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Design and synthesis of rho kinase inhibitors (III)

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Abstract—The structure–activity relationship of Rho kinase inhibitors bearing an isoquinoline scaffold was studied. *N*-(1-Benzyl-3-pyrrolidyl)-*N*-(5-isoquinolyl)amine analogues were optimized with respect to their inhibitory potencies for the enzyme and for chemotaxis. The potent analogues were further evaluated by an ex vivo test in which the selected compounds were orally administered to rats, and the Rho kinase inhibitory potency observed in the rat serum was evaluated 3 h after the administration. Compound **23g** showed a high level of Rho kinase inhibitory activity in the rat serum and was stable in an in vitro metabolic test using a microsomal cytochrome preparation. The (*R*)-isomer of **23g** displayed a higher level of inhibitory potency than the (*S*)-isomer in a cell-free kinase assay and in the cell migration assay ($IC_{50}^{ENZ} = 25$ nM and $IC_{50}^{MCP} = 1$ μM). The (*R*)-isomer successfully inhibited the phosphorylation of MBS (myosin-binding subunit) in cells.

1. Introduction

Rho kinase is a serine/threonine protein kinase that is composed of the kinase domain at the N-terminal followed by the coiled-coil domain, the Rho-binding domain, and the PH-like domain at the C-terminal. Rho kinase is one of the best-studied Rho effectors.[1] Regulation of the phosphorylation of myosin light chain (MLC) by Rho is mediated by the activation of Rho kinase. Rho kinase in the free state is auto-inhibited. Association of the GTP-binding form of Rho with the Rho-binding domain of Rho kinase induces a structural change and the enzyme is activated.

The activated Rho kinase directly phosphorylates MLC, and simultaneously Rho kinase phosphorylates the myosin-binding subunit of myosin phosphatase, thereby inactivating the phosphatase. These direct and indirect effects act in coordination to regulate the phosphorylation state of MLC that results in the contraction of smooth muscle cells and stress fiber formation in non-muscle cells.[1]

Rho kinase inhibitors would hold great potential as novel therapies for a number of diseases because the kinase plays an important role in stress fiber formation, focal adhesion, and smooth muscle contraction as described above. It was shown that Fasudil dihydrochloride (HA-1077), which has been used clinically as a cerebrovascular contraction inhibitor,[2] is a potent Rho kinase inhibitor and that the action of HA-1077 could be explained by the inhibition of Rho kinase. Evidence is accumulating that Rho kinase inhibitors would be useful for the treatment of myocardial ischemia,[3] hypertension,[4] penile corpus cavernosum,[5] glaucoma,[6] cancer migration,[7] and kidney diseases.[8,9] Recently, it has been suggested that the inhibition of Rho kinase might accelerate the regeneration of nerve fibers after spinal-cord injury and might be effective in neurological disorders such as spinal-cord injury, Alzheimer's disease, and multiple sclerosis.[10] Recent progress in the development of Rho kinase inhibitors and their potential applications have been reviewed.[11]

Keywords: Rho kinase; Inhibitor; Structure–activity relationship; Myosin-binding subunit phosphorylation.
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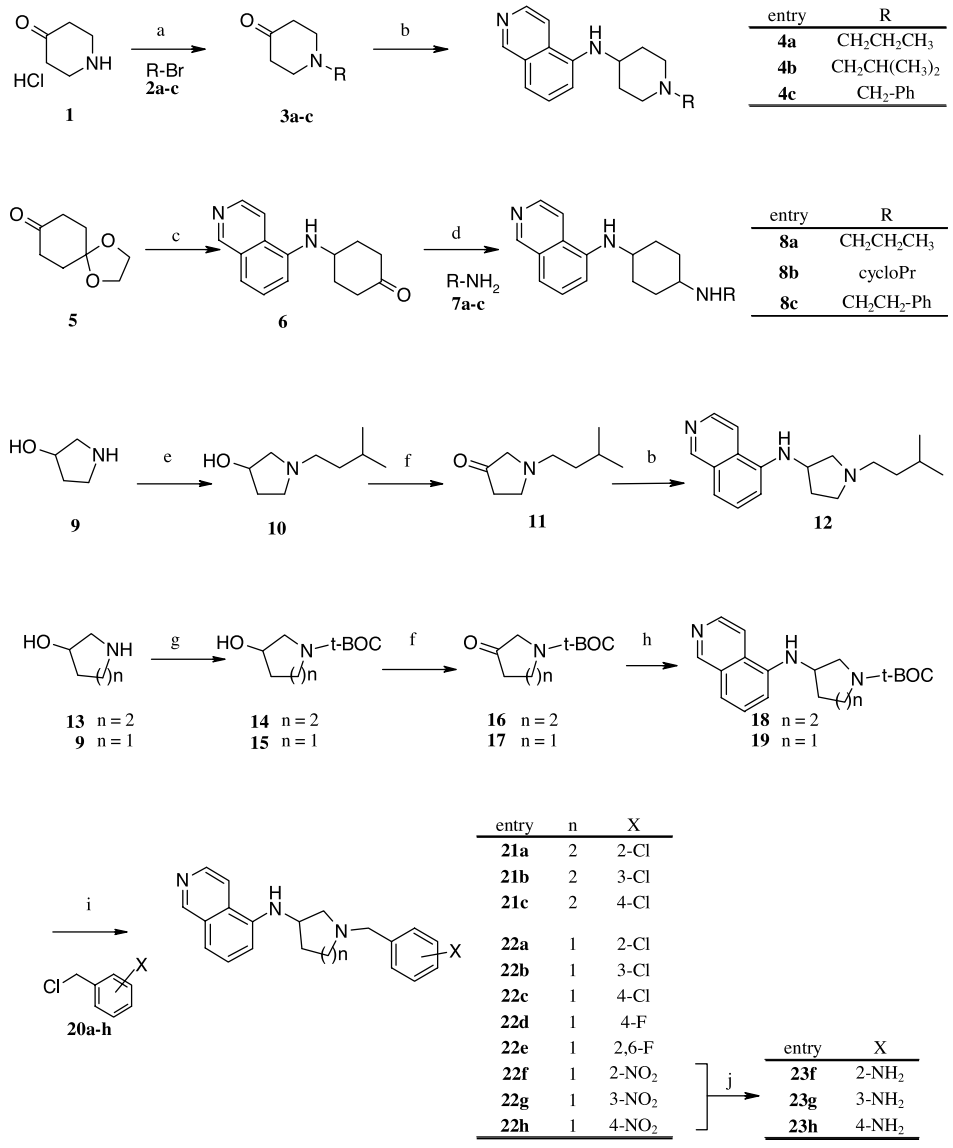
In order to develop potent and selective Rho kinase inhibitors, we carried out a structure-based drug design of inhibitors using a homology model of the kinase and have succeeded in identifying the seed compounds of several different scaffolds.[12] We have reported the properties and the structural optimization of 1*H*-indazole analogues.[13] Here we report on our investigation of the structure–activity relationship (SAR) of isoquinoline analogues.

2. Chemistry

The intermediates, 1-alkyl-4-piperidone **3a**–**c**, were obtained by alkylation of 4-piperidone hydrochloride **1** with several alkyl bromides **2a**–**c** (Scheme 1). *N*-(1-Alkyl-4-piperidyl)-*N*-(5-isoquinolyl)amine analogues **4a**–**c** were prepared by reductive alkylation of 5-aminoisoquinoline with **3a**–**c** using Ti $(OiPr)_4$ as a Lewis acid catalyst and $NaBH_4$ as a reducing agent.

Reductive alkylation of 5-aminoisoquinoline with 1,4-cyclohexanedione monoethylene ketal **5** using the BH_3–pyridine complex in the presence of a catalytic amount of acetic acid gave the corresponding compound. This step was then followed by hydrolysis of the ketal group with 50% acetic acid to give the intermediate **6**. 1,4-Diaminocyclohexane analogues **8a**–**c** were prepared by reductive amination of **6** with several alkyl amines using $NaBH(OAc)_3$.

