Novel Malonamide Derivatives as $\alpha_v\beta_3$ Antagonists. Syntheses and Evaluation of 3-(3-Indolin-1-yl-3-oxopropanoyl)aminopropanoic Acids on Vitronectin Interaction with $\alpha_v\beta_3$

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In attempt to find novel integrin $\alpha_v\beta_3$ antagonists, we selected SC65811 and its guanidine analogue (1) as lead compounds. Modification of the glycine part of SC65811 led to a new series of malonamide derivatives that exhibited $\alpha_v\beta_3$ inhibitory activity. Among them, (*R*,*S*)-3-{3-[6-(3-benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoic acid (43a) showed not only potent activity with an IC_{50} value of 3.0 nM but also good selectivity for $\alpha_v\beta_3$ relative to $\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$, and $\alpha_v\beta_5$ with IC_{50} values of 19000, 11000, and 14 nM, respectively. Furthermore, optimization of 43a led to the most potent $\alpha_v\beta_3$ antagonist, (*R*,*S*)-3-(3-{6-[(4,5-dihydro-1*H*-imidazol-2-yl)amino]indolin-1-yl}-3-oxopropanoylamino)-3-(quinolin-3-yl)propanoic acid (43l) with an IC_{50} value of 0.42 nM. The synthesis and the structure–activity relationships of these malonamide derivatives are presented.

Key words $\alpha_v\beta_3$ antagonist; malonamide; indoline; selectivity

The integrins are transmembrane heterodimeric glycoproteins that mediate cell adhesion, migration and cellular signaling and are formed by various combinations of at least 16α subunits and 8β subunits.[3] The integrin families are often classified based on the β subunits.

The β_3 integrins consist of $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) and $\alpha_v\beta_3$. Among them, $\alpha_{IIb}\beta_3$, which is known as the receptor for fibrinogen, the von Willebrand factor and fibronectin, is expressed on the platelet membrane and has received considerable attention as a drug target due to its important role in platelet aggregation which is a significant mechanism of thrombosis.[4] The other β_3 integrin $\alpha_v\beta_3$ which is known as vitronectin receptor is distributed in various cell types, such as platelets, endothelial cells, melanoma cells, smooth muscle cells and osteoclasts.[5] Because $\alpha_v\beta_3$ plays an important role in angiogenesis, migration of muscular smooth muscle cells and adhesion of osteoclast to the bone matrix, inhibition of $\alpha_v\beta_3$ is an attractive target for the treatment of the disease involving neovascularization, such as rheumatoid arthritis and cancer, restenosis following percutaneous transluminal coronary angioplasty (PTCA) and osteoporosis.[6]

The arginine–glycine–asparatic acid (RGD) sequence, which commonly exists within the surface loops of β_3 integrin ligands is estimated as the minimal sequence necessary for binding to β_3 integrins, and regarded as a template for low-molecular-weight β_3 integrin antagonists. As for $\alpha_{IIb}\beta_3$ antagonists, a study of RGD-containing tri- and tetrapeptides led to the discovery of cyclic RGD-containinng peptides, *e.g.*, SK&F 106760.[7] Although SK&F 106760 displayed potent *in vivo* antiaggregatory activity following intravenous infusion,[8] peptidic antagonists are generally thought to lack the activity and duration after oral administration. As a result of earnest studies of nonpeptidic RGD mimetics, several orally active $\alpha_{IIb}\beta_3$ antagonists were discoverd.[9] In the research on nonpeptidic $\alpha_{IIb}\beta_3$ antagonists, it has been proved that a basic part such as an arginine mimetic and an acidic part such as an asparatic acid mimetic are indispensable. On the contrary, a wide variety of structures can be available for central scaffolds in place of the peptide backbone.[10] For example, benzene, phenoxymethyl lactam, isoxazoline, benzodiazepine rings and succinamide moiety are respectively adopted as central scaffolds for tirofiban, fradafiban, roxifiban, lotrafiban and xemilofiban.[5] Among them, tirofiban has been launched, moreover, fradafiban and roxifiban are currently undergoing clinical trials.

Recently, like $\alpha_{IIb}\beta_3$ antagonists, nonpeptidic $\alpha_v\beta_3$ antagonists having various central scaffolds, such as benzene, benzodiazepine, piperazine, isoxazoline, indazole, hydantoin and glycine, have been reported.[11—17] On the basis of these findings, in attempt to find more potent and selective nonpeptidic $\alpha_v\beta_3$ antagonists, we have focused on the identification of novel central scaffolds. In the beginning, we selected SC65811 and its guanidine analogue (**1**) as lead compounds because they had potent and selective affinity for $\alpha_v\beta_3$ and had a simple structure consisting of a benzylphenylurea or phenylguanidine moiety, glycine and β-amino propanoic acid moieties as a basic part, a central scaffold and an acidic part, respectively. Furthermore, it is noteworthy that the benzylphenylurea moiety of SC65811 seems to act as a basic part instead of guanidine, in spite of the fact that the urea moiety shows no basicity.[18] We initially investigated a modification of a glycine moiety of SC65811. As a result of some modifications, we have discovered a 1-(3-oxopropanoyl)indoline moiety, as a novel central scaffold for $\alpha_v\beta_3$ antagonists. Subsequently, optimization of both the basic and acidic parts led to the identification of 3-[3-(indolin-1-yl)-3-oxopropanoylamino]propanoic acid derivatives as novel potent and selective $\alpha_v\beta_3$ antagonists.

In this paper, we wish to report the synthesis and structure–activity relationships of the novel malonamide derivatives.

Chemistry

The synthetic routes to the malonamide derivatives are summarized in Chart 1. Treatment of 3-nitroaniline (**2a**) and *N*-methyl-3-nitroaniline (**2b**) with ethyl malonyl chloride in the presence of triethylamine (Et_3N) gave compounds **3a** and

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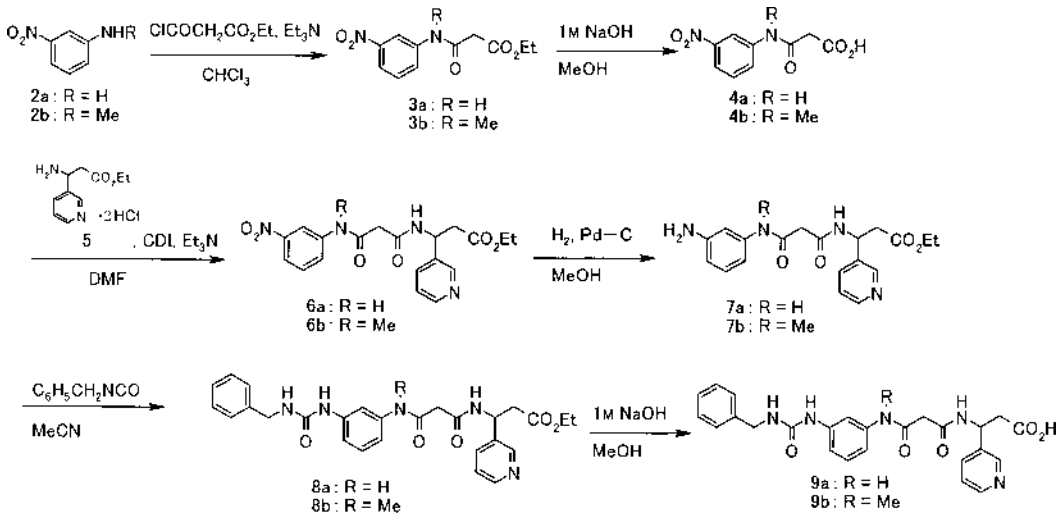


Chart 1

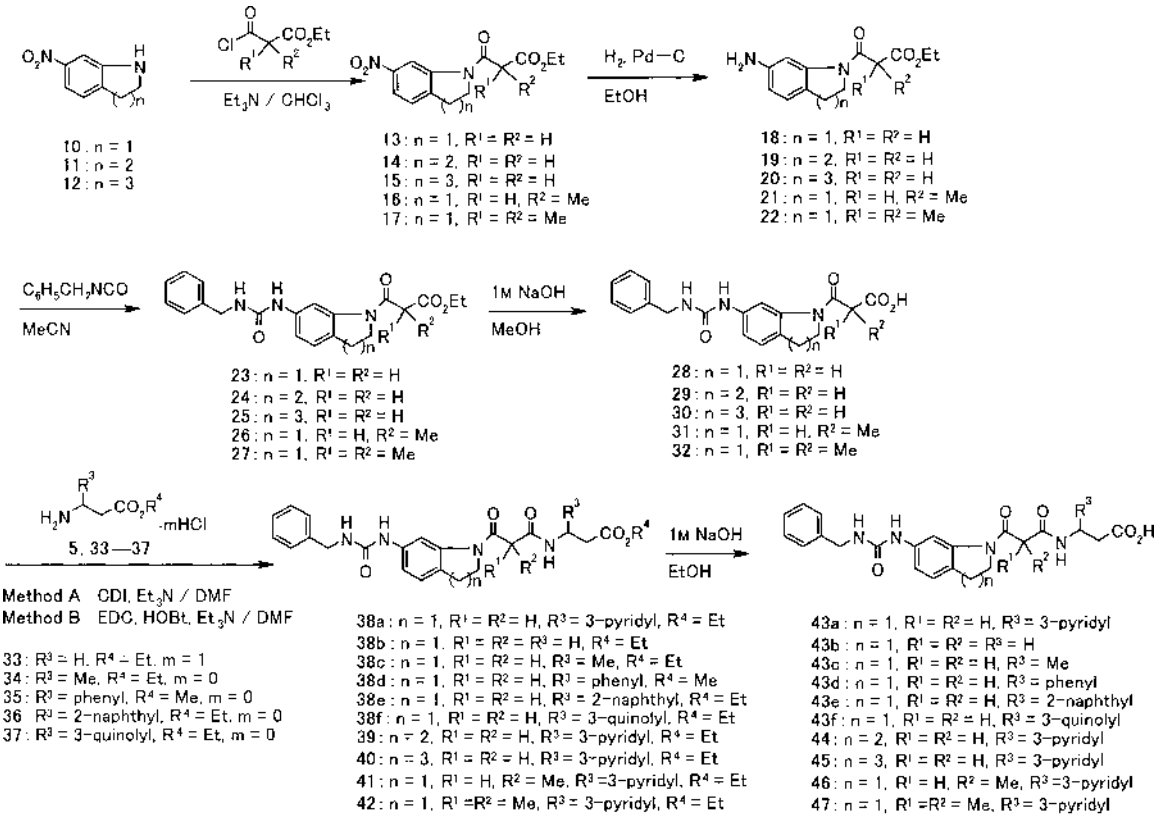


Chart 2

3b respectively. Saponification of compounds **3a** and **3b** furnished the corresponding carboxylic acids **4a** and **4b** which were condensed with (*R*,*S*)-ethyl 3-amino-3-(pyridin-3-yl)propanoate dihydrochloride (**5**)[17a] in the presence of 1,1′-carbonyldiimidazole (CDI) in *N*,*N*′-dimethylformamide (DMF) to give compounds **6a** and **6b**. Compounds **6a** and **6b** were converted into aniline derivatives **7a** and **7b** by catalytic hydrogenation in the presence of 10% palladium on carbon (Pd–C). Treatment of compounds **7a** and **7b** with benzyl isocyanate in acetonitrile gave benzylurea derivatives **8a** and **8b**, respectively. Subsequent hydrolysis of **8a** and **8b** under basic condition gave the desired malonamide derivatives **9a** and **9b**, respectively.

The preparation of indoline derivatives **43a**—**f**, **46** and **47**, tetrahydroquinoline derivative **44** and tetrahydrobenzo[*b*]-azepine derivative **45** are shown in Chart 2. Compounds **10**—**12** were converted into ethyl malonamide derivatives **13**—**17**, then the nitro group of compounds **13**—**17** were converted into benzylurea in a manner similar to that described above. Saponification of compounds **23**—**27** furnished the corresponding carboxylic acid derivatives **28**—**32** which were condensed with *β*-alanine derivatives **5**, **33**—**37** in the presence of CDI (method A) or 1-(3-dimethylaminopropyl)-3′-ethylcarbodiimide hydrochloride (EDC) and 1-hy-

