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Design and synthesis of Rho kinase inhibitors (II)

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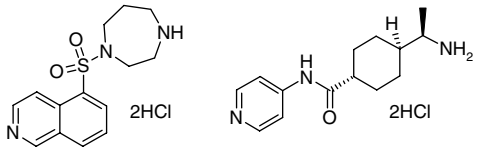
Abstract—In a previous study, we identified several structurally unrelated scaffolds of the Rho kinase inhibitor using pharmacophore information obtained from the results of a high-throughput screening and structural information from a homology model of Rho kinase. 1*H*-Indazole is one of the candidate scaffolds on which a new series of potent Rho kinase inhibitors could be developed. In this study, the detailed structure–activity relationship of 1*H*-indazole analogues was studied. During this study, we found that the cell-free enzyme inhibitory potential of Rho kinase inhibitors having the 1*H*-indazole scaffold did not necessarily correlate with their inhibitory potential toward the chemotaxis of cultured cells. The choice of the linker substructure was shown to be an important factor for the 1*H*-indazole analogues to inhibit the chemotaxis of cells. Optimization of the 1*H*-indazole inhibitors with respect to the in vitro inhibition of monocyte chemotaxis induced by MCP-1 was carried out. The inhibitory potential was improved both in the cell-free enzyme assay and in the chemotaxis assay.

1. Introduction

The small GTPase, Rho, is involved in a variety of cellular functions, including cell migration and cell adhesion. Rho kinase, which is also designated ROCK II, is the best characterized among the Rho effectors.[1] Rho kinase is considered to be a potential target kinase where an inhibitor could be of therapeutic value. For example, it is known that Rho kinase inhibitors can inhibit the migration of monocyte cells induced by MCP-1 (monocyte chemoattractant protein 1). Such Rho kinase inhibitors could be useful compounds for the treatment of atherosclerotic plaque formation and inflammation.[2,3] Several Rho kinase inhibitors have been reported as illustrated in Figure 1. Fasudil dihydrochloride (HA-1077)[4] has been used clinically as a cerebrovascular contraction inhibitor.[5] It is also under clinical studies for the treatment of cardiac remodeling because the inhibition of Rho kinase is expected to suppress the migration of vascular smooth muscle cells.[3] Uehata and colleagues have reported pyridine compounds having a cyclohexanecarboxyamide or benzamide moiety. Among them, Y-27632 (Fig. 1) was representative as a specific Rho kinase inhibitor, by which it was demonstrated that the Rho kinase inhibitor might be useful in the prevention of invasion and metastasis of cancer cells.[6]



HA-1077 (Fasudil) **Y-27632**

Figure 1. Known Rho kinase inhibitors.

We have previously reported several novel chemical scaffolds as potential platforms for developing specific Rho kinase inhibitors based on a homology model of the kinase.[7] *N*-(1*H*-5-indazolyl)-*N*′-benzylurea **1** (Fig. 2) is one of the new inhibitors designed in the previous study. The ligand-binding pocket of Rho kinase is composed of three regions, namely the A, F and D regions (Fig. 2). The A region is the bottom of the

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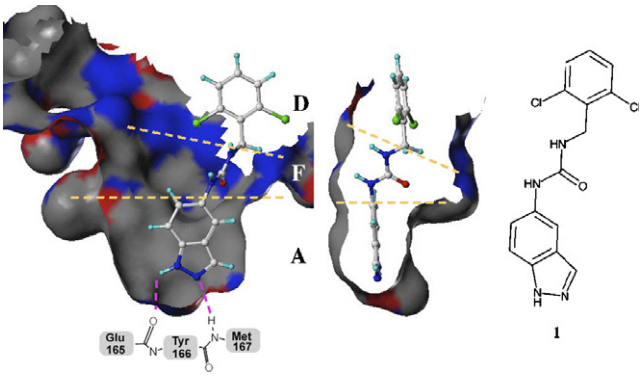


Figure 2. Orthographic view of the ligand-binding pocket of Rho kinase. A docking model of the 1*H*-indazole inhibitor **1** is shown. The pocket is composed of three regions, A, F, and D. The possible hydrogen-bonding sites are colored on the surface representation of the pocket (blue, donor sites; red, acceptor sites). The backbone NH group of Met167 is the hydrogen bond donor site of Rho kinase that interacts with the N2 of the 1*H*-indazole ring. The backbone C=O group of Glu165 acts as the hydrogen bond acceptor site. Hydrogen bonds between the Rho kinase and **1** suggested by the docking simulation are indicated by purple dashed lines.

pocket and offers an essential hydrogen bond donor (the backbone NH of Met167) which forms a hydrogen bond with the N2 of the 1*H*-indazole ring. The 1*H*-indazole scaffold fits the flat shape of this region. The urea moiety in the F region is a linker substructure that connects the 1*H*-indazole ring and the hydrophobic substructure in the D region.

The inhibitory potency of the **1** analogues, such as **3a**–**c** (Table 1), determined by the cell-free Rho kinase enzyme assay (IC_{50}^{ENZ}) was in the 100–300 nM range. However, these compounds exhibited no inhibitory potential for the chemotaxis of CCR2 over-expressing lymphoma cells induced by MCP-1 ($IC_{50}^{MCP} > 100$ μM). In contrast, HA-1077 showed a weak but significant inhibitory activity in the chemotaxis assay with an IC_{50}^{MCP} value of 35 μM. The IC_{50}^{ENZ} of HA-1077 determined for the cell-free enzyme was 180 nM and was similar to that of the analogues of **1**. Species having comparative IC_{50}^{ENZ} exhibited a distinct difference in IC_{50}^{MCP}. Biophysical properties other than the enzyme inhibitory potential appeared necessary for the 1*H*-indazole analogues to possess a high biological efficacy. The aim of this study was the structural optimization of the 1*H*-indazole derivatives based on the chemotaxis assay.

2. Chemistry

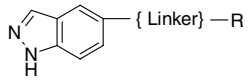
Analogues with various linker substructures between the 1*H*-indazole ring and the hydrophobic moiety were synthesized from 5-amino-1*H*-indazole **2** as shown in Scheme 1. A urea analogue **3a** was prepared by reacting **2** with benzylisocyanate at 110 °C. Compounds **3b** and **3c** were also synthesized by reacting **2** with 2,6-difluoro- or 2,4-dichlorobenzylisocyanate which were prepared from 2,6-difluoro- or 2,4-dichlorophenylacetic acid using diphenylphosphoryl azide under the Curtius rearrangement conditions, respectively.[8] Compound **4** was prepared as follows: first, **2** was acylated with 2-chloroacetylchloride, then the resulting α-chloroamide was coupled with *N*-methylbenzylamine using K_2CO_3 as a base in CH_3CN at room temperature. Finally, the amide group was reduced by treatment with the BH_3–THF complex in THF at room temperature. Compounds **5a**–**c** were prepared as follows: **2** was treated with 1-benzyl-4-piperidone, 1-benzyl-3-pyrrolidone, or 1-benzyl-3-piperidone. The resulting Schiff bases were reduced by the BH_3–pyridine complex in the presence of a catalytic amount of acetic acid. Compound **6** was prepared by reducing the Schiff base formed by **2** with 1-benzyl-4-piperidinecarbaldehyde. The aldehyde was prepared by the oxidation of 1-benzyl-4-piperidinemethanol with the sulfur trioxide–trimethylamine complex (SO_3–Me_3N) in DMSO at room temperature. Compounds **7a** and **7b** were synthesized by the condensation of **2** with *N*-benzylisonipecotic acid or *N*-benzylnipecotic acid using HOBT and WSC-HCl. Compound **8** was prepared by the reaction of **2** and 1-benzyl-4-aminopiperidine using triphosgene with Et_3N in $CHCl_3$ at room temperature. Compound **9** was prepared as follows: 1,4-cyclohexanedione monoethylene ketal was reacted with **2**, and the deprotection of the ketal group was then carried out under acidic conditions. The resulting ketone was reacted with benzylamine and reduced by $NaBH(OAc)_3$ to give **9**. Compound **9** was obtained as a mixture of two diastereomers in the ratio of ca. 1:1. Each diastereomer (**9a** and **9b**) was separated using reverse-phase chromatography. Compound **10** was prepared from **5a**. Compound **5a** was acetylated with Ac_2O using a catalytic amount of DMAP in pyridine. The resulting diacetylated intermediate of **5a** was hydrolyzed under acidic conditions. The N1 acetyl group was successfully removed to produce **10**.

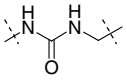
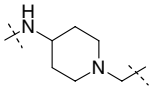
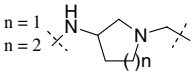
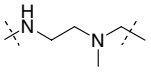
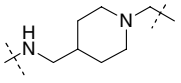
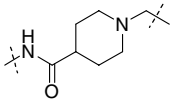
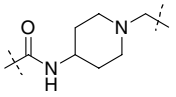
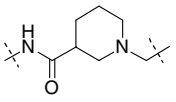
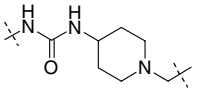
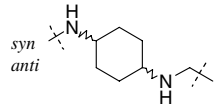
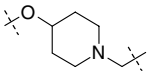
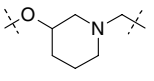
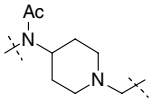
The syntheses of analogues having a 1*H*-indazole moiety other than **2** are shown in Scheme 2. The cyclization of 2-methylanilines (**11** and **14**) to 1*H*-indazole was performed using the method of Ruchart and Hassmann.[9] 4-Amino-3-cresol **11** was treated with acetic anhydride and then reacted with isoamylnitrite to give *N*,*O*-diacetyl-5-hydroxy-1*H*-indazole. Deacetylation afforded 5-hydroxy-1*H*-indazole **12**, which was then converted to compounds **13a** or **13b** under Mitsunobu conditions.[10] The intermediate, 1*H*-indazole-5-carboxylic acid **15**, was synthesized by the Ruchart and Hassmann cyclization.[9] Condensation of intermediate **15** with 1-benzyl-4-aminopiperidine was carried out for the preparation of **16** using WSC-HCl and HOBT in DMF at room temperature.

