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Article

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Discovery of novel pyrazole-based selective aldosterone synthase (CYP11B2) inhibitors: a new template to coordinate the heme-iron motif of CYP11B2

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Sohyaku, Innovative Research Division, Mitsubishi Tanabe Pharma Corporation, 2-2-50, Kawagishi, Toda, Saitama, 335-8505, Japan

ABSTRACT

It is necessary for aldosterone synthase (CYP11B2) inhibitors to have both high potency and high selectivity over 11β-hydroxylase (CYP11B1), a critical enzyme for cortisol synthesis. Previous studies have reported a number of CYP11B2 inhibitors, most of which have an imidazole or pyridine ring to coordinate the heme-iron motif of CYP11B2; however, highly selective inhibitors of human CYP11B2 are still needed. To expand the selectivity in humans, we

explored alternative templates and found that pyrazoles were suitable templates for CYP11B2 inhibitors. Investigation of pyrazoles, especially *N*-alkyl pyrazoles, as a new template to coordinate the heme-iron motif led to a potent and highly selective CYP11B2 inhibitor **28** with an aldosterone-lowering effect at 1 mg/kg dosing in cynomolgus monkeys.

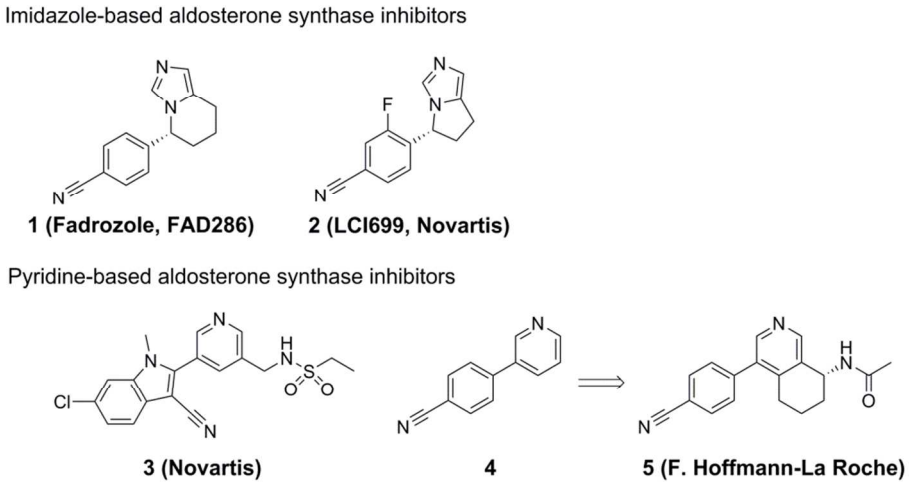
Introduction

Aldosterone is an endogenous ligand of mineralocorticoid receptors and is a key component of the rennin-angiotensin-aldosterone system. Aldosterone is primarily produced in the zona glomerulosa of the adrenal cortex and plays a vital role in the regulation of fluid and electrolyte homeostasis. Recent studies suggest that aldosterone is not only an exacerbation factor of hypertension but also a risk factor for cardiovascular disorders.[1] Aldosterone synthase (CYP11B2) is known to mediate the last three steps of aldosterone synthesis from 11-deoxycorticosterone to aldosterone, and plasma aldosterone levels are regulated by this enzymatic activity at the adrenal cortex.[2] Therefore, inhibition of CYP11B2 may be an attractive approach to the treatment of aldosterone related diseases by lowering plasma aldosterone levels. However, human CYP11B2 is 93% homologous to the 11β-hydroxylase (CYP11B1), an enzyme essential for cortisol synthesis, and this high homological identity has made it challenging to discover selective inhibitors of CYP11B2 over CYP11B1.[3]

CYP11B2 contains a heme-iron motif at its active site.[4] All nonsteroidal CYP11B2 inhibitors have an sp2-hybridized nitrogen atom to coordinate the active site heme-iron motif.[5] The crystal structure of CYP11B2 in complex with FAD286 (**1**, Figure 1) (PDB ID: 4FDH), the R-enantiomer of the aromatase (CYP19A1) inhibitor Fadrozole, revealed the interaction between the imidazole nitrogen atom of FAD286 (**1**) and the heme-iron motif.[4,6] LCI-699 (**2**, Figure 1),