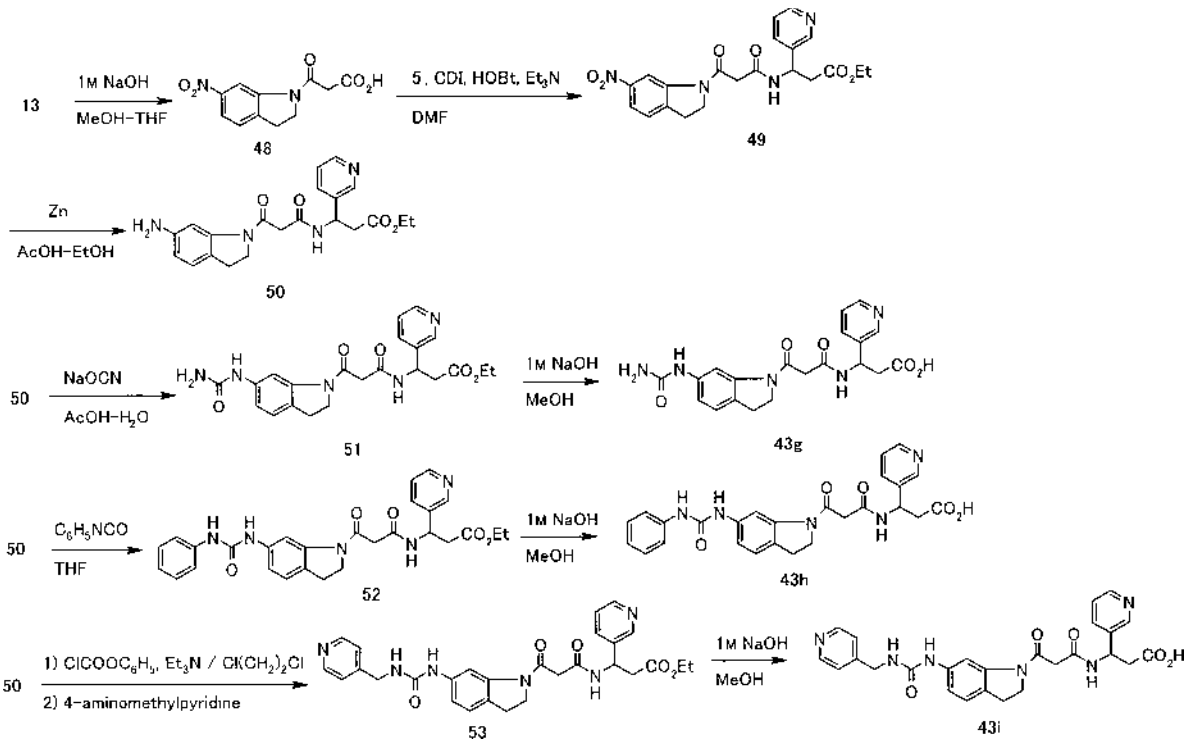


Chart 3

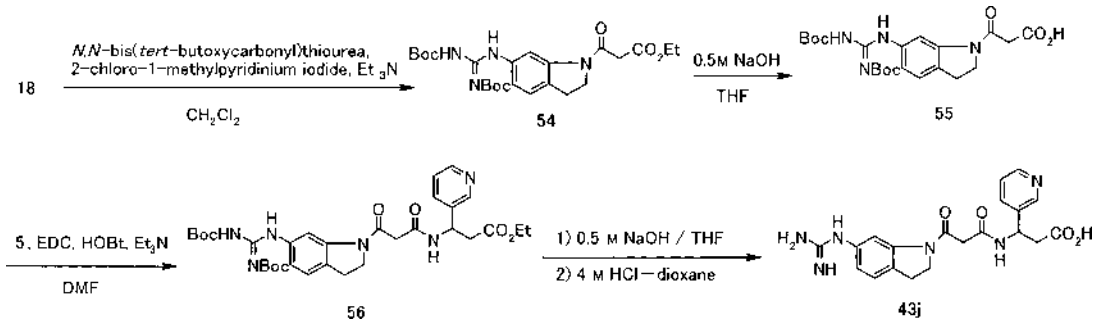


Chart 4

droxybenzotriazole (HOBt) (method B) to give the desired ethyl propanoate derivatives **38a**—**f** and **39**—**42**. Hydrolysis of compounds **38a**—**f** and **39**—**42** afforded indoline **43a**—**f**, **46** and **47**, tetrahydroquinoline **44** and tetrahydrobenzo[*b*]-azepine **45**, respectively.

Chart 3 illustrates the preparation of indoline derivatives having various substituents instead of the benzyl group on the urea moiety of compound **43a**. Compound **13** was converted into compound **49** in a manner similar to that described above. Hydrogenation of compound **49** in the presence of zinc powder in acetic acid–ethanol yielded the aniline **50**. Treatment of **50** with sodium cyanate in acetic acid–H_2O,[19] or with phenylisocyanate in tetrahydrofuran (THF), and then subsequent hydrolysis afforded urea derivatives **43g** and **43h**. Conversion of compound **50** into a phenyl carbamate with phenyl chloroformate, and subsequent treatment with a small excess of 4-aminomethylpyridine gave pyridin-4-ylmethylurea derivative **53**.[20] Subsequent hydrolysis of **53** under basic condition gave the desired propanoic acid derivative **43i**.

An indoline derivative containing guanidine instead of benzylurea of **43a** was prepared according to the method shown in Chart 4. Guanylation of compound **18** with *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea in the presence of 2-chloro-1-methylpyridine iodide and Et_3N in dichloromethane[21] afforded the *tert*-butoxycarbonyl (Boc) protected guanidine derivative **54**. Careful hydrolysis of **54** with 0.5 M NaOH in THF followed by coupling with compound **5** in the presence of EDC and HOBt furnished ethyl propanoate derivative **56**. Hydrolysis of the ethyl ester and removal of the Boc group afforded the desired guanidine derivative **43j**.

Cyclic guanidine derivatives (**43k**, **l**) were synthesized according to the method shown in Chart 5. Treatment of compound **18** with 1-*tert*-butoxycarbonyl-2-(3,5-dimethylpyrazolyl)-4,5-dihydro-1*H*-imidazole (**57**) in acetonitrile followed by hydrolysis afforded 3-oxopropanoic acid having a Boc-protected cyclic guanidine moiety **59**.[22] As described in Chart 4, compound **59** was coupled with compound **5** or **37** to give ethyl propanoate derivatives **60** or **61**, respectively. Hydrolysis and removal of the Boc group of compounds **60**

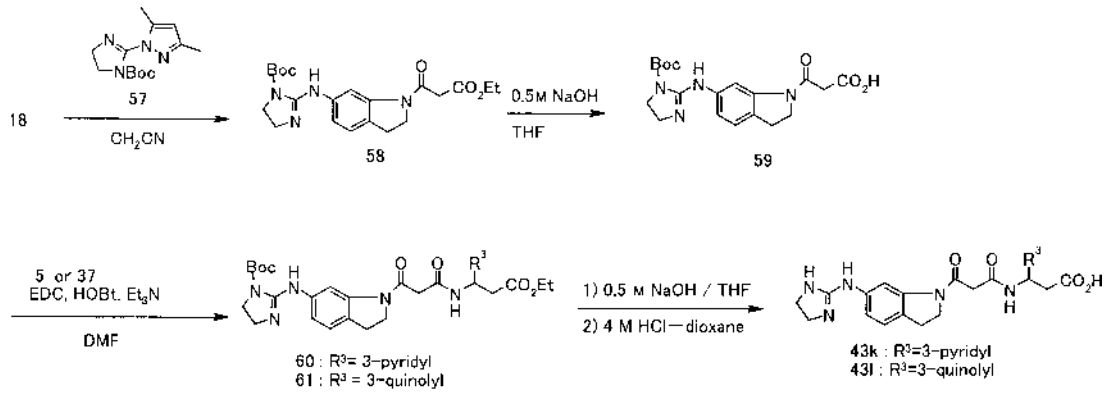
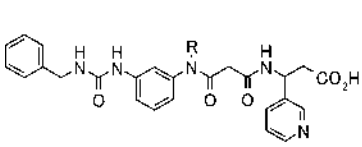
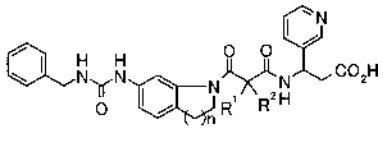


Chart 5

Table 1. Inhibitory Activity of Compounds (**9a**, **9b**, **43a**, **44**—**47**) on Vitronectin Binding with $\alpha_v\beta_3$



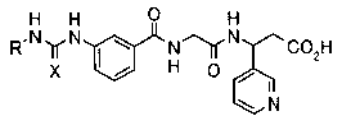
9a, b



43a, 44—47

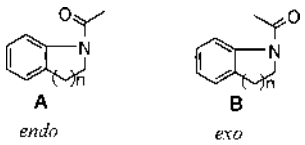
Compd. No.	R	R^1	R^2	*n*	IC_{50} (nM)[a]
SC65811					0.86
9a	H	—	—	—	14
9b	Me	—	—	—	600
43a	—	H	H	1	3.0
44	—	H	H	2	9.8
45	—	H	H	3	120
46	—	H	Me	1	>1000
47	—	Me	Me	1	>1000

a) Biotinylated vitronectin was allowed to bind purified human $\alpha_v\beta_3$ in the presence of test compounds. Binding activity data are presented from one determination (duplicate). The peptide RGDS was included for reference in every experimet. The concentration necessary for half-maximal inhibition of ligand is shown as IC_{50}. See experimental section.



SC65811: R=$C_6H_5CH_2$, X=O
1: R=H, X=NH

Fig. 1. Chemical Structure of SC65811 and Guanidine Analogue (**1**)



A *endo* B *exo*

n = 1—3

Fig. 2. The Direction of the Amide Bond of *N*-Acylindoline, *N*-Acyltetrahydroquinoline and *N*-Acyltetrahydrobenzo[*b*]azepine

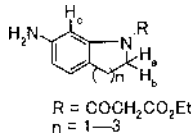
and **61** afforded **43k** and **43l**, respectively.

Results and Discussion

The activity of test compounds was evaluated in an $\alpha_v\beta_3$ binding assay. Biotinylated vitronectin was allowed to bind purified $\alpha_v\beta_3$ in the presence of the test compounds, and IC_{50} values are shown in Tables 1 and 4.

From our efforts to modify the glycine part of SC65811 with various structures, it was revealed that malonamide derivative **9a** had modest activity (Table 1, IC_{50}=14 nM). Moreover, an indoline derivative **43a**, which was a cyclic analogue of **9a**, showed an enhanced activity (IC_{50}=3.0 nM). Methylation of the amide nitrogen of **9a**, however, resulted in much decreased potency (**9b** IC_{50}=600 nM). The reason of this result is unclear, but we assumed that decrease of the activity of **9b** was due to the change of the stereochemistry of its amide bond. As indicated in the study on benzanilide derivatives reported by Saito *et al.*,[23] the conformation of amide group of **9a** is most likely to be *trans*, whereas introduction of a methyl group onto amide nitrogen (**9b**) might change its conformation into *cis*. In the case of malonamide analogues, the *trans* conformation of the amide bond would be much favorable for potent activity. In the case of **43a**, the conformation of the amide group of **43a** is likely to be *endo* conformation (Fig. 2A), which is corresponding to the *trans* conformation of **9a**, as indicated in the studies on *N*-acylindolines.[24] Conversion of the indoline moiety with tetrahydroquinoline and tetrahydrobenzo[*b*]azepine, affording **44** and **45**, provided approximately a 3-fold and 30-fold decrease in activity. The reasons of these results might also be related to the con-

Table 2. Chemical Shifts of ^{1}H-NMR Spectrum of Compounds **18**—**20** in $CDCl_3$



Compd. No.	*n*	Chemical shifts (δ, ppm)[a]		
		H_a	H_b	H_c
18	1	4.06 (t, *J*=8.2 Hz)		7.67 (d, *J*=2.1 Hz)
19	2	3.78 (br t, *J*=6.6 Hz)		6.52 (br)
20	3	2.69—2.78 (m)	4.63—4.70 (m)	6.51 (d, *J*=2.4 Hz

a) See experimental section.

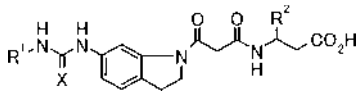
formation of amide groups of tetrahydroquinoline and tetrahydrobenzo[*b*]azepine. Hassner and Amit revealed the direction of the conformation of amide groups of *N*-acyltetrahydroquinoline and *N*-acyltetrahydrobenzoazepine based on the ^{1}H-NMR study.[25] According to their report, *N*-acylbenzoazepines exist almost entirely in an exo conformation (Fig. 2B), and *N*-acyltetrahydroquinolines is able to accommodate both the *endo* and the *exo* conformation which are dependent on the bulkiness of the acyl substituents. They found that *N*-acyltetrahydrobenzoazepines exhibit a downfield shift for the equatorial hydrogen at the 2 position of the tetrahydrobenzoazepine ring and that neither *N*-acylindoline nor *N*-acyltetrahydroquinoline exhibit this phenomenon. The downfield shift in *N*-acyltetrahydrobenzoazepine can be accounted for by a chair conformation of the 7-membered ring in which the amide carbonyl assumes coplanarity with the equatorial hydrogen at the 2-position. In the case of our compounds, to clarify the downfield shift, the ^{1}H-NMR spectrum of compound **20** was compared with those of compounds **18** and **19**, because the ^{1}H-NMR spectra of these compounds were simpler than those of compounds **43a**, **44**, and **45** and were easy to analyze (Table 2). As a result, the phenomenon of the downfield shift of the equatorial hydrogen at the 2-position was observed in compound **20** but not in compounds **18** and **19**. Furthermore, a downfield shift of the 7-position proton (H_c) in the indoline ring of **18** was observed but not in compounds **19** and **20**. This phenomenon was due to a shielding effect by the carbonyl group attached to the nitrogen atom of the indoline ring. These results suggest that compound **18**, corresponding to compound **43a**, preferred an *endo* conformation; on the contrary, compound **20**, corresponding to compound **45**, preferred an *exo* conformation. In the case of compound **19**, corresponding to compound **44**, broad signals of ^{1}H-NMR spectra might indicate the possibility of both the *endo* and *exo* conformation. That is, the conformation of the amide groups might be important for the activity by fixing the benzylurea and carboxylic acid moieties in a suitable position. Introduction of methyl group(s) into the active methylene of **43a** resulted in a loss of activity (**46**, **47**). These results indicated that methylation might cause unfavorable change in the conformation of the molecule presumably due to a steric repulsion between hydrogen atoms at 2-position of indoline and methyl group(s) of compounds **46** and **47**, and benzylurea and carboxylic acid could not fit in a suitable position. Furthermore, compound **43a** was found to

Table 3. Effect of Compounds SC65811 and **43a** on Vitronectin, Fibronectin and Fibrinogen Interaction with $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$ and $\alpha_v\beta_5$

Compd. No.	IC_{50} (nM)[a]			
	$\alpha_v\beta_3$	$\alpha_{IIb}\beta_3$	$\alpha_5\beta_1$	$\alpha_v\beta_5$
SC65811	0.86	54000	150	4.3
43a	3.0	19000	11000	14

a) Biotinylated vitronectin fibronectin, and fibrinogen were allowed to bind purified human $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$ $\alpha_5\beta_1$ and $\alpha_v\beta_5$, in the presence of test compounds. The concentration necessary for half-maximal inhibition of ligand is shown as IC_{50}. See experimental section. Binding activity data are presented from one determination (duplicate).