The intermediate, 3-methyl-1*H*-indazole **18**, was synthesized by diazotization of 2-aminoacetophenone **17**. A nitro group was introduced at the 5 position of **18** to yield the intermediate **19**, which was reduced to an aminoindazole **20**. Compound **21a** was obtained by reacting the intermediate **20** with 1-benzyl-3-piperidone. Compound **21b** was synthesized according to the literature.[7]

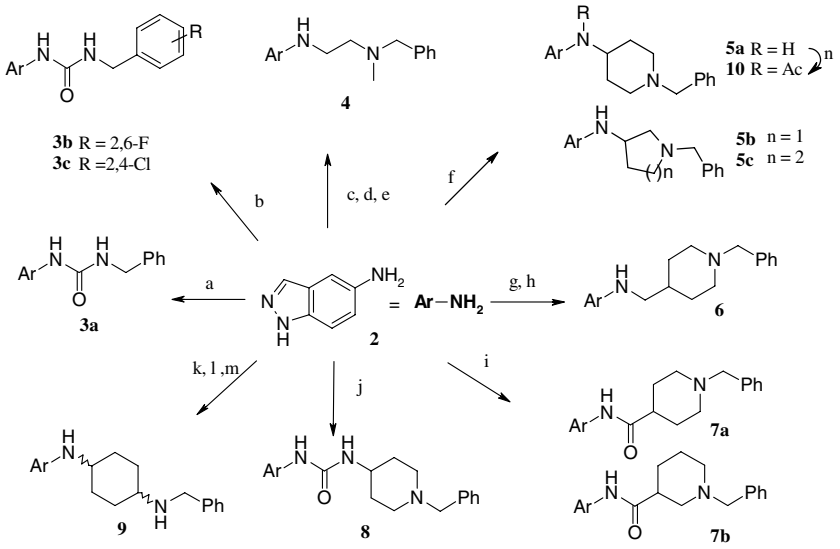
Scheme 3 shows the synthesis of *N*-(3-piperidyl)-*N*-(1*H*-5-indazolyl)amine analogues with substitutions at N-1 of the piperidine moiety. Analogues **24a**–**o** were

Table 1. Modification of linker moiety

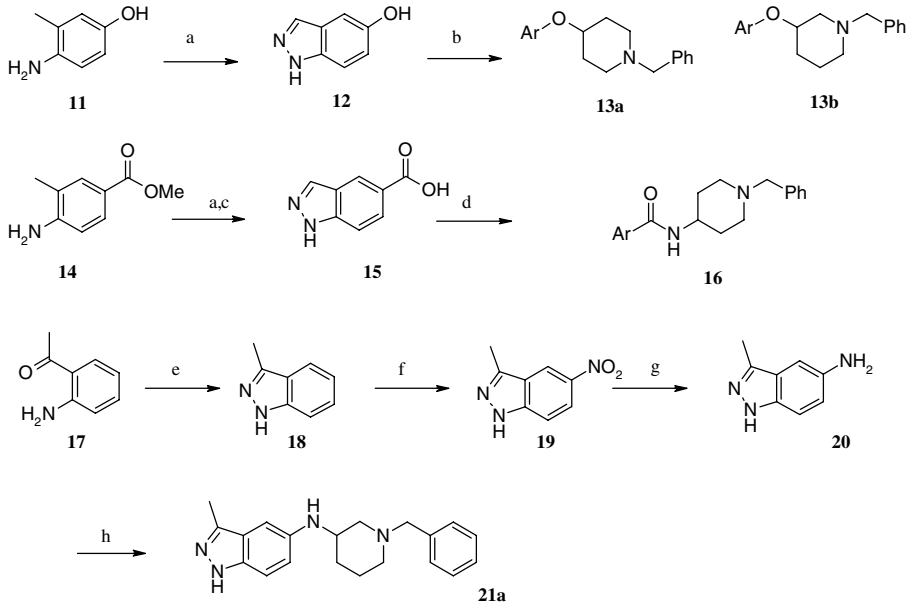


Compound	Rho kinase IC_{50}^{ENZ} (nM)	CCR2/MCP1 IC_{50}^{MCP} (μM)	{Linker}	R
HA-1077 (Fasudil)	180	35		
1	20	>100		2,6-Cl–Ph
3a	260	>100		Ph
3b	85	>100		2,6-F–Ph
3c	220	>100		2,4-Cl–Ph
5a	240	30		Ph
5b	32	10		Ph
5c	20	2		Ph
4	7900	nt		Ph
6	>10,000	nt		4-F–Ph
7a	136	nt		Ph
16	540	nt		Ph
7b	18	1		Ph
8	>10,000	nt		Ph
9a	320	nt		Ph
9b	520	nt		Ph
13a	100	6		Ph
13b	10	1		Ph
10	>10,000	>100		Ph

nt, not tested.

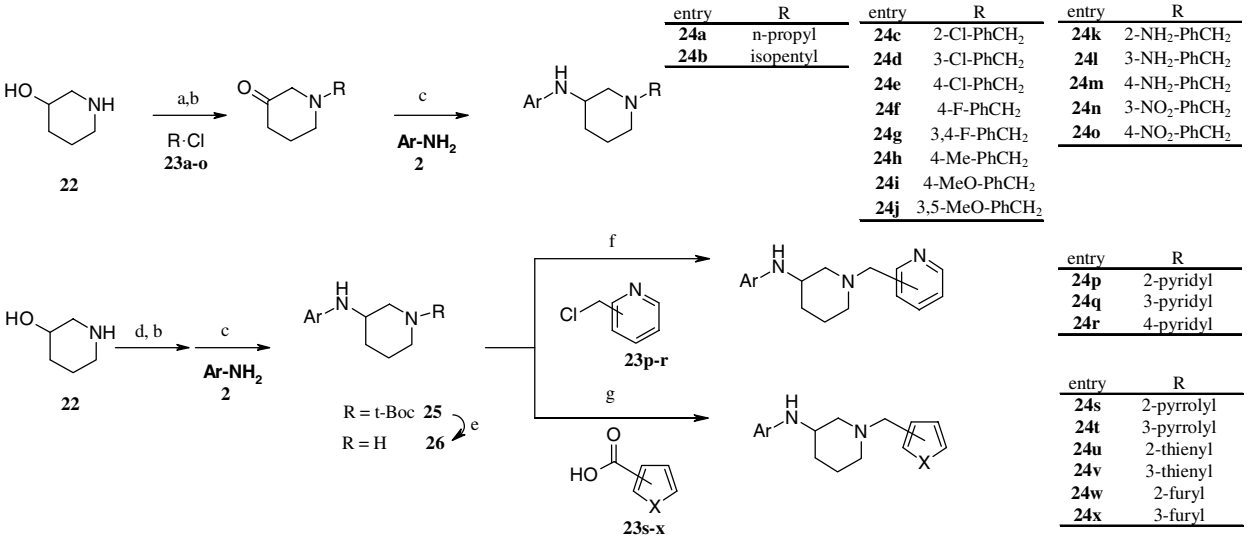


Scheme 1. Synthetic routes of 1*H*-indazole analogues. The 1*H*-5-indazolyl group is abbreviated to Ar. Reagents and conditions: (a) benzylisocyanate, DMF; (b) 2,6-difluorophenylacetic acid or 2,4-dichlorophenylacetic acid, $(PhO)_2PON_3$, Et_3N; (c) 2-chloroacetylchloride, K_2CO_3, EtOAc, H_2O, rt; (d) *N*-methylbenzylamine, K_2CO_3, CH_3CN, rt; (e) (1) BH_3–THF, THF, 60 °C, 1 h; (2) 1 N HCl aq, 60 °C; (f) 1-benzyl-4-piperidone, 1-benzyl-2-pyrrolidinone, or 1-benzyl-3-piperidone, BH_3-pyridine, MeOH, AcOH, rt; (g) (1-benzyl-4-piperidiyl)methanol, SO_3–Me_3N, DMSO, Et_3N, rt; (h) **2**, $NaBH(OAc)_3$, MeOH, rt; (i) *N*-benzylnipecotic acid or *N*-benzylisonipecotic acid, WSC-HCl, HOBT, DMF, rt; (j) triphosgene, Et_3N, $CHCl_3$, rt, then 1-benzylpiperidin-4-amine; (k) 1,4-cyclohexane dione monoethylene ketal, BH_3–pyridine, MeOH, AcOH, rt; (l) 50% AcOH, 80 °C; (m) benzylamine, $NaBH(OAc)_3$, rt; (n) (1) Ac_2O, pyridine, DMAP, rt; (2) 6 N HCl aq.



Scheme 2. Synthetic routes of analogues having a 1*H*-indazole moiety other than 5-amino-1*H*-indazole. The 1*H*-5-indazolyl group is abbreviated to Ar. Reagents and conditions: (a) (1) Ac_2O, AcOK, $CHCl_3$, 0 °C, then isoamylnitrite, 80 °C; (2) 6 N HCl aq, MeOH, 40 °C; (b) 1-benzyl-4-hydroxypiperidine or 1-benzyl-3-hydroxypiperidine, DEAD, PPh_3, THF, rt; (c) 3N NaOH aq; (d) (1-benzyl-4-piperidyl)-amine, WSC-HCl, HOBT, DMF, rt; (e) 50% H_2SO_4, $NaNO_2$, then $SnCl_2$, 0 °C; (f) 50% H_2SO_4, $NaNO_3$, 0 °C; (g) 50% HCl, $SnCl_2$, rt; (h) 1-benzyl-3-piperidone, BH_3–pyridine, MeOH, AcOH, rt.

prepared as follows: alkylchlorides **23a**–**b** and substituted benzylchlorides **23c**–**o** were reacted with 3-hydroxypiperidine **22**. The secondary alcohols were then oxidized to ketones. The Schiff bases formed by the ketones with 5-amino-1*H*-indazole **2** were reduced using BH_3-pyridine to give **24a**–**o**. Analogues **24p**–**r** and **24s**–**x** were prepared as follows: the *t*-Boc-protected *N*-(3-pyperidyl)-*N*-(1*H*-5-indazolyl)amine **25** was first synthesized, then *t*-Boc was removed by treating **25** with TFA. The resulting *N*-(3-piperidyl)-*N*-(1*H*-5-indazolyl)amine **26** was converted to analogues **24p**–**r** by a substitution reaction with alkylchlorides **23p**–**r**, or to analogues **24s**–**x** by



Scheme 3. Synthetic routes of *N*-(3-piperidyl)-*N*-(1*H*-5-indazolyl)amine analogues. The 1*H*-5-indazolyl group is abbreviated to Ar. Reagents and conditions: (a) **23a–o**, K_2CO_3, CH_3CN, rt; (b) SO_3–Me_3N, DMSO, Et_3N, rt; (c) **2**, BH_3–pyridine, MeOH, AcOH, rt; (d) $(t\text{-BuOCO})_2O$, 3 N NaOH aq rt; (e) TFA, $CHCl_3$, rt; (f) **23p–r**, K_2CO_3, CH_3CN, rt; (g) (1) **23s–x**, WSC-HCl, HOBT, DMF, rt; (2) BH_3–THF, THF, 60 °C, 1 h, then 1 N HCl aq, 60 °C.

amide coupling with aryl carboxylates **23s–x** followed by reduction with the BH_3–THF complex.

3. Structure optimization

The cell-free Rho kinase assay was performed according to the methods described in a previous report[7] using the ribosomal S6 kinase substrate, S6 231–239, as the substrate. The chemotaxis assay was performed using CCR2-overexpressing human-derived histiocyte lymphoma (U937) cells and MCP-1 by the Boyden Chamber method.[11] HA-1077 was prepared by the published method and used as the positive control.[12]

3.1. Inhibitory potency of 1*H*-indazole analogues for chemotaxis

In a previous study, we carried out a structure-based design of Rho kinase inhibitors.[7] One important bit of information obtained in the initial study was that several different substructures could be introduced as the linker moiety at the 5 position of 1*H*-indazole scaffold to produce potent Rho kinase inhibitors.

In this study, the 1*H*-indozole inhibitors were initially tested for their inhibition of MCP-1-induced cell migration of CCR2 over-expressing U937 cells. HA-1077 is used as a positive control because HA-1077 is a compound in clinical usage that has the required biophysical properties. The inhibitory potential of HA-1077 in a cell-free enzyme assessment, IC_{50}^{ENZ}, was 180 nM. HA-1077 inhibited the chemotaxis of CCR2/U937 cells with an IC_{50}^{MCP} of 35 μM. However, compounds **3a–c** that carry the urea substructure exhibited poor inhibition of chemotaxis in spite of the fact that they had comparable IC_{50}^{ENZ} values with HA-1077 (Table 1). On the other hand, compounds **5a–c** were found to be potent inhibitors of the chemotaxis (Table 1). This difference might be due to the differing biophysical properties of **3a–c** and **5a–c**.

We assumed that water-solubility might be a good surrogate index for the inhibitors' ability to inhibit the chemotaxis of CCR2/U937 cells. In fact, the solubility of **3b** was poor; 5 μg/mL in WFI (water for injection, Otsuka Pharmaceutical, Japan), 5 μg/mL in JP1st (0.086 N HCl containing 34 mM NaCl), and less than 1 μg/mL in JP2nd (50 mM KH_2PO_4, 24 mM NaOH). The solubility of **5a** was 794 μg/mL in WFI, 881 μg/mL in JP1st, and 495 μg/mL in JP2nd. Inhibitors potent in cell-free enzyme inhibition but having extremely poor water-solubility would be less potent in inhibiting Rho kinase inside the cells.