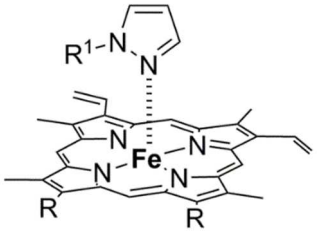
the first CYP11B2 inhibitor investigated in humans, also has an imidazole structure. Administration of LCI-699 (**2**) to healthy humans and patients with primary aldosteronism, essential hypertension, and resistant hypertension significantly reduced the level of aldosterone in both plasma and urine.[7] However, the selectivity over CYP11B1 was not enough to avoid impairment of cortisol biosynthesis. Since LCI-699 (**2**) entered clinical studies, additional studies have reported a variety of CYP11B2 inhibitors to improve selectivity over CYP11B1. As shown in Figure 1, most of the reported inhibitors have one of two templates to coordinate the heme-iron motif: imidazole-based inhibitors[8] like FAD286 (**1**) and LCI-699 (**2**), or pyridine-based inhibitors[9] including tetrahydroisoquinoline-based inhibitors derived from substituted pyridine structures. Among a number of reported pyridine-based inhibitors, compound **3** reported by Novartis displayed high potency and selectivity over CYP11B1 and is in phase 1 clinical trials. Hoffman-la-Roche also investigated pyridine-based inhibitors, represented by compound **4**, which have potent CYP11B2 inhibitory activity but poor selectivity over CYP11B1. Based on a homology model of CYP11B2 and CYP11B1, optimization of the 5,6,7,8-tetrahydroisoquinoline ring led to the discovery of a potent CYP11B2 inhibitor **5**, which has high selectivity over CYP11B1 in cynomolgus monkeys. A further optimized tetrahydroisoquinoline-based inhibitor is in phase 1 clinical trials.[10] The pyridine-based inhibitors **3** and **5** are potent and considerably selective over CYP11B1 in cynomolgus monkeys, but less selective in humans. To expand the selectivity in humans, we explored alternative templates other than imidazoles and pyridines and found that pyrazole was a suitable template for CYP11B2 inhibitors.

Figure 1. Structures of representative aldosterone synthase inhibitors **1**−**5**.



Because pyrazole is a *N*-containing heterocycle having different physicochemical properties compared with imidazole and pyridine, including weak basicity and hydrogen-bond acceptor ability,[11] and has been applied to potent scaffolds in medicinal chemistry,[12] we investigated it as a template to coordinate the heme-iron motif of CYP11B2.[13] In a study of CYP2E1 metabolism, Jones *et al*. experimentally determined the inhibition constant of *N*-containing heterocycles for CYP2E1, and revealed that 1*H*-pyrazole derivatives bind tightly to the heme-iron motif.[14] Meanwhile, *N*-alkyl pyrazoles have a substituent ortho to the nitrogen that could coordinate the heme-iron motif (Figure 2), but few studies have been reported on CYP11B2 inhibitors having an ortho substituent.[15] Previous studies evaluating CYP-targeted inhibitors have reported that introduction of a substituent ortho to the nitrogen effectively improves selectivity over human drug-metabolizing CYP enzymes and other metalloenzymes.[16] We found that pyrazoles, especially *N*-alkyl pyrazoles, were effective new templates to coordinate the heme-iron motif, were suitable for CYP11B2 inhibition, and discovered potent and highly selective CYP11B2 inhibitors.