Table 4. Inhibitory Activity of Compounds (**43a**—**l**) on Vitronectin Binding with $\alpha_v\beta_3$



Compd. No.	X	R^1	R^2	IC_{50} (nM)[a]
SC65811				0.86
1				0.76
43a	O	Benzyl	3-Pyridyl	3.0
43g	O	H	3-Pyridyl	110
43h	O	Phenyl	3-Pyridyl	280
43i	O	4-Pyridylmethyl	3-Pyridyl	3.6
43j	NH	H	3-Pyridyl	7.8
43k		$N(CH_2)_2$	3-Pyridyl	1.1
43b	O	Benzyl	H	560
43c	O	Benzyl	Me	130
43d	O	Benzyl	Phenyl	22
43e	O	Benzyl	2-Naphthyl	36
43f	O	Benzyl	3-Quinolyl	1.4
43l		$N(CH_2)_2$	3-Quinolyl	0.42

a) See corresponding footnote to Table 1.

show modest to high selectivity for $\alpha_v\beta_3$ versus $\alpha_{IIb}\beta_3$, $\alpha_5\beta_1$ and $\alpha_v\beta_5$ (Table 3). These results suggest that *N*-malonyl indoline is appropriate as a novel central scaffold for potent and selective $\alpha_v\beta_3$ antagonists.

We next examined the evaluation of the urea part (Table 4). By changing the urea subsutituent of the compound **43a** with 4-pyridylmethyl group (**43i**), the activity was maintained, while substitution with hydrogen atom (**43g**) and phenyl group (**43h**) decreased the activities. In the case of Searl's compounds, both SC65811 and guanidine derivative **1** were equipotent, however, as for our compounds, guanidine derivative **43j** was 2-fold less active than **43a**. Cyclic guanidine derivative **43k** was approximately 3-fold more potent than benzylurea **43a**, as the results reported by other groups.[11,16] The reasons for the difference in affinity for $\alpha_v\beta_3$ of guanidine derivatives are not clear at present but may be due to the lipophilicity or steric bulkiness of the guanidine moiety. Subsequently, we focused on the optimization of β substituent of propanoic acid moiety (Table 4). Substitution of the pyridine moiety of **43a** with hydrogen atom (**43b**) or methyl group (**43c**) resulted in significant loss of activity. Furthermore, phenyl derivative **43d** and naphthyl derivative **43e** were also less active than compound **43a**. Interestingly, quinolyl derivative **43f** was 2-fold more active than compound **43a**. These results suggest that nitrogen atom of the

quinoline ring may play an important role in the activity. Finally, the combination of the cyclic guanidine moiety instead of benzylurea and quinolyl group as a β substituent of propanoic acid moiety led to the most potent compound **43l** with an IC_{50} value of 0.42 nM.

Conclusions

In order to find novel potent and selective $\alpha_v\beta_3$ antagonists, we discovered the novel 1-(3-oxopropanoyl)indoline derivatives as $\alpha_v\beta_3$ antagonists with potent activity and good selectivity by means of modification of peptidic backbone of SC65811. Further efforts to discover novel $\alpha_v\beta_3$ antagonists are ongoing.

Experimental

All melting points were determined on a Yanagimoto MP-3 melting point apparatus and without correction. ^{1}H-NMR spectra were taken on a JEOL JNM-LA300 or JEOL JNM-EX400 spectrometer. Chemical shifts are given in ppm relative to that of Me_4Si (δ=0) in $CDCl_3$ or dimethylsulfoxide-d_6 (DMSO-d_6) as an internal standard. The abbreviations for the signal patterns are as follows: s: singlet, d: doublet, t: triplet, q: quartet, quint: quintet, br: broad, m: multiplet. Column chromatography was carried out on silica gel (Wakogel C-200 or Merck Silica gel 60) or ODS-A 120—230/70. FAB-MS were obtained with a JEOL JMS-DX300 mass spectrometer.

***N*-Methyl-3-nitroaniline (2b)**[26] To a solution of 3-nitroaniline (4.14 g, 30 mmol) in 1,2-dichloroethane (50 ml) was added trifluoroacetic anhydride (18.9 g, 90 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h, then the solvent was removed *in vacuo.* The residue was dissolved in 2-butanone (100 ml). To the solution, potassium carbonate (K_2CO_3, 8.30 g, 60 mmol) and methyl iodide (12.8 g, 90 mmol) were added. The reaction mixture was heated at 60 °C for 2 h and then was filtered. The filtrate was concentrated *in vacuo*, and the resulting residue was dissolved in methanol (250 ml) and H_2O (50 ml). To the solution, K_2CO_3 (4.14 g, 30 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. The mixture was extracted with chloroform ($CHCl_3$). The extract was washed with saturated brine and dried over anhydrous magnesium sulfate ($MgSO_4$). The solvent was removed *in vacuo.* The residue was purified by column chromatography with hexane–ethyl acetate (AcOEt) (5 : 1, v/v) to yield **2b** (4.40 g, 28.9 mmol, 96%) as an orange needle, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 2.90 (3H, d, J=5.1 Hz), 4.06 (1H, br), 6.86 (1H, dd, J=8.1, 2.4 Hz), 7.25—7.31 (1H, m), 7.38—7.39 (1H, m), 7.53 (1H, dd, J=8.1, 2.4 Hz). GC-MS *m/z*: 152 (M^+).

Ethyl *N*-(3-Nitrophenyl)malonamate (3a)[27] To a solution of 3-nitroaniline (4.10 g, 30 mmol) and Et_3N (3.50 g, 35 mmol) in $CHCl_3$ (150 ml) was added dropwise a solution of ethyl malonyl chloride (5.00 g, 33 mmol) in $CHCl_3$ (30 ml) at 0 °C. The reaction mixture was stirred at room temperature for 4 h, then poured into water. The mixture was extracted with $CHCl_3$. The extract was washed with saturated brine and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo.* The residue was purified by column chromatography with $CHCl_3$–methanol (100 : 1, v/v) to yield **3a** as a pale yellow oil (6.80 g, 27.0 mmol, 90%). ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, t, J=7.2 Hz), 3.52 (2H, s), 4.29 (2H, q, J=7.2 Hz), 7.50 (1H, t, J=8.1 Hz), 7.94—8.00 (2H, m), 8.44—8.46 (1H, m). FAB-MS *m/z*: 253 [$(M^+ + H)^+$].

Ethyl *N*-methyl-*N*-(3-nitrophenyl)malonamate (**3b**) was similarly prepared from **2b** and ethyl malonyl chloride as yellow oil. Yield: 79%. ^{1}H-NMR ($CDCl_3$) δ: 1.23—1.29 (3H, m), 3.24 (2H, s), 3.37 (3H, s), 4.13—4.18 (2H, m), 7.64—7.65 (2H, m), 8.15—8.16 (1H, m), 8.24 (1H, m). FAB-MS *m/z*: 267 [$(M^+ + H)^+$].

***N*-(3-Nitrophenyl)malonamic Acid (4a)**[27] A mixture of compound **3a** (4.70 g, 19,0 mmol), 1 M NaOH (40 ml) and methanol (50 ml) was stirred at room temperature for 2 h, then concentrated *in vacuo.* The residue was acidified with 1 M HCl (100 ml) and extracted with AcOEt. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give *N*-(3-nitrophenyl)malonamic acid (3.60 g, 16.1 mmol, 85%) as a colorless solid, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 3.41 (2H, s), 7.62 (1H, t, J=8.1 Hz), 7.87—7.95 (2H, m), 8.62 (1H, t, J=2.1 Hz), 12.74 (1H, br). FAB-MS *m/z*: 223 [$(M^+ - H)^+$].

N-Methyl-*N*-(3-nitrophenyl)malonamic acid (**4b**) was similarly prepared from **3b** as a pale yellow solid, which was used for the next reaction without further purification. Yield: 75%. ^{1}H-NMR ($CDCl_3$) δ: 3.18 (2H, s), 3.40 (3H, s), 7.62 (1H, d, J=7.8 Hz), 7.69—7.75 (1H, m), 8.13—8.15 (1H, m), 8.62 (1H, t, J=8.1 Hz), 8.32 (1H, d, J=7.8 Hz). FAB-MS *m/z*: 229 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-(3-Nitrophenylcarbamoylacetylamino)-3-(pyridin-3-yl)-propanoate (6a) A mixture of **4a** (1.55 g, 6.9 ml), CDI (1.20 g, 7.5 mmol), and DMF (15 ml) was stirred at 0 °C for 1 h. To the mixture was added a solution of (*R*,*S*)-ethyl 3-amino-3-(pyridin-3-yl)propanoate dihydrochloride[15] (**5**, 1.80 g, 6.90 mmol) in DMF (20 ml) and Et_3N (1.40 g, 14.0 mmol) and stirred at room temperature for 16 h. The reaction mixture was then diluted with H_2O and extracted with AcOEt. The extract was washed with saturated aqueous sodium bicarbonate ($NaHCO_3$), saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo.* The solvent was removed *in vacuo.* The residue was purified by column chromatography with $CHCl_3$–methanol (50 : 1, v/v) to yield **6a** (6.80 g, 4.47 mmol, 65%) as a colorless powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (3H, t, J=6.9 Hz), 2.87 (2H, d, J=7.2 Hz), 3.31 (2H, s), 4.03 (2H, q, J=6.9 Hz), 5.22—5.30 (1H, m), 7.37 (2H, dd, J=7.8, 4.8 Hz), 7.61 (1H, t, J=8.1 Hz), 7.76—7.80 (1H, m), 7.86—7.94 (2H, m), 8.46—8.48 (1H, m), 8.56 (1H, d, J=2.1 Hz), 8.61—8.63 (1H, m), 8.77 (1H, d, J=8.4 Hz), 10.57 (1H, s). FAB-MS *m/z*: 401 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-[methyl-(3-nitrophenyl)carbamoylacetylamino]-3-(pyridin-3- yl)propanoate (**6b**) was similarly prepared from **4b** and **5** as an amorphous powder, which was used for the next reaction without further purification. Yield: 77%. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=6.9 Hz), 2.83—2.98 (2H, m), 3.12 (2H, s), 3.36 (3H, s), 4.11 (2H, q, J=7.2 Hz), 5.42—5.59 (1H, m), 7.25—7.30 (1H, m), 7.55—7.70 (3H, m), 8.10 (1H, br), 8.25 (1H, br d, J=7.8 Hz), 8.52 (1H, dd, J=4.8, 2.1 Hz), 8.60 (1H, d, J=2.1 Hz), 8.56 (1H, br d, J=7.2 Hz). FAB-MS *m/z*: 415 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-[2-(3-Aminophenylcarbamoyl)acetylamino]-3-(pyridin-3-yl)propanoate (7a) A mixture of compound **6a** (1.78 g, 4.45 mmol), 10% Pd–C (100 mg) and methanol (50 ml) was stirred under atmospheric pressure of hydrogen at room temperature for 5 h. The catalyst was removed by filtration on celite, and the filtrate was concentrated to give **7a** (1.66 g, 4.45 mmol, quant.) as a pale brown powder, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, t, J=7.2 Hz), 2.85 (1H, dd, J=15.6, 6.3 Hz), 2.93 (1H, dd, J=15.6, 6.3 Hz), 3.37 (2H, Abq, J=16.1 Hz), 4.08 (2H, q, J=7.2 Hz), 5.44—5.51 (1H, m), 6.43 (1H, dd, J=7.2, 2.4 Hz), 6.73—6.77 (1H, m), 7.03—7.09 (2H, m), 7.23—7.28 (2H, m), 7.64—7.68 (1H, m), 8.20 (1H, br d, J=8.1 Hz), 8.51 (1H, dd, J=4.8, 1.8 Hz), 8.64 (1H, q, J=2.1 Hz), 9.16 (1H, s). FAB-MS *m/z*: 371 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{2-[(3-aminophenyl)methyl]carbamoylacetylamino}-3-(pyridin-3-yl)propanoate (**7b**) was similarly prepared from **6b** as a pale brown powder, which was used for the next reaction without further purification. Yield: 96%. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=6.9 Hz), 2.87 (1H, dd, J=15.9, 6.6 Hz), 2.95 (1H, dd, J=15.9, 6.6 Hz), 3.16 (1.3H, s), 3.20 (0.7H, s), 3.26 (2H, s), 3.28 (1H, s), 4.10 (2H, q, J=6.9 Hz), 5.41—5.51 (1H, m), 6.43—6.51 (1H, m), 6.62—6.70 (1H, m), 6.88—7.35 (2H, m), 7.77—7.79 (1H, m), 8.52—8.64 (2H, m), 8.98—9.00 (1H, m). FAB-MS *m/z*: 385 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-[3-(3-Benzylureido)phenylcarbamoylacetylamino]-3-(pyridin-3-yl)propanoate (8a) To the solution of **7a** (890 mg, 2.4 mmol) in acetonitrile (50 ml) was added benzylisocyanate (960 mg, 7.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h, then the solvent was removed *in vacuo.* The residue was purified by column chromatography with $CHCl_3$–methanol (20 : 1, v/v) to yield **8a** (1.20 g, 2.38 mmol, 99%) as a colorless powder which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.13 (3H, t, J=7.2 Hz), 2.76 (1H, dd, J=15.6, 6.3 Hz), 2.87 (1H, dd, J=15.6, 6.6 Hz), 3.45 (2H, s), 4.01 (2H, q, J=7.2 Hz), 4.35 (2H, d, J=5.1 Hz), 5.88—5.92 (1H, m), 7.03—7.48 (10H, m), 7.57—7.60 (1H, m), 7.80 (1H, br), 8.42 (1H, dd, J=4.8, 1.8 Hz), 8.59 (1H, d, J=2.1 Hz), 8.71 (1H, d, J=7.5 Hz), 9.91 (1H, s). FAB-MS *m/z*: 504 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-[3-(3-benzylureido)methylphenylcarbamoylacetylamino]-3-(pyridin-3-yl)propanoate (**8b**) was similarly prepared from **7b** as a colorless powder, which was used for the next reaction without further purification. Yield 64%. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7.2 Hz), 2.80 (1H, dd, J=15.6, 6.9 Hz), 2.88 (1H, dd, J=15.6, 6.9 Hz), 3.11 (2H, d, J=1.2 Hz), 3.23 (3H, s), 4.09 (2H, q, J=7.2 Hz), 4.38 (2H, d, J=5.7 Hz), 5.32—5.39 (1H, m), 5.79 (1H, br t, J=5.7 Hz), 6.64—6.67 (1H, m), 7.07—7.33 (9H, m), 7.61—7.63 (1H, m), 7.72 (1H, s), 8.46 (1H, dd, J=6.1, 1.5 Hz), 8.51 (1H, d, J=2.1 Hz), 8.85 (1H, d, J=8.1 Hz). FAB-MS *m/z*: 518 [$(M^+ + H)^+$].