The intermediate**10** was prepared by reaction with 3-hydroxypyrrolidine **9** and 1-chloro-3-methylbutane. The corresponding ketone **11** was synthesized by oxida-

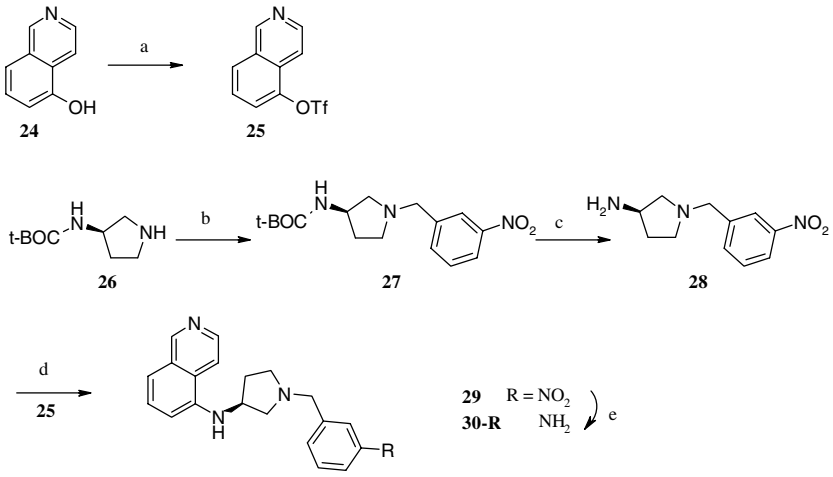


Scheme 1. Synthetic routes of isoquinoline analogues. Reagents and conditions: (a) K_2CO_3, CH_3CN, rt, then reflux; (b) 5-aminoisoquinoline, $Ti(OiPr)_4$, rt then MeOH, $NaBH_4$, rt; (c) (1) 5-aminoisoquinoline, BH_3/pyridine, AcOH, MeOH, rt; (2) 50% AcOH, reflux; (d) $NaBH(OAc)_3$, MeOH, rt; (e) 1-chloro-3-methylbutane, K_2CO_3, DMF, 0 °C; (f) SO_3–Me_3N, DMSO, Et_3N, rt; (g) di-*tert*-butyl dicarbonate, THF, 3 N NaOH aq, rt; (h) (1) 5-aminoisoquinoline, Na_2SO_4, AcOH, rt; (2) $NaBH(OAc)_3$, rt; (i) (1) 95% TFA, $CHCl_3$, rt; (2) K_2CO_3, CH_3CN, rt; (j) $SnCl_2/H_2O$, HCl, reflux.

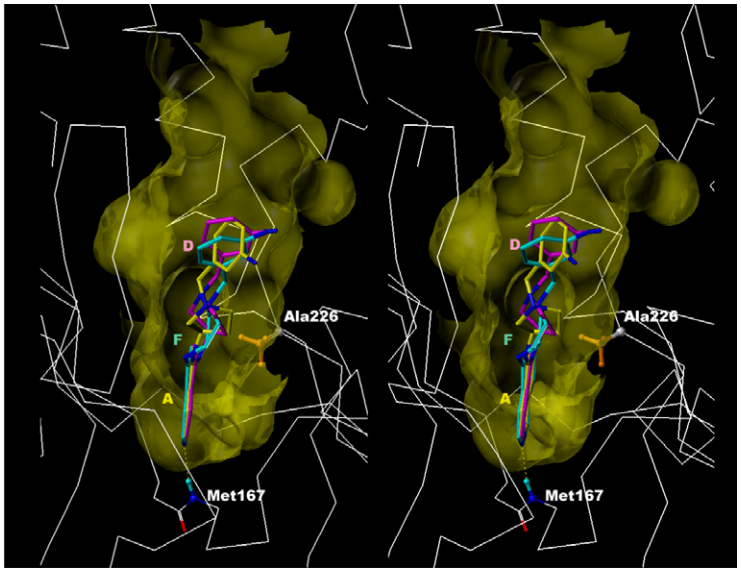
tion of **10** using the sulfur trioxide-trimethylamine complex (SO_3–Me_3N) in DMSO at room temperature. **12** was prepared by reductive alkylation of 5-aminoisoquinoline with **11** using Ti $(OiPr)_4$and $NaBH_4$.

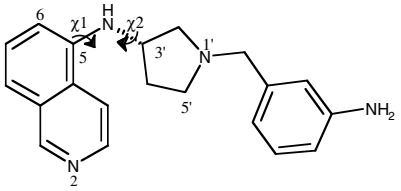
The secondary amines, 3-hydroxypiperidine **13** and 3-hydroxypyrrolidine **9**, were protected by the *tert*-butoxycarbonyl (*t*-Boc) group (**14** and **15**). The ketones **16** and **17** were synthesized by oxidation of **14** and **15** using the same method for the synthesis of **11**. Compounds **18** and **19** were, respectively, obtained from **16** and **17** by reductive alkylation with 5-aminoisoquinoline using $NaBH(OAc)_3$ at room temperature in glacial acetic acid. Under other conditions such as $Ti(OiPr)_4/NaBH_4$ or BH_3/Pyridine/AcOH, this alkylation resulted in a low yield (< 5%). The *t*-Boc group was of **18** and **19** were removed using TFA. The *N*-alkylated compounds **21a**–**c** and **22a**–**h** were then prepared by alkylation with substituted benzylchlorides. The amines **23f**–**h** were synthesized by reduction of the corresponding nitro compounds **22f**–**h** with tin(II) chloride monohydrate.

The enantiomers of **23g** were synthesized as shown in Scheme 2. Trifluoromethanesulfonate **25** was prepared from 5-hydroxyisoquinoline **24** by treatment with trifluoromethanesulfonic anhydride. (3*R*)-(+)-3-(*t*-Boc-amino)-pyrrolidine **26** was allowed to react with 3-nitrobenzylchloride to afford **27**. The intermediate **28** was prepared by cleavage of the *t*-Boc group of **27**. Compound **29** was synthesized from **28** and **25** by the



Scheme 2. Synthesis of **30-R**. Reagents and conditions: (a) Tf_2O, $CHCl_3$, pyridine, rt; (b) 3-nitrobenzylchloride, K_2CO_3, CH_3CN, rt; (c) TFA, $CHCl_3$, rt; (d) cat. $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 80 °C; (e) $SnCl_2/H_2O$, HCl, reflux.

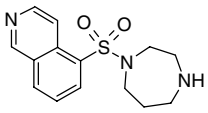
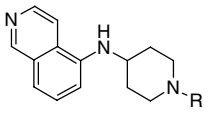
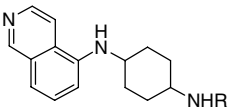
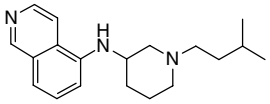
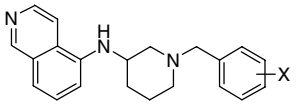
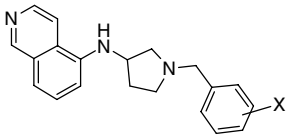




	model		
	a	**b**	**c**
color of carbon atoms	cyan	magenta	yellow
packering position of pyrrolidine ring	5'	1'	3'
pseudochirality of N1'	S	S	S
χ1 (6-5-N-3')	-72	-17	-20
χ2 (5-N-3'-2')	87	94	154

Figure 1. Docking models of **30-R**. The molecular surface of the ligand-binding pocket was visualized. Three representative conformer models of **30-R**, **a**–**c**, are shown in a superimposed view. In the A region, the isoquinoline ring took a constant position, and the essential hydrogen bond between the backbone NH of Met167 and the N2 of isoquinoline was well conserved (yellow dashed line). In the F region, different conformations were possible for the pyrrolidine ring. The torsion angles that determine the orientation of the pyrrolidine ring relative to the isoquinoline ring (χ_1 and χ_2) were not conserved. However, the center of the mass of the ring was conserved in the center of the F region. The sidechain methyl group of Ala226 is shown.

Table 1. Inhibitory potencies of isoquinoline derivatives

Entry	Rho kinase IC_{50}^{ENZ} (nM)		Isoquinoline analogues
HA-1077	180		
		R	
4a	345	$CH_2CH_2CH_3$	
4b	210	$CH_2CH(CH_3)_2$	
4c	140	CH_2-Ph	
		R	
8a	180	$CH_2CH_2CH_3$	*anti*
	180		*syn*
8b	70	CycloPr	*anti* 
	195		*syn*
8c	440	CH_2CH_2-Ph	*anti*
	580		*syn*
12	270		
		X	
21a	210	2-Cl	
21b	265	3-Cl	
21c	185	4-Cl	
		X	
22a	100	2-Cl	
22b	65	3-Cl	
22c	65	4-Cl	
22d	95	4-F	
22e	75	2,6-F	
23f	205	2-NH_2	
23g	275	3-NH_2	
23h	235	4-NH_2	

Buchwald-Hartwig reaction with a catalytic amount of $Pd(OAc)_2$ and (±)-BINAP in the presence of Cs_2CO_3 at 80 °C in toluene in a moderate yield.[14] The corresponding amine **30-R** was obtained from **29** by reduction with tin(II) chloride monohydrate. The other isomer, **30-S**, was synthesized from (3S)-(−)-3-(*t*-Boc-amino)-pyrrolidine in the same manner.