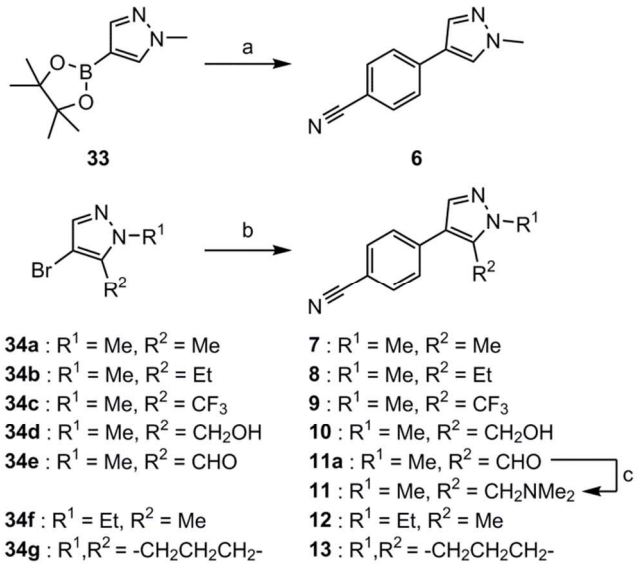
Figure 2. Illustration of the *N*-alkyl pyrazoles coordinating to the heme-iron motif.



Chemistry

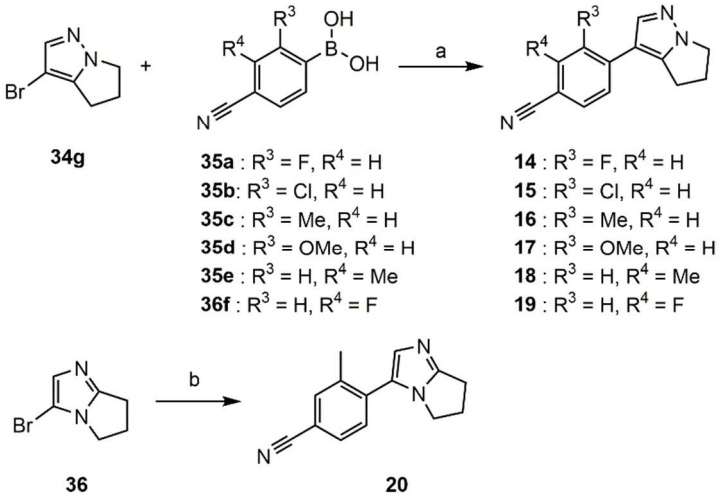
The synthesis of a series of *N*-alkyl pyrazole compounds **6**−**13** is shown in Scheme 1. Commercially available *N*-methylpyrazole-4-boronic acid pinacol ester (**33**) was reacted with 4-bromobenzonitrile under Suzuki-Miyaura coupling conditions using a microwave reactor to produce compound **6**. Similarly, Suzuki-Miyaura coupling of 4-cyanophenylboronic acid with various 1,5-disubstituted 4-bromopyrazoles **34a**−**34g** produced compounds **7**−**10**, **11a**, **12** and **13**, respectively. Reductive amination of aldehyde **11a** with dimethylamine produced compound **11**. Tri substituted benzene compounds **14**−**19** and imidazole compound **20** were also prepared under Suzuki-Miyaura conditions (Scheme 2).

Scheme 1. Synthesis of aldosterone synthase inhibitors **6**−**13**.



Reagents and conditions: (a) 4-bromobenzonitrile, $Pd(PPh_3)_4$, $NaHCO_3$, DME, H_2O, reflux 5 h, 78%; (b) 4-cyanophenylboronic acid, $Pd(OAc)_2$, 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl, K_2CO_3, MeCN, H_2O, MW, 100 °C, 30 min or 4-cyanophenylboronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane, H_2O, MW, 150 °C, 10 min, 57%−75%; (c) dimethylamine, $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 18 h, 67%.