A docking study of the 1*H*-indazole inhibitors **5a–c** indicated that aminopiperidine or aminopyrrolidine would show a goof fit with the F region of the ligand-binding pocket of the Rho kinase, the shape of which is spherical and spacious (Fig. 2). These cyclic aliphatic amines were considered appropriate linker substructures both for the interaction with the enzyme by full occupation of the F region and for the physical properties of the inhibitor. Enzyme inhibition and a suitable degree of solubility would be necessary for the inhibitors to reach and inhibit Rho kinase in cells. Compound **4** possessed a good degree of solubility but lost its affinity for the ligand-binding pocket of the enzyme due to the loss of shape complementarities with the F region (Table 1).

3.2. Optimization of the linker substructure

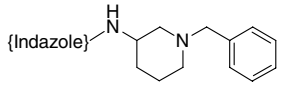
To develop potent 1*H*-indazole inhibitors effective within cells, we first focused on modifying the linker moiety of **1** (Fig. 2) by considering its effects on the solubility and the shape complementarities with the spherical F region. The results are shown in Table 1. Compound **6**, in which the linker length was extended by the insertion of

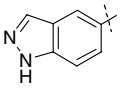
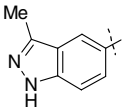
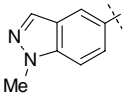
a methylene unit, lost its enzyme inhibition, but an extension by an amide bond (**7a**, and **16**) retained the inhibitory potency, although the potency was not very good. By adjusting the topology of the benzyl group (**7b**), a good enzyme inhibitory potential was recovered along with an inhibitory potential for chemotaxis. Further extension with a urea substructure was not successful (**8**). In compounds **9a** and **9b**, the amine nitrogen atom was placed outside the ring. These compounds were active but with a low potency. Compounds **13a** and **13b**, in which the nitrogen atom between the1*H*-indazole ring and the cyclic amine of **5a** or **5c** was replaced with an oxygen atom, exhibited good IC_{50}^{ENZ} and IC_{50}^{MCP} values. Compound **10** was the N-acetylated analogue of **5a**. As shown in the previous study,[7] introduction of a substitution on the nitrogen atom between the 1*H*-indazole ring and cyclic amine abolished the cell-free enzyme inhibitory activity, because the substitution would not allow **10** to dock to the ligand-binding pocket. This compound was tested by the chemotaxis assay to determine if cells might incorporate this compound and if subsequent hydrolysis might produce an active **5a** inside the cells. This intent was not successful. The IC_{50}^{MCP} of **10** was quite inferior to the IC_{50}^{MCP} of **5a**.

Pyrrolidine and piperidine seemed to be desirable linker substructures. Table 2 compares the IC_{50}^{ENZ} and IC_{50}^{MCP} of analogues of **5a**–**c** in order to investigate the effect of ring size and substitutions of the cyclic amines. The analogues having the 1,3-disubstituted piperidine (**5c** and **24c**–**e**) ring as the linker indicated higher potency than the **5a** and **5b** analogues.

We next examined the effect of modification on the 1*H*-indazole ring of **5c**. Table 3 shows the effects of the substitution group at the 1 and 3 positions of the 1*H*-indazole ring. Substitution with a methyl group at the 1 position (**21b**) diminished the activity. We have reported the structure–activity relationship of 3-amino-1-benzyl-piperidine derivatives in a previous study.[7] A difference observed between the 3-amino-1-benzyl-piperidine derivatives and the 4-amino-1-benzyl-piperidine derivatives was the effect

Table 3. Modification of I*H*-indazole ring



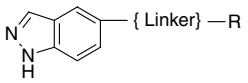
Compound	Rho kinase IC_{50}^{ENZ} (nM)	{Indazole}
5c	20	
21a	150	
21b	>10,000	

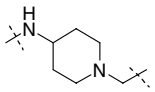
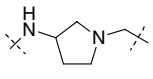
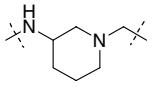
of the substitution at the 3 position of the 1*H*-indazole ring (**21a**). Introduction of a methyl group at the 3 position decreased the activity from an IC_{50}^{ENZ} of 20 nM (**5c**) to an IC_{50}^{ENZ} of 150 nM (**21a**), while the decrease in the potency of the corresponding 4-amino-1-benzyl-piperidine derivative, *N*-(1-benzyl-4-piperidyl)-*N*-(3-methyl-1*H*-5-indazolyl)amine, was more than 40-fold; the IC_{50}^{ENZ} of **5a** was 240 nM, while the IC_{50}^{ENZ} of the 3-methylated **5a** was higher than 10 μM.[7] It seemed that modification of the 1*H*-indazole ring cannot effectively enhance the inhibitory potency for the analogues employing the piperidine ring as the linker substructure.

3.3. Optimization of the hydrophobic substructure for the D region

Finally, the hydrophobic group for the D region of **5c** was optimized. The results are illustrated in Table 4. The replacement of the benzyl group with an *n*-propyl group (**24a**) or isopentyl group (**24b**) decreased the Rho kinase inhibitory activity (IC_{50}^{ENZ} **24a**; 780 nM, **24b**; 340 nM), suggesting that aromatic hydrophobic

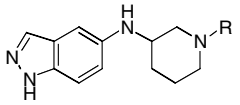
Table 2. Cyclic amine linkers



Compound	Rho kinase IC_{50}^{ENZ} (nM)	CCR2/MCP1 IC_{50}^{MCP} (μM)	{Linker}	R
5a	240	30		Ph
5a2	440	nt		2-Cl–Ph
5a3	290	nt		3-Cl–Ph
5a4	290	nt		4-Cl–Ph
5b	32	10		Ph
5b2	45	7		2-Cl–Ph
5b3	20	5		3-Cl–Ph
5b4	24	1		4-Cl–Ph
5c	20	2		Ph
24c	13	8		2-Cl–Ph
24d	19	8		3-Cl–Ph
24e	11	1		4-Cl–Ph

nt, not tested.

Table 4. Effect of the distal hydrophobic group



Compound	Rho kinase IC_{50}^{ENZ} (nM)	CCR2/MCP1 IC_{50}^{MCP} (μM)	R
5c	20	2	$PhCH_2$
24a	780	nt	*n*-Propyl
24b	340	nt	Isopentyl
24c	13	8	2-Cl–$PhCH_2$
24d	19	8	3-Cl–$PhCH_2$
24e	11	1	4-Cl–$PhCH_2$
24f	25	4	4-F–$PhCH_2$
24g	10	1	3,4-F–$PhCH_2$
24h	9	1	4-Me–$PhCH_2$
24i	16	1	4-MeO–$PhCH_2$
24j	80	>30	3,5-MeO–$PhCH_2$
24k	85	1	2-NH_2–$PhCH_2$
241	35	3	3-NH_2–$PhCH_2$
24m	10	2	4-NH_2–$PhCH_2$
24n	3	19	3-NO_2–$PhCH_2$
24o	87	19	4-NO_2–$PhCH_2$
24p	240	nt	2-PyridylCH_2
24q	72	20	3-PyridylCH_2
24r	190	>30	4-PyridylCH_2
24s	11	10	2-PyrrolylCH_2
24t	>1000	nt	3-PyrrolylCH_2
24u	5	6	2-ThienylCH_2
24v	3	1	3-ThienylCH_2
24w	65	5	2-FurylCH_2
24x	180	nt	3-FurylCH_2

nt, not tested.

groups were more suitable for maintaining the activity in the low nanomolar range than aliphatic groups. This might be because the D region has a cleft-like shape. The introduction of a chlorine atom at the 2, 3, or 4 position of the phenyl ring did not affect the cell-free Rho kinase inhibitory activity very significantly (IC_{50}^{ENZ} **24c**; 13 nM, **24d**; 19 nM, and **24e**; 11 nM). With regard to the inhibition of the chemotaxis, an analogue substituted at the 4 position (**24e**) was the best (IC_{50}^{MCP} 1 μM), whereas analogues that were substituted at the 2 or 3 position had a slightly decreased inhibitory potential for the chemotaxis (IC_{50}^{MCP} 8 μM). The 4-fluoro- (**24f**) and 3,4-difluoro- (**24g**) analogues showed an IC_{50}^{MCP} of 1 μM. Analogues substituted with methyl (**24h**) or methoxyl groups (**24i**) at the 4 position exhibited a similar potency. A topological effect was observed in the IC_{50}^{ENZ} of the analogues substituted with a less hydrophobic amino group (IC_{50}^{ENZ} **24k**; 85 nM, **24l**; 35 nM, **24m**; 10 nM). However, in contrast to the chlorine-substituted analogues, there was little difference in the IC_{50}^{MCP} values of these amine-substituted analogues. Analogues with a nitro group substitution, **24n** and **24o**, exhibited lower IC_{50}^{MCP} values than expected from their IC_{50}^{ENZ}.

Replacement of the phenyl ring in the D region with 3-pyridine and 2-pyrrole led to a moderate change in the Rho kinase inhibitory activities (IC_{50}^{ENZ} **24q**; 72 nM, **24s**; 11 nM), but their inhibitory activities for the chemotaxis were not improved (IC_{50}^{MCP} **24q**; 20 μM, **24s**; 10 μM). Good inhibitory potential in the cell-free Rho kinase inhibition and inhibition of the migration of CCR2/U937 cells was observed in analogues that carried the 2- or 3-thiophene ring (**24u** and **24v**). The 2-furyl analogue, **24w**, exhibited a higher IC_{50}^{MCP} value than expected from the IC_{50}^{ENZ}, while the IC_{50}^{ENZ} of the 3-furyl analogue, **24x**, was low. A similar relationship was observed in the pyrrole analogues, **24s** and **24t**.

4. Conclusion

The inhibitory potential of the 1*H*-indazole analogues against chemotaxis did not correlate directly with their enzyme inhibitory potential, suggesting that biophysical properties are relevant for in vivo efficacy.

We optimized the 1*H*-indazole derivatives based on the inhibition of chemotaxis of CCR2 over-expressing cells. First, we found that inhibitors employing the piperidine and pyrrolidine linkers displayed an inhibitory activity in the MCP-1/CCR2 chemotaxis assay as well as in the cell-free Rho kinase enzyme assay, while those employing the urea linker failed to exhibit inhibition in the chemotaxis assay. Second, we optimized the substitution group on the 1*H*-indazole ring and substructures for the D region of the ligand-binding pocket of the Rho kinase. 1*H*-indazole inhibitors with IC_{50}^{ENZ} values in the low nanomolar range were attained.

Rho kinase has been reported to be involved in the chemotaxis of eosinophiles.[13] Compound **24g** was submitted to the eotaxin/CCR3 chemotaxis assay. The IC_{50} value of **24g** for the inhibition of the chemotaxis of CCR3 over-expressing cells induced by eotaxin ($IC_{50}^{Eotaxin}$) was 0.7 μM, while the $IC_{50}^{Eotaxin}$ of HA-1077 was 9.8 μM. This result indicated that Rho kinase inhibitors could suppress a wide range of leucocyte migrations. A series of these 1*H*-indazole Rho kinase inhibitors might be useful for the treatment of inflammation associated with leucocyte migrations.

We observed that a variety of substructures could be utilized for the 5-substituted 1*H*-indazole scaffold.[7] Indeed, other Rho kinase inhibitors having 1*H*-indazole scaffolds have been reported.[14] The chemical structures of these inhibitors differ largely in the linker substructure. Accordingly, their biophysical properties would also differ. 1*H*-Indazole analogues might be a good source to seek Rho kinase inhibitors with a high biological efficacy.