3. Structure optimization

The cell-free Rho kinase assay was performed according to the methods described in a previous report.[12] A chemotaxis assay was performed by the Boyden Chamber method using CCR2-overexpressing human-derived histiocyte lymphoma (U937) cells and MCP-1.[13] HA-1077 was prepared by the published method[15] and was used as the positive control.

3.1. Enzyme inhibitory potency

The ligand-binding pocket of Rho kinase is composed of three regions, namely the A, F, and D regions. The A region is the bottom of the pocket and offers the essential hydrogen bond donor (the backbone NH of Met167, Fig. 1). Planar aromatic scaffolds that have a hydrogen bond acceptor atom inside or outside of the aromatic ring, such as pyridine, indazole, isoquinoline, phthalimide, and benzamide, were found compatible with the flat shape of the A region.[12] The F region is spherical and spacious. In the case of inhibitors employing 1*H*-indazole as the substructure for the A region, docking simulation indicated that several chemical fragments could possibly occupy the F region. Actually, 1*H*-indazole derivatives that have 3-aminopiperidine, 4-aminopiperidine, 3-aminopyrrolidine, and urea as the substructures occupying the F region were all potent inhibitors.[12,13] In

contrast, as the isoquinoline ring occupies more space than a 1*H*-indazole ring in the A region, the choice of the substructure in the F region was expected to be limited. In fact, it was demonstrated that the use of urea or amide substructures leads to the loss of the inhibitory activity of isoquinoline analogues ($IC_{50}^{ENZ} > 10\ \mu M$), and that a linker substructure consisting of an amine nitrogen atom and a saturated ring was suitable.[12] Considering the results from the previous study[12], we examined the enzyme inhibitory potency of isoquinoline analogues carrying a cyclic aliphatic ring of different chemical structures.

In Table 1, the kinase inhibitory potency of isoquinoline derivatives having 4-aminopiperidine (**4a**–**c**), 1,4-diaminocyclohexane (**8a**–**c**), 3-aminopiperidine (**12** and **21a**–**c**), and 3-aminopyrrolidine (**22a**–**h**) substructures is shown. 4-Aminopiperidine and 3-aminopiperidine derivatives showed potency comparative to that of HA-1077 (**4a**–**c**, **12**, and **21a**–**c**). In the case of 1,4-diaminocyclohexane derivatives, only **8b** (*anti*) showed improved potency, and other derivatives had comparative potency. The 3-aminopyrrolidyl substructure (**22a**–**c**; $IC_{50}^{ENZ} < 100$ nM) was more favorable than the 3-piperidyl substructure (**12**, **21a**–**c**; $IC_{50}^{ENZ} > 100$ nM). This preference of an aminopyrrolidine substructure over an aminopiperidine substructure was the opposite of the SAR found in the 1*H*-indazole analogues.[13]

We examined the effect of the substitutions on the phenyl ring of 3-aminopyrrolidine analogues. Analogues substituted with halogen atoms (**22a**–**e**) indicated a higher potency than **23f**–**h**, which had an amino group on the phenyl ring (Table 1). Hydrophobic substitution on the phenyl ring would be advantageous for interaction with the enzyme.

3.2. Chemotaxis inhibitory potency

Potent isoquinoline-pyrrolidine derivatives also showed good CCR2/MCP-1 chemotaxis inhibitory potency (Table 2). Among the compounds **21c** and **22a**–**e**, the Rho kinase inhibition potency roughly correlated with the inhibition potency of chemotaxis;

$$\log(IC_{50}^{MCP}) = 0.69\log(IC_{50}^{ENZ}) - 0.17 (r^2 = 0.61) \quad (1)$$

This fact would suggest that these halogenated analogues have nearly equal permeability into the cells. On the other hand, compounds **23f**–**h**, which have a less hydrophobic amino group on the phenyl ring indicated IC_{50}^{MCP} values comparative with those of **21c** and **22a**–**e**. The inhibitory potency (IC_{50}^{MCP}) of compounds **23f**–**h** is several times the value expected from their in vitro enzyme inhibitory potency (IC_{50}^{ENZ}) based on Eq. 1. Hydrophilic substitution on the phenyl ring would be advantageous for cell permeability, but not for enzyme inhibition potency. We had previously observed a similar SAR during the course of the structure optimization of 1*H*-indazole inhibitors.[13]

Table 2. Properties of isoquinoline analogues

Compound	Rho kinase IC_{50}^{EHZ} (nM)	CCR2/MCP-1 IC_{50}^{MCP} (μM)	Ex vivo (%)
HA-1077	180	35	20
21c	185	23	18
22a	100	17	0
22b	65	14	11
22c	65	11	0
22d	95	22	4
22e	75	10	2
23f	205	14	9
23g	275	20	57
23h	235	15	15

3.3. Ex vivo test

To estimate their efficacy in vivo, we measured the inhibitory potency of the serum 3 h after oral administration of the isoquinoline inhibitors to SD rats at doses of 30 mg/kg (ex vivo test). The inhibition rate (%) was calculated relative to that of the control rats (water was administered). The result is shown in Table 2. The inhibition rate of HA-1077 was 20%. Compounds **22a**–**e** have better IC_{50}^{ENZ} and IC_{50}^{MCP} values than HA-1077, but showed poorer inhibition (<20%). In contrast, compound **23g** with an amino group at the 3-position on the phenyl ring showed superior inhibition (57%). The analogues having an amino group at the 2- or 4-position on the phenyl ring showed weak inhibition in the ex vivo test (**23f**: 9%, **23h**: 15%), though they indicated a sufficient inhibitory potency for the enzyme reaction and for chemotaxis as did **23g**. It was considered that the solubility or permeability of the compounds was not the major reason for this difference.

Thus, the metabolic stability of the inhibitors was evaluated using a rat liver microsome preparation. Compounds were incubated with an excess of microsome and NADPH for 30 min. The residual concentration of **23g** was 67%, while that for HA-1077 it was 45%. Analogues that exhibited poorer efficacy in the ex vivo test all indicated a higher susceptibility to microsome oxidation (<15%). Stability against metabolism by liver cytochromes might be a possible explanation why the serum of the rats administered with **23g** retained good inhibitory potency. However, because the results of the ex vivo test would reflect many complex pharmacokinetic processes (for example, absorption, distribution, and the possible existence of active metabolites), we should refrain from attributing the good ex vivo property of **23g** only to its metabolic stability.

3.4. Profile of the (*R*)-isomer of 23g

Compound **23g** has a chiral center on the C3 of the pyrrolidine ring. As shown in Table 3, the (*R*)-isomer, **30-R**, has 12-fold Rho kinase inhibitory activity and 30-fold chemotaxis inhibitory activity relative to the (*S*)-isomer,

Table 3. Properties of **30-R** and **30-S**

Compound	Rho kinase IC_{50}^{EHZ} (nM)	CCR2/MCP-1 IC_{50}^{MCP} (μM)	Ex vivo (%)		
			1h	4h	8h
30-R	25	1	52	42	26
30-S	310	30	30	17	0

30-S. Compound **30-R** fortunately indicated higher ex vivo efficacy. Although a time-dependent decrease in inhibition was shown in the ex vivo test, sufficient inhibition (26%) was observed even after 8 h (Table 3).

The docking study of **30-R** (Fig. 1) indicated that the isoquinoline ring fully occupies the A region of the ligand-binding pocket and that the N2 atom of the isoquinoline ring interacts with NH of Met167. The stereochemistry of C3 of the pyrrolidine ring, which links the nitrogen atom of 5-aminoisoquinoline to its ring system, is important. It is noted that all stable conformers of **30-R** could certainly occupy the volume of the ligand-binding pocket. Such a conformational advantage was not possible with the (*S*)-isomer. Only a proportion of the stable conformers of the (*S*)-isomer could be docked into the pocket. The conformational freedom in the ligand-binding pocket might explain the higher potency of the (*R*)-isomer than the (*S*)-isomer.

