse-phase ODS column (Sensyu Pak ODS-H-5301 20 × 300 mm), a mobile phase of acetonitrile/water (5/95–10/90), and a flow rate of 10 mL/min. Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC aluminum sheets silica gel 60 F_{254}, and detected by UV quenching at 254 nm or by spraying with phosphomolybdic acid or ninhydrin. All analytical samples were found to be homogeneous on TLC.

^{1}H NMR spectra were recorded on a JEOL FX90Q or a JEOL JNM-EX400 spectrometer and chemical shifts are

given in ppm (δ) from tetramethylsilane as the internal standard. Mass spectra were performed with a JEOL JMS-AX505W (EI, CI), a JEOL JMS-HX110 (FD, FAB), or a JEOL JMS-700 (HRMS) spectrometer. IR spectra were recorded on a HITACHI 270–30 or HORIBA FT-720 spectrometer.

5.2. Anticoagulant activity. Plasma recalcification time (PRCT)

Plasma was mixed with saline (100 μL) or a saline solution of the inhibitor in a glass tube and incubated for 2 min at 37 °C. Coagulation was started with the addition of 20 mM $CaCl_2$ solution (100 μL). Anticoagulant activity was evaluated with the plasma clotting time doubling concentration (CT2).

5.3. Anti-fXa activity

Anti-fXa activities were measured by using chromogenic substrate S-2222 (KabiVitrum) and human fXa. fXa was obtained from the activation of factor X (Enzyme Research Laboratories, Inc.) by Russell viper venom. Saline (20 μL) or a saline solution of the inhibitor and 1 mM S-2222 (100 μL) were mixed with 0.1 M Tris–0.2 M NaCl buffer pH 8.4 (360 μL). The reaction was started with the addition of 0.5 unit/mL human fXa solution (20 μL), and the mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 60% AcOH (100 μL), and the optical densities (OD) were measured (405 nm)

$$\text{Anti-fXa activity (inhibition\%)} = 1 - (\text{OD of the inhibitor/OD of saline control}) \times 100.$$

The IC_{50} value was obtained by plotting the inhibitor concentrations against the anti-fXa activity (inhibition%) on statistical probability paper.

5.4. Anti-fIIa activity

Saline (100 μL; Tris–HCl buffered to pH 7.45) (TBS) containing fibrinogen (6 mg/mL; Type 1, Daiichi Pure Chemicals Co., Ltd) was mixed with saline or a saline solution of the inhibitors (100 μL). After the addition of a solution of thrombin (100 μL; 4 units/mL: Sankyo Co., Ltd) to the above mixture, the clotting time was measured at 37 °C, and then a calibration curve was prepared. Antithrombin activities (inhibition%) were obtained by measuring the clotting time using solutions of inhibitors in saline (100 μL). The IC_{50} value was obtained from the percentage of inhibition.

5.5. Ex vivo anticoagulant activity on oral administration

Male Wistar rats (200–250 g) were fasted overnight. Synthetic compounds were dissolved in water and administered orally to rats with a stomach tube. Fifteen minutes after administration, rats were anesthetized with sodium thiopental (100 mg/kg, ip). Blood samples were collected from the juglar vein (in the presence of trisodium citrate) at several time points. After these blood samples were centrifuged, the platelet-poor plasma samples were used for measuring their activated thromboplastin times (APTT).

5.6. APTT

Plasma (20 μL) and saline (20 μL) or a saline solution of the inhibitors were mixed with Platern Plus Activator (Enzyme Research Laboratories, Inc.) (20 μL) in the process tube, and the coagulation was started by the addition of 20 mM $CaCl_2$ (20 μL).

5.7. [(5-Cyano-1-benzothien-2-yl)methyl](triphenyl)phosphonium chloride 1a

To a stirred and ice-cooled solution of compound **7a** (4.0 g, 21 mmol) in Et_2O (100 mL)–pyridine (10 drops) was added a solution of $SOCl_2$ (5.5 g, 46 mmol) in Et_2O (5.0 mL). After stirring for 2 h at room temperature, the reaction mixture was poured onto ice-cold water and extracted with benzene. The separated organic layer was washed with satd $NaHCO_3$ aq, dried over $MgSO_4$, and concentrated. The solution of the residue and triphenylphosphine (7.2 g, 28 mmol) in xylene (100 mL) was heated at reflux for 10 h. After cooling the reaction mixture, collecting the precipitate gave a colorless powder (6.3 g, 64%). Mp > 250 °C. ^{1}H NMR ($CDCl_3$): δ 6.70 (2H, d, $J = 15.1$ Hz), 7.30–8.10 (19H, m). MS (FAB): m/z 434 $(M)^+$. HRMS (FAB) calcd for $C_{28}H_{21}NPS$: 434.1132. Found: 434.1136. IR (ATR): 2813, 2742, 2217, 1434, 1112, 1062, 890, 831 cm^{-1}.

5.8. [(6-Cyano-1-benzothien-2-yl)methyl](triphenyl)phosphonium chloride 1b

Starting with **7b** and following the procedure for the preparation of **1a** gave **1b** (yield, 64%) as colorless needle crystals. Mp > 250 °C. ^{1}H NMR ($CDCl_3$): δ 6.5 (2H, d, $J = 17$ Hz), 7.20–8.00 (19H, m). MS (FAB): m/z 434 $(M)^+$. HRMS (FAB) calcd for $C_{28}H_{21}NPS$: 434.1132. Found: 434.1108.

5.9. [(5-Cyano-1*H*-indol-2-yl)methyl](triphenyl)phosphonium bromide 1d

Starting with **11a** and following the procedure for the preparation of **1h** gave **1d** (yield, 51%) as a colorless amorphous mass. ^{1}H NMR ($CDCl_3$): δ 5.42 (2H, d, $J = 14.2$ Hz), 7.50–7.75 (19H, m). MS (FAB): m/z 417 $(M)^+$. HRMS (FAB) calcd for $C_{28}H_{22}N_2P$: 417.1521. Found: 417.1549. IR (ATR): 3135, 2215, 1436, 1319, 1112, 804 cm^{-1}.

5.10. [(6-Cyano-1*H*-indol-2-yl)methyl](triphenyl)phosphonium bromide 1e

Starting with **13a** and following the procedure for the preparation of **1h** gave **1e** (yield, 51%) as a colorless powder. Mp 255–259 °C. ^{1}H NMR ($CDCl_3$): δ 5.83 (2H,

d, $J = 14.4$ Hz), 7.15–7.80 (19H, m). MS (FAB): m/z 417 $(M)^+$. HRMS (FAB) calcd for $C_{28}H_{22}N_2P$: 417.1521. Found: 417.1521. IR (ATR): 3054, 2964, 2215, 1436, 1307, 1110, 817 cm^{-1}.

5.11. [(6-Cyano-1-methyl-1*H*-indol-2-yl)methyl](triphenyl)phosphonium bromide 1f

Starting with **13b** and following the procedure for the preparation of **1h** gave **1f** (yield, 77%) as a pale yellow amorphous mass. ^1H NMR (DMSO-d_6): δ 3.10–3.30 (3H, m), 5.53 (2H, br d, $J = 15.4$ Hz), 7.20–8.10 (19H, s). MS (FAB): m/z 431 $(M)^+$. HRMS (FAB) calcd for $C_{29}H_{24}N_2P$: 431.1677. Found: 431.1656. IR (ATR): 2892, 2829, 2769, 2223, 1513, 1473, 1438, 1432, 1340, 1182, 1108, 995, 908, 831 cm^{-1}.

5.12. [(5-Cyano-1-ethyl-1*H*-indol-2-yl)methyl](triphenyl)phosphonium bromide 1g

Starting with **11b** and following the procedure for the preparation of **1h** gave **1g** (yield, 70%) as a colorless powder. Mp > 270 °C. ^1H NMR ($CDCl_3$+CD_3OD): δ 1.12 (3H, t, $J = 7.2$ Hz), 3.75–3.85 (2H, m), 5.29 (2H, br d, $J = 13.9$ Hz), 7.30–7.95 (19H, s). MS (FAB): m/z 445 $(M)^+$. HRMS (FAB) calcd for $C_{30}H_{26}N_2P$: 445.1834. Found: 445.1841. IR (ATR): 2987, 2842, 2778, 2219, 1481, 1436, 1340, 1110, 921, 800 cm^{-1}.