5. Experimental

Commonly used abbreviations; AcOH (acetic acid), DEAD (diethyl azodicarboxylate), DMAP (*N*, *N*-dimethyl-4-aminopyridine), DMF (*N*,*N*-dimethylformamide), DMSO (dimethylsulfoxide), EtOAc (ethyl acetate), EtOH (ethanol), HOBT (5-hydroxybenzotriazole), MeOH (methanol), *t*-Boc (*tert*-butoxycarbonyl group), TFA (trifluoroacetic acid), THF (tetrahydrofuran), ODS (octadecylated silica gel), WSC-HCl (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride),

BSA (bovine serum albumin), CCR2 (C–C chemokine receptor type 2 or MCP-1 receptor), MCP (monocyte chemoattractant protein).

5.1. Chemotaxis assays

Mouse-derived CCR2 overexpressing human-derived histiocyte lymphoma (U937) cells were suspended in a 0.1% BSA-containing RPMI 1640 medium (10^7 cells/mL). A test compound was added, and the solution was incubated for 20 min. Five hundred microliters each of MCP-1 (1 nM) diluted in RPMI 1640 medium supplemented with 0.1% BSA was placed in the wells of 24-well plates. A 24-well microchemotaxis chamber (Chemotaxicell™, Kurabo Co., Osaka) was placed thereon, and 200 μL of the cell suspension was added to the top of the layer, followed by migration under a 5% CO_2 atmosphere at 37 °C for 1 h. The number of cells which had migrated to the lower chamber was counted with a particle count analyzer (CDA-500, SYSMEX Co.), and the percent inhibition of migration was calculated using the following equation; Migration inhibition (%) = {1 − (the number of migrated cells in the presence of the test compound/the number of migrated cells in the absence of the test compound)} × 100. Chemokines were purchased from Peprotech (London, UK).

5.2. Chemistry

Analytical data were recorded for the compounds described below using the following general procedures. 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 on internal chloroform as 7.24 ppm in deuterochloroform. Coupling constants (J) were recorded in Hertz. Mass spectra (MS) were recorded using a PLATFORM-LC (Micromass) spectrometer. Melting points were taken using an electrothermal melting point apparatus and are uncorrected.

5.2.1. *N*-Benzyl-*N*′-(1*H*-5-indazolyl)urea (3a). To a solution of 5-amino-1*H*-indazole **2** (66 mg, 0.50 mmol) in toluene (1 mL) and DMF (1 drop) was added benzylisocyanate (0.061 mL, 0.50 mmol). The reaction mixture was stirred at 110 °C for 3 h and quenched with water. The reaction mixture was extracted with EtOAc. The EtOAc layer was washed with water and saturated NaCl aq, dried over anhydrous Na_2SO_4, and filtered. The filtrate was then concentrated. The crude was purified by silica gel column chromatography eluting with $CHCl_3$/MeOH (95:5) to give the title compound (21 mg, 16% yield) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.31 (d, J = 5.9 Hz, 2H), 6.53 (t, J = 5.9 Hz, 1H), 7.21–7.28 (m, 2H), 7.29–7.36 (m, 4H), 7.41 (d, J = 8.8 Hz, 1H), 7.85 (s, 1H), 7.93 (s, 1H,), 8.46 (s, 1H), 12.84 (s, 1H). MS (ESI) m/z 267 $(M+1)^+$.

5.2.2. *N*-(2,6-Difluorobenzyl)-*N*′-(1*H*-5-indazolyl)urea (3b). To a mixture of 2,6-difluorophenylacetic acid (129 mg, 0.75 mmol) and triethylamine (0.4 mL) in toluene (2 mL), diphenylphosphoryl azide (248 mg, 0.90 mmol) was added dropwise at rt. The reaction mixture was stirred at 110 °C for 1 h followed by the addition of 5-amino-1*H*-indazole **2** (100 mg, 0.75 mmol) and DMF (0.2 mL). The resulting mixture was stirred at 110 °C for 3 h and diluted with EtOAc and water. The resulting precipitate was filtered and dried to give the title compound as a colorless solid (14 mg, 0.05 mmol, 6% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.39 (d, J = 5.8 Hz, 2H), 6.51 (t, J = 5.7 Hz, 1H), 7.10 (t, J = 8.2 Hz, 2H), 7.21 (dd, J = 2.0 Hz, 8.8 Hz, 1H), 7.35–7.44 (m, 2H), 7.83 (d, J = 1.2 Hz, 1H), 7.92 (d, J = 1.0 Hz, 1H), 8.36 (s, 1H), 12.8 (s, 1H). MS (ESI) m/z 303 $(M+1)^+$.

5.2.3. *N*-(2,4-Dichlorobenzyl)-*N*′-(1*H*-5-indazolyl)urea (3c). Compound **3c** was prepared from 2,4-dichlorophenylacetic acid in a manner similar to that described for **3b** with a yield of 8% as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.35 (d, J = 5.9 Hz, 2H), 6.40 (t, J = 5.9 Hz, 1H), 7.26 (dd, J = 1.8 Hz, 8.9 Hz, 1H), 7.39–7.47 (m, 3H), 7.56 (d, J = 1.7 Hz, 1H), 7.85 (s, 1H), 7.93 (s, 1H), 8.63 (s, 1H), 12.8 (s, 1H). MS (ESI) m/z 334 $(M-1)^+$, 336 $(M+1)^+$.

5.2.4. *N*1-Benzyl-*N*1-methyl-*N*2-(1*H*-5-indazolyl)-1,2-ethanediamine (4). To a mixture of 5-amino-1*H*-indazole **2** (3.99 g, 30 mmol) and K_2CO_3 (8.28 g, 60 mmol) in THF/water (50:50 mL), 2-chloroacetylchloride (10.60 g, 50 mmol) was added dropwise at rt. The reaction mixture was stirred at rt for 18 h, evaporated to remove the THF, and diluted with EtOAc. The resulting precipitate, crude *N*-(1*H*-5-indazolyl)-2-chloroacetamide, was filtered and dried. To a mixture of the precipitate (105 mg, 0.5 mmol) and K_2CO_3 (276 mg, 2 mmol) in CH_3CN (1 mL), a solution of *N*-methylbenzylamine (121 mg, 1.0 mmol) in CH_3CN (1 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 to give *N*-(1*H*-5-indazolyl)-2-(methylbenzylamino)acetamide. The residue was suspended in THF (1 mL), and the BH_3–THF complex (2.5 mL, 2.5 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at 60 °C for 3 h and evaporated to remove the THF. HCl (1 N) aq (1 mL) was added to the residue. The resulting mixture was stirred at 60 °C for 1 h and basified by the addition of saturated $NaHCO_3$ aq at 0 °C. 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. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a dense yellow oil (57 mg, 0.20 mmol, 40% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.40 (s, 3 H), 2.89 (t, J = 5.6 Hz, 2H), 3.19 (t, J = 5.2 Hz, 2H), 3.76 (s, 2H), 6.74 (s, 1H), 6.78 (d, J = 8.8 Hz, 1H), 7.18–7.24 (m, 6H), 7.81 (s, 1H). MS (ESI) m/z 281 $(M+1)^+$.

5.2.5. *N*-(1-Benzyl-4-piperidyl)-*N*-(1*H*-5-indazolyl)amine (5a). To a mixture of 1-benzyl-4-piperidone (635 mg, 3.4 mmol), 5-amino-1*H*-indazole **2** (532 mg, 4.0 mmol), and AcOH (0.20 mL) in MeOH (10 mL), the BH_3–pyridine complex (0.51 mL, 5.1 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 reaction mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a yellow solid (1.00 g, 3.2 mmol, 82% yield). Mp 166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46–1.59 (m, 2H), 2.05–2.12 (m, 2H), 2.15–2.25 (m, 2H), 2.85–2.93 (m, 2H), 3.27–3.37 (m, 1H), 3.56 (s, 2H), 6.77–6.82 (m, 2H), 7.24–7.35 (m, 6H), 7.88 (s, 1H). MS (ESI) *m*/*z* 307 $(M+1)^+$.

5.2.6. *N*-[1-(2-Chlorobenzyl)-4-piperidyl]-*N*-(1*H*-5-indazolyl)amine (5a2). To a mixture of 4-piperidone hydrochloride monohydrate (77 mg, 0.50 mmol) and K_2CO_3 (138 mg, 1.0 mmol) in CH_3CN (1.0 mL), a solution of 2-chlorobenzylchloride (81 mg, 0.50 mmol) in CH_3CN (0.5 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 concentrated to give 1-(2-chlorobenzyl)-4-piperidone. To a mixture of the residue, 5-amino-1*H*-indazole **2** (53 mg, 0.40 mmol), and AcOH (0.02 mL) in MeOH (1.0 mL), a BH_3–pyridine complex (0.05mL, 0.50 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 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 yellow solid (109 mg, 0.32 mmol, 80% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.47–1.60 (m, 2H), 2.06–2.16 (m, 2H), 2.25–2.36 (m, 2H), 2.87–2.97 (m, 2H), 3.30–3.40 (m, 1H), 3.67 (s, 2H), 6.78–6.84 (m, 2H), 7.16–7.34 (m, 3H), 7.35 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 6.8 Hz, 1H), 7.88 (s, 1H). MS (ESI) *m*/*z* 341 $(M^+ + 1)$.

5.2.7. *N*-[1-(3-Chlorobenzyl)-4-piperidyl]-*N*-(1*H*-5-indazolyl)amine (5a3). The compound **5a3** was prepared from 3-chlorobenzylchloride in a manner similar to that described for the compound **5a2** with a yield of 79% as yellow dense oil.^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.45–1.58 (m, 2H), 2.05–2.14 (m, 2H), 2.15–2.25 (m, 2H), 2.82–2.90 (m, 2H), 3.28–3.37 (m, 1H), 3.52 (s, 2H), 6.78–6.83 (m, 2H), 7.18–7.26 (m, 3H), 7.29 (d, J = 8.5 Hz, 1H), 7.35 (s, 1H), 7.88 (s, 1H). MS (ESI) *m*/*z* 341 $(M^+ + 1)$.

5.2.8. *N*-[1-(4-Chlorobenzyl)-4-piperidyl]-*N*-(1*H*-5-indazolyl)amine (5a4). The compound **5a4** was prepared from 4-chlorobenzylchloride in a manner similar to that described for the compound **5a2** with a yield of 79% as yellow dense oil.^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.45–1.59 (m, 2H), 2.05–2.13 (m, 2H), 2.13–2.25 (m, 2H), 2.81–2.91 (m, 2H), 3.25–3.37 (m, 1H), 3.51 (s, 2H), 6.78–6.82 (m, 2H), 7.27–7.32 (m, 5H), 7.87 (s, 1H). MS (ESI) *m*/*z* 341 $(M^+ + 1)$.

5.2.9. *N*-(1-Benzyl-3-pyrrolidyl)-*N*-(1*H*-5-indazolyl)amine (5b). Compound **5b** was prepared from 1-benzyl-3-pyrrolidone in a manner similar to that described for **5a** with a yield of 35% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.66–1.78 (m, 1H), 2.30–2.41 (m, 1H), 2.44–2.53 (m, 1H), 2.61–2.38 (m, 1H), 2.77–2.87 (m, 2H), 3.66 (s, 2H), 4.00–4.08 (m, 1H), 6.73–6.76 (m, 1H), 6.77–6.83 (m, 1H), 7.24–7.36 (m, 6H), 7.88 (s, 1H). MS (ESI) *m*/*z* 293 $(M+1)^+$.

5.2.10. *N*-[1-(2-Chlorobenzyl)-3-pyrrolidyl]-*N*-(1*H*-5-indazolyl)amine (5b2). The compound **5b2** was prepared from 3-hydroxypyrrolidine and 2-chlorobenzylchloride in a manner similar to that described for the compound **24g** with a yield of 41% as yellow dense oil. 1H NMR (CDCl3, 400 MHz) δ ppm: 1.66–1.75 (m, 1H), 2.23–2.34 (m, 1H), 2.44–2.53 (m, 1H), 2.63–2.70 (m, 1H), 2.77–2.88 (m, 2H), 3.73 (s, 2H), 3.95–4.03 (m, 1H), 6.68–6.71 (m, 1H), 6.72–6.77 (m, 1H), 7.11–7.18 (m, 2H), 7.20–7.24 (m, 1H), 7.26–7.30 (m, 1H), 7.40 (d, J = 6.8 Hz, 1H), 7.82 (s, 1H). MS (ESI) *m*/*z* 327 $(M+1)^+$.