(*R*,*S*)-3-[3-(3-Benzylureido)phenylcarbamoylacetylamino]-3-(pyridin-

Table 5. Physical Properties of Compounds **9a**, **9b**, **43a**—**l** and **44**—**47**

Compd. No	Formula	Analysis (%) Calcd (Found) C	H	N	Yield (%)	^{1}H-NMR (δ)[a]	FAB-MS *m/z*:	Recrystn. solvent[b]	mp (°C)
9a	$C_{25}H_{25}N_5O_5 \cdot 0.5H_2O$	61.97 (61.70	5.41 5.27	14.45 14.39)	48	2.73—2.83 (2H, m), 3.27 (2H, d, *J*=1.9 Hz), 4.29 (2H, d, *J*=5.8 Hz), 5.18—5.24 (1H, m), 6.54 (1H, br t, *J*=5.8 Hz), 7.13—7.37 (9H, m), 7.69 (1H, s), 7.76 (1H, dt, *J*=7.8, 2.0 Hz), 8.45 (1H, dd, *J*=4.4, 2.0 Hz), 8.57 (1H, d, *J*=2.0 Hz), 8.59 (1H, s), 8.69 (1H, d, *J*=7.8 Hz), 10.04 (1H, s), 12.36 (1H, s)	476 [(M+H)$^+$]	M–EA	181—182
9b	$C_{26}H_{27}N_5O_5 \cdot 0.5H_2O$	62.64 (62.66	5.66 5.68	14.05 13.88)	76	2.72 (2H, br d, *J*=6.8 Hz), 3.02 (2H, br), 3.13 (3H, s), 4.30 (2H, d, *J*=5.8 Hz), 5.11—5.16 (1H, m), 6.70 (1H, br), 6.81 (1H, t, *J*=7.8 Hz), 7.22—7.42 (9H, m), 7.70 (1H, d, *J*=7.8 Hz), 8.42—8.53 (3H, m), 8.73 (1H, s), 12.32 (1H, s)	490 [(M+H)$^+$]	—	Amorphous
43a	$C_{27}H_{27}N_5O_5 \cdot H_2O$	62.42 (62.46	5.63 5.32	13.48 13.47)	Quant.	2.78 (2H, br d, *J*=7.4 Hz), 3.06 (2H, br t, *J*=8.6 Hz), 3.44 (2H, s), 3.90—4.15 (2H, m), 4.28 (2H, d, *J*=5.9 Hz), 5.21—5.26 (1H, m), 6.45 (1H, br t, *J*=5.9 Hz), 7.06 (1H, d, *J*=7.8 Hz), 7.20—7.39 (7H, m), 7.79 (1H, d, *J*=7.8 Hz), 7.99 (1H, s), 8.47 (1H, d, *J*=4.4 Hz), 8.60 (1H, d, *J*=3.0 Hz), 8.57 (1H, d, *J*=7.8 Hz), 12.37 (1H, br)	502 [(M+H)$^+$]	M–EA	208—210
43b	$C_{22}H_{24}N_4O_5$	62.25 (62.00	5.70 5.72	13.20 13.01)	94	2.41 (2H, t, *J*=6.9 Hz), 3.03 (2H, br t, *J*=8.4 Hz), 3.28 (2H, t, *J*=6.6 Hz), 3.38 (2H, s), 4.08 (2H, t, *J*=8.4 Hz), 4.28 (2H, d, *J*=6.0 Hz), 6.45 (1H, br t, *J*=6.0 Hz), 7.06 (1H, d, *J*=7.8 Hz), 7.21—7.36 (6H, m), 8.01 (1H, s), 8.12 (1H, br t, *J*=5.4 Hz), 8.57 (1H, s), 12.24 (1H, br)	425 [(M+H)$^+$]	M–T	203—205
43c	$C_{23}H_{26}N_4O_5$	63.00 (62.62	5.98 5.90	12.78 12.82)	82	1.11 (3H, d, *J*=6.9 Hz), 2.30 (1H, dd, *J*=15.7, 7.4 Hz), 2.47 (1H, dd, *J*=15.7, 6.4 Hz), 3.03 (2H, br), 3.35 (2H, ABq, *J*=15.1 Hz), 4.06—4.13 (3H, m), 4.28 (2H, d, *J*=5.9 Hz), 6.44 (1H, br t, *J*=5.9 Hz), 7.06 (1H, d, *J*=7.8 Hz), 7.22—7.35 (6H, m), 8.02 (1H, d, *J*=1.5 Hz), 8.09 (1H, d, *J*=7.8 Hz), 8.58 (1H, s), 12.19 (1H, s)	437 [(M−H)$^+$]	M–EA	213—214
43d	$C_{28}H_{28}N_4O_5 \cdot 0.5H_2O$	66.00 (66.09	5.74 5.69	11.00 10.99)	77	2.69 (1H, dd, *J*=15.6, 7.2 Hz), 2.75 (1H, dd, *J*=15.6, 7.8 Hz), 3.00 (2H, br t, *J*=8.4 Hz), 3.42 (2H, br), 3.96—4.12 (2H, m), 4.28 (2H, d, *J*=5.7 Hz), 5.17—5.25 (1H, m), 6.44 (1H, t, *J*=5.7 Hz), 7.05 (1H, d, *J*=8.1 Hz), 7.21—7.37 (11H, m), 7.99 (1H, d, *J*=1.8 Hz), 8.58 (1H, s), 8.66 (1H, d, *J*=8.1 Hz), 12.28 (1H, br)	499 [(M−H)$^+$]	M–EA	216—218
43e	$C_{32}H_{30}N_4O_5$	69.80 (69.82	5.49 5.45	10.18 10.32)	84	2.83 (2H, d, *J*=7.4 Hz), 2.96—3.06 (2H, m), 3.46 (2H, ABq, *J*=15.6 Hz), 3.99—4.12 (2H, m), 4.28 (2H, d, *J*=5.9 Hz), 5.35—5.41 (1H, m), 6.44 (1H, br t, *J*=5.9 Hz), 7.05 (1H, d, *J*=7.8 Hz), 7.22—7.35 (6H, m), 7.47—7.54 (3H, m), 7.86—7.90 (4H, m), 8.01 (1H, d, *J*=2.0 Hz), 8.58 (1H, s), 8.77 (1H, d, *J*=7.8 Hz), 12.30 (1H, s)	551 [(M+H)$^+$]	M–T	221—223
43f	$C_{31}H_{29}N_5O_5 \cdot 0.4H_2O$	66.63 (66.62	5.37 5.27	12.53 12.70)	90	2.90—3.02 (4H, m), 3.48 (2H, br), 3.99—4.12 (2H, m) 4.29 (2H, d, *J*=5.9 Hz), 5.37—5.44 (1H, m), 6.46 (1H, br t, *J*=5.9 Hz), 7.05 (1H, d, *J*=8.3 Hz), 7.22—7.35 (6H, m), 7.59—7.63 (1H, m), 7.73—7.77 (1H, m), 7.98—8.02 (3H, m), 8.34 (1H, d, *J*=1.4 Hz), 8.60 (1H, s), 8.86 (1H, d, *J*=7.8 Hz), 8.96 (1H, d, *J*=1.9 Hz), 12.40 (1H, br)	552 [(M+H)$^+$]	M–T	222—224
43g	$C_{20}H_{21}N_5O_5 \cdot 0.7H_2O$	56.65 (56.33	5.32 5.44	16.52 16.80)	46	2.79 (2H, d, *J*=7.5 Hz), 3.00 (2H, br t, *J*=8.4 Hz), 3.44 (2H, s), 3.95—4.12 (2H, m), 5.19—5.26 (1H, m), 5.71 (2H, s), 7.04 (1H, d, *J*=8.1 Hz), 7.28—7.39 (2H, m), 7.77 (1H, d, *J*=7.5 Hz), 7.94 (1H, s), 8.46 (1H, d, *J*=4.8 Hz), 8.54 (1H, s), 8.58 (1H, s), 8.74 (1H, d, *J*=8.1 Hz), 12.40 (1H, br)	412 [(M+H)$^+$]	M–T	179—181
43h	$C_{26}H_{25}N_5O_5 \cdot H_2O$	61.77 (61.71	5.38 5.25	13.85 13.80)	29	2.80 (2H, br d, *J*=6.6 Hz), 3.01—3.07 (2H, m), 3.46 (2H, s), 3.99—4.12 (2H, m), 5.20—5.28 (1H, q, *J*=7.8 Hz), 6.96 (1H, br t, *J*=6.9 Hz), 7.11 (1H, d, *J*=7.8 Hz), 7.22—7.50 (6H, m), 7.77 (1H, d, *J*=7.2 Hz), 8.07 (1H, s), 8.46 (2H, br), 8.59 (1H, s), 8.70—8.78 (2H, m), 12.36 (1H, br)	488 [(M+H)$^+$]	M–EA	212—214
43i	$C_{26}H_{26}N_6O_5 \cdot 1.5H_2O$	58.97 (59.25	5.52 5.40	15.87 15.78)	59	2.79 (2H, d, *J*=7.2 Hz), 3.01 (2H, br t, *J*=8.1 Hz), 3.44 (2H, s), 3.97—4.12 (2H, m), 4.31 (2H, d, *J*=6.0 Hz), 5.19—5.26 (1H, m), 6.58 (1H, br t, *J*=6.0 Hz), 7.06 (1H, d, *J*=8.4 Hz), 7.27—7.36 (4H, m), 7.77 (1H, d, *J*=7.8 Hz), 8.00 (1H, s), 8.46 (1H, d, *J*=3.9 Hz), 8.50 (2H, d, *J*=5.4 Hz) 8.58 (1H, s), 8.73 (1H, s), 8.75 (1H, s) 12.38 (1H, br)	503 [(M+H)$^+$]	M–EA	168—170
43j	$C_{20}H_{22}N_6O_4 \cdot H_2O$	56.07 (56.05	5.65 5.55	19.62 19.89)	27	2.43—2.46 (2H, m), 3.00—3.02 (2H, m), 3.35—3.46 (2H, m), 3.84—3.96 (1H, m), 4.11—4.15 (1H, m), 5.14—5.24 (1H, m), 6.81—6.95 (2H, m), 7.20—7.35 (2H, m), 7.66—7.92 (5H, m), 8.40 (1H, dd, *J*=4.4, 14 Hz), 8.51 (1H, d, *J*=10.3 Hz), 8.80—8.90 (1H, m), 13.33 (1H, br)	411 [(M+H)$^+$]	M–T	238—240

Table 5. (continued)

Compd. No	Formula	Analysis (%) Calcd (Found) C	H	N	Yield (%)	^{1}H-NMR (δ)[a]	FAB-MS *m/z*:	Recrystn. solvent[b]	mp (°C)
43k	$C_{22}H_{24}N_6O_4 \cdot 2.9H_2O$	54.07 (54.06	6.15 5.95	17.20 17.17)	71	2.90—3.11 (4H, m), 3.47—3.78 (6H, m), 4.07—4.22 (2H, m), 5.12—5.32 (1H, m), 6.80 (1H, br), 6.97 (1H, d, J=7.8 Hz), 7.18—7.36 (2H, m), 7.67—7.73 (1H, m), 8.38—8.44 (1H, m), 8.51 (1H, br), 8.78—8.94 (1H, m)	437 $[(M+H)^+]$	M–T	196—198
43l	$C_{26}H_{26}N_6O_4 \cdot 1.5H_2O \cdot 1.0CH_4O$	59.44 (59.14	6.10 5.72	15.40 15.29)	25	2.56—2.78 (2H, m), 2.95—3.18 (2H, m), 3.45—3.88 (6H, m), 4.00—4.23 (2H, m), 5.32—5.52 (1H, m), 6.75—6.87 (1H, m), 6.90—7.32 (2H, m), 7.42—7.75 (3H, m), 7.85—8.03 (3H, m), 8.12—8.29 (1H, m), 8.86—9.04 (2H, m)	487 $[(M+H)^+]$	M–E	225—228
44	$C_{28}H_{29}N_5O_5 \cdot 0.2H_2O$	64.78 (64.91	5.71 5.87	13.49 13.23)	84	1.81 (2H, qn, J=6.3 Hz), 2.60 (2H, t, J=6.6 Hz), 2.74 (2H, d, J=7.2 Hz), 3.47 (2H, br), 3.60—3.64 (2H, m), 4.28 (2H, d, J=5.7 Hz), 5.13—5.20 (1H, m), 6.58 (0.5H, br), 7.01 (1H, d, J=7.5 Hz), 7.16—7.36 (8H, m), 7.50 (0.5H, br), 7.72 (1H, d, J=6.9 Hz), 8.44 (1H, br d, J=4.8 Hz), 8.52 (2H, d, J=12.6 Hz), 8.60 (1H, d, J=3.3 Hz), 12.33 (1H, s)	514 $[(M-H)^+]$	M–EA	193—194
45	$C_{29}H_{31}N_5O_5 \cdot 0.8H_2O$	64.03 (64.03	6.04 5.91	12.87 12.87)	57	1.22 (1H, br), 1.69 (2H, br), 1.84 (1H, br), 2.52—2.73 (3H, m), 2.85 (1H, dd, J=17.1, 15.1 Hz), 3.08 (1H, dd, J=15.1, 12.2 Hz), 3.29 (2H, s), 4.28—4.31 (2H, m), 4.43—4.48 (1H, d, J=13.2 Hz), 5.07—5.14 (1H, m), 6.63—6.68 (1H, m), 7.07 (0.5H, d, J=8.3 Hz), 7.14 (0.5H, d, J=8.3 Hz), 7.19—7.37 (8H, m), 7.61—7.64 (0.5H, m), 7.70—7.74 (0.5H, m), 8.42—8.57 (3H, m), 8.60 (1H, s), 12.30 (1H, s)	528 $[(M-H)^+]$	—	Amorphous
46	$C_{28}H_{29}N_5O_5 \cdot 0.5H_2O$	64.11 (64.28	5.76 5.56	13.35 13.29)	89	1.22 (2H, d, J=6.8 Hz), 1.28 (1H, d, J=7.4 Hz), 2.79 (2H, d, J=7.3 Hz), 2.94—3.04 (2H, m), 3.57—3.66 (1H, m), 3.94—4.14 (2H, m), 4.28—4.29 (2H, m), 5.14—5.25 (1H, m), 6.47 (1H, br), 7.00—7.07 (1H, m), 7.22—7.38 (7H, m), 7.71—7.56 (1H, m), 8.02 (1/3H, s), 8.06 (2/3H, s), 8.51—8.55 (2H, m), 8.72—8.78 (1H, m), 12.40 (1H, br)	514 $[(M-H)^+]$	—	Amorphous
47	$C_{29}H_{31}N_5O_5 \cdot H_2O$	63.61 (63.30	6.07 5.83	12.79 12.65)	60	1.31 (3H, s), 1.33 (3H, s), 2.71—2.93 (4H, m), 3.62—3.78 (2H, m), 4.28 (2H, d, J=5.9 Hz), 5.22—5.28 (1H, m), 6.49 (1H, br t, J=5.9 Hz), 7.03 (1H, d, J=7.8 Hz), 7.17—7.40 (7H, m), 7.78 (1H, d, J=7.8 Hz), 8.17 (1H, d, J=1.9 Hz), 8.47—8.56 (4H, m), 12.37 (1H, br)	528 $[(M-H)^+]$	M–W	206—210

a) ^{1}H-NMR spectrum of all compounds were measured in DMSO-d_6. *b*) M=methanol, E=diethyl ether, EA=ethyl acetate, T=THF, W=water.