Crystal structures of ROCK-I,[16] an isoform of Rho kinase, and Rho kinase[17] have been elucidated recently. It seemed that good use of the F region is important for inhibitors to have a high affinity for and selectivity against Rho kinase. The F region of Rho kinase is wider than c-AMP dependent protein kinase A (PKA) because the residue corresponding to Ala226 of Rho kinase is Thr183 in PKA (Fig. 1). A mutation study of PKA[18] strongly suggested that Ala226 is the key residue that affects the inhibitor's affinity and selectivity between PKA and Rho kinase.

3.5. Inhibition of MBS phosphorylation

Rho kinase phosphorylates the myosin-binding subunit (MBS) of myosin phosphatase and thereby inactivates the phosphatase activity. The sites of phosphorylation of MBS by Rho kinase are Thr697, Ser854, Thr855, and several other residues. By using an antibody that specifically recognizes MBS phosphorylated at Ser854, it has been found that the stimulation of MDCK epithelial cells with tetradecanoylphorbol-13-acetate (TPA) induced the phosphorylation of MBS under conditions where membrane ruffling and cell migration were induced.[19] As shown in Figure 2, phosphorylation of MBS induced by TPA was inhibited by pretreatment with **30-R** in a dose dependent manner. It should be noted that the inhibition occurred at a concentration between 1 and 10 μM. These concentrations correspond to the IC_{50}^{MCP} of **30-R**.

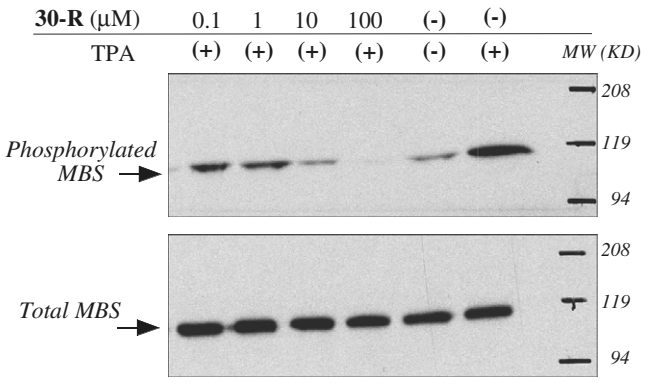


Figure 2. Inhibition of Rho kinase by **30-R**. Serum-deprived MDCK II cells pretreated with **30-R** were stimulated with 10 nM TPA for 10 min. The cell lysates were resolved by SDS–PAGE followed by immunoblot analysis with anti-phosphorylated-MBS antibody (top) or anti-MBS antibody (bottom). Arrowheads indicate the position of phosphorylated MBS and total MBS.

4. Conclusion

The Rho kinase inhibitory potency of the serum of rats administered with isoquinoline analogues was measured and **23g** was identified as an optimized lead Rho kinase inhibitor having in vivo efficacy. The (*R*)-isomer, **30-R**, had 12- to 30-fold Rho kinase inhibitory potency relative to the other isomer ($IC_{50}^{ENZ} = 25$ nM, $IC_{50}^{MCP} = 1$ μM). **30-R** exhibited enhanced metabolic stability. It inhibited MBS-phosphorylation in cells. Thus, **30-R** would be a useful molecular probe to study the function of Rho kinase in vivo. Application of **30-R** to several disease animal models is underway and will be reported elsewhere.

5. Experimental

Commonly used abbreviations: Ab (antibody), CCR-2 (C–C chemokine receptor type 2), MBS (myosin-binding domain), MCP-1 (monocyte chemoattractant protein 1), TPA (tetradecanoylphorbol-13-acetate), PBS (Na_2HPO_4 81 mM, KH_2PO_4 15 mM, 137 mM NaCl, 2.7 mM KCl, pH 7.4), DMEM (Dulbecco's modified Eagle's medium), DTT (dithiothreitol), PVDF (polyvinylidene fluoride), SDS (sodium dodecylsulfonate), TBS (Tris–HCl buffer 25 mM, pH 7.4, containing 137 mM NaCl and 23 mM KCl), Ac (acetyl group), AcOH (acetic acid), cycloPr (cyclopropyl group), DMF (*N*,*N*-dimethylformamide), DMSO (dimethylsulfoxide), EtOAc (ethyl acetate), EtOH (ethanol), MeOH (methanol), ODS (octadecylated silica gel), TFA (trifluoroacetic acid), *t*-Boc (*tert*-butoxycarbonyl group), Tf (trifluoromethane sulfonyl group), THF (tetrahydrofuran).

5.1. Serum Rho kinase inhibitory activity (ex vivo test)

Rho kinase inhibitors (30 mg/kg) were orally administered to SD rats ($N = 4$). The blood samples were obtained from the tail artery 3 h after the administration. The serum was collected by centrifugation and frozen at −20 °C until analysis. The serum samples were diluted 8-fold in water, and the Rho kinase inhibitory activity of the samples was determined. The inhibition rate (%) was determined by comparison of the inhibitory activity of the serum of the control rats (water was administered).

5.2. Rat liver microsomal assays

The reaction mixture consisted of 100 μL of microsomal protein (prepared from rat liver, protein concentration

5 mg/mL), 100 μL of NADPH generation cofactors (80 mM glucose-6-phosphate, 3.3 mM NADP, and 10 U/mL of glucose-6-phosphate dehydrogenase in 60 mM $MgCl_2$), 350 μL of 250 mM potassium phosphate buffer (pH 7.4), 350 μL of water, and 100 μL of the inhibitor (100 μM). The final concentration of the test compound was 10 μM in a total reaction volume of 1000 μL. The mixture was pre-incubated for 5 min at 37 °C. The reaction was initiated by the addition of NADPH generation cofactor solution and incubated for 30 min. The reaction was terminated by the addition of EtOAc (6 mL). An internal standard was added to the mixture. The organic phase (4 mL) was extracted, dried, and diluted with the appropriate solvent. Drug concentrations were determined by LC analysis (Inatsil-ODS-3, GL-Science, ϕ 4.6 × 250 mm; flow rate, 1.0 mL/min; Temp, 40 °C; UV 240 nm; isocratic mobile phase consisting of appropriate ratios of CH_3CN and 100 mM ammonium acetate) and were corrected by comparison with the internal standard.

5.3. Myosin-binding subunit phosphorylation assay

Serum-deprived MDCK II cells (5×10^5/ 60 mm dish) in DMEM (4 mL) were treated with various doses of Rho kinase inhibitors in PBS (300 μL). The cells were incubated for 10 min, followed by the addition of TPA (tetradecanoylphorbol-13-acetate, 200 nM, 250 μL) at 37 °C for 10 min. The cells were washed with PBS while cooled on ice and treated with 10% trichloroacetic acid containing 2 mM DTT. Cells were scraped off while cooled on the ice and centrifuged. The pellet was washed with acetone containing 2 mM DTT, suspended in SDS-buffer, sonicated, and centrifuged. The supernatant was boiled at 100 °C for 5 min and stored at −20 °C. The specimen (40 μL) was subjected to 8% SDS–polyacrylamide electrophoresis. Proteins were transferred electrophoretically to a PVDF membrane. Membranes were treated with skimmed milk and successively treated with anti-pS854 Ab (a polyclonal rabbit antibody to MBS phosphorylated at Ser854) or anti-MBS Ab (a polyclonal rabbit anti-MBS antibody). They were then treated with TBS buffer containing 0.1% Tween 20, skimmed milk, and anti-rabbit IgG-HRP (horseradish peroxidase) for 1 h. After washing with TBS buffer, detection was accomplished with an enhanced chemiluminescence (ECL+) detection system. Details of the assay are described elsewhere.[15]

5.4. Docking simulation of 30-R with Rho kinase homology model

The docking simulation was carried out using Flexi Dock available in the SYBYL software package in a manner similar to that described in a previous report.[12] The Flexi Dock algorithm allows ligand molecule single bond rotation but ring conformation is fixed. Thus, when a ligand contains a flexible ring system, multiple models of the ligand should be considered one by one. **30-R** was such a case. Many conformational models could be expected for the five-member ring (pyrrolidine ring) of **30-R**. Ring puckering (up and down) at the five positions of the pyrrolidine ring and the pseudo-chirality at the two nitrogen atoms produced 40 (2 × 5 × 2 × 2) conformational models for the 1-benzyl-3-aminopyrrolidine substructure. Forty corresponding models of 1-benzyl-3-aminopyrrolidine were built, and stable structures were searched using the GA Conformational Search of the SYBYL package for each of the 40 models. Because the GA Search employs the same conformation generation algorithm as Flexi Dock, the ring conformation and stereochemistry of the models are not altered. After minimization by Maxmin2 (Tripos force field), the models were further optimized using a semi empirical molecular orbital method (MOPAC) with a structure optimization option. After this calculation, some structures converged to identical structures. Ten stable conformational models were finally obtained for the 1-benzyl-3-aminopyrrolidine substructure. Based on the 10 substructure models, the corresponding models of **30-R** were built. These models were subjected to Flexi Dock simulation.