5.2.11. *N*-[1-(3-Chlorobenzyl)-3-pyrrolidyl]-*N*-(1*H*-5-indazolyl)amine (5b3). The compound **5b3** was prepared from 3-hydroxypyrrolidine and 3-chlorobenzylchloride in a manner similar to that described for the compound **24g** with a yield of 72% as yellow dense oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.68–1.79 (m, 1H), 2.30–2.41 (m, 1H), 2.42–2.51 (m, 1H), 2.61–2.67 (m, 1H), 2.76–2.86 (m, 2H), 3.62 (s, 2H), 4.01–4.08 (m, 1H), 6.74–6.77 (m, 1H), 6.79–6.83 (m, 1H), 7.18–7.26 (m, 3H), 7.29 (d, J = 8.8 Hz, 1H), 7.35 (s, 1H), 7.89 (s, 1H). MS (ESI) *m*/*z* =327 $(M^+ + 1)$.

5.2.12. *N*-[1-(4-Chlorobenzyl)-3-pyrrolidyl]-*N*-(1*H*-5-indazolyl)amine (5b4). The compound **5b4** was prepared from 3-hydroxypyrrolidine and 4-chlorobenzylchloride in a manner similar to that described for the compound **24g** with a yield of 67% as yellow dense oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.67–1.77 (m, 1H), 2.30–2.41 (m, 1H), 2.41–2.50 (m, 1H), 2.59–2.65 (m, 1H), 2.75–2.85 (m, 2H), 3.61 (s, 2H), 4.00–4.07 (m, 1H), 6.73–6.76 (m, 1H), 6.77–6.82 (m, 1H), 7.24–7.30 (m, 5H), 7.88 (s, 1H). MS (ESI) *m*/*z* =327 $(M^+ + 1)$.

5.2.13. *N*-(1-Benzyl-3-piperidyl)-*N*-(1*H*-5-indazolyl)amine (5c). Compound **5c** was prepared from 1-benzyl-3-piperidone in a manner similar to that described for **5a** with a yield of 78% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40–1.60 (m, 2H), 1.70–1.80 (m, 2H), 2.10–2.50 (m, 4H), 3.16–3.22 (m, 2H), 3.45 (s, 1H), 6.75 (m, 2H), 7.15–7.28 (m, 6H), 7.78 (s, 1H). MS (ESI) *m*/*z* 307 $(M+1)^+$.

5.2.14. *N*-[1-(4-Fluorobenzyl)-4-piperidyl]methyl-*N*-(1*H*-5-indazolyl)amine (6). To a mixture of 4-piperidylmethanol (345 mg, 3.0 mmol) and K_2CO_3 (414 mg, 3.2 mmol) in CH_3CN (2 mL), a solution of 4-fluorobenzylchloride (378 mg, 3.0 mmol) in CH_3CN (2 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 5 h and diluted with EtOAc. The resulting mixture was filtered through Celite. The filtrate was then concentrated to give 1-(4-fluorobenzyl)-4-piperidylmethanol. This material was dissolved in anhydrous DMSO (2 mL), and triethylamine (0.5 mL) was added. Sulfur trioxide–trimethylamine complex (700 mg, 5 mmol) was then

added to the mixture 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 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 1-(4-fluorobenzyl)-4-piperidylcarbaldehyde. This intermediate was dissolved in MeOH (3 mL), and 5-amino-1*H*-indazole **2** (276 mg, 2.0 mmol) was added. $NaBH(OAc)_3$ (666 mg, 3.0 mmol) was then 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 $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated. The residual oil was purified by silica gel chromatography eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a dense yellow oil (328 mg, 1.0 mmol, 50% yield in this step). ^1H NMR ($CDCl_3$, 400 MHz) δ 1.03–1.15 (m, 1H), 1.62–1.77 (m, 2H), 1.80–1.87 (m, 1H), 1.93–2.20 (m, 3H), 2.85–2.93 (m, 1H), 3.00 (d, J = 6.6 Hz, 2H), 3.03–3.12 (m, 1H), 3.60 (d, J = 16.6 Hz, 1H), 6.69 (s, 1H), 6.71 (dd, J = 8.8 Hz, 1H), 6.90–6.98 (m, 2H), 7.21–7.28 (m, 3H), 7.83 (s, 1H) MS (ESI) m/z 339 $(M+1)^+$.

5.2.15. 1-Benzyl-*N*-(1*H*-5-indazolyl)piperidine-4-carboxamide (7a). To a solution of isonipecotic acid (129 mg, 1.0 mmol) in 3 N NaOH aq (1 mL), a solution of benzylchloride (127 mg, 1.0 mmol) in DMF (1 mL) was added dropwise at rt. The reaction mixture was stirred at rt for 2 h followed by a portionwise addition of 5-amino-1*H*-indazole **2** (133 mg, 1.0 mmol), HOBT (230 mg, 1.5 mmol), WSC-HCl (288 mg, 1.5 mmol), and dimethylaminopyridine (5 mg) 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 $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then 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 yellow solid (86 mg, 0.26 mmol, 26% yield). Mp 218 °C. ^1H NMR (CD_3OD, 400 MHz) δ 1.76–85 (m, 4H), 2.02–2.12 (m, 2H), 2.32 (dt, J = 7.7 Hz, 15.4 Hz, 1H), 2.93 (d, J = 11.7 Hz, 2H), 3.50 (s, 2H), 7.19 (dd, J = 2.8 Hz, 5.1 Hz, 1H), 7.22–7.28 (m, 4H), 7.36 (dd, J = 9.0 Hz, 17.6 Hz, 2H), 7.88 (s, 1H), 7.93 (s, 1H). MS (ESI) m/z 335 $(M+1)^+$.

5.2.16. 1-Benzyl-*N*-(1*H*-5-indazolyl)piperidine-3-carboxamide (7b). Compound **7b** was prepared from nipecotic acid in a manner similar to that described for **7a** with a yield of 35% as a yellow solid. Mp 175 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.53–1.65 (m, 2H), 1.69–1.82 (m, 1H), 2.00–2.16 (m, 2H), 2.26–2.34 (m, 1H), 2.59–2.65 (m, 1H), 2.91–3.01 (m, 1H), 3.06–3.15 (m, 1H), 3.50 (d, J = 10.7 Hz, 2H), 7.21–7.38 (m, 7H), 7.96 (s, 1H), 7.98 (s, 1H). MS (ESI) m/z 335 $(M+1)^+$.

5.2.17. *N*-(1-Benzyl-4-piperidyl)-*N*′-(1*H*-5-indazolyl)urea (8). To a mixture of 4-amino-1-benzylpiperidine (95 mg, 0.50 mmol) and triethylamine (0.25 mL) in $CHCl_3$ (1 mL), a solution of triphosgene (148 mg, 0.50 mmol) in $CHCl_3$ (1 mL) was added dropwise at rt. The mixture was stirred at rt for 30 min and followed by the portionwise addition of 5-amino-1*H*-indazole **2** (67 mg, 0.50 mmol) at rt. 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 $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was purified by silica gel chromatography, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a yellow solid (128 mg, 0.13 mmol, 73% yield). Mp 90 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ 1.40–154 (m, 1H), 1.58–1.70 (m, 1H), 1.90–1.98 (m, 1H), 1.98–2.06 (m, 1H), 2.10–2.23 (m, 2H), 2.77–2.88 (m, 2H), 3.50 (s, 2H), 4.90–5.00 (m, 1H), 6.98 (d, J = 8.3 Hz, 1H), 7.27–7.33 (m, 6H), 7.91 (s, 1H), 8.23 (d, J = 8.8 Hz, 1H). MS (ESI) m/z 350 $(M+1)^+$.

5.2.18. *N*1-Benzyl-*N*4-(1*H*-5-indazolyl)-1,4-cyclohexanediamine (9a and 9b). To a mixture of 1,4-cyclohexane monoethyleneketal (3.90 g, 25 mmol), 5-amino-1*H*-indazole **2** (2.66 g, 20 mmol), and AcOH (0.5 mL), a BH_3–pyridine complex (2.50 mL, 25 mmol) was added dropwise at 0 °C. The mixture was stirred at rt for 18 h and concentrated for the removal of MeOH. The residue was dissolved in 50% AcOH (50 mL). The reaction mixture was stirred at 80 °C for 3 h and quenched by the addition of saturated $NaHCO_3$ aq. The reaction mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was purified by silica gel chromatography, eluting with $CHCl_3$ to give 4-(1*H*-5-indazolylamino)-1-cyclohexanone (3.21 g, 14 mmol). To a mixture of 4-(1*H*-5-indazolylamino)-1-cyclohexanone (57 mg, 0.25 mmol), benzylamine (52 mg, 0.5 mmol) in MeOH (1 mL), $NaBH(OAc)_3$ (111 mg, 0.5 mmol) was 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 $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to reverse-phase ODS HPLC (YMC-Pack ODS-AQ, 20 mm X 150 mm), eluting with 5% TFA aq/acetonitrile (80:20) to give the title compound as a dense yellow oil (14 mg, syn isomer **9a**, 24 mg, anti-isomer **9b**). Syn isomer: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.50–1.82 (m, 8H), 2.68 (tt, J = 3.9 Hz, 7.6 Hz, 1H), 3.44–3.50 (m, 1H), 3.90 (s, 2H), 6.70–6.77 (m, 2H), 7.20–7.30 (m, 6H), 7.80 (s, 1H). MS (ESI) m/z 321 $(M+1)^+$. Anti-isomer: ^1H NMR ($CDCl_3$, 400 MHz) δ 1.05–1.32 (m, 4H), 1.95–2.03 (m, 2H), 2.05–2.20 (m, 2), 2.52 (tt, J =3.9, 11.0 Hz, 1H), 3.20 (tt, J = 307, 11.0 Hz, 1H), 3.70 (s, 2H), 6.68–6.77 (m, 2H), 7.22 (d, J = 8.8 Hz, 1H), 7.25–7.30 (m, 5H), 7.81 (s, 1H). MS (ESI) m/z 321 $(M+1)^+$.

5.2.19. *N*1-(1-Benzyl-4-piperidyl)-*N*1-(1*H*-5-indazolyl)acetamide (10). To a mixture of *N*-(1-benzyl-4-piperidyl)-*N*-(1*H*-5-indazolyl)amine **5a** (307 mg, 1.0 mmol) and pyridine (190 mg, 2.4 mmol) and DMAP (15 mg, 0.12 mmol) in $CHCl_3$ (1 mL), acetic anhydride (245 mg,

2.4 mmol) was added dropwise at rt. 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 $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The crude material was dissolved in MeOH (1 mL). To the solution was added HCl–MeOH (1 mL). The reaction mixture was stirred at rt for 5 h and basified 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. The residual oil was purified by silica gel chromatography, eluting with $CHCl_3$ to give the title compound as a dense yellow oil (321 mg, 0.95 mmol, 95% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.23–1.40 (m, 1H), 1.50–1.70 (m, 1H), 1.70–1.80 (m, 1H), 1.82–1.92 (m, 1H), 2.02 (s, 3H), 2.11–2.28 (m, 2H), 2.91–3.08 (m, 2H), 3.48 (d, J = 12.7 Hz, 1H), 3.55 (d, J = 13.0 Hz, 1H), 4.66–4.76 (m,1H), 6.92–6.98 (m, 1H), 7.20–7.27 (m, 6H), 7.52 (s, 1H), 8.05 (s, 1H). MS (ESI) m/z 349 $(M+1)^+$.