3-yl)propanoic Acid (9a) A mixture of compound **8a** (560 mg, 1.1 mmol), 1 M NaOH (5 ml) and ethanol (25 ml) was stirred at room temperature for 1 h, then concentrated *in vacuo.* The residue was acidified with 1 M HCl (5 ml) , then concentrated *in vacuo.* The residue was purified by column chromatography using ODS-A with H_2O–methanol (1 : 1, v/v) to yield **9a** (410 mg, 0.84 mmol, 76%) as a colorless powder, which was recrystallized from methanol–AcOEt. Physical data for **9a** are listed in Table 5.

(*R*,*S*)-3-[3-(3-Benzylureidophnyl)methylcarbamoyl]acetylamino-3-(pyridin-3-yl)propanoic acid (**9b**) was similarly prepared from **8b**. Physical data for **9b** are listed in Table 5.

Ethyl 3-(6-Nitroindolin-1-yl)-3-oxopropanoate (13) To a solution of **10** (1.52 g, 9.26 mmol) and Et_3N (3.50 g, 10.2 mmol) in $CHCl_3$ (150 ml) was added dropwise a solution of ethyl malonyl chloride (1.53 g, 10.2 mmol) in $CHCl_3$ (20 ml) at 0 °C. The reaction mixture was stirred at room temperature for 14 h, then poured into water. The mixture was extracted with $CHCl_3$. The extract was washed with 1 M HCl and saturated brine, then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo.* The residue was washed by diethylether to give **13** (2.25 g, 8.09 mmol, 87%) as a yellow powder, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7.3 Hz), 3.31 (2H, t, J=8.6 Hz), 3.59 (2H, s), 4.14—4.38 (4H, m), 7.30 (1H, d. J=8.1 Hz), 7.94 (1H, dd, J=8.1, 2.3 Hz), 9.03 (1H, d, J=2.3 Hz). FAB-MS *m/z*: 279 $[(M^+ + H)^+]$.

Compounds **14** and **15** were similarly prepared from compounds **11**[28] and **12**,[29] and these compounds were used for the next reaction without further purification.

Ethyl 3-(7-Nitro-1,2,3,4-tetrahydroquinolin-1-yl)-3-oxopropanoate (**14**): Yellow solid. Yield: 80%. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J=6.9 Hz), 1.93 (2H, quint, J=6.4 Hz), 2.86 (2H, t, J=6.4 Hz), 3.73 (2H, t, J=6.4 Hz), 3.80 (2H, s), 4.10 (2H, q, J=6.9 Hz), 7.47 (1H, d, J=8.4 Hz), 7.95 (1H, dd, J=8.4, 2.1 Hz), 8.60 (1H, br). FAB-MS *m/z*: 293 $[(M^+ + H)^+]$.

Ethyl 3-(8-Nitro-2,3,4,5-tetrahydrobenzo[*b*]azepin-1-yl)-3-oxopropanoate (**15**): Ivory solid. Yield: 68%. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7.5 Hz), 1.31—1.47 (1H, m), 1.78—1.90 (1H, m), 1.95—2.13 (2H, m), 2.67 (1H, ddd, J=13.8, 11.7, 2.0 Hz), 2.80—3.05 (2H, m), 3.25 (2H, s), 4.11 (2H, q, J=7.5 Hz), 4.77 (1H, m), 7.45 (1H, d, J=8.1 Hz), 8.10 (1H, d, J=2.1 Hz), 8.13 (1H, dd, J=8.1, 2.1 Hz). FAB-MS *m/z*: 307 $[(M^+ + H)^+]$.

Ethyl 2-Methyl-3-(6-nitroindolin-1-yl)-3-oxopropanoate (16) To a solution of ethyl 2-carboethoxypropanoate[30] (3.00 g, 20.5 mmol) in benzene (30 ml) was added thionyl chloride ($SOCl_2$, 4.88 g, 41.0 mmol). The reaction mixture was heated at reflux for 1.5 h and concentrated *in vacuo.* The residue was added to benzene (30 ml) and concentrated *in vacuo* to give a colorless oil. To a solution of **10** (1.00 g, 6.09 mmol) and Et_3N (3.11 g, 30.8 mmol) in $CHCl_3$ (15 ml) was added dropwise a solution of the above oil in $CHCl_3$ (15 ml) at 0 °C. The reaction mixture was stirred at room temperature for 14 h, then poured into water. The mixture was extracted with AcOEt. The extract was washed with 1 M HCl, saturated $NaHCO_3$ and saturated brine, then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo.* The residue was purified by column chromatography with hexane–AcOEt (3 : 1, v/v) to give **16** (1.45 g, 4.96 mmol, 81%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=6.8 Hz), 1.35 (3H, d, J=6.8 Hz), 3.29 (2H, m), 3.97 (1H, q, J=6.8 Hz), 4.10—4.17 (2H, m), 4.19—4.26 (2H, m), 4.33—4.39 (1H, m), 7.52 (1H, d, J=8.3 Hz), 7.96 (1H, dd, J=8.3, 2.5 Hz), 8.23 (1H, d, J=2.5 Hz). FAB-MS *m/z*: 293 $[(M^+ + H)^+]$.

Ethyl 2,2-dimethyl-3-(6-nitro-2,3-dihydroindol-1-yl)-3-oxopropanoate (**17**) was similarly prepared from 2-carboethoxy-2-methylpropanoic acid[31] and compound **10** as a yellow solid, which was used for the next reaction without further purification. Yield: 98%. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J=7.4 Hz), 1.45 (6H, s), 3.26 (2H, t, J=8.3 Hz), 3.97 (2H, t, J=8.3 Hz), 4.20 (2H, q, J=7.4 Hz), 7.53 (1H, d, J=8.3 Hz), 7.97 (1H, dd, J=8.3, 2.0 Hz), 8.87 (1H, d, J=2.0 Hz). FAB-MS *m/z*: 307 $[(M^+ + H)^+]$.

Ethyl 3-(6-Aminoindolin-1-yl)-3-oxopropanoate (18) A mixture of compound **13** (1.70 g, 6.11 mmol), 10% Pd–C (170 mg) and ethanol (34 ml)

was stirred under atmospheric pressure of hydrogen at room temperature for 1 h. The catalyst was removed by filtration on Celite, and the filtrate was concentrated to give **18** (1.51 g, 6.11 mmol, quant.) as a yellow powder, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.1 Hz), 3.08 (2H, t, J=8.2 Hz), 3.52 (2H, s), 3.66 (2H, br), 4.06 (2H, t, J=8.2 Hz), 4.24 (2H, q, J=7.1 Hz), 6.37 (1H, dd, J=8.2, 2.1 Hz), 6.94 (1H, d, J=8.2 Hz), 7.67 (1H, d, J=2.1 Hz). FAB-MS m/z: 249 [$(M^+ + H)^+$].

Compounds **19**—**22** were prepared in a manner similar to that described for **18** from compounds **14**—**17**, and these compounds were used for the next reaction without further purification.

Ethyl 3-(7-Amino-1,2,3,4-tetrahydroquinolin-1-yl)-3-oxopropanoate (**19**): Pale yellow oil. Yield: quant. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.1 Hz), 1.94 (2H, quint, J=6.6 Hz), 2.62 (2H, t, J=6.6 Hz), 3.62 (2H, s), 3.78 (2H, br t, J=6.6 Hz), 4.16 (2H, q, J=7.1 Hz), 6.49 (1H, dd, J=8.2, 2.4 Hz), 6.52 (1H, br), 6.93 (1H, d, J=8.2 Hz). FAB-MS m/z: 263 [$(M^+ + H)^+$].

Ethyl 3-(8-Nitro-2,3,4,5-tetrahydrobenzo[*b*]azepin-1-yl)-3-oxopropanoate (**20**): Ivory powder. Yield: quant. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7.2 Hz), 1.28—1.34 (1H, m), 1.74—2.00 (3H, m), 2.53—2.78 (3H, m), 3.26 (2H, Abq, J=15.3 Hz), 3.68 (2H, br), 4.13 (2H, q, J=7.2 Hz), 4.63—4.70 (1H, m), 6.51—6.57 (3H, m), 7.00 (1H, d, J=8.1 Hz). FAB-MS m/z: 277 [$(M^+ + H)^+$].

Ethyl 3-(6-Aminoindolin-1-yl)-2-methyl-3-oxopropanoate (**21**): Colorless powder. Yield: 75%. ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, t, J=6.8 Hz), 1.30 (3H, d, J=6.9 Hz), 2.96 (2H, t, J=8.3 Hz), 3.86 (1H, q, J=6.9 Hz), 4.10—4.17 (4H, m), 4.97 (2H, br), 6.24 (1H, dd, J=7.8, 2.1 Hz), 6.86 (1H, d, J=7.8 Hz), 7.44 (1H, d, J=2.1 Hz). FAB-MS m/z: 263 [$(M^+ + H)^+$].

Ethyl 3-(6-Aminoindolin-1-yl)-2,2-dimethyl-3-oxopropanoate (**22**): Colorless powder. Yield: 95%. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J=6.9 Hz), 1.40 (6H, s), 2.89 (2H, t, J=7.8 Hz), 3.74 (2H, t, J=7.8 Hz), 4.17 (2H, q, J=6.9 Hz), 4.96 (2H, br), 6.24 (1H, dd, J=7.8, 2.0 Hz), 6.85 (1H, d, J=7.8 Hz), 7.46 (1H, d, J=2.0 Hz). FAB-MS m/z: 277 [$(M^+ + H)^+$].

Ethyl 3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoate (23) To the solution of **18** (1.51 g, 6.11 mmol) in acetonitrile (30 ml) was added benzyl isocyanate (900 mg, 6.76 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h, then the solvent was removed *in vacuo* and the residue was washed with 2-propanol and diethylether to give **23** (2.15 g, 5.64 mmol, 92%) as a colorless solid, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.21 (3H, t, J=7.2 Hz), 3.04 (2H, t, J=8.1 Hz), 3.65 (2H, s), 4.03—4.18 (4H, m), 4.28 (2H, d, J=5.7 Hz), 6.45 (1H, t, J=5.7 Hz), 7.07 (1H, d. J=8.1 Hz), 7.20—7.36 (6H, m), 8.03 (1H, s), 8.57 (1H, s). FAB-MS m/z: 382 [$(M^+ + H)^+$].

Compounds **24**—**27** were prepared in a manner similar to that described for **23** from compounds **19**—**22**, and these compounds were used for the next reaction without further purification.

Ethyl 3-[(3-Benzylureido)-1,2,3,4-tetrahydroquinolin-1-yl]-3-oxopropanoate (**24**): Colorless powder. Yield: quant. ^{1}H-NMR (DMSO-d_6) δ: 1.14 (3H, br t, J=6.3 Hz), 1.85 (2H, quint., J=6.3 Hz), 2.62 (2H, t, J=6.3 Hz), 3.64—3.67 (4H, m), 4.04 (2H, br), 4.29 (2H, d, J=5.8 Hz), 6.58 (1H, br), 7.03 (1H, d. J=8.3 Hz), 7.14—7.52 (7H, m), 8.53 (1H, s). FAB-MS m/z: 396 [$(M^+ + H)^+$].

Ethyl 3-[8-(3-Benzylureido)-2,3,4,5-tetrahydrobenzo[*b*]azepin-1-yl]-3-oxopropanoate (**25**): Colorless powder. Yield: 84%. ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, t, J=7.2 Hz), 1.25—1.33 (1H, m), 1.74—2.00 (3H, m), 2.56—2.77 (3H, m), 3.25 (2H, Abq, J=15.6 Hz), 4.00 (2H, q, J=7.2 Hz), 4.23 (2H, d, J=5.7 Hz), 4.54—4.61 (1H, m), 5.80 (1H, t, J=5.7 Hz), 7.00 (1H, d, J=2.1 Hz), 7.11 (1H, d, J=8.7 Hz), 7.23—7.35 (5H, m), 7.52 (1H, dd, J=8.4, 2.4 Hz). FAB-MS m/z: 410 [$(M^+ + H)^+$].

Ethyl 3-[6-(3-Benzylureido)indolin-1-yl]-2-methyl-3-oxopropanoate (**26**): Amorphous powder. Yield: 93%. ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, t, J=6.8 Hz), 1.31 (3H, d, J=7.3 Hz), 3.06 (2H, t, J=8.3 Hz), 3.89 (1H, q, J=7.3 Hz), 4.02—4.24 (4H, m), 4.28 (2H, d, J=5.8 Hz), 6.47 (1H, t, J=5.8 Hz), 7.22—7.35 (6H, m), 8.09 (1H, d, J=2.0 Hz), 8.55 (1H, s). FAB-MS m/z: 396 [$(M^+ + H)^+$].