5.5. Chemistry

Proton NMR spectra were recorded using a Lambda 400 (Nippon Densi Datum, JEOL); chemical shifts were recorded in ppm (δ) based on the internal tetramethylsilane standard as 0 ppm or an internal chloroform as 7.24 ppm in deuterochloroform. Coupling constants (J) were recorded in Hz. Mass spectra (MS) were recorded using a PLATFORM-LC (Micromass) spectrometer. HA-1077 was prepared by the published method.[15]

5.5.1. *N*-(1-Propyl-4-piperidyl)-*N*-(5-isoquinolyl)amine (4a). To a mixture of 4-piperidone monohydrate **1** (300 mg, 2.6 mmol) and K_2CO_3 (540 mg, 3.9 mmol) in CH_3CN (5 mL), a solution of *n*-propylbromide **2a** (359 mg, 2.7 mmol) in CH_3CN (2 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 18 h and diluted with EtOAc. The mixture was filtered through Celite to give 1-propyl-4-piperidone **3a**. The filtrate was concentrated. A mixture of the crude **3a** and 5-aminoisoquinoline (208 mg, 1.4 mmol) was dissolved in titanium tetraisopropoxide (5 mL), and the reaction mixture was stirred at rt for 30 min. The mixture was diluted with MeOH (5 mL), and sodium borohydride (101 mg, 2.6 mmol) was added at 0 °C. The mixture was stirred at rt for 3 h, filtered through Celite, and quenched by the addition of saturated $NaHCO_3$ aq. The mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by silica gel column chromatography, eluting with $CHCl_3$–MeOH (95:5), to give the title compound as a dense yellow oil (101 mg, 0.37 mmol, 26% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.85 (t, J = 7.3 Hz, 3H), 1.47 (q, J = 7.6 Hz, 2H), 1.57 (dq, J = 4.2 Hz, 10.7 Hz, 2H), 2.05–2.18 (m, 4H), 2.28 (t, J = 7.8 Hz, 2H), 2.87 (t, J = 12.2 Hz, 2H), 3.38–3.50 (m, 1H), 6.71 (d, J = 7.6 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.46 (d, J = 6.1 Hz, 1H), 8.39 (d, J = 5.9 Hz, 1H), 9.07 (s, 1H). MS (ESI) m/z 270 $(M+1)^+$.

5.5.2. *N*-(1-Isobutyl-4-piperidyl)-*N*-(5-isoquinolyl)amine (4b). The compound **4b** was prepared from 1-bromo-2-methylpropane **2b** in a manner similar to that described for the compound **4a** with a yield of 35% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.84

(d, J = 6.6 Hz, 6H), 1.56 (dq, J = 3.2 Hz, 10.5 Hz, 2H), 1.68-1.77 (m, 1H), 2.06 (d, J = 7.3 Hz, 4H), 2.09 (d, J = 10.7 Hz, 2H), 2.79 (d, J = 11.9 Hz, 2H), 3.38–3.45 (m, 1H), 6.70 (d, J = 7.6 Hz, 1H), 7.21 (d, J = 8.1 Hz, 1H), 7.37 (t, J = 7.8 Hz, 1H), 7.45 (d, J = 5.9 Hz, 1H), 8.38 (d, J = 5.9 Hz, 1H), 9.07 (s, 1H). MS (ESI) m/z = 284 (M^++1).

5.5.3. *N*-(1-Benzyl-4-piperidyl)-*N*-(5-isoquinolyl)amine (4c). The compound **4c** was prepared from benzylbromide **2c** in a manner similar to that described for the compound **4a** with a yield of 25% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.5–1.7 (m, 4H), 2.1–2.2 (m, 2H), 2.2–2.3 (m, 2H), 3.59 (s, m, 3H), 6.77 (d, J = 7.8 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.2–7.4 (m, 5H), 7.43 (dd, J = 7.8 Hz, 1H), 7.52 (d, J = 6.0 Hz, 1H), 8.46 (d, J = 6.0 Hz, 1H), 9.14 (s, 1H). MS (ESI) m/z 318 $(M+1)^+$.

5.5.4. *N*1-Propyl-*N*4-(5-isoquinolyl)-1,4-cyclohexanediamine (8a). To a mixture of 1,4-cyclohexanedione monoethyleneketal **5** (6.20 g, 40 mmol), 5-aminoisoquinoline (4.33 g, 30 mmol), and AcOH (0.5 mL) in MeOH (50 mL), BH_3–pyridine complex (4.0 mL, 40 mmol) was added dropwise at 0 °C. The mixture was stirred at rt for 18 h and concentrated for removal of MeOH. The residue was dissolved in 50% AcOH (50 mL). The solution was stirred at 80 °C for 3 h and concentrated, then quenched by the addition of saturated $NaHCO_3$ aq. The aqueous layer was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by silica gel column chromatography, eluting with $CHCl_3$, to give 4-(isoquinolin-5-ylamino)-cyclohexanone **6** (5.80 g, 24 mmol).

To a mixture of **6** (60 mg, 0.25 mmol) and propylamine **7a** (52 mg, 0.5 mmol) in MeOH (1 mL), $NaBH(OAc)_3$ (111 mg, 0.5 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 18 h and quenched by the addition of saturated $NaHCO_3$ aq. The aqueous layer was extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was subjected to a reverse-phase HPLC (ODS), eluting with 5%TFA aq–CH_3CN (4:1), to give the title compound as a dense yellow oil (18 mg, *syn* isomer; 22 mg, *anti* isomer). *Anti* isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, J = 7.4 Hz, 3H), 1.18–1.32 (m, 4H), 1.41–1.52 (m, 2H), 1.94–2.06 (m, 2H), 2.14–2.16 (m, 2H), 2.44–2.58 (m, 2H), 2.57 (t, J =7.5 Hz, 2H), 3.31–3.44 (m, 1H), 4.06–4.20 (m, 1H), 6.70 (d, J = 7.6 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.38 (t, J = 7.7 Hz, 1H), 7.44 (t, J = 6.1 Hz, 1H), 8.38 (t, J = 5.9 Hz, 1H), 9.07 (s, 1H); MS (ESI) m/z 284 $(M+1)^+$. *Syn* isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, J = 7.3 Hz, 3H), 1.40–1.50 (m, 2H), 1.50–1.60 (m, 2H), 1.68–1.76 (m, 4H), 1.80–1.90 (m, 2H), 2.57 (t, J =7.3 Hz, 2H), 2.58–2.68 (m, 1H), 3.60–3.70 (m, 1H), 4.33–4.45 (m, 1H), 6.68 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 7.6 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.47 (t, J = 5.8 Hz, 1H), 8.37 (t, J = 6.1 Hz, 1H), 9.07 (s, 1H). MS (ESI) m/z 284 $(M+1)^+$.

5.5.5. *N*1-Cyclopropyl-*N*4-(5-isoquinolyl)-1,4-cyclohexanediamine (8b). The compound **8b** was prepared from cyclopropylamine **7b** in a manner similar to that described for the compound **8a** as a dense yellow oil. *Anti* isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ −0.16 to −0.09 (m, 2H), −0.04 to 0.20 (m, 2H), 0.75–0.93 (m, 4H), 1.58–1.72 (m, 3H), 1.75–1.85 (m, 2H), 2.16–2.27 (m, 1H), 2.87–3.03 (m, 1H), 3.60–3.85 (m, 1H), 6.29 (d, J = 7.6 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 7.06 (t, J = 8.0 Hz, 1H), 7.16 (t, J = 6.1 Hz, 1H), 8.08 (t, J = 5.8 Hz, 1H), 8.76 (s, 1H); MS (ESI) m/z 282 $(M+1)^+$. *Syn* isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ −0.04 to 0.02 (m, 2H), 0.07–0.13 (m, 2H), 0.90–1.58 (m, 8H), 1.68–1.78 (m, 1H), 2.48 (tt, J = 3.9 Hz, 7.8 Hz, 1H), 3.28–3.40 (m, 1H), 3.95–4.13 (m, 1H), 6.39 (d, J = 7.6 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 7.06 (t, J = 8.0 Hz, 1H), 7.16 (t, J = 6.1 Hz, 1H), 8.08 (d, J = 5.8 Hz, 1H), 8.76 (s, 1H). MS (ESI) m/z 282 $(M+1)^+$.