5.2.20. 1*H*-5-Indazolol (12). To a mixture of 4-amino-3-cresol **11** (12.3 g, 100 mmol) and potassium acetate (24.4 g, 244 mmol) in $CHCl_3$ (200 mL), acetic anhydride (47.1 mL, 450 mmol) was added dropwise at 0 °C. The resulting mixture was stirred at rt for 30 min, heated to 80 °C, followed by the dropwise addition of isoamylnitrite (12.7 g, 110 mmol) at 80 °C. The reaction mixture was stirred at 80 °C for 18 h and quenched by the addition of saturated $NaHCO_3$ aq. The reaction mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to flash chromatography on silica gel eluting with $CHCl_3$–MeOH to give *N*,*O*-diacetyl-5-indazolol, which was then treated with HCl–MeOH (200 mL) at 80 °C for 5 h and evaporated to remove HCl–MeOH. The resulting mixture was basified with saturated $NaHCO_3$ aq. The mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The precipitate was washed with $CHCl_3$ to give the title compound as a brown solid (7.99 g, 60 mmol, 60% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.95 (d, J = 8.8 Hz, 1H), 7.03 (s, 1H), 7.31 (d, J = 8.8 Hz, 1H), 7.89 (s, 1H).

5.2.21. 1-Benzyl-4-piperidyl-(1*H*-5-indazolyl)ether (13a). To a mixture of 1*H*-5-indazolol **12** (52 mg, 0.40 mmol), 1-benzyl-4-hydroxypiperidine (96 mg, 0.50 mmol), and triphenylphosphine (131 mg, 0.50 mmol) in THF (1 mL), a solution of DEAD (87 mg, 0.50 mmol) in toluene (1 mL) 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 reaction mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to silica gel column chromatography eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a yellow solid (35 mg, 0.11 mmol, 28% yield). Mp 90 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.71–184 (m, 2H), 1.92–2.00 (m, 2H), 2.20–2.30 (m, 2H), 2.65–2.75 (m, 2H), 3.48 (s, 2H), 4.16–4.28 (m, 1H), 6.96–7.03 (m, 1H), 7.07–7.09 (m, 1H), 7.20–7.28 (m, 5H), 7.30 (d, J = 9.0 Hz, 1H), 7.89 (s, 1H). MS (ESI) m/z 308 $(M+1)^+$.

5.2.22. 1-Benzyl-3-piperidyl-(1*H*-5-indazolyl)ether (13b). Compound **13b** was prepared from 1-benzyl-3-hydroxypiperidine in a manner similar to that described for **13a** with a yield of 33% as dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46–1.57 (m, 1H), 1.57–1.70 (m, 1H), 1.73–1.88 (m, 1H), 2.05–2.15 (m, 1H), 2.15–2.34 (m, 2H), 2.63–2.68 (m, 1H), 3.04 (m, 2H), 3.63 (s, 1H), 4.26–4.38 (m, 1H), 7.02 (d, J = 9.0 Hz, 1H), 7.13 (s, 1H), 7.20–7.35 (m, 6H), 7.88 (s, 1H). MS (ESI) m/z 308 $(M+1)^+$.

5.2.23. 1*H*-Indazol-5-carboxylic acid (15). To a mixture of methyl 4-amino-3-methylbenzoate **14** (0.85 g, 5.2 mmol) and potassium acetate (1.47 g, 15 mmol) in $CHCl_3$ (20 mL), acetic anhydride (1.42 mL, 15 mmol) was added dropwise at 0 °C. The resulting mixture was stirred at rt for 30 min, heated to 80 °C, followed by the dropwise addition of isoamyl nitrite (1.17 g, 10 mmol) at 80 °C. The reaction mixture was stirred at 80 °C for 18 h and quenched by the addition of saturated $NaHCO_3$ aq. The reaction mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was purified by silica gel column chromatography, eluting with $CHCl_3$–MeOH to give the intermediate, N1-acetyl-indazol-5-carboxylic acid. The intermediate was hydrolyzed in 3 M NaOH aq (5 mL)–MeOH (5 mL) at rt for 18 h and evaporated to remove MeOH and water. The residual solid was subjected to reverse-phase chromatography on ODS, eluting with 0.5% TFA aq/CH_3CN (95:5) to give the title compound as a yellow solid (0.32 g, 2.0 mmol, 38% yield).

5.2.24. *N*5-(1-Benzyl-4-piperidyl)-1*H*-5-indazolecarboxyamide (16). To a mixture of 4-amino-1-benzylpiperidine (280 mg, 1.47 mmol), 1*H*-5-indazolecarboxylic acid **15** (243 mg, 1.81 mmol), HOBT (306 mg, 2.0 mmol), and dimethylaminopyridine (5 mg) in DMF (3 mL) was added WSC-HCl (383 mg, 2.0 mmol) 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 $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then 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 yellow solid (42 mg, 0.13 mmol, 9% yield). Mp 126 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.60–1.80 (m, 2H), 1.95–2.08 (m, 2H), 2.20–2.32 (m, 2H), 2.90–2.98 (m, 2H), 3.07 (s, 2H), 3.80–3.86 (m, 1H), 7.16–7.40 (m, 5H), 7.46 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 8.8 Hz, 1H), 8.07 (s, 1H), 8.14 (s, 1H). MS (ESI) m/z 335 $(M+1)^+$.

5.2.25. *N*-(1-Benzyl-3-piperidyl)-*N*-(3-methyl-1*H*-5-indazolyl)amine (21a). (1) 5-Amino-3-methyl-1*H*-indazole (**20**). To a suspension of 2′-aminoacetophenone **17**

(1.35 g, 10 mmol) in 50% H_2SO_4 aq (20 mL), sodium nitrite (0.82 g, 12 mmol) was slowly added at 0 °C. The resulting mixture was stirred at rt for 1 h, followed by the addition of tin(II)chloride dihydrate (6.76 g, 30 mmol). The reaction mixture was stirred at 0 °C for 1 h and diluted with water. The white solid was collected by filtration and dried to give 3-methyl-1*H*-indazole **18** (1.20 g, 9 mmol).

Compound **18** was suspended in 50% H_2SO_4 aq (5 mL) and sodium nitrate (0.85 g, 10 mmol) at 0 °C. The mixture was stirred at rt for 1 h and quenched by the addition of saturated $NaHCO_3$ aq. The reaction mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual solid was washed with $CHCl_3$ to give 3-methyl-5-nitro-1*H*-indazole **19**. The nitro group of **19** was reduce to an amino group: **19** was suspended in 50% HCl aq (10 mL) and tin(II) chloride dihydrate (4.1 g, 18 mmol) was added slowly at 0 °C. The resulting mixture was stirred at rt for 5 h and quenched by the addition of saturated $NaHCO_3$ aq. The reaction mixture was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual solid was washed with $CHCl_3$ to give **20** with a yield of 88% as a brown solid.

(2) Compound **21a** was prepared from 5-amino-3-methyl-1*H*-indazole **20** and 1-benzyl-3-piperidone in a manner similar to that described for **5a** with a yield of 55% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44–1.61 (m, 3H), 1.65–1.80 (m, 2H), 2.20–2.30 (m, 1H), 2.43 (s, 3H), 2.72–2.81 (m, 2H), 3.48 (d, J = 4.1 Hz, 2H), 3.53–3.60 (m, 1H), 6.65 (d, J = 2.0 Hz, 1H), 6.73 (dd, J = 2.2 Hz, 8.8 Hz, 1H), 7.14 (d, J = 8.8 Hz, 1H), 7.22–7.30 (m, 5H). MS (ESI) m/z 321 $(M+1)^+$.

5.2.26. *N*-(1-Benzyl-3-piperidyl)-*N*-(1-methyl-5-indazolyl)amine (21b). Compound **21b** was synthesized in the same way as that in the Ref. 7.

5.2.27. *N*-(1-Propyl-3-piperidyl)-*N*-(1*H*-5-indazolyl)amine (24a). Compound **24a** was prepared from 3-bromopropane in a manner similar to that described for **24g** with a yield of 35% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90 (t, J = 7.6 Hz, 3H), 1.51–2.07 (m, 4H), 2.07–2.21 (m, 4H), 2.36–2.40 (m, 2H), 2.96–2.99 (m, 2H), 3.29–3.36 (m, 1H), 6.75–6.81 (m, 2H), 7.25 (d, J = 12.0 Hz, 1H), 7.88 (s, 1H). MS (ESI) m/z 259 $(M+1)^+$.

5.2.28. *N*-(1-Isopentyl-3-piperidyl)-*N*-(1*H*-5-indazolyl)-amine (24b). Compound **24b** was prepared from 1-chloro-3-methylbutane in a manner similar to that described for **24g** with a yield of 32% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89–0.91 (m, 6H), 1.35–1.41 (m, 3H), 1.54–1.76 (m, 6H), 2.32–2.37 (m, 3H), 2.45–2.50 (m, 1H), 3.55–3.63 (m, 1H), 6.82–6.97 (m, 2H), 7.28–7.32 (m, 1H), 7.88 (s, 1H). MS (ESI) m/z 287 $(M+1)^+$.

5.2.29. *N*-[1-(2-Chlorobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24c). Compound **24c** was prepared from 2-chlorobenzylchloride in a manner similar to that described for **24g** with a yield of 63% as a yellow solid. Mp 72 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42–1.74 (m, 4H), 2.31–2.53 (m, 3H), 2.65–2.75 (m, 1H), 3.51–3.60 (m, 3H), 6.71–6.79 (m, 2H), 7.07–7.18 (m, 2H), 7.21 (d, J = 8.8 Hz, 1H), 7.28 (dd, J = 1.7 Hz, 7.6 Hz, 1H), 7.34–7.42 (m, 1H), 7.78 (s, 1H). MS (ESI) m/z 341 $(M+1)^+$.

5.2.30. *N*-[1-(3-Chlorobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24d). Compound **24d** was prepared from 3-chlorobenzylchloride in a manner similar to that described for **24g** with a yield of 46% as a yellow solid. Mp 85 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49–1.65 (m, 2H), 1.68–1.78 (m, 2H), 2.33–2.54 (m, 3H), 2.60–2.73 (m, 1H), 3.42–3.54 (m, 2H), 3.54–3.64 (m, 1H), 6.78–6.86 (m, 2H), 7.16–7.22 (m, 3H), 7.27 (d, J = 8.8 Hz, 1H), 7.34 (s, 1H), 7.85 (s, 1H). MS (ESI) m/z 341 $(M+1)^+$.

5.2.31. *N*-[1-(4-Chlorobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24e). Compound **24e** was prepared from 4-chlorobenzylchloride in a manner similar to that described for **24g** with a yield of 29% as a yellow solid. Mp 139 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43–1.57 (m, 2H), 1.62–1.74 (m, 2H), 2.20–2.40 (m, 3H), 2.63–2.70 (m, 1H), 3.33–3.48 (m, 2H), 3.48–3.58 (m, 1H), 6.72–6.78 (m, 2H), 7.18–7.24 (m, 5H), 7.79 (s, 1H). MS (ESI) m/z 341 $(M+1)^+$.

5.2.32. *N*-[1-(4-Fluorobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24f). Compound **24f** was prepared from 4-fluorobenzylchloride in a manner similar to that described for **24g** with a yield of 56% as a yellow solid. Mp 166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43–1.58 (m, 2H), 1.60–1.75 (m, 2H), 2.20–2.40(m, 3H), 2.60–2.75 (m, 1H), 3.34–3.47 (m, 2H), 3.47–3.58 (m, 1H), 6.72–6.78 (m, 2H), 6.89–6.96 (m, 2H), 7.19–7.25 (m, 3H), 7.79 (s, 1H). MS (ESI) m/z 325 $(M+1)^+$.