5.13. [(6-Cyano-1-ethyl-1*H*-indol-2-yl)methyl](triphenyl)phosphonium bromide 1h

To a stirred and ice-cooled solution of compound **13c** (14 g, 61 mmol) in THF (200 mL) was added CaI_2 (34 g) and $NaBH_4$ (10 g). After stirring for 2 h at room temperature, to the mixture were added glacial acetic acid and AcOEt. The separated organic layer was dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography with $CHCl_3$/MeOH (98/2) as an eluent, providing alcohol **14c**. To a stirred and ice-cooled solution of the obtained alcohol **14c** in CH_2Cl_2 (200 mL) was added PBr_3 (6.0 mL). After stirring for 1 h at room temperature, to the mixture was added ice-cold water and the mixture was neutralized with Na_2CO_3. The separated organic layer was dried over Na_2SO_4. The solution of the residue and triphenylphosphine (7.2 g, 28 mmol) in CH_2Cl_2 was heated at reflux for 1 h. After cooling the reaction mixture, collecting the precipitate gave **1h** as a colorless powder (17 g, 59%). Mp > 270 °C. ^1H NMR ($CDCl_3$): δ 1.00–1.15 (3H, m), 3.75–3.85 (2H, m), 5.75 (2H, br d, $J = 13.7$ Hz), 7.25–7.85 (19H, s). MS (FAB): m/z 445 $(M)^+$. HRMS (FAB) calcd for $C_{30}H_{26}N_2P$: 445.1834. Found: 445.1812. IR (ATR): 2985, 2840, 2773, 2213, 1714, 1436, 1334, 1187, 1108, 871, 835 cm^{-1}.

5.14. Ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(5-cyano-1-benzothien-2-yl)propanoate 3a

To a solution of phosphonium salt **1a** (3.0 g, 6.4 mmol) and *tert*-butyl (3*S*)-3-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-pyrrolidinecarboxylate (**2**) (2.6 g, 7.0 mmol) in dry THF (50 mL)–EtOH (50 mL) was added DBU (1.1 g, 7.0 mmol) at room temperature. The mixture was stirred for 1 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with toluene/ethyl acetate (19/1) as an eluent, providing ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(5-cyano-1-benzothien-2-yl)-propenoate as a mixture of *E* and *Z* forms. The mixture of *E* and *Z* and 10% palladium on carbon (50% wet) (5.0 g) in THF (50 mL)–EtOH (50 mL) was shaken at room temperature under a current of hydrogen (1 atm). After filtration of the catalyst, followed by evaporation of the filtrate, the residue was purified by silica gel column chromatography with toluene/ethyl acetate (19/1) as an eluent, yielding a viscous oil (2.2 g, 68%). ^1H NMR ($CDCl_3$): δ 1.17 (3H, t, $J = 7.0$ Hz), 1.47 (9H, s), 1.90–2.20 (2H, m), 3.10–3.95 (7H, m), 4.10 (2H, q, $J = 7.0$ Hz), 4.84 (1H, br), 6.81 (2H, d, $J = 9.0$ Hz), 7.20 (1H, s), 7.25 (2H, d, $J = 9.0$ Hz), 7.44 (1H, dd, $J = 9.0$, 1.6 Hz), 7.81 (1H, dd, $J = 9.0$, 1.6 Hz), 7.94 (1H, d, $J = 1.6$ Hz).

5.15. Ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(6-cyano-1-benzothien-2-yl)propanoate 3b

Starting with **1b** and following the procedure for the preparation of **3a** gave **3b** (yield, 81%) as a colorless viscous oil. ^1H NMR ($CDCl_3$): δ 1.17 (3H, t, $J = 7.5$ Hz), 1.46 (9H, s), 1.90–2.30 (2H, m), 3.20–3.75 (6H, m), 3.80–3.95 (1H, m), 4.10 (2H, q, $J = 7.5$ Hz), 4.80–5.00 (1H, br), 6.75–7.00 (2H, m), 7.04 (1H, s), 7.15–7.40 (2H, m), 7.50–7.85 (2H, m), 8.04 (1H, s).

5.16. Ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(5-cyano-1*H*-benzimidazol-2-yl)propanoate 3c

To a stirred solution of compound **10** (1.0 g, 5.6 mmol) in 1,2-dichloroethane (30 mL) was added triphenylphosphine (2.2 g, 8.4 mmol). The reaction mixture was heated at 140 °C for 1 h. Removal of the solvent gave crude phosphonium salt **1c**. To a solution of phosphonium salt **1c** and *tert*-butyl (3*S*)-3-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-pyrrolidinecarboxylate **2** (2.0 g, 5.6 mmol) in dry THF (20 mL)–EtOH (20 mL) was added DBU (1.1 g, 7.2 mmol) at room temperature. The mixture was stirred for 72 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with $CHCl_3$/EtOH (98/2) as an eluent, providing ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(5-cyano-1*H*-benzimidazol-2-yl)propenoate as a mixture of *E* and *Z* forms. The mixture of *E* and *Z* and $PdO(H_2O)BaSO_4$ (palladium oxide hydrate barium sulfate)[10] (1.5 g) in THF (50 mL)–EtOH (50 mL) was shaken at room temperature under a current of hydrogen (1 atm). After filtration of the catalyst, followed by evaporation of the filtrate, the residue was purified by silica gel column chromatography with $CHCl_3$/EtOH (99/1) as an eluent, yielding a colorless

amorphous form (0.32 g, 11%). ^{1}H NMR ($CDCl_3$): δ 1.14 (3H, t, $J = 7.0$ Hz), 1.48 (9H, s), 1.90–2.30 (2H, m), 3.05–3.90 (7H, m), 4.12 (2H, q, $J = 7.0$ Hz), 4.00–4.30 (1H, br), 4.70–4.95 (1H, br), 6.79 (2H, d, $J = 8.8$ Hz), 7.19 (2H, d, $J = 8.8$ Hz), 7.35–8.10 (3H, m). MS (FD): m/z 505 $(M+H)^+$.

5.17. 3-(5-Amidino-1-benzothien-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5a

A solution of **3a** (2.2 g, 4.2 mmol) in dry EtOH (100 mL) was saturated with HCl gas with ice cooling and was left to stand for 24 h at room temperature. After distilling off the solvent and HCl, the resulting residue was dissolved in ethanolic ammonia solution (14% w/v), and the whole mixture was left to stand for 24 h at room temperature. After removal of the solvent, the resulting residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 5/95). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give ethyl 3-(5-amidino-1-benzothien-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoate as a colorless amorphous solid (1.9 g, 83%). A solution of ethyl 3-(5-amidino-1-benzothien-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoate (1.2 g, 2.2 mmol) in 2 N HCl was heated at reflux for 30 min. After evaporation of the solvent, the residue was purified by preparative HPLC. After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (0.70 g, 62%). ^{1}H NMR (DMSO-d_6): δ 1.90–2.40 (2H, br), 3.00–4.10 (7H, m), 5.14 (1H, br), 6.93 (2H, d, $J = 8.2$ Hz), 7.28 (1H, s), 7.33 (2H, d, $J = 8.2$ Hz), 7.70 (1H, d, $J = 8.8$ Hz), 8.09 (1H, d, $J = 8.8$ Hz), 8.26 (1H, s), 9.24 (2H, br), 9.47 (2H, br), 9.00–10.20 (2H, br). IR (KBr): 1674, 1509, 1239 cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_3O_3S{\cdot}2HCl{\cdot}1.5H_2O$: C, 51.87; H, 5.54; N, 8.25. Found: C, 52.10; H, 5.92; N, 8.32.

5.18. 3-(6-Amidino-1-benzothien-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5b

Starting with **3b** and following the procedure for the preparation of **5a** gave **5b** (yield, 34%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 2.00–2.30 (2H, m), 3.00–4.05 (7H, m), 5.09 (1H, br s), 6.96 (2H, d, $J = 8.3$ Hz), 7.20–7.40 (3H, m), 7.60–7.80 (1H, m), 8.42 (1H, s), 9.00–9.80 (6H, m). MS (FAB): m/z 410 $(M+H)^+$. HRMS (FAB) calcd for $C_{22}H_{24}N_3O_3S$: 410.1538. Found: 410.1541. IR (ATR): 1725, 1680, 1509, 1435 cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_3O_3S{\cdot}2HCl{\cdot}H_2O$: C, 52.80; H, 5.44; N, 8.40. Found: C, 53.00; H, 5.64; N, 8.57.

5.19. 3-(5-Amidino-1*H*-benzimidazol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5c

Starting with **3c** and following the procedure for the preparation of **5a** gave **5c** (yield, 51%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.98–2.28 (2H, br), 3.00–4.80 (7H, m), 5.00–5.20 (1H, br), 6.93 (2H, d, $J = 9.0$ Hz), 7.34 (2H, d, $J = 9.0$ Hz), 7.91 (2H, s), 8.28 (1H, s), 9.36 (2H, br), 9.61 (2H, br), 9.40–10.10 (2H, br). MS (FAB): m/z 394 $(M+H)^+$. HRMS (FAB) calcd for $C_{21}H_{24}N_5O_3$: 394.1879. Found: 394.1859. IR (KBr): 1725, 1680, 1509, 1435 cm^{-1}. Anal. Calcd for $C_{21}H_{23}N_5O_3{\cdot}3HCl{\cdot}1.5H_2O$: C, 47.60; H, 5.52; N, 13.22. Found: C, 47.34; H, 5.80; N, 13.31.