Ethyl 3-[6-(3-Benzylureido)indolin-1-yl]-2,2-dimethyl-3-oxopropanoate (**27**): Colorless powder. Yield: 99%. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J=6.8 Hz), 1.41 (6H, s), 3.00 (2H, t, J=7.8 Hz), 3.81 (2H, t, J=7.8 Hz), 4.18 (2H, q, J=6.8 Hz), 4.28 (2H, d, J=5.9 Hz), 6.49 (1H, t, J=5.9 Hz), 7.07 (1H, d, J=8.3 Hz), 7.19—7.35 (6H, m), 8.14 (1H, d, J=2.0 Hz), 8.54 (1H, s). FAB-MS m/z: 410 [$(M^+ + H)^+$].

3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoic Acid (28) A mixture of the urea **23** (2.06 g, 5.40 mmol), 1 M NaOH (18.9 ml) and methanol (60 ml) and THF (60 ml) was stirred at room temperature for 2 h, then the reaction mixture was acidified with 1 M HCl (20 ml) and concentrated *in vacuo.* After addition of H_2O to the residue, resulting precipitates were collected and washed with H_2O to give **28** (1.91 g, 5.40 mmol, quant.) as a colorless powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 3.03 (2H, t, J=8.4 Hz), 3.53 (2H, s), 4.07 (2H, t, J=8.4 Hz), 4.27 (2H, d, J=5.7 Hz), 6.47 (1H, t, J=6.0 Hz), 7.05 (1H, d. J=8.1 Hz), 7.22—7.35 (6H, m), 8.01 (1H, s), 8.59 (1H, s), 12.76 (1H, br). FAB-MS m/z: 354 [$(M^+ + H)^+$].

Compounds **29**—**32** were prepared in a manner similar to that described for **28** from compound **24**—**27**, and these compounds were used for the next reaction without further purification.

3-[7-(3-Benzylureido)-1,2,3,4-tetrahydroquinolin-1-yl]-3-oxopropanoic Acid (**29**): Colorless powder. Yield: 68%. ^{1}H-NMR (DMSO-d_6) δ: 1.86 (2H, quint., J=6.3 Hz), 2.62 (2H, t, J=6.3 Hz), 3.57 (2H, br), 3.65 (2H, t, J=6.3 Hz), 4.29 (2H, d, J=5.8 Hz), 6.56 (1H, br), 7.03 (1H, d. J=8.3 Hz), 7.17—7.48 (7H, m), 8.51 (1H, s), 12.55 (1H, s). FAB-MS m/z: 368 [$(M^+ + H)^+$].

3-[8-(3-Benzylureido)-2,3,4,5-tetrahydrobenzo[*b*]azepin-1-yl]-3-oxopropanoic Acid (**30**): Colorless powder. Yield: 83%. ^{1}H-NMR (DMSO-d_6) δ: 1.24 (1H, br), 1.72 (2H, br), 1.84—1.92 (1H, m), 2.53—2.78 (3H, m), 3.11 (2H, s), 4.29 (2H, br), 4.47 (1H, br d, J=13.2 Hz), 6.67 (1H, t, J=6.0 Hz), 7.14 (1H, d, J=8.4 Hz), 7.22—7.34 (7H, m), 8.62 (1H, s), 12.44 (1H, s). FAB-MS m/z: 382 [$(M^+ + H)^+$].

(*R*,*S*)-3-[6-(3-Benzylureido)indolin-1-yl]-2-methyl-3-oxopropanoic Acid (**31**): Ivory powder. Yield: 93%. ^{1}H-NMR (DMSO-d_6) δ: 1.28 (3H, d, J=7.3 Hz), 3.06 (2H, t, J=8.3 Hz), 3.78—3.81 (1H, m), 3.82—4.12 (1H, m), 4.14—4.22 (1H, m), 4.28 (2H, d, J=5.9 Hz), 6.49 (1H, t, J=5.9 Hz), 7.07 (1H, d, J=7.8 Hz), 7.22—7.35 (6H, m), 8.09 (1H, d, J=1.9 Hz), 8.55 (1H, s), 12.71 (1H, br). FAB-MS m/z: 368 [$(M^+ + H)^+$].

3-[6-(3-Benzylureido)indolin-1-yl]-2,2-dimethyl-3-oxopropanoic Acid (**32**): Pale yellow powder. Yield: 99%. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (6H, s), 3.01 (2H, t, J=8.3 Hz), 3.91 (2H, t, J=8.3 Hz), 4.29 (2H, d, J=5.9 Hz), 6.48 (1H, t, J=5.9 Hz), 7.07 (1H, d, J=7.8 Hz), 7.20—7.35 (6H, m), 8.13 (1H, d, J=1.9 Hz), 8.54 (1H, s), 13.03 (1H, br). FAB-MS m/z: 380 [$(M^+ - H)^+$].

Method A. (*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoate (38a) A mixture of **28** (1.30 g, 3.70 mmol), CDI (0.72 g, 4.40 mmol), and DMF (20 ml) was stirred at 0 °C for 1 h. To the mixture was added a solution of **5** (1.20 g, 4.40 mmol) in DMF (10 ml) and Et_3N (1.11 g, 11 .0 mmol) and stirred at room temperature for 4 h. The reaction mixture was then diluted with H_2O (100 ml), and resulting precipitates were collected and washed with H_2O to give **38a** (1.40 g, 2.64 mmol, 71%) as an ivory solid, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, t, J=7.1 Hz), 2.87 (2H, d, J=7.9 Hz), 3.01 (2H, br t, J=8.3 Hz), 3.43 (2H, s), 3.98—4.11 (4H, m), 4.27 (2H, d, J=5.9 Hz), 5.23—5.29 (1H, m), 6.43 (1H, br t, J=5.9 Hz), 7.06 (1H, d, J=8.3 Hz), 7.21—7.39 (7H, m), 7.78 (1H, br d, J=8.3 Hz), 7.98 (1H, d, J=1.4 Hz), 8.46 (1H, dd, J=3.9, 1.0 Hz), 8.59 (2H, s), 8.76 (1H, d, J=7.8 Hz). FAB-MS m/z: 530 [$(M^+ + H)^+$].

Compound **38c** and **39** were prepared following a procedure similar to Method A, and were used for the next reaction without further purification.

(*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-methylpropanoate (**38c**): This compound was prepared from **28c** and **34** as an ivory powder. Yield 57%. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, d, J=6.8 Hz), 1.17 (3H, t, J=6.8 Hz), 2.38 (1H, dd, J=15.1, 7.3 Hz), 2.52 (1H, dd, J=15.1, 6.4 Hz), 3.03 (2H, br t, J=8.3 Hz), 3.35 (2H, s), 4.02—4.24 (5H, m), 4.27 (2H, d, J=6.0 Hz), 6.45 (1H, br t, J=6.0 Hz), 7.06 (1H, d, J=7.8 Hz), 7.21—7.36 (6H, m), 8.01 (1H, d, J=1.8 Hz), 8.02 (1H, d, J=7.8 Hz), 8.56 (1H, s). FAB-MS m/z: 467 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{3-[7-(3-Benzylureido)-1,2,3,4-tetrahydroquinolin-1-yl]-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoate (**39**): This compound was prepared from **29** and **5** as a colorless powder. Yield: 97%. ^{1}H-NMR ($CDCl_3$) δ: 1.14 (3H, t, J=7.2 Hz), 1.88 (2H, quint., J=6.6 Hz), 2.61 (2H, t, J=6.6 Hz), 2.72 (1H, dd, J=15.9, 6.6 Hz), 2.83 (1H, dd, J=15.9, 6.6 Hz), 3.50 (1H, d, J=15.9 Hz), 3.57 (1H, d, J=15.9 Hz), 3.70—3.75 (2H, m), 4.03 (2H, q, J=7.2 Hz), 4.40 (2H, d, J=5.4 Hz), 5.26—5.33 (1H, m), 5.94 (1H, br), 6.91 (1H, br), 6.97 (1H, d, J=8.1 Hz), 7.16—7.30 (7H, m), 7.42 (1H, br), 7.58 (1H, d, J=8.1 Hz), 8.19 (1H, br), 8.44 (1H, dd, J=4.8, 1.5 Hz), 8.52 (1H, s). FAB-MS m/z: 544 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-Amino-3-(naphthalen-2-yl)propanoate (36) To a mixture of napthalene-2-aldehyde (3.12 g, 20.0 mmol), ammoniun acetate (3.86 g, 50.0 mmol) in 2-propanol (20 ml) was added malonic acid (2.08 g, 20.0 mmol) and heated at reflux for 4 h. The resulting precipitates were filtered off, and the filtrate was concentrated *in vacuo.* The residue was diluted with ethanol (30 ml) and $SOCl_2$ (2.2 ml) was added dropwise to the mixture at −20 °C. The reaction mixture was warmed to room temperature, then

heated at reflux for 3 h. The reaction mixture was concentrated *in vacuo.* To the residue were added 1 M HCl (60 ml) and extracted with AcOEt (50 ml). The aqueous layer was made alkaline (pH 9) with K_2CO_3 and extracted with AcOEt. The extract was dried over anhydrous $MgSO_4$, and the solvent was removed *in vacuo* to give **36** (810 mg, 3.33 mmol, 18%) as a colorless powder, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=6.9 Hz), 2.54 (2H, s), 2.73—2.76 (2H, m), 4.14 (2H, q, J=6.9 Hz), 4.57—4.61 (1H, m), 7.42—7.50 (3H, m), 7.80—7.83 (4H, m). FAB-MS m/z: 244 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-amino-3-(quinolin-3-yl)propanoate (**37**) was prepared in a manner similar to that described for **36** as yellow oil. Yield 17%. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, t, J=6.9 Hz), 1.94 (2H, s), 2.78 (2H, d, J=7.2 Hz), 4.15 (2H, q, J=6.9 Hz), 4.67 (1H, t, J=7.2 Hz), 7.52—7.57 (1H, m), 7.67—7.73 (1H, m) 7.81 (1H, d, J=8.1 Hz), 8.10 (1H, d, J=8.4 Hz), 8.16 (1H, d, J=1.8 Hz), 8.94 (1H, d, J=2.1 Hz). FAB-MS m/z: 245 [$(M^+ + H)^+$].

Method B. (*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}propanoate (38b) A mixture of **28** (404 mg, 1.14 mmol), HOBt (231 mg, 1.71 mmol), EDC (327 mg, 2.01 mmol), β-alanine ethyl ester hydrochloride (**33**, 263 mg, 1.71 mmol), Et_3N (403 mg, 4.00 mmol) and DMF (8 ml) was stirred at room temperature for 17 h. After addition of H_2O to the residue, the resulting precipitates were collected and washed with H_2O to yield **38b** (403 mg, 0.89 mmol, 78%) as a colorless solid, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J=6.9 Hz), 2.45 (2H, t, J=7.2 Hz), 3.03 (2H, br t, J=8.1 Hz), 3.31 (2H, t, J=7.2 Hz), 3.37 (2H, s), 4.03—4.10 (4H, m), 4.28 (2H, d, J=6.0 Hz), 6.45 (1H, br t, J=6.0 Hz), 7.06 (1H, d, J=7.8 Hz), 7.21—7.36 (6H, m), 8.01 (1H, s), 8.12 (1H, br t, J=5.4 Hz), 8.57 (1H, s). FAB-MS m/z: 453 [$(M^+ + H)^+$].

Compound **38d**—**f** and **40**—**42** were prepared following a procedure similar to Method B, and were used for the next reaction without further purification.

(*R*,*S*)-Methyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-pheylpropanoate (**38d**): This compound was prepared from **28** and **35**[32] as a colorless powder. Yield quant. ^{1}H-NMR (DMSO-d_6) δ: 2.75—2.90 (2H, m), 3.00—3.10 (2H, m), 3.43 (2H, d, br), 3.56 (3H, s), 3.95—4.20 (2H, m), 4.30 (2H, d, J=5.7 Hz), 5.20—5.35 (1H, m), 6.45 (1H, br t, J=5.7 Hz), 7.05 (1H, d, J=8.1 Hz), 7.20—7.38 (11H, m), 7.99 (1H, d, J=1.8 Hz), 8.59 (1H, s), 8.67 (1H, d, J=8.1 Hz). FAB-MS m/z: 515 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-(naphthalen-2-yl)propanoate (**38e**): This compound was prepared from **28** and **36** as an amorphous powder. Yield 64%. ^{1}H-NMR ($CDCl_3$) δ: 1.07 (3H, t, J=7.3 Hz), 2.85—2.99 (4H, m), 3.34 (2H, br), 3.87—4.02 (4H, m), 4.37—4.49 (2H, m), 5.58—5.68 (2H, m), 6.96—6.98 (1H, br), 7.20—7.25 (7H, m), 7.37—7.43 (4H, m), 7.76—7.73 (3H, m), 7.87 (1H, br), 8.73 (1H, d, J=8.3 Hz). FAB-MS m/z: 579 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-(quinolin-3-yl)propanoate (**38f**): This compound was prepared from **28** and **37** as an amorphous powder. Yield 83%. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, t, J=7.3 Hz), 2.89 (2H, br), 2.92 (1H, dd, J=15.6, 6.4 Hz), 3.03 (1H, dd, J=15.6, 7.3 Hz), 3.35 (2H, ABq, J=16.1 Hz), 3.84 (2H, br), 3.93—4.05 (2H, m), 4.42 (2H, d, J=5.8 Hz), 5.62—5.70 (1H, m), 5.94 (1H, t, J=5.8 Hz), 6.89 (1H, d, J=7.3 Hz), 7.18—7.29 (4H, m), 7.41—7.47 (2H, m), 7.60—7.66 (3H, m), 7.74 (1H, s), 7.86—7.90 (1H, m), 7.97 (1H, d, J=8.7 Hz), 8.50 (1H, d, J=1.9 Hz), 8.91 (1H, d, J=8.3 Hz), 9.06 (1H, d, J=2.5 Hz). FAB-MS m/z: 580 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{3-[8-(3-Benzylureido)-2,3,4,5-tetrahydrobenzo[*b*]azepin-1-yl]-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoate (**40**): This compound was prepared from **30** and **5** as an amorphous powder. Yield 73%. ^{1}H-NMR (DMSO-d_6) δ: 1.06 (1.5H, t, J=7.2 Hz), 1.07 (1.5H, t, J=7.2 Hz), 1.23 (1H, br), 1.69 (2H, br), 1.99 (1H, m), 2.53—2.68 (1H, m), 2.75—2.79 (2H, m), 2.98 (1H, dd, J=15.1, 9.8 Hz), 3.07 (1H, dd, J=15.1, 10.3 Hz), 3.29 (2H, s), 3.94—4.00 (2H, m), 4.26—4.30 (2H, m), 4.43—4.48 (1H, m), 5.11—5.18 (1H, m), 6.63—6.67 (1H, m), 7.07 (0.5H, d, J=8.3 Hz), 7.14 (0.5H, d, J=8.3 Hz), 7.20—7.37 (9H, m), 7.62—7.65 (0.5H, m), 7.71—7.74 (0.5H, m), 8.43—8.48 (2H, m), 8.60 (1H, s). FAB-MS m/z: 558 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-2-methyl-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoate (**41**): This compound was prepared from **31** and **5** as a colorless powder. Yield: 48%. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, t, J=7.2 Hz), 1.26 (3H, d, J=6.8 Hz), 2.73—2.83 (1H, m), 2.86 (2H, d, J=7.8 Hz), 2.94—3.02 (1H, m), 3.58—3.64 (1H, m), 3.96—4.06 (4H, m), 4.27 (2H, d, J=5.8 Hz), 5.18—5.24 (1H, m), 6.46 (1H, br t, J=5.8 Hz), 7.01 (1H, d, J=7.8 Hz), 7.21—7.39 (7H, m), 7.76 (1H, d, J=8.3 Hz), 8.03 (1H, s), 8.46 (1H, dd, J=4.4, 1.5 Hz), 8.50 (1H, s), 8.56 (1H, d, J=1.5 Hz), 8.73 (1H, d, J=8.3 Hz). FAB-MS m/z: 544 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-{3-[6-(3-Benzylureido)indolin-1-yl]-2,2-dimethyl-3-oxopropylamino}-3-(pyridin-3-yl)propanoate (**42**): This compound was prepared from **32** and **5** as a colorless powder. Yield: 63%. ^{1}H-NMR (DMSO-d_6) δ: 1.05 (3H, t, J=7.2 Hz), 1.32 (3H, s), 1.33 (3H, s), 2.70—2.86 (3H, m), 2.97 (1H, dd, J=15.6, 10.3 Hz), 3.59—3.71 (2H, m), 3.87—3.97 (2H, m), 4.27 (2H, d, J=5.9 Hz), 5.24—5.30 (1H, m), 6.48 (1H, br t, J=5.9 Hz), 7.03 (1H, d, J=7.9 Hz), 7.16 (2H, dd, J=7.8, 2.0 Hz), 7.22—7.26 (1H, m), 7.29—7.35 (5H, m), 7.75 (1H, ddd, J=7.8, 3.5, 1.9 Hz), 8.18 (1H, d, J=1.9 Hz), 8.45 (1H, d, J=3.5 Hz), 8.50 (1H, s), 8.53 (2H, d, J=7.9 Hz). FAB-MS m/z: 558 [$(M^+ + H)^+$].