5.5.6. *N*1-Phenylethyl-*N*4-(5-isoquinolyl)-1,4-cyclohexanediamine (8c). The compound **8c** was prepared from 2-phenylethylamine **7c** in a manner similar to that described for the compound **8a** as a dense yellow oil. *Anti* isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.15–1.30 (m, 2H), 1.92–2.03 (m, 2H), 2.12–2.25 (m, 2H), 2.45–2.55 (m, 1H), 2.76 (t, J = 7.1 Hz, 2H), 2.88 (t, J =7.1 Hz, 2H), 3.29–3.42 (m, 1H), 4.05–4.18 (m, 1H), 6.69 (d, J = 7.6 Hz, 1H), 7.12–7.26 (m, 6H), 7.37 (d, J = 7.9 Hz, 1H), 7.43 (t, J = 6.1 Hz, 1H), 8.37 (d, J = 6.1 Hz, 1H), 9.06 (s, 1H); MS (ESI) m/z 346 $(M+1)^+$. *Syn* isomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46–1.58 (m, 2H), 1.64–1.74 (m, 4H), 1.76–1.86 (m, 2H), 2.65 (tt, J = 3.7 Hz, 8.1 Hz, 1H), 2.76 (tt, J = 6.8 Hz, 7.1 Hz, 2H), 3.60–3.70 (m, 1H), 4.28–4.42 (m, 1H), 6.69 (d, J = 7.6 Hz, 1H), 7.10–7.26 (m, 6H), 7.37 (t, J = 7.9 Hz, 1H), 7.46 (d, J = 6.1 Hz, 1H), 8.38 (d, J = 6.1 Hz, 1H), 9.07 (s, 1H). MS (ESI) m/z 346 $(M+1)^+$.

5.5.7. *N*-(1-Isopentyl-3-pyrrolidyl)-*N*-(5-isoquinolyl)amine (12). To a mixture of 3-hydroxypyrrolidine **9** (1.0 g, 10 mmol) and K_2CO_3 (2.68 g, 20 mmol) in DMF (10 mL), a solution of 1-chloro-3-methylbutane (1.07 g, 10 mmol) in CH_3CN (5 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 18 h and diluted with EtOAc. The resulting mixture was filtered through Celite. The filtrate was then concentrated to give 1-isopentyl-3-hydroxypyrrolidine **10**. To a solution of **10** and triethylamine (1.78 mL) in anhydrous DMSO (8 mL), a sulfur trioxide-trimethylamine complex (2.45 g, 23 mmol) was slowly added at 0 °C. The reaction mixture was stirred at rt for 18 h and quenched by the addition of saturated $NaHCO_3$ aq. The reaction mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated to give crude 1-isopentyl-3-pyrrolidone **11** (1.55 g). 775 mg of the crude **11** and 5-aminoisoquinoline (304 mg, 1.9 mmol) were dissolved in titanium tetraisopropoxide (5 mL), and the reaction mixture was stirred at rt for 30 min. The mixture was diluted with MeOH (5 mL), and sodium borohydride (101 mg, 2.6 mmol) was added at 0 °C. The mixture was stirred at rt for 3 h, filtered through

Celite, and quenched by the addition of saturated $NaHCO_3$ aq. The mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by silica gel column chromatography, eluting with $CHCl_3$–MeOH (95:5), to give the title compound as a dense yellow oil (172 mg, 0.56 mmol, 29% yield). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.52–1.80 (m, 6H), 2.61–2.71 (m, 2H), 3.68 (s, 2H), 3.76–3.84 (m, 1H), 5.06–5.19 (m, 1H), 6.63–6.72 (m, 3H), 7.24 (s, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.52 (d, J = 6.0 Hz, 1H), 8.47 (d, J = 6.0 Hz, 1H), 9.07 (s, 1H). MS (ESI) m/z 284 $(M+1)^+$.

5.5.8. 3-(Isoquinolin-5-ylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (18). To a solution of 3-hydroxypiperidine **13** (10.0 g, 100 mmol) in 3 M NaOH aq (100 mL), a solution of di-*tert*-butyl dicarbonate (25.0 g, 120 mmol) in THF (100 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 1 h and evaporated to remove THF. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. To a mixture of the residue and triethylamine (20 mL) in anhydrous DMSO (100 mL), a sulfur trioxide-trimethylamine complex (44.4 g, 200 mmol) was added portionwise at 0 °C. The reaction mixture was stirred at rt for 18 h and quenched by the addition of saturated $NaHCO_3$ aq. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The crude mixture was purified by silica gel column chromatography, eluting with $CHCl_3$ to give 3-oxo-piperidine-1-carboxylic acid *tert*-butyl ester **16** (15.6 g). To a mixture of the crude **16** (3.7 g, 20 mmol), 5-aminoisoquinoline (2.5 g, 17 mmol), and anhydrous Na_2SO_4 (14.2 g, 100 mmol) in AcOH (100 mL), $NaBH(OAc)_3$ (4.5 g, 20 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 18 h and evaporated to remove AcOH. The resulting mixture was basified with saturated $NaHCO_3$ aq. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by flash chromatography on silica gel, eluting with $CHCl_3$–MeOH (95:5), to give the title compound as a dense yellow oil (3.7 g, 12 mmol, 60% yield). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H), 1.48–1.68 (m, 1H), 1.78–1.83 (m, 2H), 1.90–2.10 (m, 1H), 3.10–3.32 (m, 2H), 3.52–3.65 (m, 2H), 3.92–3.98 (m, 1H), 6.86 (d, J = 7.6 Hz, 1H), 7.30 (d, J = 8.3 Hz, 1H), 7.45 (t, J = 7.9 Hz, 1H), 7.54 (d, J = 5.8 Hz, 1H), 8.42 (d, J = 5.8 Hz, 1H), 9.13 (s, 1H). MS (ESI) m/z = 328 $(M^+ + 1)$.

5.5.9. 3-(Isoquinolin-5-ylamino)-pyrrolidine-1-carboxylic acid *tert*-butyl ester (19). The compound **19** was prepared from 3-hydroxypyrrolidine **9** in a manner similar to that described for the compound **18** with a yield of 65% as a dense yellow oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 9H), 1.75–1.94 (m, 1H), 2.02–2.10 (m, 1H), 3.35–3.55 (m, 3H), 3.75–3.86 (m, 1H), 4.17–4.24 (m, 1H), 4.75–4.90 (m, 1H), 6.91 (d, J = 7.6 Hz, 1H), 7.44 (d, J = 8.3 Hz, 1H), 7.58 (t, J = 7.9 Hz, 1H), 7.80–7.90 (m, 1H), 8.42 (d, J = 6.4 Hz, 1H), 9.20 (s, 1H). MS (ESI) m/z = 314 $(M^+ + 1)$.

5.5.10. *N*-[1-(2-Chlorobenzyl)-3-piperidyl]-*N*-(5-isoquinolyl)amine (21a). To a solution of 3-(5-isoquinolylamino)-1-piperidinecarboxylic acid *tert*-butyl ester **18** (66 mg, 0.20 mmol) in $CHCl_3$ (1 mL), TFA (1 mL) was added dropwise at rt. The reaction mixture was stirred at rt for 3 h. The mixture was concentrated under a reduced pressure. CH_3CN (1 mL) and K_2CO_3 (69 mg, 0.50 mmol) were added to the residue, and 2-chlorobenzylchloride **20a** (40 mg, 0.25 mmol) in CH_3CN (0.5 mL) was added dropwise at rt. The reaction mixture was stirred at rt for 18 h. The reaction was quenched by the addition of water (2 mL). The mixture was concentrated under a reduced pressure to remove CH_3CN, and the residue was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residue was dissolved in a minimum amount of $CHCl_3$–MeOH (10:1) and briefly purified using a small silica gel column. The crude material was concentrated and subjected to chromatography on silica gel, eluting first with $CHCl_3$, then with $CHCl_3$–MeOH (10:1) to give the titled compound as a dense yellow oil (19 mg, 0.054 mmol, 27% yield). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.50–1.90 (m, 5H), 2.30–2.40 (m, 1H), 2.65–2.80 (m, 1H), 2.75 (m, 1H), 3.62 (d, J = 13.4 Hz, 1H), 3.67 (d, J = 13.4 Hz, 1H), 3.82 (br s, 1H), 6.70 (d, J = 7.8 Hz, 1H), 7.21–7.27 (m, 3H), 7.39–7.43 (m, 3H), 7.60 (d, J = 5.9 Hz, 1H), 8.47 (d, J = 5.9 Hz, 1H), 9.13 (s, 1H). MS (ESI) m/z = 353 $(M^+ + 1)$.