5.20. 3-(5-Amidino-1*H*-indol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5d

To a solution of phosphonium salt **1d** (5.0 g, 10 mmol) and *tert*-butyl (3*S*)-3-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-pyrrolidinecarboxylate **2** (3.7 g, 10 mmol) in dry THF (50 mL)–MeOH (50 mL) was added DBU (1.8 g, 12 mmol) at room temperature. The mixture was stirred for 2 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with CH_2Cl_2/acetone (99/1) as an eluent, providing the ester as a mixture of *E* and *Z* forms. The mixture of *E* and *Z* and $PdO(H_2O)BaSO_4$ (palladium oxide hydrate barium sulfate) (5.0 g) in THF (50 mL)–MeOH (100 mL) was shaken at room temperature under a current of hydrogen (1 atm). After filtration of the catalyst, followed by evaporation of the filtrate, the residue was purified by silica gel column chromatography with CH_2Cl_2/acetone (98/2) as an eluent, providing an ester compound. A solution of the ester compound in dry EtOH (150 mL)–CH_2Cl_2 (100 mL) was saturated with HCl gas with ice cooling and left to stand for 24 h at room temperature. After distilling off the solvents and HCl, the resulting residue was dissolved in ethanolic ammonia solution (14% w/v) (100 mL), and the whole mixture was left to stand for 24 h at room temperature. After removal of the solvent, a solution of the resulting residue in 2 N HCl (200 mL) was heated at reflux for 30 min. After evaporation of the solvent, the resulting residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 7/93). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (2.3 g, 47%). ^{1}H NMR (DMSO-d_6): δ 2.00–2.35 (2H, m), 2.90–3.80 (6H, m), 4.00–4.30 (1H, m), 5.00–5.30 (1H, br), 6.37 (1H, s), 7.00 (2H, d, $J = 8.0$ Hz), 7.40 (2H, d, $J = 8.0$ Hz), 7.60 (2H, s), 8.10 (1H, s), 8.90–10.10 (6H, m), 11.6 (1H, s). MS (FAB): m/z 393 $(M+H)^+$. HRMS (FAB) calcd for $C_{22}H_{25}N_4O_3$: 393.1927. Found: 393.1934. IR (KBr): 1720, 1670 cm^{-1}.

5.21. 3-(6-Amidino-1*H*-indol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5e

Starting with **1e** and following the procedure for the preparation of **5d** gave **5e** (yield, 10%) as a brown amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.90–2.30 (2H, m), 2.70–4.50 (4H, m), 5.16 (1H, br s), 6.36 (1H, s), 7.00 (2H, d, $J = 8.0$ Hz), 7.25–7.80 (4H, m), 7.96 (1H, s), 9.20–9.50 (6H, m), 11.80 (1H, s). MS (FAB): m/z 393 $(M+H)^+$. HRMS (FAB) calcd for $C_{22}H_{25}N_4O_3$: 393.1927. Found: 393.1934. IR (KBr): 3064, 1668, 1542, 1509 cm^{-1}.

5.22. 3-(6-Amidino-1-methyl-1*H*-indol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5f

Starting with **1f** and following the procedure for the preparation of **5d** gave **5f** (yield, 50%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 2.00–2.40 (2H, m), 3.00–3.90 (6H, m), 3.79 (3H, s), 4.00–4.30 (1H, m), 5.15–5.35 (1H, m), 6.50 (1H, s), 7.10 (2H, d, $J = 7.9$ Hz), 7.25–7.75 (4H, m), 8.35 (1H, s), 8.90–9.90 (6H, m). MS (FAB): m/z 407 (M+H)$^+$. HRMS (FAB) calcd for $C_{23}H_{27}N_4O_3$: 407.2083. Found: 407.2091. IR (KBr): 1720, 1665, 1615 cm^{-1}. Anal. Calcd for $C_{23}H_{26}N_4O_3{\cdot}2HCl{\cdot}0.5H_2O$: C, 56.56; H, 5.98; N, 11.47. Found: C, 56.89; H, 5.99; N, 11.50.

5.23. 3-(5-Amidino-1-ethyl-1*H*-indol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5g

Starting with **1g** and following the procedure for the preparation of **5d** gave **5g** (yield, 6.8%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.10–1.40 (3H, m), 1.95–2.30 (2H, m), 2.90–4.60 (9H, m), 5.10–5.30 (1H, br), 6.37 (1H, s), 6.92 (2H, d, $J = 8.0$ Hz), 7.30–7.80 (4H, m), 8.17 (1H, s), 8.90–10.10 (6H, m). MS (FAB): m/z 421 (M+H)$^+$. HRMS (FAB) calcd for $C_{24}H_{29}N_4O_3$: 421.2240. Found: 421.2249. IR (ATR): 3392, 1722, 1668, 1610, 1552, 1510, 1468, 1404, 1348, 1238, 1178 cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_4O_3{\cdot}2HCl{\cdot}1.5H_2O$: C, 55.38; H, 6.39; N, 10.77. Found: C, 55.35; H, 6.17; N, 10.65.

5.24. 3-(6-Amidino-1-ethyl-1*H*-indol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 5h

Starting with **1h** and following the procedure for the preparation of **5d** gave **5h** (yield, 46%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.10–1.40 (3H, m), 1.95–2.30 (2H, m), 2.90–3.80 (6H, m), 3.90–4.45 (3H, m), 5.00–5.20 (1H, br), 6.37 (1H, s), 6.92 (2H, d, $J = 8.0$ Hz), 7.30–7.70 (4H, m), 8.11 (1H, s), 8.90–10.10 (6H, m). MS (FAB): m/z 421 (M+H)$^+$. HRMS (FAB) calcd for $C_{24}H_{29}N_4O_3$: 421.2240. Found: 421.2245. IR (KBr): 1713, 1668, 1614 cm^{-1}. Anal. Calcd for $C_{24}H_{28}N_4O_3{\cdot}2HCl{\cdot}2H_2O$: C, 54.44; H, 6.47; N, 10.58. Found: C, 54.17; H, 6.50; N, 10.53.

5.25. 3-(5-Amidino-1-benzothien-2-yl)-2-{4-[(3*S*)-1-ethanimidoylpyrrolidin-3-yloxy]phenyl}propanoic acid 6a

To a stirred and ice-cooled solution of compound **5a** (2.0 g, 3.8 mmol) in H_2O (40 mL) was added ethyl acetimidate hydrochloride (2.4 g, 19 mmol) in small portions while the reaction mixture was maintained at pH 7.5–8.5 with 1 N NaOH aq. After stirring for further 15 min with cooling in an ice bath, the reaction mixture was adjusted to pH 1.0 and concentrated to dryness. The obtained residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 5/95). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (0.70 g, 33%). ^{1}H NMR (DMSO-d_6): δ 2.00–2.50 (5H, m), 3.10–4.20 (7H, m), 4.96 (1H, br), 6.93 (2H, d, $J = 7.9$ Hz), 7.29 (1H, s), 7.34 (2H, d, $J = 7.9$ Hz), 7.73 (1H, d, $J = 8.3$ Hz), 8.10 (1H, d, $J = 8.3$ Hz), 8.30 (1H, s), 8.50–9.30 (1H, br), 9.37 (2H, br), 9.54 (3H, br). MS (FAB): m/z 451 (M+H)$^+$. HRMS (FAB) calcd for $C_{24}H_{27}N_4O_3S$: 451.1804. Found: 451.1784. IR (KBr): 1674, 1509, 1449 cm^{-1}.

5.26. 3-(6-Amidino-1-ethyl-1*H*-indol-2-yl)-2-{4-[(3*S*)-1-ethanimidoylpyrrolidin-3-yloxy]phenyl}propanoic acid 6h

A solution of compound **5h** (1.2 g, 2.3 mmol) in EtOH–$SOCl_2$ (2.0 mL) was heated at reflux for 1 h. After evaporation of the solvent, the residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 10/90–20/80), providing an ester derivative. The obtained ester derivative was dissolved in EtOH (30 mL). To the stirred and ice-cooled solution were added triethylamine (1.0 mL, 7.2 mmol) and ethyl acetimidate hydrochloride (0.20 g, 1.6 mmol). After stirring for 3 h at room temperature, the solvent was distilled off. A solution of the obtained residue in 2 N HCl (50 mL) was heated at reflux for 30 min. After evaporation of the solvent, the residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 5/95–10/90). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (0.95 g, 75%). ^{1}H NMR (DMSO-d_6): δ 1.31 (3H, t, $J = 7.1$ Hz), 2.15–2.35 (2H, m), 2.29 (3H, d, $J = 12.7$ Hz), 3.10–3.20 (1H, m), 3.40–4.00 (5H, m), 4.10–4.20 (1H, m), 4.20–4.40 (2H, m), 5.19 (1H, d, $J = 25.9$ Hz), 6.38 (1H, s), 6.90–7.05 (2H, m), 7.35–7.45 (2H, m), 7.48 (1H, d, $J = 8.9$ Hz), 7.62 (1H, d, $J = 8.5$ Hz), 8.16 (1H, s), 8.57 (1H, d, $J = 35.6$ Hz), 9.05 (2H, s), 9.30 (2H, s), 9.37 (2H, d, $J = 15.1$ Hz). MS (FAB): m/z 461 (M+H)$^+$. IR (ATR): 3052, 1668, 1610, 1508, 1463, 1340, 1234, 1172, 1087, 821 cm^{-1}. Anal. Calcd for $C_{26}H_{31}N_5O_3{\cdot}2HCl{\cdot}H_2O$: C, 56.52; H, 6.39; N, 12.68. Found: C, 56.31; H, 6.43; N, 12.68.