5.2.33. *N*-[1-(3,4-Difluorobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24g). To a mixture of 3-hydroxypiperidine (71 mg, 0.7 mmol) and K_2CO_3 (268 mg, 2.0 mmol) in DMF (1 mL), a solution of 3,4-difluorobenzylchloride (119 mg, 0.7 mmol) in CH_3CN (0.5 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-(3,4-difluorobenzyl)-3-hydroxypiperidine. To a mixture of the residue, triethylamine (0.25 mL) in anhydrous DMSO (1 mL) was slowly added a sulfur trioxide–trimethylamine complex (209 mg, 2.0 mmol) 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 1-(3,4-difluorobenzyl)-piperidine-3-one. To a mixture of this intermediate, 5-amino-1*H*-indazole **2** (53 mg, 0.4 mmol), and AcOH (0.02 mL) in MeOH (1 mL), the BH_3–pyridine complex (0.05 mL,

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 mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a yellow solid (85 mg, 0.25 mmol, 63% yield in this step). Mp 166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30–1.40 (m, 1H), 1.59–1.69 (m, 1H), 1.74–1.80 (m, 1H), 1.91–2.00 (m, 2H), 2.18 (t, J = 7.0 Hz, 1H), 2.58–2.68 (m, 1H), 2.93 (t, J = 10.0 Hz, 1H), 3.30–3.32 (m, 1H), 3.48 (q, J = 8.0 Hz, 2H), 6.85 (s, 1H), 6.92 (dd, J = 2.2, 8.8 Hz, 1H), 7.07–7.10 (m, 1H), 7.14 (dd, J = 8.1 Hz, 10.2 Hz, 1H), 7.26 (ddd, J = 2.0, 8.1, 10.5 Hz, 1H), 7.31 (d, J = 8.8 Hz, 1H), 7.77 (s, 1H). MS (ESI) m/z 342 $(M+1)^+$.

5.2.34. *N*-[1-(4-Methylbenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24h). Compound **24h** was prepared from 4-methylbenzylchloride in a manner similar to that described for **24g** with a yield of 22% as a yellow solid. Mp 158 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42–1.57 (m, 2H), 1.60–1.72 (m, 2H), 2.26 (s, 3H), 2.24–2.40 (m, 3H), 2.62–2.75 (m, 1H), 3.35–3.48 (m, 2H), 3.48–3.58 (m, 1H), 6.71–6.78 (m, 2H), 7.05 (d, J = 7.8 Hz, 2H), 7.14 (d, J = 8.1 Hz, 2H), 7.21 (d, J = 8.8 Hz, 1H), 7.79 (s, 1H). MS (ESI) m/z 321 $(M+1)^+$.

5.2.35. *N*-[1-(4-Methoxybenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24i). Compound **24i** was prepared from 4-methoxybenzylchloride in a manner similar to that described for **24g** with a yield of 19% as a yellow solid, Mp 184 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43–1.56 (m, 2H), 1.60–1.73 (m, 2H), 2.23–2.40 (m, 3H), 2.61–2.80 (m, 1H), 3.33–3.47 (m, 2H), 3.48–3.58 (m, 1H), 3.72 (s, 3H), 6.74–6.78 (m, 2H), 6.78 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 1H), 7.79 (s, 1H). MS (ESI) m/z 337 $(M+1)^+$.

5.2.36. *N*-[1-(3,5-Dimethoxybenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24j). Compound **24j** was prepared from 3,5-dimethoxybenzylchloride in a manner similar to that described for **24g** with a yield of 38% as a yellow solid. Mp 205 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40–1.57 (m, 2H), 1.62–1.75 (m, 2H), 2.20–2.43 (m, 3H), 2.70–2.80 (m, 1H), 3.35–3.47 (m, 2H), 3.50–3.60 (m, 1H), 3.73 (s, 6H), 6.29 (s, 1H), 6.45 (s, 2H), 6.72–6.79 (m, 2H), 7.22 (d, J = 8.5 Hz, 1H), 7.79 (s, 1H). MS (ESI) m/z 367 $(M+1)^+$.

5.2.37. *N*-[1-(2-Aminobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24k). Compound **24k** was prepared from *N*-[1-(2-nitrobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)-amine in a manner similar to that described for **24l** with a yield of 90% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.35–1.68 (m, 2H), 1.73–1.95 (m, 2H), 2.00–2.35 (m, 3H), 2.35–2.62 (m, 1H), 2.80–2.96 (m, 1H), 3.45–3.37 (m, 2H), 6.65 (d, J = 6.8 Hz, 1H), 6.67 (d, J = 7.6 Hz, 1H), 6.79 (s, 1H), 6.98 (d, J = 6.8 Hz, 1H), 7.09 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 9.3 Hz, 1H), 7.86 (s, 1H). MS (ESI) m/z 322 $(M+1)^+$.

5.2.38. *N*-[1-(3-Aminobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24l). To a solution of *N*-[1-(3-nitrobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine **24n** (88 mg, 0.25 mmol) in concd HCl (0.25 mL) and MeOH (0.50 mL), tin(II)chloride dihydrate (113 mg, 0.50 mmol) was slowly added at rt. The resulting mixture was stirred at rt for 3 h and basified by the addition of saturated $NaHCO_3$ aq. The mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to flash chromatography on aluminum oxide, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a dense yellow oil (64 mg, 0.23 mmol, 92% yield in this steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.42–1.68 (m, 3H), 1.70–1.88 (m, 1H), 2.35–2.47 (m, 3H), 2.60–2.80 (m, 1H), 3.38–3.50 (m, 2H), 3.55–3.70 (m, 1H), 6.58 (d, J = 8.5 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 6.71 (d, J = 7.3 Hz, 1H), 6.82 (br s, 2H), 7.09 (t, J = 7.7 Hz, 1H), 7.29 (d, J = 8.8 Hz, 1H), 7.86 (s, 1H). MS (ESI) m/z 322 $(M+1)^+$.

5.2.39. *N*-[1-(4-Aminobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24m). Compound **24m** was prepared from *N*-(1*H*-5-Indazolyl)-*N*-[1-(3-nitrobenzyl)-3-piperidyl]amine **24o** in a manner similar to that described for **24l** with a yield of 99% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.53–1.65 (m, 2H), 1.65–1.80 (m, 2H), 2.26–2.49 (m, 3H), 2.70–2.82 (m, 1H), 3.45 (d, J = 9.3 Hz, 2H), 3.61 (br s, 1H), 6.58 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 6.82 (s, 1H), 6.83 (d, J = 8.8 Hz, 1H), 7.09 (t, J = 7.6 Hz, 1H), 7.25 (s, 1H), 7.29 (d, J = 8.6 Hz, 1H), 7.86 (s, 1H). MS (ESI) m/z 322 $(M+1)^+$.

5.2.40. *N*-[1-(3-Nitrobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24n). Compound **24n** was prepared from 3-nitrobenzylchloride in a manner similar to that described for **24g** with a yield of 43% as a yellow solid. Mp 80 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52–1.66 (m, 2H), 1.71–1.84 (m, 2H), 2.26–2.50 (m, 3H), 2.70–2.80 (m, 1H), 3.52–3.65 (m, 3H), 6.77–6.85 (m, 2H), 7.27 (d, J = 8.8 Hz, 1H), 7.44 (dd, J = 7.8, 8.1 Hz, 1H), 7.63 (d, J = 6.8 Hz, 1H), 7.83 (s, 1H), 8.07 (d, J = 8.3 Hz, 1H), 8.23 (s, 1H). MS (ESI) m/z 352 $(M+1)^+$.

5.2.41. *N*-[1-(4-Nitrobenzyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24o). Compound **24o** was prepared from 4-nitrobenzylchloride in a manner similar to that described for **24g** with a yield of 25% as a yellow solid. Mp 85 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.51–1.68 (m, 2H), 1.69–1.86 (m, 2H), 2.26–2.47 (m, 3H), 2.71–2.83 (m, 1H), 3.52–3.65 (m, 3H), 6.79–6.83 (m, 2H), 7.28 (d, J = 9.5 Hz, 2H), 7.49 (d, J = 8.5 Hz, 1H), 7.84 (s, 1H), 8.15 (d, J = 8.8 Hz, 2H). MS (ESI) m/z 352 $(M+1)^+$.

5.2.42. *N*-[1-(2-Pyridylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24p). To a solution of *tert*-butyl 3-(1*H*-5-indazolylamino)-1-piperidinecarboxylate **25** (93 mg, 0.30 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 (138 mg, 1.0 mmol) and CH_3CN (1 mL) were added.

To the mixture, a solution of 2-chloromethyl-pyridine (50 mg, 0.30 mmol) in CH_3CN (0.5 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 18 h and quenched by the addition of water. 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. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a dense yellow oil (80 mg, 0.26 mmol, 87% yield in this step). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50–1.72 (m, 2H), 1.73–1.88 (m, 2H), 2.32–2.62 (m, 3H), 2.80–2.93 (m, 1H), 3.60–380 (m, 3H), 6.82 (s, 1H), 6.84 (d, J = 8.5 Hz, 1H), 7.16 (t, J = 5.7 Hz, 1H), 7.28 (d, J = 8.5 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.65 (t, J = 7.6 Hz, 1H), 7.86 (s, 1H), 8.55 (d, J = 4.9 Hz, 1H). MS (ESI) m/z 308 $(M+1)^+$.

5.2.43. *N*-[1-(3-Pyridylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24q). Compound **24q** was prepared from 3-chloromethyl-pyridine in a manner similar to that described for **24p** with a yield of 67% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52–1.65 (m, 2H), 1.70–1.84 (m, 2H), 2.30–2.50 (m, 3H), 2.73–2.85 (1H), 3.50–3.68 (m, 3H), 6.82 (s, 1H), 6.83 (d, J = 8.8 Hz, 1H), 7.23–7.27 (m, 1H), 7.29 (d, J = 8.8 Hz, 1H), 7.70 (br s, 1H), 7.86 (s, 1H), 8.51 (d, J = 4.6 Hz, 1H), 8.56 (s, 1H). MS (ESI) m/z 308 $(M+1)^+$.

5.2.44. *N*-[1-(4-Pyridylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24r). Compound **24r** was prepared from 4-chloromethyl-pyridine in a manner similar to that described for **24p** with a yield of 68% as a dense yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.54–1.67 (m, 2H), 1.74–1.85 (m, 2H), 2.32–2.50 (m, 3H), 2.76–2.87 (m, 1H), 3.54 (dd, J = 14.3, 25.3 Hz, 2H), 3.65 (br s, 1H), 6.83 (s, 1H), 6.84 (d, J = 7.8 Hz, 1H), 7.27 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.1 Hz, 1H), 7.87 (s, 1H), 8.54 (d, J = 5.9 Hz, 2H). MS (ESI) m/z 308 $(M+1)^+$.

5.2.45. *N*-[1-(1*H*-2-Pyrrolylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24s). To a solution of *tert*-butyl 3-(1*H*-5-indazolylamino)-1-piperidinecarboxylate **25** (82 mg, 0.25 mmol) in $CHCl_3$ (1 mL), TFA (1 mL) was added dropwise at rt. The mixture was stirred at rt for 3 h and concentrated. To the residue, pyrrole-2-carboxylic acid (56 mg, 0.50 mmol), HOBT (77 mg, 0.5 mmol), triethylamine (0.2 mL) and dimethylaminopyridine (5 mg) in CH_3CN (1 mL) were added. WSC-HCl (86 mg, 0.5 mmol) was then 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 a solution of the residue in THF (1 mL) was added the BH_3–THF (2.0 mL, 2.0 mmol) at 0 °C. The resulting mixture was stirred at 80 °C for 3 h. After cooling to rt, the reaction mixture was acidified by the addition of 1 N HCl aq and stirred at 80 °C for 1 h. The reaction mixture was basified by the addition of saturated $NaHCO_3$ aq. The mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. The residual oil was subjected to flash chromatography on silica gel, eluting with $CHCl_3$/MeOH (95:5) to give the title compound as a yellow solid (12 mg, 0.04 mmol, 16% yield in three steps). Mp 93 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25–1.58 (m, 2H), 1.64–1.82 (m, 2H), 2.15–2.34 (m, 2H), 2.38–2.48 (m, 1H), 2.70–2.84 (m, 1H), 3.49 (d, J = 15.8 Hz, 2H), 3.63 (s, 1H), 5.96 (s, 1H), 6.03 (s, 1H), 6.67 (s, 1H), 6.74 (s, 1H), 6.75 (d, J = 3.4 Hz, 1H), 7.21 (d, J = 9.5 Hz, 1H), 7.79 (s, 1H). MS (ESI) m/z 296 $(M+1)^+$.