5.5.11. *N*-[1-(3-Chlorobenzyl)-3-piperidyl]-*N*-(5-isoquinolyl)amine (21b). Compound **21b** was prepared from 3-chlorobenzylchloride **20b** in a manner similar to that described for the compound **21a** with a yield of 33% as a dense yellow oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.45–1.81 (m, 5H), 2.35–2.50 (m, 1H), 2.60–2.68 (m, 2H), 3.48 (d, J = 13.4 Hz, 1H), 3.60 (d, J = 13.4 Hz, 1H), 3.80 (br s, 1H), 6.73 (d, J = 7.8 Hz, 1H), 7.26–7.30 (m, 4H), 7.42 (t, J = 7.8 Hz, 1H), 7.44 (s, 1H), 7.57 (d, J = 5.9 Hz, 1H), 8.50 (d, J = 5.9 Hz, 1H), 9.14 (s, 1H). MS (ESI) m/z = 353 $(M^+ + 1)$.

5.5.12. *N*-[1-(4-Chlorobenzyl)-3-piperidyl]-*N*-(5-isoquinolyl)amine (21c). Compound **21c** was prepared from 4-chlorobenzylchloride **20c** in a manner similar to that described for the compound **21a** with a yield of 36% as a dense yellow oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.45–1.76 (m, 5H), 2.30–2.45 (m, 1H), 2.46–2.60 (m, 1H), 2.61–2.75 (m, 1H), 3.45–3.60 (m, 2H), 3.80 (br s, 1H), 6.73 (d, J = 8.2 Hz, 1H), 7.25–7.35 (m, 4H), 7.42 (t, J = 8.2 Hz, 1H), 7.50–7.58 (m, 2H), 8.49 (d, J = 6.0 Hz, 1H), 9.15 (s, 1H). MS (ESI) m/z = 353 $(M^+ + 1)$.

5.5.13. *N*-[1-(2-Chlorobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22a). To a solution of **19** (62 mg, 0.20 mmol) in $CHCl_3$ (1 mL), TFA (1 mL) was added dropwise at rt. The resulting mixture was stirred at rt for 3 h and concentrated. To the residue, K_2CO_3 (69 mg, 0.50 mmol) and CH_3CN (1 mL) were added. A solution of 2-chlo-

robenzylchloride **20a** (40 mg, 0.25 mmol) in CH_3CN (0.5 mL) was then added dropwise at 0 °C. The reaction mixture was stirred at rt for 18 h and quenched by the addition of water. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was subjected to flash chromatography on silica gel, eluting $CHCl_3$–MeOH (95:5), to give the title compound as a dense yellow oil (28 mg, 0.08 mmol, 42% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.98–2.10 (m, 1H), 2.40–2.52 (m, 1H), 2.70–2.90 (m, 1H), 3.00–3.86 (m, 3H), 4.00–4.15 (m, 2H), 4.25–4.35 (m, 1H), 6.59 (d, J = 7.6 Hz, 1H), 7.20–7.28 (m, 3H), 7.32–7.38 (m, 2H), 7.63–7.83 (m, 2H), 8.44 (d, J = 5.8 Hz, 1H), 9.07 (s, 1H). MS (ESI) m/z = 339 (M^++1).

5.5.14. *N*-[1-(3-Chlorobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22b). Compound **22b** was prepared from 3-chlorobenzylchloride **20b** in a manner similar to that described for the compound **22a** with a yield of 21% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00–2.10 (m, 1H), 2.45–2.55 (m, 1H), 2.70–2.80 (m, 1H), 3.00–3.45 (m, 3H), 3.80–3.90 (m, 2H), 4.25–4.36 (m, 1H), 6.64 (d, J = 7.6 Hz, 1H), 7.28–7.34 (m, 3H), 7.37–7.43 (m, 3H), 7.75–7.85 (m, 1H), 8.48 (d, J = 6.1 Hz, 1H), 9.12 (s, 1H). MS (ESI) m/z = 339 (M^++1).

5.5.15. *N*-[1-(4-Chlorobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22c). Compound **22c** was prepared from 4-chlorobenzylchloride **20c** in a manner similar to that described for the compound **22a** with a yield of 15% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.00–2.10 (m, 1H), 2.45–2.55 (m, 1H), 2.60–2.90 (m, 1H), 3.00–3.40 (m, 3H), 3.85–4.00 (m, 2H), 4.28–4.50 (m, 1H), 6.62 (d, J = 7.3 Hz, 1H), 7.28–7.44 (m, 6H), 7.80–8.00 (m, 1H), 8.48 (d, J = 6.1 Hz, 1H), 9.12 (s, 1H). MS (ESI) m/z = 339 (M^++1).

5.5.16. *N*-[1-(4-Fluorobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22d). Compound **22d** was prepared from 4-fluorobenzylchloride **20d** in a manner similar to that described for the compound **22a** with a yield of 27% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80–2.10 (m, 1H), 2.40–2.53 (m, 1H), 2.57–2.75 (m, 1H), 2.90–3.25 (m, 3H), 3.75–3.88 (m, 2H), 4.21–4.31 (m, 1H), 6.64 (d, J = 7.6 Hz, 1H), 7.03 (t, J = 8.5 Hz, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.35–7.43 (m, 3H), 7.65–7.78 (m, 1H), 8.47 (d, J = 6.1 Hz, 1H), 9.12 (s, 1H). MS (ESI) m/z = 322 (M^++1).

5.5.17. *N*-[1-(2,6-Difluorobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22e). Compound **22e** was prepared from 2,6-difluorobenzylchloride **20e** in a manner similar to that described for the compound **22a** with a yield of 50% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78–1.90 (m, 1H), 2.35–2.45 (m, 1H), 2.52–2.65 (m, 1H), 2.88–3.00 (m, 3H), 3.90 (s, 2H), 4.11–4.20 (m, 1H), 4.75–4.85 (m, 1H), 6.66 (d, J = 7.6 Hz, 1H), 6.85–6.95 (m, 3H), 7.29 (d, J = 8.3 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.58 (d, J = 6.1 Hz, 1H), 8.44 (d, J = 6.1 Hz, 1H), 9.13 (s, 1H). MS (ESI) m/z = 340 (M^++1).

5.5.18. *N*-[1-(2-nitrobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22f). This compound was prepared from 2-nitrobenzylchloride **20f** in a manner similar to that described for the compound **22a** with a yield of 38% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.72–1.88 (m, 1H), 2.10–2.50 (m, 2H), 2.56–2.85 (m, 2H), 2.85–3.02 (m, 1H), 3.99 (s, 2H), 4.05–4.17 (m, 1H), 6.65 (d, J = 7.6 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.40 (t, J = 7.8 Hz, 1H), 7.45–7.60 (m, 2H), 7.67 (s, 1H), 7.75–7.90 (m, 2H), 8.49 (d, J = 6.0 Hz, 1H), 9.12 (s, 1H). MS (ESI) m/z = 349 (M^++1).

5.5.19. *N*-[1-(3-nitrobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22g). This compound was prepared from 3-nitrobenzylchloride **20g** in a manner similar to that described for the compound **22a** with a yield of 38% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.78–1.90 (m, 1H), 2.40–2.57 (m, 2H), 2.72–2.79 (m, 1H), 2.85–2.96 (m, 2H), 3.77 (s, 2H), 4.14–4.24 (m, 1H), 4.55–4.67 (m, 1H), 6.69 (d, J = 7.6 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.58 (t, J = 6.1 Hz, 1H), 7.69 (d, J = 6.7 Hz, 1H), 8.11 (d, J = 7.3 Hz, 1H), 8.23 (s, 1H), 8.47 (d, J = 6.1 Hz, 1H), 9.14 (s, 1H). MS (ESI) m/z = 349 (M^++1).