5.27. (5-Cyano-1*H*-benzimidazol-2-yl)methyl chloride 10

To a stirred solution of compound **8** (3.42 g, 25.7 mmol) in dry ethanol (100 mL) was added chloroacetimino ethyl ether hydrochloride (3.42 g, 25.7 mmol). The reaction mixture was heated at reflux for 3 h. After removal of the solvent, the residue was partitioned between AcOEt and water. The separated organic layer was dried over $MgSO_4$. After concentration of the solvent, the precipitate was collected and dried to give a colorless powder (2.7 g, 55%). Mp 144–146 °C. ^{1}H NMR ($CDCl_3$): δ 4.83 (2H, s), 7.48 (2H, d, $J = 7.1$ Hz), 7.57 (2H, d, $J = 7.1$ Hz), 7.95 (1H, s). IR (KBr): 2224, 1626, 1455, 1422, 1311 cm^{-1}. No further purification was attempted on this compound, which was used directly in the next step.

5.28. Ethyl 5-cyano-1-ethyl-1*H*-indole-2-carboxylate 11b

Starting with **11a** and following the procedure for the preparation of **13c** gave **11b** (yield, 95%) as a colorless

amorphous mass. ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, $J = 7.2$ Hz), 1.43 (3H, t, $J = 7.2$ Hz), 4.40 (2H, q, $J = 7.2$ Hz), 4.64 (2H, q, $J = 7.2$ Hz), 7.36 (1H, s), 7.46 (1H, d, $J = 8.8$ Hz), 7.50–7.60 (1H, m), 8.00–8.10 (1H, m). MS (FAB): m/z 243 $(M+H)^+$. HRMS (FAB) calcd for $C_{14}H_{15}N_2O_2$: 243.1134. Found: 243.1142. IR (KBr): 2220, 1714 cm^{-1}.

5.29. Methyl 6-cyano-1-methyl-1*H*-indole-2-carboxylate 13b

Starting with **13a** and following the procedure for the preparation of **13c** gave **13b** (yield, 87%) as a colorless amorphous mass. ^{1}H NMR (DMSO-d_6): δ 3.92 (3H, s), 4.10 (3H, s), 7.42 (1H, s), 7.52 (1H, dd, $J = 8.0$, 1.5 Hz), 7.98 (1H, d, $J = 8.0$ Hz), 8.30–8.40 (1H, br). MS (FAB): m/z 215 $(M+H)^+$. HRMS (FAB) calcd for $C_{12}H_{11}N_2O_2$: 215.0821. Found: 215.0808. IR (KBr): 2220, 1730, 1720 cm^{-1}. Anal. Calcd for $C_{12}H_{10}N_2O_2$: C, 67.28; H, 4.71; N, 13.08. Found: C, 67.23; H, 4.97; N, 12.99.

5.30. Methyl 6-cyano-1-ethyl-1*H*-indole-2-carboxylate 13c

To a stirred and ice-cooled solution of compound **13a** (15 g, 75 mmol) in DMF (100 mL) was added NaH (60% in oil) (3.1 g). After stirring for 20 min at room temperature, to the reaction mixture was added EtI (6.8 mL, 85 mmol) followed by stirring for 2 h at room temperature. To the mixture were added water and AcOEt/toluene. The separated organic layer was dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography with $CHCl_3$ as an eluent, yielding a colorless amorphous mass (14 g, 83%). ^{1}H NMR ($CDCl_3$): δ 1.42 (3H, t, $J = 7.5$ Hz), 3.97 (3H, s), 4.65 (2H, q, $J = 7.5$ Hz), 7.30–7.45 (2H, m), 7.70–7.90 (2H, m). MS (FAB): m/z 229 $(M+H)^+$. HRMS (FAB) calcd for $C_{13}H_{13}N_2O_2$: 229.0977. Found: 229.0975. IR (KBr): 2220, 1720, 1512 cm^{-1}.

5.31. Diethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)malonate 17

To a stirred solution of diethyl 2-(4-hydroxyphenyl)malonate **15** (4.7 g, 19 mmol), *tert*-butyl (3*R*)-3-hydroxy-1-pyrrolidinecarboxylate **16** (4.7 g, 25 mmol) and triphenylphosphine (6.58 g, 25 mmol) in anhydrous THF (150 mL) was added diethyl azodicarboxylate (4.37 g, 25 mmol) at room temperature. The resultant solution was stirred for 1 day. Additional *tert*-butyl-(3*R*)-3-hydroxy-1-pyrrolidinecarboxylate **16** (1.0 g, 5.3 mmol) and triphenylphosphine (1.6 g, 6.1 mmol) and diethyl azodicarboxylate (1.0 g, 5.7 mmol) were added to the reaction mixture. The resultant solution was stirred for a further 1 day at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with the solvent system toluene/AcOEt (19/1), providing a colorless oil (5.5 g, 69%). ^{1}H NMR ($CDCl_3$): δ 1.25 (6H, t, $J = 7.1$ Hz), 1.49 (9H, s), 2.00–2.10 (2H, m), 3.35–3.70 (4H, m), 4.20 (2H, q, $J = 7.1$ Hz), 4.52 (1H, s), 4.75–4.95 (1H, m), 6.82 (2H, d, $J = 10$ Hz), 7.28 (2H, d, $J = 10$ Hz). MS (FAB): m/z 444 $(M+Na)^+$. IR (KBr): 2977, 1731, 1691, 1509, 1402, 1365, 1241, 1216, 1162, 1114, 1 9129 cm^{-1}. Anal. Calcd for $C_{22}H_{31}NO_7$: C, 62.69; H, 7.41; N, 3.32. Found: C, 62.84; H, 7.53; N, 3.28.

5.32. 2-(Bromomethyl)-1,3-benzothiazole-5-carbonitrile 19a

To a solution of 2-methyl-1,3-benzothiazole-6-carbonitrile **18a** (7.46 g, 43 mmol) in CCl_4 (250 mL) were added NBS (7.62 g, 43 mmol) and 2,2′-azobisisobutylonitrile (0.15 g, 0.91 mmol). The reaction mixture was refluxed and irradiated with light for 1 night. The resultant suspension was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography with toluene as an eluent, yielding colorless prismatic crystals (2.18 g, 20%). Mp 185–186 °C. ^{1}H NMR ($CDCl_3$): δ 4.83 (2H, s), 7.67 (1H, dd, $J = 9.1$, 1.7 Hz), 7.97 (1H, d, $J = 9.1$ Hz), 8.15 (1H, d, $J = 1.7$ Hz). IR (KBr): 2232 cm^{-1}. Anal. Calcd for $C_9H_5BrN_2S$: C, 42.71; H, 1.99; N, 11.07. Found: C, 42.55; H, 1.98; N, 11.00.

5.33. 2-(Bromomethyl)-1,3-benzothiazole-6-carbonitrile 19b

Starting with **18b** and following the procedure for the preparation of **19a** gave **19b** (yield, 49%) as pale yellow prismatic crystals. Mp 117–119 °C. ^{1}H NMR ($CDCl_3$): δ 4.84 (2H, s), 7.77 (1H, dd, $J = 10$, 1.7 Hz), 8.02 (1H, d, $J = 10$ Hz), 8.20 (1H, d, $J = 1.7$ Hz). IR (KBr): 2224 cm^{-1}. Anal. Calcd for $C_9H_5BrN_2S$: C, 42.71; H, 1.99; N, 11.07. Found: C, 42.52; H, 2.00; N, 11.20.