(*R*,*S*)-3-{3-[6-(3-Benzylureido)indolin-1-yl]-3-oxopropanoylamino}-3-pyridin-3-ylpropanoic Acid (43a) A mixture of compound **38a** (950 mg, 1.80 mmol), 1 M NaOH (5.4 ml) and ethanol (19 ml) was heated at 60 °C for 1 h, then concentrated *in vacuo.* The residue was acidified with 1 M HCl (5.4 ml), and the resulting precipitates were collected and washed with H_2O to yield **43a** (902 mg, 1.80 mmol, quant.) as a colorless powder, which was recrystallized from methanol–AcOEt. Physical data for **43a** are listed in Table 5.

Compounds **43b**—**f** and **44**—**47** were prepared in a manner similar to that described for **43a** from compounds **38b**—**f** and **39**—**42**. Physical data for **43b**—**f** and **44**—**47** are listed in Table 5.

3-(6-Nitroindolin-1-yl)-3-oxopropanoic Acid (48) A mixture of compound **13** (10.1 g, 36.3 mmol), 1 M NaOH (54 ml) and methanol (150 ml)–THF (100 ml) was stirred at room temperature for 2 h. The reaction mixture was added to 1 M HCl (55 ml) and extracted with AcOEt. The extract was washed with saturated brine and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo.* The residue was washed with AcOEt–diethyl ether to give **48** (7.96 g, 31.8 mmol, 88%) as a yellow powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 3.28 (2H, t, J=8.4 Hz), 3.63 (2H, s), 4.22 (2H, t, J=8.4 Hz), 7.51 (1H, d. J=8.4 Hz), 7.94 (1H, dd, J=8.4, 2.1 Hz), 8.79 (1H, br), 12.81 (1H, s). FAB-MS m/z: 251 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-[3-(6-Nitroindolin-1-yl)-3-oxopropanoylamino]-3-(pyridin-3-yl)propanoate (49) A mixture of **48** (3.89 mg, 15.5 mmol), HOBt (2.73 mg, 20.2 mmol), EDC (3.87 g, 20.2 mmol), **5** (5.40 g, 20.2 mmol), Et_3N (6.30 g, 62.0 mmol) and DMF (40 ml) was stirred at room temperature for 19 h, then concentrated *in vacuo.* The residue was diluted with H_2O and extracted with AcOEt. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo.* The residue was added to 2-propanol and hexane to give **49** (5.83 g, 13.7 mmol, 88%) as brown solid, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (3H, t, J=6.9 Hz), 2.88 (2H, d, J=7.5 Hz), 3.26 (2H, t, J=8.4 Hz), 3.51 (2H, s), 4.02 (2H, q, J=6.9 Hz), 4.21 (2H, t, J=8.4 Hz), 5.23—5.30 (1H, m), 7.36—7.40 (1H, m), 7.50 (1H, d. J=8.1 Hz), 7.78—7.81 (1H, m), 7.93 (1H, dd, J=8.1, 2.1 Hz), 8.47—8.48 (1H, m), 8.59 (1H, s), 8.79—8.82 (2H, m). FAB-MS m/z: 427 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-[3-(6-Aminoindolin-1-yl)-3-oxopropanoylamino]-3-(pyridin-3-yl)propanoate (50) A mixture of the **49** (5.82 g, 13.6 mmol), zinc powder (4.46 g, 68.2 mmol), acetic acid (12 ml) and ethanol (60 ml) was heated at 50 °C for 2 h, then concentrated *in vacuo.* The residue was diluted with AcOEt, and neutralized with 1 M NaOH, and filtered through celite. The filtrate was extracted with AcOEt. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo.* The residue was added to AcOEt–hexane to give **50** (3.54 g, 8.93 mmol, 65%) as yellow solid, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (3H, t, J=6.9 Hz), 2.84—2.94 (4H, m), 3.40 (2H, s), 3.91—4.06 (4H, m), 4.96 (2H, s), 5.22—5.30 (1H, m), 6.21 (1H, dd, J=7.5, 1.8 Hz),6.83 (1H, d, J=7.5 Hz), 7.34—7.42 (2H, m), 7.78 (1H, d, J=7.8 Hz), 8.47 (1H, d, J=4.8 Hz), 8.58 (1H, br), 8.74 (1H, d, J=8.1 Hz). FAB-MS m/z: 397 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-Pyridin-3-yl-3-[3-(6-ureidoindolin-1-yl)-3-oxopropanoylamino]propanoate (51) A mixture of **50** (304 mg, 0.77 mmol), sodium cyanate (100 mg, 1.54 mmol), acetic acid (1.5 ml) and H_2O (3 ml) was heated at 60 °C for 2 h. The reaction mixture was concentrated *in vacuo.* The residue was purified by column chromatography with $CHCl_3$–methanol (10 : 1, v/v) to give **51** (280 mg, 0.64 mmol, 83%) as a brown powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (3H, t, J=6.9 Hz), 2.87 (2H, d, J=7.5 Hz), 3.00 (2H, t, J=8.1 Hz), 3.43 (2H, s), 3.98—4.10 (4H, m), 5.23—5.30 (1H, m), 5.72 (2H, s), 7.04 (1H, d, J=8.1 Hz), 7.28—7.40 (2H, m), 7.78 (1H, d, J=8.1 Hz), 7.94 (1H, s), 8.47 (1H, dd, J=4.8, 1.5 Hz), 8.55 (1H, s), 8.59 (1H, s), 8.76 (1H, d, J=8.1 Hz). FAB-MS m/z: 440 [$(M^+ + H)^+$].

(*R*,*S*)-3-(Pyridin-3-yl)-3-[3-(6-ureidoindolin-1-yl)-3-oxopropanoylamino]propanoic Acid (43g) A mixture of **51** (274 mg, 0.62 mmol), 1 M

NaOH (2.2 ml) and methanol (6 ml) was stirred at room temperature for 16 h. The reaction mixture was added to 1 M HCl (2.2 ml), then concentrated *in vacuo.* The residue was purified by column chromatography using ODS-A with H_2O–methanol (6 : 4, v/v) to yield **43g** (120 mg, 0.29 mmol, yield 47%) as a colorless powder, which was recrystallized from methanol–THF. Physical data for **43g** are listed in Table 5.

(*R*,*S*)-Ethyl 3-{3-[6-(3-Phenylureido)indolin-1-yl]-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoate (52) A mixture of **50** (304 mg, 0.77 mmol), phenyl isocyanate (101 mg, 0.85 mmol), and THF (9 ml) was stirred at room temperature for 1 h. The reaction mixture was diluted with diethyl ether (30 ml), and the precipitated solids were collected and washed with diethyl ether to give **52** (348 mg, 0.67 mmol, yield 88%) as pale yellow powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.12 (3H, t, J=6.9 Hz), 2.88 (2H, d, J=7.8 Hz), 3.04 (2H, t, J=8.4 Hz), 3.45 (2H, s), 4.00—4.11 (4H, m), 5.23—5.31 (1H, m), 6.96 (1H, t, J=7.2 Hz), 7.11 (1H, d, J=8.1 Hz), 7.24—7.32 (3H, m), 7.37 (1H, dd, J=7.8, 4.8 Hz), 7.43 (2H, d, J=8.4 Hz), 7.77 (1H, d, J=7.8 Hz), 8.06 (1H, s), 8.46—8.48 (2H, m), 8.60 (1H, d, J=1.8 Hz), 8.71 (1H, s), 8.77 (1H, d, J=8.1 Hz). FAB-MS m/z: 516 [$(M^+ + H)^+$].

(*R*,*S*)-3-{3-[6-(3-Phenylureido)indolin-1-yl]-3-oxopropanoylamino}-3-(pyridin-3-yl)propanoic Acid (43h) A mixture of **52** (350 mg, 0.68 mmol), 1 M NaOH (2.4 ml) and methanol (7 ml) was stirred at room temperature for 16 h.The reaction mixture was added to 1 M HCl (2.4 ml), then concentrated *in vacuo.* The residue was washed with H_2O and methanol–AcOEt to give **43h** (99 mg, 0.20 mmol, 30%) as a pale yellow powder, which was recrystallized from methanol–AcOEt. Physical data for **43h** are listed in Table 5.

(*R*,*S*)-Ethyl 3-(Pyridin-3-yl)-3-(3-{6-[3-(pyridin-4-yl)methylureido]indolin-1-yl}-3-oxopropanoylamino)propanoate (53) To a solution of **50** (400 mg, 1.01 mmol) and Et_3N (133 mg, 1.31 mmol) in 1,2-dichloroethane (12 ml) was added phenyl chloroformate (174 mg, 1.11 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 40 min., then 4-aminomethylpyridine (175 mg, 1.62 mmol) was added to the mixture, and the reaction mixture was stirred at the same temperature for 3 h. To the reaction mixture was added 1 M NaOH (1.2 ml) and H_2O (5 ml), and the mixture was refluxed for 1 h. The precipitated solid was collected and washed with H_2O to give **53** (343 mg, 0.65 mmol, 64%) as yellow powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.11 (3H, t, J=6.9 Hz), 2.87 (2H, d, J=7.2 Hz), 3.01 (2H, t, J=8.4 Hz), 3.43 (2H, s), 3.98—4.07 (4H, m), 4.31 (2H, d, J=5.4 Hz), 5.22—5.30 (1H, m), 6.55—6.60 (1H, m), 7.06 (1H, d, J=7.8 Hz), 7.16—7.28 (4H, m), 7.36 (1H, dd, J=7.8, 4.8 Hz), 7.78 (1H, d, J=8.1 Hz), 8.00 (1H, s), 8.45—8.50 (3H, m), 8.58 (1H, s), 8.74 (1H, s). FAB-MS m/z: 531 [$(M^+ + H)^+$].

(*R*,*S*)-3-(Pyridin-3-yl)-3-(3-{6-[3-(pyridin-4-yl)methylureido]indolin-1-yl}-3-oxopropanoylamino)propanoic Acid (43i) A mixture of **53** (335 mg, 0.63 mmol), 1 M NaOH (2.2 ml) and methanol (10 ml) was stirred at room temperature for 16 h. The reaction mixture was added to 1 M HCl (2.2 ml), then concentrated *in vacuo.* The residue was purified by column chromatography using ODS-A with H_2O–methanol (1 : 1, v/v) to yield **43i** (196 mg, 0.39 mmol, yield 59%) as a yellow powder, which was recrystallized from methanol–AcOEt. Physical data for **43i** are listed in Table 5.

Ethyl 3-{6-[*N*,*N*′-Bis(*tert*-butoxycarbonyl)guanidino]indolin-1-yl}-3-oxopropanoate (54) To a solution of **18** (500 mg, 2.01 mmol), *N*,*N*′-bis(*tert*-butoxycarbonyl)thiourea (666 mg, 2.41 mmol) and Et_3N (447 mg, 4.42 mmol) in CH_2Cl_2 (20 ml) were added to 2-chloro-1-methylpyridinium iodide (616 mg, 2.41 mmol) and the reaction mixture was stirred at room temperature for 17 h, then diluted with sat. $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with saturated brine and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo.* The residue was purified by column chromatography with hexane–AcOEt (3 : 1, v/v) to yield **54** (830 mg, 1.69 mmol, 81%) as a colorless solid, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.3 Hz), 1.49 (9H, s), 1.52 (9H, s), 3.18 (2H, t, J=7.3 Hz), 3.54 (2H, s), 4.09 (2H, t, J=8.3 Hz), 4.25 (2H, q, J=7.3 Hz), 6.93 (1H, d, J=8.3 Hz), 7.76 (1H, dd, J=7.8, 2.0 Hz), 8.10 (1H, d, J=1.5 Hz), 10.3 (1H, s), 11.6 (1H, s). FAB-MS m/z: 491 [$(M^+ + H)^+$].