5.2.46. *N*-[1-(1*H*-3-Pyrrolylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24t). Compound **24t** was prepared from pyrrole-3-carboxylic acid in a manner similar to that described for **24s** with a yield of 22% as a yellow solid. Mp 187 °C. ^{1}H NMR (CD_3OD, 400 MHz) δ 1.46–1.65 (m, 4H), 1.73–1.81 (m, 2H), 2.01–2.11 (m, 2H), 2.75–3.00 (m, 1H), 3.25–3.40 (m, 1H), 4.16 (d, J = 13.2 Hz, 2H), 6.22 (s, 2H), 6.64 (s, 2H), 6.84 (d, J = 8.3 Hz, 1H), 7.03 (s, 1H), 7.23 (d, J = 9.0 Hz, 1H), 7.68 (s, 1H). MS (ESI) m/z 296 $(M+1)^+$.

5.2.47. *N*-[1-(2-Thienylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24u). Compound **24u** was prepared from thiophene-2-carboxylic acid in a manner similar to that described for compound **24s** with a yield of 19% as a yellow solid. Mp 136 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52–1.63 (m, 2H), 1.64–1.80 (m, 2H), 1.88–1.97 (m, 1H), 2.35–2.55 (m, 1H), 2.72–2.78 (m, 2H), 3.14–3.24 (m, 2H), 3.56–3.64 (m, 1H), 6.80 (s, 1H), 6.82 (d, J = 8.8 Hz, 1H), 6.91 (dd, J = 3.4, 5.1 Hz, 1H), 7.21 (d, J = 5.1 Hz, 1H), 7.27 (d, J = 8.8 Hz, 1H), 7.84 (s, 1H). MS (ESI) m/z 313 $(M+1)^+$.

5.2.48. *N*-[1-(3-Thienylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24v). Compound **24v** was prepared from thiophene-3-carboxylic acid in a manner similar to that described for **24s** with a yield of 23% as a yellow solid. Mp 149 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.48–1.65 (m, 2H), 1.67–1.80 (m, 2H), 2.24–2.50 (m, 3H), 2.68–2.84 (m, 1H), 3.49–3.64 (m, 3H), 6.80 (s, 1H), 6.79–6.83 (m, 1H), 7.05 (d, J = 4.4 Hz, 1H), 7.10 (s, 1H), 7.25 (s, 1H), 7.27 (d, J = 6.8 Hz, 1H), 7.85 (s, 1H). MS (ESI) m/z 313 $(M+1)^+$.

5.2.49. *N*-[1-(2-Furylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24w). Compound **24w** was prepared from furan-2-carboxylic acid in a manner similar to that described for **24s** with a yield of 4% as a yellow solid. Mp 167 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40–1.52 (m, 1H), 1.54–1.66 (m, 1H), 1.68–1.84 (m, 2H), 1.98–2.30 (m, 1H), 2.30–2.40 (m, 1H), 2.46–2.60 (m, 1H), 2.80–2.94 (m, 1H), 3.57 (d, J =12.9 Hz, 2H), 3.54–3.62 (m, 1H), 6.17 (d, J = 2.9 Hz, 1H), 6.29 (dd, J = 1.2, 3.2 Hz, 1H), 6.78–6.83 (m, 2H), 7.27 (d, J = 9.5 Hz, 1H), 7.36 (s, 1H), 7.84 (s, 1H). MS (ESI) m/z 297 $(M+1)^+$.

5.2.50. *N*-[1-(3-Furylmethyl)-3-piperidyl]-*N*-(1*H*-5-indazolyl)amine (24x). Compound **24x** was prepared from furan-3-carboxylic acid in a manner similar to that described for **24s** with a yield of 24% as a yellow solid. Mp 149 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45–1.65 (m, 2H), 1.67–1.80 (m, 2H), 2.22–2.52 (m, 3H), 2.70–

2.84 (m, 1H), 3.41 (d, $J = 10.3$ Hz, 2H), 3.55–3.64 (m, 1H), 6.38 (s, 1H), 6.80 (s, 1H), 6.81 (d, $J = 3.9$ Hz, 1H), 7.27 (d, $J = 8.8$ Hz, 1H), 7.31 (s, 1H), 7.36 (s, 1H), 7.85 (s, 1H). MS (ESI) m/z 297 $(M+1)^+$.

5.2.51. *tert*-Butyl 3-(1*H*-5-indazolylamino)-1-piperidinecarboxylate (25). To a mixture of 3-hydroxypiperidine (1.01 g, 10 mmol) in 3 N NaOH aq (10 mL), a solution of di-*tert*-butyl dicarbonate (2.40 g, 11 mmol) in THF (10 mL) was added dropwise at 0 °C. The reaction mixture was stirred at rt for 1 h and evaporated to remove the THF. The aqueous layer was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then concentrated. To a mixture of the residue and triethylamine (2 mL) in anhydrous DMSO (10 mL), the sulfur trioxide–trimethylamine complex (4.44 g, 20 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 a mixture of the residue, 5-amino-1*H*-indazole **2** (0.98 g, 7.4 mmol), and AcOH (0. 2 mL) in MeOH (10 mL), the BH_3–pyridine complex (1.0 mL, 10 mmol) was slowly added at 0 °C. The reaction mixture was stirred at rt for 18 h. The reaction was quenched by the addition of saturated $NaHCO_3$ aq. The mixture was extracted with $CHCl_3$. The combined organic layer was dried over anhydrous Na_2SO_4 and filtered. The filtrate was then 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 (2.30 g, 7.20 mmol, 72% yield in three steps). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H), 1.83–2.02 (m, 2H), 2.15–2.25 (m, 1H), 3.30–3.56 (m, 4H), 3.98–4.10 (m, 1H), 4.40–4.46 (m, 1H), 6.77–6.81 (m, 2H), 7.30 (d, $J = 8.3$ Hz, 1H), 7.88 (s, 1H).

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References and notes

1. (a) Fukata, Y.; Amano, M.; Kaibuchi, K. *Trends Pharmacol. Sci.* **2001**, 22; (b) Matsui, T.; Amano, M.; Yamamoto, T.; Chihara, K.; Nakafuku, M.; Ito, M.; Nakano, T.; Okawa, K.; Iwamatsu, A.; Kaibuchi, K. *EMBO J.* **1996**, *15*, 2208; (c) Ishizaki, T.; Maekawa, M.; Fujisawa, K.; Okawa, K.; Iwamatsu, A.; Fujita, A.; Watanabe, N.; Saito, Y.; Kakizuka, A.; Morii, N.; Narumiya, S. *EMBO J.* **1996**, *15*, 1885.
2. (a) Saito, H.; Minamiya, Y.; Saito, S.; Ogawa, J. *J. Leuk. Biol.* **2002**, *72*, 829; (b) Liu, L.; Schwartz, B. R.; Lin, N.; Winn, R. K.; Harlan, J. M. *J. Immunol.* **2002**, *169*, 2330; (c) Hauert, A. B.; Martinelli, S.; Marone, C.; Niggli, V. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 838; (d) Ashida, N.; Arai, H.; Yamasaki, M.; Kita, T. *J. Biol. Chem.* **2001**, *276*, 16555; (e) Niggli, V. *FEBS Lett.* **1999**, *445*, 69.
3. (a) Masumoto, A.; Mohri, M.; Shimokawa, H.; Urakami, L.; Usui, M.; Takeshita, A. *Circulation* **2002**, *105*, 1545; (b) Shimokawa, H. *J. Cardiovasc. Pharmacol.* **2002**, *39*, 319.
4. (a) Satoh, S.; Suzuki, Y.; Harada, T.; Ikegaki, I.; Asano, T.; Shibuya, M.; Sugita, K.; Hidaka, H. *Eur. J. Pharmacol.* **1992**, *220*, 243; (b) Shibuya, M.; Suzuki, Y.; Sugita, K.; Saito, I.; Sasaki, T.; Takakura, K.; Nagata, I.; Kikuchi, H.; Takemae, T.; Hidaka, H.; Nakashima, M. *J. Neurosurg.* **1992**, *76*, 571.
5. Tsutiya, M.; Asano, T.; Sako, K.; Yonemasu, Y. *Jpn. Pharmacol. Ther*. **1992**, Suppl. 20, s1525. and references cited therein.
6. (a) Uehata, M.; Ishizaki, T.; Satoh, H.; Ono, T.; Kawahara, T.; Morishita, T.; Tamakawa, H.; Yamagami, K.; Inui, J.; Maekawa, M.; Narumiya, S. *Nature* **1997**, *389*, 990; (b) Itoh, K.; Yoshioka, K.; Akedo, H.; Uehata, M.; Ishizaki, T.; Narumiya, S. *Nat. Med.* **1999**, *5*, 221; (c) Ishizaki, T.; Uehata, M.; Tamechika, I.; Keel, J.; Nonomura, K.; Maekawa, M.; Narumiya, S. *Am. Soc. Pharmacol. Exp. Ther.* **2000**, *57*, 976; (d) Narumiya, S.; Ishizaki, T.; Uehata, M. *Methods Enzymol.* **2000**, *325*, 273.
7. Takami, A.; Iwakubo, M.; Okada, Y.; Kawata, T.; Odai, H.; Takahasi, N.; Shindo, K.; Kimura, K.; Tagami, Y.; Miyake, M.; Fukushima, K.; Inagaki, M.; Amano, M.; Kaibuchi, K.; Iijima, H. *Bioorg. Med. Chem.* **2004**, *12*, 2115.
8. Crutius Thornton, T. J. *Synthesis* **1990**, 295.
9. Ruchardt, C.; Hassmann, V. *Liebigs Ann. Chem.* **1980**, 908.
10. Mitsunobu, O. *Synthesis* **1981**, 1.
11. Ohashi, H.; Takei, M.; Ide, Y.; Ishii, H.; Kita, H.; Gleich, G. J.; Ishikawa, M.; Fukamachi, H. *Int. Arch. Allergy Immunol.* **1999**, *118*, 44.
12. Morikawa, A.; Sone, T.; Asano, T. *Chem. Pharm. Bull.* **1992**, *40*, 770.
13. Adachi, T.; Vita, R.; Sannohe, S.; Stafford, S.; Alam, R.; Kayaba, H.; Chihara, J. *J. Immunol.* **2000**, *167*, 4609.
14. (a) Nagarathnam, D.; Asgari, D.; Shao, J.; Liu, X.-G.; Khire, U.; Wang, C.; Hart, B.; Boyer, S.; Weber, O.; Lynch, M.; Bankston, D. WO 02/076976 A2; (b) Imazaki, N.; Kitano, M.; Ohashi, N.; Matsui, K. WO 02/100833 A1; (c) Hu, E.; Lee, D. *Expert Opin. Ther. Targets* **2005**, *9*, 715; (d) Mueller, B. K.; Mack, H.; Teush, N. *Nat. Rev. Drug Discovery* **2005**, *4*, 387.