5.34. 3-(5-Amidino-1,3-benzothiazol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 21a

To a stirred and ice-cooled solution of compound **17** (6.3 g, 15 mmol) in THF (80 mL) was added NaH (60% in oil) (0.60 g). After stirring for a few minutes at room temperature, to the reaction mixture was added THF (100 mL) solution of 2-(bromomethyl)-1,3-benzothiazole-5-carbonitrile **19a** (3.2 g, 13 mmol) and stirred for 3 days at room temperature. To the mixture were added ice-cooled concentrated HCl and AcOEt. The separated organic layer was washed with water and dried over Na_2SO_4. After removal of the solvent, the residue was purified by silica gel column chromatography with $CHCl_3$ as an eluent, yielding a yellow oil **20a** (7.7 g, quant.). A solution of **20a** (7.7 g, 14 mmol) in dry EtOH (250 mL) was saturated with HCl gas with ice cooling and left to stand for 24 h at room temperature. After distilling off the solvents and HCl, the resulting residue was dissolved in ethanolic ammonia solution (14% w/v), and the whole was left to stand for 24 h at room temperature. After removal of the solvent, a solution of the resulting residue in 2 N HCl (100 mL) was heated at reflux for 1.5 h. After evaporation of the solvent, the

residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 5/95) and purified by preparative HPLC. After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (1.1 g, 17%). ^{1}H NMR (DMSO-d_6): δ 2.00–2.30 (2H, br), 2.80–4.40 (7H, m), 5.00–5.20 (1H, br), 6.95 (2H, d, $J = 8.3$ Hz), 7.34 (2H, d, $J = 8.3$ Hz), 7.70–7.90 (1H, m), 8.29 (1H, d,$J = 8.3$ Hz), 8.35–8.45 (1H, m), 9.15–9.70 (6H, m). MS (FAB): m/z 411 $(M+H)^+$. IR (KBr): 3030, 1678, 1600, 1510 cm^{-1}. Anal. Calcd for $C_{21}H_{22}N_4O_3S{\cdot}2HCl{\cdot}H_2O$: C, 50.31; H, 5.23; N, 11.17. Found: C, 50.17; H, 5.58; N, 11.12.

5.35. 3-(6-Amidino-1,3-benzothiazol-2-yl)-2-{4-[(3*S*)-pyrrolidin-3-yloxy]phenyl}propanoic acid 21b

Starting with **19b** (3.3 g, 13 mmol) and following the procedure for the preparation of **21a** gave **21b** (510 mg, 7.8%) as a pale brown amorphous solid. ^{1}H NMR (DMSO-d_6): δ 2.00–2.30 (2H, m), 3.00–4.35 (7H, m), 5.00–5.20 (1H, br), 6.95 (2H, d, $J = 8.3$ Hz), 7.35 (2H, d, $J = 8.3$ Hz), 7.90 (2H, dd,$J = 8.3$, 1.3 Hz), 8.13 (1H, d, $J = 8.3$ Hz), 8.60 (1H, d, $J = 1.3$ Hz), 9.20–9.80 (6H, m). MS (FAB): m/z 411 $(M+H)^+$. HRMS (FAB) calcd for $C_{21}H_{23}N_4O_3$: 411.1491. Found: 411.1513. IR (KBr): 3010, 1677, 1608 cm^{-1}. Anal. Calcd for $C_{21}H_{22}N_4O_3S{\cdot}2HCl{\cdot}1.5H_2O$: C, 49.41; H, 5.33; N, 10.98. Found: C, 49.23; H, 5.29; N, 10.95.

5.36. 3-(5-Amidino-1,3-benzothiazol-2-yl)-2-{4-[(3*S*)-1-ethanimidoylpyrrolidin-3-yloxy]phenyl}propanoic acid 22a

To a stirred and ice-cooled solution of compound **21a** (0.70 g, 1.4 mmol) in H_2O (15 mL) was added ethyl acetimidate hydrochloride (4.0 g, 3.2 mmol) in small portions while the reaction mixture was maintained at pH 7.5–8.5 with 1 N NaOH aq. After stirring for a further 3 h with cooling on an ice bath, the reaction mixture was adjusted to pH 1.0 and concentrated to dryness. The obtained residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 5/95) and then preparative HPLC (acetonitrile/H_2O). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (0.49 g, 63%). ^{1}H NMR (DMSO-d_6): δ 2.26, 2.30 (total 3H, each s), 2.80–4.35 (9H, m), 5.10–5.30 (1H, br), 6.99 (2H, d, $J = 7.9$ Hz), 7.31 (2H, d, $J = 7.9$ Hz), 7.88 (2H, d, $J = 7.9$ Hz), 8.25 (1H, d, $J = 7.9$ Hz), 8.40–8.70 (2H, m), 9.33, 9.55 (total 4H, br s). IR (KBr): 3100, 1674, 1510 cm^{-1}. Anal. Calcd for $C_{23}H_{25}N_5O_3S{\cdot}2HCl{\cdot}2.2H_2O$: C, 48.97; H, 5.61; N, 12.42. Found: C, 49.19; H, 5.28; N, 12.07.

5.37. *tert*-Butyl 4-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-piperidinecarboxylate 24a

To a stirred solution of ethyl 2-(4-hydroxyphenyl)-2-oxoacetate **31** (5.0 g, 26 mmol), *tert*-butyl 4-hydroxy-1-piperidinecarboxylate **32a** (5.2 g, 26 mmol) and triphenylphosphine (10 g, 39 mmol) in anhydrous THF (40 mL) was added diethyl azodicarboxylate (6.7 g, 39 mmol) at room temperature. The resultant solution was stirred for 24 h. After removal of the solvent, the residue was purified by silica gel column chromatography with the solvent system hexane/AcOEt (7/3–1/1), providing a yellow oil (5.6 g, 58%). ^{1}H NMR ($CDCl_3$): δ 1.35 (3H, t, $J = 6$ Hz), 1.49 (9H, s), 1.80–2.00 (4H, m), 3.20–4.00 (4H, m), 4.46 (2H, q, $J = 6$ Hz), 4.60–4.80 (1H, m), 7.01 (2H, d, $J = 8$ Hz), 8.04 (2H, d, $J = 8$ Hz). MS (FAB): m/z 378 $(M+H)^+$. HRMS (FAB) calcd for $C_{20}H_{28}NO_6$: 378.1917. Found: 378.1872. IR (ATR): 2975, 2935, 2871, 1673, 1594, 1421, 1365, 1257, 1232, 1207, 1159, 1022, 970, 848 cm^{-1}.

5.38. *tert*-Butyl (3*R*)-3-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-pyrrolidinecarboxylate 24b

Starting with **32b** and following the procedure for the preparation of **24a** gave **24b** (yield, 80%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.40 (3H, t, $J = 7.2$ Hz), 1.46 (9H, s), 2.00–2.30 (2H, m), 3.40–3.70 (4H, m), 4.43 (2H, q, $J = 7.2$ Hz), 4.90–5.10 (1H, m), 6.95 (2H, d, $J = 9.0$ Hz), 7.99 (2H, d, $J = 9.0$ Hz). MS (EI): m/z 363 $(M+H)^+$. HRMS (EI) calcd for $C_{19}H_{25}NO_6$: 363.1682. Found: 363.1691.

5.39. *tert*-Butyl (2*S*)-2-[4-(2-ethoxy-2-oxoacetyl)phenoxy]methyl-1-pyrrolidinecarboxylate 24c

Starting with **32c** and following the procedure for the preparation of **24a** gave **24c** (yield, 46%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.41 (3H, t, $J = 10.7$ Hz), 1.47 (9H, s), 1.65–2.15 (4H, br), 3.20–3.50 (2H, br), 3.80–4.30 (3H, m), 4.43 (2H, q, $J = 10.7$ Hz), 7.0 (2H, d, $J = 9.5$ Hz), 7.95 (2H, d, $J = 9.5$ Hz). MS (FAB): m/z 378 $(M+H)^+$. HRMS (FAB) calcd for $C_{20}H_{28}NO_6$: 378.1917. Found: 378.1888. IR (ATR): 2975, 2879, 1733, 1675, 1596, 1571, 1509, 1388, 1365, 1307, 1263, 1203, 1157, 1106, 1016, 977, 842 cm^{-1}.

5.40. Ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(7-cyano-2-naphthyl)propanoate 25d

To a solution of phosphonium salt **23** (8.40 g, 16.5 mmol) and *tert*-butyl (3*S*)-3-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-pyrrolidinecarboxylate **2** (5.00 g, 13.8 mmol) in dry THF (100 mL)–EtOH (100 mL) was added DBU (2.51 g, 16.5 mmol) at room temperature. The mixture was stirred for 3 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with hexane/ethyl acetate (7/3) as an eluent, providing ethyl 2-(4-{[(3*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(7-cyano-2-naphthyl)propenoate as a mixture of *E* and *Z* forms. The mixture of *E* and *Z* and $PdO(H_2O)BaSO_4$ (palladium oxide hydrate barium sulfate) (2.0 g) in THF (80 mL)–EtOH (80 mL) was shaken at room temperature under a current of hydrogen (1 atm). After filtration

of the catalyst, followed by evaporation of the filtrate, the residue was purified by silica gel column chromatography with hexane/ethyl acetate (7/3) as an eluent, yielding a colorless viscous oil (6.24 g, 90%). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, $J = 7.0$ Hz), 1.47 (9H, s), 2.00–2.20 (2H, m), 3.00–3.30 (1H, m), 3.40–3.70 (4H, m), 3.80–4.20 (4H, m), 4.84 (1H, m), 6.80–8.20 (10H, m). MS (EI): m/z 514 M^+. HRMS (EI) calcd for $C_{31}H_{34}N_2O_5$: 514.2468. Found: 514.2483.