3-{6-[*N*,*N*′-Bis(*tert*-butoxycarbonyl)guanidino]indolin-1-yl}-3-oxopropanoic Acid (55) A mixture of **54** (490 mg, 1.00 mmol), 0.5 M NaOH (3.0 ml) and THF (3 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*; then the pH of the reaction mixture was adjusted 5 with 0.5 M HCl at 0 °C. The mixture was extracted with AcOEt. The extract was washed with H_2O and saturated brine and dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* and the residue was triturated with diethyl ether–diisopropyl ether to yield **55** (410 mg, 0.89 mmol, 89%) as a colorless solid, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (18H, br), 3.22 (2H, t, J=8.1 Hz), 3.47 (2H, s), 4.07 (2H, t, J=8.1 Hz), 7.18 (1H, d, J=8.4 Hz), 7.48 (1H, dd, J=8.4, 2.1 Hz), 8.47 (1H, d, J=2.1 Hz), 10.35 (1H, br). FAB-MS m/z: 463 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-(3-{6-[*N*,*N*′-Bis(*tert*-butoxycarbonyl)guanidino]indolin-1-yl}-3-oxopropanoylamino)-3-(pyridin-3-yl)propanoate (56) A mixture of **55** (1.21 g, 2.60 mmol), HOBt (350 mg, 2.60 mmol), EDC (500 mg, 2.60 mmol), **5** (700 mg, 2.60 mmol), Et_3N (1.05 g, 10.4 mmol) and DMF (20 ml) was stirred at room temperature for 12 h, then concentrated *in vacuo.* The residue was diluted with H_2O and extracted with AcOEt. The extract was washed with saturated brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo.* The residue was purified by column chromatography with $CHCl_3$–methanol (100 : 1, v/v) to give **56** (1.16 g, 1.82 mmol, 70%) as a colorless amorphous powder, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, J=7.2 Hz), 1.49 (9H, s), 1.55 (9H, s), 2.91 (1H, d, J=6.6 Hz), 2.95 (1H, d, J=6.6 Hz), 3.16 (2H, t, J=8.1 Hz), 3.44 (2H, ABq, J=17.4 Hz), 4.09—4.15 (4H, m), 5.49—5.60 (1H, m), 7.16 (1H, d, J=8.1 Hz), 7.24—7.28 (1H, m), 7.66—7.73 (2H, m), 8.13 (1H, d, J=1.8 Hz), 8.51 (1H, dd, J=4.8, 1.8 Hz), 8.63 (1H, d, J=1.8 Hz), 8.84 (1H, d, J=8.1 Hz), 10.29 (1H, br), 11.65 (1H, br). FAB-MS m/z: 639 [$(M^+ + H)^+$].

(*R*,*S*)-3-[(6-Guanidinoindolin-1-yl)-3-oxopropanoylamino]-3-(pyridin-3-yl)propanoic Acid (43j) A mixture of **56** (640 mg, 1.00 mmol), 0.5 M NaOH (3.0 ml) and THF (3 ml) was stirred at room temperature for 16 h. The reaction mixture was concentrated *in vacuo*, then added to H_2O and 0.5 M HCl (3.0 ml) at 0 °C. The mixture was extracted with AcOEt. The extract was dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo.* The residue was added to 4 M HCl–dioxane (15 ml). The mixture was stirred at room temperature for 2 h and concentrated *in vacuo.* The residue was purified by column chromatography using ODS-A with H_2O–methanol (9 : 1, v/v) to yield a colorless amorphous solid, which was crystallized from H_2O–methanol to give **43j** (120 mg, 0.29 mmol, yield 29%) as a colorless solid. The analytical sample was recrystallized from methanol–THF. Physical data for **43j** are listed in Table 5.

1-*tert*-Butoxycarbonyl-(3,5-dimethylpyrazol-2-yl)-4,5-dihydro-1*H*-imidazole (57) To a solution of potassium *tert*-butoxide (KO^{t}Bu, 4.03 g, 35.9 mmol) in DMF (80 ml) was added 2-(3,5-dimethylpyrazolyl)-4,5-dihydroimidazole hydrobromide (4.00 g, 16.3 mmol) at −5 °C. To the mixture was added di-*tert*-butoxydicarbonate [$(Boc)_2O$, 3.92 g, 18.0 mmol] at the same temperature, and the reaction mixture was stirred at 0 °C for 2 h. After addition of KO^{t}Bu (1.83 g, 16.3 mmol) and $(Boc)_2O$ (3.56 g, 16.3 mmol), and the reaction mixture was stirred at room temperature for 21 h. The reaction mixture was diluted with H_2O and extracted with AcOEt. The extract was dried over anhydrous $MgSO_4$ and the solvent was removed *in vacuo* to give **57** as pale yellow powder (4.13 g, 15.6 mmol, 96%), which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (9H, s), 2.24 (3H, s), 2.33 (3H, s), 3.90—4.07 (2H, m), 4.02—4.07 (2H, m), 5.91 (1H, s). FAB-MS m/z: 265 [$(M^+ + H)^+$].

Ethyl 3-{6-[(1-*tert*-Butoxycarbonyl-4,5-dihydro-1*H*-imidazole-2-yl)-amino]indolin-1-yl}-3-oxopropanoic Acid (58) To a solution of **57** (1.64 g, 6.20 mmol) in acetonitrile (30 ml) was added **18** (1.40 g, 5.60 mmol) at room temperature. The reaction mixture was stirred at the same temperature for 2 d and heated at 60 °C for 12 h. The solvent was removed *in vacuo* and the residue was purified by column chromatography with $CHCl_3$–methanol (9 : 1, v/v) to yield **58** (530 mg, 1.27 mmol, 53%) as a pale yellow powder, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7.2 Hz), 1.52 (9H, s), 3.42 (2H, t, J=8.4 Hz), 3.53 (2H, s), 3.70—3.89 (4H, m), 4.07 (2H, t, J=8.4 Hz), 4.24 (2H, q, J=7.2 Hz), 7.09 (1H, d, J=8.1 Hz), 7.68 (1H, dd, J=8.1, 2.1 Hz), 8.17 (1H, br), 9.43 (1H, br). FAB-MS m/z: 417 [$(M^+ + H)^+$].

3-{6-[(1-*tert*-Butoxycarbonyl-4,5-dihydro-1*H*-imidazole-2-yl)amino]-indolin-1-yl}-3-oxopropanoic Acid (59) A mixture of **58** (520 mg, 1.25 mmol), 0.5 M NaOH (3.8 ml) and THF (20 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated *in vacuo*, then added to H_2O and 0.5 M HCl (3.8 ml) at 0 °C. The mixture was concentrated *in vacuo.* The residue was purified by column chromatography using ODS-A with H_2O–methanol (7 : 3, v/v) to yield **59** (220 mg, 0.57 mmol, 45%) as a colorless amorphous powder, which was used for the next reaction without further purification. ^{1}H-NMR (DMSO-d_6) δ: 1.51 (9H, s), 3.04 (2H, t, J=8.4 Hz), 3.46 (2H, s), 3.61—3.72 (4H, m), 4.09 (2H, t, J=8.4 Hz), 7.10 (1H, d, J=8.1 Hz), 7.50 (1H, d, J=8.1 Hz), 8.12 (1H, s). FAB-MS m/z: 387 [$(M^+ - H)^-$].

(*R*,*S*)-Ethyl 3-(3-{6-[(4,5-Dihydro-1*H*-imidazol-2-yl)amino]indolin-1-yl}-3-oxopropanoylamino)-3-(pyridin-3-yl)propanoate (60) A mixture of **59** (190 mg, 0.50 mmol), HOBt (70 mg, 0.52 mmol), EDC (100 mg, 0.52 mmol), **5** (130 mg, 0.49 mmol), Et_3N (152 mg, 1.5 mmol) and DMF (5 ml) was stirred at room temperature for 12 h, then concentrated *in vacuo.* The residue was purified by column chromatography with $CHCl_3$–methanol (30 : 1, v/v) to give **60** (190 mg, 0.34 mmol, 68%) as a colorless amorphous solid, which was used for the next reaction without further purification. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, J=7.2 Hz), 1.55 (9H, s), 2.89 (1H, dd, J=15.6, 6.6 Hz), 2.98 (1H, dd, J=15.6, 6.6 Hz), 3.13 (2H, t, J=8.4 Hz), 3.44 (2H, ABq, J=17.1 Hz), 3.71—3.85 (4H, m), 4.04—4.19 (4H, m), 5.45—5.56 (1H, m), 7.10 (1H, d, J=8.1 Hz), 7.61 (1H, dd, J=8.1, 1.8 Hz), 7.60—7.64 (1H, m), 7.60—7.64 (1H, m), 7.70—7.75 (1H, m), 8.23 (1H, d, J=1.8 Hz), 8.51 (1H, dd, J=7.8, 2.1 Hz), 8.63 (1H, d, J=2.1 Hz), 8.93 (1H, d, J=7.8 Hz), 9.42 (1H, br). FAB-MS m/z: 565 [$(M^+ + H)^+$].

(*R*,*S*)-Ethyl 3-(3-{6-[(4,5-Dihydro-1*H*-imidazol-2-yl)amino]indolin-1-yl}-3-oxopropanoylamino)-3-(quinolin-3-yl)propanoate (61) This compound was prepared in the manner similar as **60** from compounds **59** and **37** in the absence of Et_3N. Yield 33%. Colorless amorphous powder. ^{1}H-NMR (DMSO-d_6) δ: 1.06 (3H, t, J=6.9 Hz), 1.49 (9H, s), 2.97—3.08 (4H, m), 3.61—3.72 (6H, m), 4.03 (2H, q, J=6.6 Hz), 4.09—4.17 (2H, m), 5.45 (1H, dd, J=14.1, 7.4 Hz), 6.88—6.93 (1H, m), 7.11—7.29 (2H, m), 7.59—7.78 (2H, m), 7.93—8.03 (2H, m), 8.34 (1H, s), 8.97 (2H, s), 9.25 (1H, d, J=7.8 Hz). FAB-MS m/z: 615 [$(M^+ + H)^+$].

(*R*,*S*)-3-(3-{6-[(4,5-Dihydro-1*H*-imidazol-2-yl)amino]indolin-1-yl}-3-oxopropanoylamino)-3-(pyridin-3-yl)propanoate (43k) A mixture of the **60** (150 mg, 0.27 mmol) and 4 M HCl–dioxane (5 ml) was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo.* The residue was added to H_2O (2 ml) and 1 M NaOH (1 ml). The mixture was stirred at room temperature for 1 h, then added to 1 M HCl (1.2 ml). The mixture was concentrated *in vacuo.* The residue was purified by column chromatography using ODS-A with H_2O–methanol (8 : 2, v/v) to yield **43k** (220 mg, 0.11 mmol, yield 44%) as a colorless amorphous solid, which was recrystallized from methanol–THF. Physical data for **43k** are listed in Table 5.

Compound **43l** was prepared in a manner similar to that described for **43k** from compound **61**. Physical data for **43l** are listed in Table 5.

Authentic Materials SC65811 and **1** were prepared in our company by a literatural procedure.[17a)]

Preparation of Integrins The $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$ integrins were separated from the human placenta using a murine anti-human β_3 integrin monoclonal antibody and purified by murine anti-human $\alpha_{IIb}\beta_3$ monoclonal antibody C4G1[33)] for $\alpha_v\beta_3$, P1F6 (anti-$\alpha_v\beta_5$ monoclonal antibody)-Affi-Gel 10 (Chemicon, Temecula, CA) for $\alpha_v\beta_5$ and fibronectin cell-binding domain (CBD)-agarose (Takara, Kyoto, Japan) for $\alpha_5\beta_1$. The $\alpha_{IIb}\beta_3$ was separated from human platelets and purified by the method of Fitzgerald *et al.*[34)] without Sephacryl S-300 gel filtration.

Biotinylation of Adhesive Proteins Human vitronectin (Asahi Techno-glass, Tokyo, Japan), fibronectin (Iwaki Glass, Funabashi, Japan) and fibrinogen (Sigma, St. Louis, MO, U.S.A.), were commercially purchased. These adhesive proteins were biotinylated by adding N-hydroxysuccinimido (NHS)-biotin (Pierce, Rockford, MA, U.S.A.) to their solutions to a final concentration of 0.2 mg/ml and allowing the mixture to stand for 2 h at room temperature. Excess biotin ester was removed by gel chromatography.

Adhesive Proteins Binding Assays to Purified Human Integrins Purified integrins (0.1 ml/well) were dispensed onto 96-well microtiter plates at 0.5 μg/ml for $\alpha_v\beta_3$, $\alpha_5\beta_1$, 1 μg/ml for $\alpha_{IIb}\beta_3$, and 0.2 μg/ml for $\alpha_v\beta_5$ in 20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$ [Tris-buffered saline (TBS)], and the plates were incubated overnight at room temperature. The plates were blocked for 2 h at 37 °C in TBS containing 3.5% bovin serum albumin (BSA) before incubation with biotinylated adhesive proteins (vitronectin, 1 μg/ml; fibrinogen, 5 μg/ml; fibronectin, 2 μg/ml) at 0.1 ml/well, in the absence or presence of an increasing concentration of the compounds, for 3 h at 37 °C. After washing several times with TBS, 0.1 ml of 1000-fold diluted streptavidin biotinylated peroxidase complex (Amersham, Tokyo, Japan) was poured into each well, and the plates were incubated for 1 h at room temperature, followed by several washings. The plates were developed by adding 100 μl of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonic acid) solution (Bio-Rad, Hercules, CA, U.S.A.) to each well for 20 min before stopping the reaction by the addition of 50 μl of 2% oxalic acid. The absorbance at 415 nm was measured using a microtiter plate reader (Bio-Rad, Hercules, CA, U.S.A.).

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References and Notes

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