5.5.20. *N*-[1-(4-nitrobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (22h). This compound was prepared from 4-nitrobenzylchloride **20h** in a manner similar to that described for the compound **22a** with a yield of 29% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86–1.94 (m, 1H), 2.43–2.54 (m, 1H), 2.84–3.06 (m, 3H), 3.85 (s, 2H), 4.20–4.28 (m, 1H), 4.76–4.90 (m, 1H), 6.66 (d, J = 7.6 Hz, 1H), 7.31 (d, J = 8.3 Hz, 1H), 7.41 (t, J = 7.9 Hz, 1H), 7.57 (d, J = 8.7 Hz, 2H), 7.63 (t, J=5.8 Hz, 1H), 8.18 (d, J = 8.7 Hz, 2H), 8.46 (d, J = 5.8 Hz, 1H), 9.13 (s, 1H). MS (ESI) m/z = 349 (M^++1).

5.5.21. *N*-[1-(2-aminobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (23f). To a solution of *N*-[1-(2-nitrobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine **22f** (27 mg, 0.07 mmol) in 3 M HCl aq (1 mL), $SnCl_2/H_2O$ (113 mg, 0.50 mmol) was added dropwise at 80 °C. The reaction mixture was stirred at the same temperature for 3 h and basified by the addition of 30% wt NH_3 aq. The resulting mixture was diluted with EtOAc and filtered through Celite. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by flash chromatography on aluminum oxide, eluting with $CHCl_3$–MeOH (95:5), to give the title compound as a dense yellow oil (20 mg, 0.06 mmol, 84% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.75–1.84 (m, 1H), 2.38–2.54 (m, 2H), 2.65 (dd, J = 3.5 Hz, 10.0 Hz, 1H), 2.82 (dt, J = 5.0 Hz, 8.6 Hz, 1H), 2.87 (dd, J = 6.3 Hz, 9.8 Hz, 1H), 3.65 (d, J = 12.4 Hz, 1H), 3.69 (d, J = 12.2 Hz, 1H), 4.10–4.18 (m, 1H), 4.45–4.55 (m, 1H), 6.64–6.70 (m, 3H), 7.03 (d, J = 7.3 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H), 7.43 (t, J = 8.3 Hz, 1H), 7.52 (d, J = 5.9 Hz, 1H), 8.46 (d, J = 6.1 Hz, 1H), 9.15 (s, 1H). MS (ESI) m/z = 319 (M^++1).

5.5.22. *N*-[1-(3-aminobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (23g). Compound **23g** was prepared from *N*-[1-(3-nitrobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine **22g** in a manner similar to that described for the compound **23f** with a yield of 71% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80–1.94 (m, 1H), 2.38–2.48 (m, 1H), 2.52–2.62 (m, 1H), 2.75–2.86 (m, 1H), 2.87–3.02 (m, 2H), 3.64 (s, 2H), 4.14–4.24 (m, 1H), 4.75–4.90 (m, 1H), 658 (d, J = 7.6 Hz, 1H), 6.66 (d, J = 7.6 Hz, 1H), 6.70–6.95 (m, 2H), 7.09 (t, J = 8.1 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.59–7.66 (m, 1H), 8.46 (d, J = 6.1 Hz, 1H), 9.13 (s, 1H). MS (ESI) m/z = 319 (M^++1).

5.5.23. *N*-[1-(4-aminobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (23h). Compound **23h** was prepared from *N*-[1-(4-nitrobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine **22h** in a manner similar to that described for the compound **23f** with a yield of 88% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.80–1.90 (m, 1H), 2.38–2.47 (m, 3H), 2.72–2.90 (m, 2H), 3.62(br s, 2H), 4.18–4.21 (m, 1H), 4.25–4.32 (m, 1H), 6.56–6.71 (m, 1H), 6.65 (d, J = 8.4 Hz, 2H), 6.95–6.98 (m, 1H), 7.15 (t, J = 8.4 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.65–7.72 (m, 1H), 8.47 (d, J = 6.0 Hz, 1H), 9.14 (s, 1H). MS (ESI) m/z = 319 (M^++1).

5.5.24. (3*R*)-*N*-[1-(3-Aminobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (30-R). To a mixture of 5-hydroxyisoquinoline **24** (4.35 g, 30 mmol) and pyridine (3.16 g) in $CHCl_3$ (50 mL), trifluoromethanesulfonic anhydride (10.0 g, 35 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 3 h and quenched by the addition of saturated $NaHCO_3$ aq. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$, to give 5-isoquinolyl trifluoromethanesulfonic acid (**25**) (7.55 g, 20 mmol).

To a mixture of (3R)-(+)-3-pyrrolidylcarbamic acid *tert*-butyl ester **26** (1.86 g, 10 mmol) and K_2CO_3 (2.07 g, 15 mmol) in CH_3CN (10 mL), a solution of 3-nitrobenzylchloride (1.88 g, 11 mmol) in CH_3CN (10 mL) was added at rt. The reaction mixture was stirred at rt for 18 h, diluted with EtOAc, and filtered through Celite. The filtrate was concentrated. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$–MeOH (95:5), to give (3*R*)-(+)-[1-(3-nitrobenzyl)-3-pyrrolidyl]carbamic acid *tert*-butyl ester **27** as a dense yellow oil (2.70 g, 8.5 mmol). Compound **27** (1.00 g, 3.15 mmol) was dissolved in TFA/$CHCl_3$ (2 mL/2 mL) and stirred at rt for 3 h. After evaporation of TFA, the resulting mixture was basified with 3 N NaOH aq. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by flash chromatography on aluminum oxide, eluting with $CHCl_3$, to give 1-(3-nitrobenzyl)-3-pyrrolidylamine **28** as a dense yellow oil. A mixture of the triflate **25** (858 mg, 3.1 mmol), **28**, palladium acetate (113 mg, 0.50 mmol), racemic-BINAP (311 mg, 0.50 mmol), and Cs_2CO_3 (1.63 g, 5.0 mmol) in toluene (5 mL) was stirred at 80 °C for 18 h. The resulting mixture was diluted with EtOAc, filtered through Celite, and the filtrate was concentrated. The residual oil was purified by flash chromatography on silica gel, eluting with $CHCl_3$–MeOH (95:5), to give (3*R*)-*N*-[1-(3-nirobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine **29** as a dense yellow oil (629 mg, 1.68 mmol, 55% yield in this step). The intermediate **29** was dissolved in 3N HCl aq (3 mL). $SnCl_2/H_2O$ (1.13 g, 5.0 mmol) was added to the solution portionwise at 0 °C. The reaction mixture was stirred at 80 °C for 5 h and quenched by the addition of 30% NH_3 aq. The resulting mixture was diluted with EtOAc and filtered through Celite. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by flash chromatography on aluminum oxide, eluting with $CHCl_3$, to give the title compound as a dense yellow oil (462 mg, 1.45 mmol). ^{1}H NMR (CDCl3, 400 MHz) δ 1.80–1.94 (m, 1H), 2.38–2.48 (m, 1H), 2.52–2.62 (m, 1H), 2.75–2.86 (m, 1H), 2.87–3.02 (m, 1H), 3.64 (s, 2H), 4.14–4.24 (m, 1H), 4.75–4.90 (m, 1H), 6.58 (d, J = 7.6 Hz, 1H), 6.66 (d, J = 7.6 Hz, 1H), 6.70–6.95 (m, 2H), 7.09 (t, J = 8.1 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.41 (t, J = 7.8 Hz, 1H), 7.59–7.66 (m, 1H), 8.46 (d, J = 6.1 Hz, 1H), 9.13 (s, 1H). MS (ESI) m/z = 319 (M^++1). $[\alpha]_D^{25} = -103$ (c = 0.25/$CHCl_3$).

5.5.25. (3*S*)-*N*-[1-(3-Aminobenzyl)-3-pyrrolidyl]-*N*-(5-isoquinolyl)amine (30-S). The compound **30-S** was prepared from (3S)-(−)-3-pyrrolidylcarbamic acid *tert*-butyl ester in a manner similar to that described for the compound **30-R** as a dense yellow oil. $[\alpha]_D^{25}$ =+111 (c = 0.25/$CHCl_3$).

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