5.41. Ethyl 2-(4-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl]oxy}phenyl)-3-(7-cyano-2-naphthyl)propanoate 25a

Starting with **24a** and following the procedure for the preparation of **25d** gave **25a** (yield, 80%) as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, $J = 6.0$ Hz), 1.49 (9H, s), 1.70–2.00 (4H, m), 3.00–4.10 (9H, m), 4.45 (1H, m), 6.80–8.10 (10H, m). MS (FAB): m/z 529 $(M+H)^+$. HRMS (FAB) calcd for $C_{32}H_{37}N_2O_5$: 529.2702. Found: 529.2655. IR (ATR): 2975, 2931, 2867, 2225, 1725, 1687, 1506, 1427, 1365, 1249, 1236, 1170, 1149, 1132, 1081, 1049, 835 cm^{-1}.

5.42. Ethyl 2-(4-{[(3*R*)-1-(*tert*-butoxycarbonyl)pyrrolidin-3-yl]oxy}phenyl)-3-(7-cyano-2-naphthyl)propanoate 25b

Starting with **24b** and following the procedure for the preparation of **25d** gave **25b** (yield, 81%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.11 (3H, t, $J = 7.0$ Hz), 1.47 (9H, s), 2.00–2.20 (2H, m), 3.00–4.00 (7H, m), 4.06 (2H, q, $J = 7.0$ Hz), 4.85 (1H, m), 6.80–8.20 (10H, m). MS (FAB): m/z 515 $(M+H)^+$. HRMS (FAB) calcd for $C_{31}H_{35}N_2O_5$: 515.2546. Found: 515.2533. IR (ATR): 2975, 2875, 2225, 1720, 1681, 1508, 1411, 1365, 1245, 1228, 1170, 1106, 1068, 898, 836, 813 cm^{-1}.

5.43. Ethyl 2-(4-{[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]methoxy}phenyl)-3-(7-cyano-2-naphthyl)propanoate 25c

Starting with **24c** and following the procedure for the preparation of **25d** gave **25c** (yield, 68%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.18 (3H, t, $J = 7.0$ Hz), 1.47 (9H, s), 1.90–2.10 (4H, m), 3.20–3.60 (2H, m), 3.90–4.50 (8H, m), 6.80–8.20 (10H, m). MS (FAB): m/z 529 $(M+H)^+$. HRMS (FAB) calcd for $C_{32}H_{37}N_2O_5$: 529.2702. Found: 529.2715. IR (ATR): 2975, 2933, 2875, 2225, 1727, 1685, 1509, 1388, 1243, 1164, 1106, 1033, 840 cm^{-1}.

5.44. Ethyl 3-(7-amidino-2-naphthyl)-2-[4-(piperidin-4-yloxy)phenyl]propanoate 26a

A solution of **26a** (3.93 g, 7.43 mmol) in dry EtOH (120 mL) was saturated with HCl gas with ice cooling and left to stand for 24 h at room temperature. After distilling off the solvents and HCl, the resulting residue was dissolved in ethanolic ammonia solution (13% w/v), and the whole was left to stand for 24 h at room temperature. After removal of the solvent, the resulting residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 10/90). The solvents were removed to give a colorless amorphous solid (3.00 g, 78%). ^{1}H NMR (DMSO-d_6+HCl): δ 1.01 (3H, t, $J = 7.1$ Hz), 1.75–1.90 (2H, m), 2.05–2.15 (2H, m), 3.00–3.10 (2H, br), 3.15–3.25 (3H, m), 3.54–3.50 (1H, m), 3.90–4.00 (2H, m), 4.08 (1H, t, $J = 7.8$ Hz), 4.55–4.65 (1H, br), 6.95 (2H, d, $J = 8.3$ Hz), 7.29 (2H, d, $J = 8.3$ Hz), 7.61 (1H, d, $J = 8.3$ Hz), 7.79 (1H, d, $J = 8.8$ Hz), 7.84 (1H, s), 7.95 (1H, d, $J = 8.3$ Hz), 8.08 (1H, d, $J = 8.8$ Hz), 8.40 (1H, s), 9.00–9.20 (2H, br), 9.26 (2H, s), 9.52 (2H, s). MS (FAB): m/z 446 $(M+H)^+$. HRMS (FAB) calcd for $C_{27}H_{32}N_3O_3$: 446.2444. Found: 446.2462. IR (KBr): 3348, 3112, 2948, 2800, 2736, 1716, 1670, 1608, 1508, 1454, 1374, 1248, 1214, 1170, 1134, 1108, 1062, 1026, 1014, 856, 844, 820 cm^{-1}.

5.45. Ethyl 3-(7-amidino-2-naphthyl)-2-{4-[(3*R*)-pyrrolidin-3-yloxy]phenyl}propanoate 26d

Starting with **25d** and following the procedure for the preparation of **26a** gave **26d** (yield, 56%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.01 (3H, t, $J = 7.0$ Hz), 2.00–2.20 (2H, m), 3.10–3.80 (7H, m), 3.98 (2H, q, $J = 7.0$ Hz), 5.10 (1H, m), 6.93 (2H, d, $J = 9.0$ Hz), 7.32 (2H, d, $J = 9.0$ Hz), 7.50–8.10 (5H, m), 8.44 (1H, s), 9.41 (2H, br), 9.59 (2H, br), 9.30–10.00 (2H, br). MS (FAB): m/z 432 $(M+H)^+$. IR (KBr): 3382, 1725, 1680, 1611, 1506, 1239, 1059, 966, 915, 843 cm^{-1}. Anal. Calcd for $C_{26}H_{29}N_3O_3 \cdot 2HCl \cdot 1.7H_2O$: C, 58.36; H, 6.49; N, 7.85. Found: C, 58.13; H, 6.49; N, 7.92.

5.46. 3-(7-Amidino-2-naphthyl)-2-{4-[(3*R*)-1-ethanimidoylpyrrolidin-3-yloxy]phenyl}propanoic acid 27b

A solution of **25b** (4.6 g, 9.0 mmol) in dry EtOH (150 mL) was saturated with HCl gas with ice cooling and left to stand for 20 h at room temperature. After distilling off the solvents and HCl, the resulting residue was dissolved in ethanolic ammonia solution (14% w/v), and the whole was left to stand for 23 h at room temperature. After removal of the solvent, one portion (2.6 g) of the resulting residue (5.0 g) was purified by HP-20 column chromatography (acetonitrile/H_2O, 10/90), providing ethyl 3-(7-amidino-2-naphthyl)-2-{4-[(3*R*)-pyrrolidin-3-yloxy]phenyl}propanoate (1.0 g, 2.0 mmol). With stirring and ice cooling, to EtOH (15 mL) solution of ethyl 3-(7-amidino-2-naphthyl)-2-{4-[(3*R*)-pyrrolidin-3-yloxy]phenyl}propanoate (1.0 g, 2.0 mmol) were added triethylamine (0.60 g, 6.0 mmol) and ethyl acetimidate hydrochloride (0.49 g, 4.0 mmol). After stirring for 14 h at room temperature, the solvent was distilled off. A solution of the obtained residue in 2 N HCl (50 mL) was heated at reflux for 30 min. After evaporation of the solvent, the residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 5/95–10/90) and then preparative HPLC (acetonitrile/H_2O). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a yellow amorphous solid (0.50 g, 19%). ^{1}H NMR

(DMSO-d_6): δ 2.00–2.40 (5H, m), 2.90–4.10 (7H, m), 5.20 (1H, m), 6.93 (2H, d, $J = 8$ Hz), 7.33 (2H, d, $J = 8$ Hz), 7.56 (2H, d, $J = 8$ Hz), 7.70–8.20 (4H, m), 8.45 (1H, s), 8.50–8.80 (2H, m), 9.45 (2H, br), 9.63 (2H, br). MS (FAB): m/z 445 $(M+H)^+$. IR (KBr): 3424, 1674, 1632, 1506, 1378, 1240, 1178, 1090, 850 cm^{-1}. Anal. Calcd for $C_{26}H_{28}N_4O_3 \cdot 2HCl \cdot 0.5H_2O$: C, 59.32; H, 5.93; N, 10.64. Found: C, 59.15; H, 6.20; N, 10.24.

5.47. 3-(7-Amidino-2-naphthyl)-2-[4-(1-ethanimidoylpiperidin-4-yloxy)phenyl]propanoic acid 27a

Starting with **25a** and following the procedure for the preparation of **27b** gave **27a** (yield, 16%) as a yellow amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.50–2.10 (4H, m), 2.31 (3H, s), 3.00–4.20 (7H, m), 4.60–4.80 (1H, br), 6.95 (2H, d, $J = 9.0$ Hz), 7.31 (2H, d, $J = 9.0$ Hz), 7.50–8.50 (6H, m), 8.93 (2H, br s), 9.45 (2H, br s), 9.62 (2H, br s). MS (FAB): m/z 459 $(M+H)^+$. IR (KBr): 3064, 1678, 1608, 1504, 1376, 1240, 1178, 1032, 912, 850, 714 cm^{-1}. Anal. Calcd for $C_{27}H_{30}N_4O_3 \cdot 2HCl \cdot 1.5H_2O$: C, 58.07; H, 6.32; N, 10.03. Found: C, 57.79; H, 6.39; N, 9.99.

5.48. 3-(7-Amidino-2-naphthyl)-2-{4-[(2*S*)-1-ethanimidoylpyrrolidin-2-ylmethoxy]phenyl}propanoic acid 27c

Starting with **25c** and following the procedure for the preparation of **27b** gave **27c** (yield, 24%) as a yellow amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.90–2.60 (7H, m), 3.00–4.70 (7H, m), 5.20 (1H, m), 6.92 (2H, d, $J = 9$ Hz), 7.31 (2H, d, $J = 9$ Hz), 7.80–8.10 (5H, m), 8.45 (1H, s), 8.50–8.70 (2H, m), 9.44 (2H, br), 9.64 (2H, br). MS (FAB): m/z 459 $(M+H)^+$. IR (KBr): 3388, 1674, 1512, 1376, 1242, 1178, 848 cm^{-1}. Anal. Calcd for $C_{27}H_{30}N_4O_3 \cdot 2HCl \cdot H_2O$: C, 59.02; H, 6.24; N, 10.20. Found: C, 59.14; H, 6.42; N, 9.74.

5.49. 3-(7-Amidino-2-naphthyl)-2-{4-[(3*S*)-1-propanimidoylpyrrolidin-3-yloxy]phenyl}propanoic acid 28d

To a stirred and ice-cooled solution of compound **26d** (500 mg, 1.07 mmol) and ethyl propanimidate hydrochloride (294 mg, 2.14 mmol) in dry EtOH (10 mL) was added triethylamine (0.45 mL, 3.21 mmol). After stirring for 16 h at room temperature, the solvent was distilled off. A solution of the obtained residue in 2 N HCl (15 mL) was heated at reflux for 30 min. After evaporation of the solvent, the residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 3/97–20/80) and then preparative HPLC (acetonitrile/H_2O). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a colorless amorphous solid (350 mg, 59%). ^{1}H NMR (DMSO-d_6): δ 0.95–1.10 (3H, m), 2.00–4.10 (12H, m), 5.00–5.20 (1H, m), 6.80 (2H, d, $J = 8$ Hz), 7.26 (2H, d, $J = 8$ Hz), 7.30–8.10 (5H, m), 8.33 (1H, s), 8.40–8.70 (1H, m), 9.00–9.30 (1H, m), 9.32 (1H, br), 9.50 (1H, br). IR (KBr): 3076, 1674, 1630, 1608, 1506, 1242, 1178, 850 cm^{-1}. Anal. Calcd for $C_{27}H_{30}N_4O_3 \cdot 2HCl \cdot 1.5H_2O$: C, 58.06; H, 6.32; N, 10.03. Found: C, 57.94; H, 6.32; N, 9.77.

5.50. 3-(7-Amidino-2-naphthyl)-2-{4-[(1-propanimidoylpiperidin-4-yl)oxy]phenyl}propanoic acid 28a

Starting with **26a** and ethyl propanimidate and following the procedure for the preparation of **28d** gave **28a** (yield, 8.0%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.15 (3H, t, $J = 7.3$ Hz), 1.73 (2H, br s), 2.03 (2H, br s), 2.61 (2H, q, $J = 7.3$ Hz), 3.10–3.60 (4H, m), 3.75–3.85 (2H, m), 3.98 (1H, t, $J = 7.8$ Hz), 4.68 (1H, br s), 6.95 (2H, d, $J = 8.8$ Hz), 7.30 (2H, d, $J = 8.8$ Hz), 7.61 (1H, d, $J = 8.3$ Hz), 7.78 (1H, d, $J = 8.3$ Hz), 7.86 (1H, s), 7.96 (1H, d, $J = 8.8$ Hz), 8.08 (1H, d, $J = 8.3$ Hz), 8.79 (1H, br s), 9.23 (1H, br s), 9.26 (2H, br s), 9.53 (2H, br s). MS (FAB): m/z 473 $(M+H)^+$. HRMS (FAB) calcd for $C_{28}H_{33}N_4O_3$: 473.2553. Found: 473.2564. IR (KBr): 3040, 1670, 1608 cm^{-1}. Anal. Calcd for $C_{28}H_{32}N_4O_3 \cdot 2HCl \cdot 1.5H_2O$: C, 58.74; H, 6.51; N, 9.79. Found: C, 58.57; H, 6.44; N, 9.43.

5.51. 3-(7-Amidino-2-naphthyl)-2-{4-[(1-butanimidoylpiperidin-4-yl)oxy]phenyl}propanoic acid 29a

Starting with **26a** and ethyl butanimidate and following the procedure for the preparation of **28d** gave **29a** (yield, 53%) as a colorless amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.96 (3H, t, $J = 7.3$ Hz), 1.50–1.60 (2H, m), 1.71 (2H, br s), 2.03 (2H, br s), 2.50–2.60 (2H, m), 3.10–3.20 (1H, m), 3.70–3.85 (1H, m), 3.95–4.05 (1H, m), 4.68 (1H, br s), 6.96 (2H, d, $J = 8.8$ Hz), 7.30 (2H, d, $J = 8.8$ Hz), 7.61 (1H, d, $J = 8.3$ Hz), 7.80 (1H, d, $J = 8.3$ Hz), 7.87 (1H, s), 7.96 (1H, d, $J = 8.3$ Hz), 8.08 (1H, d, $J = 8.3$ Hz), 8.42 (1H, s), 8.86 (1H, s), 9.32 (3H, s), 9.58 (2H, s). IR (KBr): 3100, 1674, 1620, 1506 cm^{-1}. Anal. Calcd for $C_{29}H_{34}N_4O_3 \cdot 2HCl \cdot 1.5H_2O$: C, 59.38; H, 6.70; N, 9.55. Found: C, 59.40; H, 6.92; N, 9.32.

5.52. 3-(7-Amidino-2-naphthyl)-2-{4-[(3*S*)-1-butanimidoylpyrrolidin-3-yloxy]phenyl}propanoic acid 29d

Starting with **26d** and ethyl butanimidate and following the procedure for the preparation of **28d** gave **29d** (yield, 57%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 0.60–4.00 (17H, m), 4.90–5.10 (1H, m), 6.79 (2H, d, $J = 8.0$ Hz), 7.21 (2H, d, $J = 8.0$ Hz), 7.30–8.10 (5H, m), 8.30 (1H, br), 8.34 (1H, s), 8.40–8.70 (1H, m), 9.35 (1H, br), 9.55 (1H, br). IR (KBr): 3052, 1672, 1625, 1608, 1504, 1238, 1176, 848 cm^{-1}. Anal. Calcd for $C_{28}H_{32}N_4O_3 \cdot 2HCl \cdot 2H_2O$: C, 57.83; H, 6.59; N, 9.63. Found: C, 57.83; H, 6.47; N, 9.49.

5.53. 3-{7-Amidino-2-naphthyl}-2-[4-({(3*S*)-1-[imino(phenyl)methyl]pyrrolidin-3-yl}oxy)phenyl]propanoic acid 30d

Starting with **26d** and ethyl benzimidate and following the procedure for the preparation of **28d** gave **30d** (yield,

17%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6): δ 2.00–4.10 (10H, m), 4.85–5.25 (1H, m), 6.70–8.10 (14H, m), 8.32 (1H, s), 9.10–9.50 (4H, m). IR (KBr): 3044, 1672, 1606, 1504, 1238, 1178, 848 cm^{-1}. Anal. Calcd for $C_{31}H_{30}N_4O_3 \cdot 2HCl \cdot 2H_2O$: C, 60.48; H, 5.91; N, 9.10. Found: C, 60.70; H, 6.14; N, 9.12.

5.54. Ethyl 2-(4-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl]oxy}phenyl)-3-(5-cyano-1-benzothien-2-yl)propanoate 33

To a solution of phosphonium salt **1a** (4.81 g, 10.2 mmol) and *tert*-butyl 4-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-piperidinecarboxylate **24a** (3.51 g, 10.3 mmol) in dry THF (40 mL)–EtOH (40 mL) was added DBU (1.69 g, 11.1 mmol) at room temperature. The mixture was stirred for 3 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with CH_2Cl_2 as an eluent, providing ethyl 2-(4-{[1-(*tert*-butoxycarbonyl)piperidin-4-yl]oxy}phenyl)-3-(5-cyano-1-benzothien-2-yl)propenoate as a mixture of *E* and *Z* forms. The mixture of *E* and *Z* and 10%Pd on carbon (50% wet) (9.0 g) in EtOH (100 mL) was shaken at room temperature under a current of hydrogen (1 atm). After filtration of the catalyst, followed by evaporation of the filtrate, the residue was purified by silica gel column chromatography with hexane/ethyl acetate (7/3) as an eluent, to yield a yellow amorphous solid (1.92 g, 39%). ^{1}H NMR ($CDCl_3$): δ 1.10 (3H, t, $J = 6.0$ Hz), 1.50 (9H, s), 1.70–2.00 (4H, m), 3.20–4.00 (7H, m), 4.15 (2H, q, $J = 6.0$ Hz), 4.30–4.60 (1H, m), 6.80–8.10 (8H, m). MS (FAB): m/z 535 $(M+H)^+$. HRMS (FAB) calcd for $C_{30}H_{35}N_2O_5S$: 535.2267. Found: 535.2244. IR (ATR): 2956, 2863, 2227, 1729, 1689, 1506, 1425, 1365, 1245, 1226, 1201, 1164, 1124, 1027, 912, 813 cm^{-1}.

5.55. 3-(5-Amidino-1-benzothien-2-yl)-2-[4-(1-ethanimidoylpiperidin-4-yloxy)phenyl]propanoic acid 34

Starting with **33** and following the procedure for the preparation of **27b** gave **34** (yield, 6.8%) as a pale brown amorphous solid. ^{1}H NMR (DMSO-d_6): δ 1.65–2.10 (4H, m), 2.32 (3H, s), 3.20–4.00 (7H, m), 4.60–4.70 (1H, m), 6.96 (2H, d, $J = 8.3$ Hz), 7.30 (3H, m), 7.69 (1H, d, $J = 8.3$ Hz), 8.10 (1H, d, $J = 8.3$ Hz), 8.26 (1H, s), 8.95 (2H, br s), 9.32 (2H, br s), 9.52 (2H, br s). MS (FAB): m/z 465 $(M+H)^+$. IR (KBr): 3424, 1674, 1510, 1448, 1242, 1178, 1062 cm^{-1}. Anal. Calcd for $C_{25}H_{28}N_4O_3S \cdot 2HCl \cdot 1.5H_2O$: C, 53.19; H, 5.89; N, 9.92. Found: C, 53.60; H, 5.45; N, 9.45.

5.56. Ethyl 2-(4-{[(2*S*)-1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]methoxy}phenyl)-3-(5-cyano-1-benzothien-2-yl)propanoate 35

Starting with **24c** and following the procedure for the preparation of **33** gave **35** (yield, 57%) as a viscous oil. ^{1}H NMR ($CDCl_3$): δ 1.17 (3H, t, $J = 7.6$ Hz), 1.47 (9H, s), 1.70–2.10 (4H, br), 3.20–3.50 (3H, m), 3.60–4.50 (7H, m), 6.80–7.10 (4H, m), 7.10–7.50 (3H, m), 7.60–8.05 (2H, m). MS (FAB): m/z 535 $(M+H)^+$. HRMS (FAB) calcd for $C_{30}H_{35}N_2O_5S$: 535.2267. Found: 535.2239. IR (ATR): 2975, 2225, 1720, 1697, 1513, 1402, 1365, 1272, 1245, 1216, 1164, 1103, 1031, 892, 829 cm^{-1}.

5.57. 3-(5-Amidino-1-benzothien-2-yl)-2-{4-[(2*S*)-1-ethanimidoylpyrrolidin-2-ylmethoxy]phenyl}propanoic acid 36

Starting with **36** and following the procedure for the preparation of **27b** gave **36** (yield, 63%) as a pale yellow amorphous solid. ^{1}H NMR (DMSO-d_6): δ 2.05 (4H, br), 2.25 and 2.43 (total 3H, each s), 3.00–4.50 (8H, m), 6.80–7.00 (2H, m), 7.15–7.30 (3H, m), 7.65 (1H, d, $J = 8.9$ Hz), 8.10 (1H, d, $J = 8.9$ Hz), 8.25 (1H, s), 8.55 (2H, br s), 9.25 (2H, br s), 9.45 (2H, br s). MS (FAB): m/z 465 $(M+H)^+$. HRMS (FAB) calcd for $C_{25}H_{29}N_4O_3S$: 465.1960. Found: 465.1967. IR (ATR): 3132, 1672, 1612 cm^{-1}. Anal. Calcd for $C_{25}H_{28}N_4O_3S \cdot 2HCl \cdot 1.5H_2O$: C, 53.19; H, 5.89; N, 9.92. Found: C, 53.19; H, 5.79; N, 9.92.

5.58. 3-(6-Amidino-1-ethyl-1*H*-indol-2-yl)-2-[4-(1-ethanimidoylpiperidin-4-yloxy)phenyl]propanoic acid 38

To a solution of phosphonium salt **1h** (11.4 g, 21.7 mmol) and *tert*-butyl 4-[4-(2-ethoxy-2-oxoacetyl)phenoxy]-1-piperidinecarboxylate **24a** (7.50 g, 20.6 mmol) in dry THF (150 mL)–MeOH (150 mL) was added DBU (3.44 g, 22.6 mmol) at room temperature. The mixture was stirred for 17.5 h at room temperature. After removal of the solvent, the residue was purified by silica gel column chromatography with hexane/ethyl acetate (2/1) as an eluent, providing ethyl 2-{4-[1-(*tert*-butoxycarbonyl)piperidin-4-yloxy]phenyl}-3-(6-cyano-1-ethyl-1*H*-indol-2-yl)propenoate as a mixture of *E* and *Z* forms. The mixture of *E* and *Z* and $PdO(H_2O)BaSO_4$ (palladium oxide hydrate barium sulfate) (1.5 g) in MeOH (250 mL) was shaken at room temperature under a current of hydrogen (1 atm). After filtration of the catalyst, followed by concentration of the filtrate, the residue was purified by silica gel column chromatography with hexane/ethyl acetate (4/1) as an eluent, providing an ester as a colorless amorphous solid. A solution of the ester in dry EtOH (130 mL) was saturated with HCl gas with ice cooling and left to stand for 20 h at room temperature. After distilling off the solvents and HCl, the resulting residue was dissolved in ethanolic ammonia solution (14% w/v), and the whole was left to stand for 23 h at room temperature. After removal of the solvent, the resulting residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 10/90), providing ethyl 3-(6-amidino-1-ethyl-1*H*-indol-2-yl)-2-[4-(piperidin-4-yloxy)phenyl]propanoate **37**. With stirring and ice cooling, to EtOH (37 mL) solution of compound **37** were added triethylamine (2.26 g, 22.4 mmol) and ethyl acetimidate hydrochloride (1.79 g, 14.5 mmol). After stirring for 17 h at room temperature, the reaction mixture was distilled off the solvent. A

solution of the obtained residue in 2 N HCl (120 mL) was heated at reflux for 1.5 h. The reaction mixture was concentrated to dryness to give a residue. After evaporation of the solvent, the residue was purified by HP-20 column chromatography (acetonitrile/H_2O, 15/85) and then preparative HPLC (acetonitrile/H_2O). After the addition of a small amount of concentrated HCl to selected fractions, the solvents were removed to give a pale yellow amorphous solid (0.56 g, 8.1%). ^{1}H NMR (DMSO-d_6): δ 1.30 (3H, t, $J = 7.0$ Hz), 1.73–2.10 (4H, m), 2.31 (3H, s), 3.05–3.15 (1H, m), 3.30–3.80 (5H, m), 4.05 (1H, t), 4.20–4.35 (2H, m), 4.70 (1H, br s), 6.38 (1H, s), 6.97 (2H, d, $J = 8.5$ Hz), 7.37 (2H, d, $J = 8.3$ Hz), 7.48 (1H, d, $J = 8.3$ Hz), 7.61 (1H, d, $J = 8.3$ Hz), 8.14 (1H, s), 8.86 (1H, br), 9.15–9.50 (5H, m). IR (KBr): 1672, 1614, 1510, 1466, 1342 cm^{-1}. Anal. Calcd for $C_{27}H_{33}N_5O_3 \cdot 2HCl \cdot H_2O$: C, 57.24; H, 6.58; N, 12.36. Found: C, 57.42; H, 6.81; N, 12.33.

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