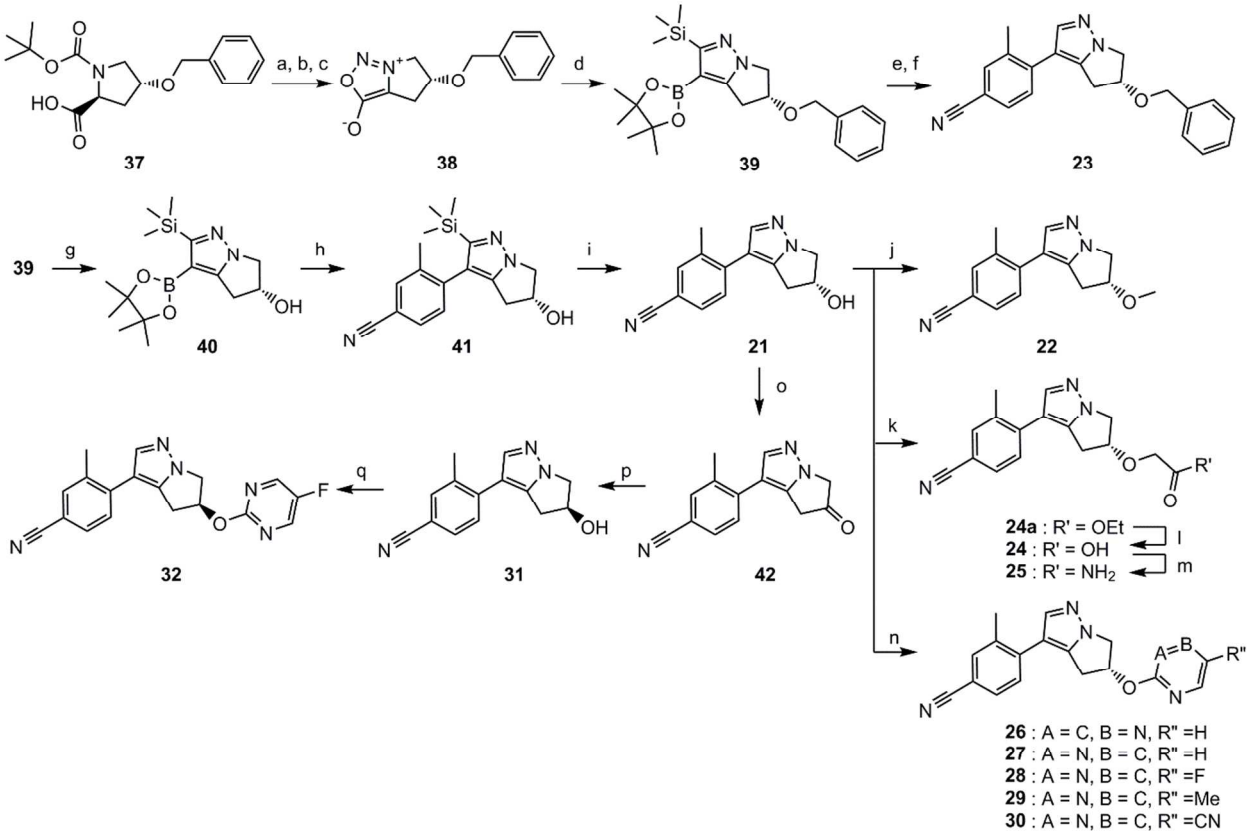
Scheme 2. Synthesis of aldosterone synthase inhibitors **14**−**20**.



Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane, H_2O, MW, 100 °C or 150 °C, 10 min, 52%−95%; (b) (4-cyano-2-methyl-phenyl)boronic acid, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane, H_2O, MW, 150 °C, 10 min, 73%.

A series of C-5 substituted 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole compounds was synthesized using the withasomnine alkaloid methodology (Scheme 3).[17] Sydnone (**38**) was synthesized in three steps from enantiomerically-pure protected hydroxyproline **37**.[17] Deprotection of the *N*-Boc group of hydroxyproline and *N*-nitrosation, followed by cyclodehydration with trifluoroacetic anhydride, produced compound **38**. Product **39** was synthesized by regioselective cycloaddition of trimethylsilyl-substituted alkynylboronate with sydnone (**38**).[17] Suzuki-Miyaura coupling of compound **39** with 4-bromo-3-methyl-benzonitrile, followed by removal of the trimethylsilyl group, produced target compound **23**. After removal of the benzyl group from **39**, Suzuki-Miyaura coupling of compound **40** with an aryl bromide, followed by desilylation of **41,** produced compound **21**. Compound **21** was treated with methyl iodide to produce compound **22**. O-alkylation of alcohol **21** with ethyl chloroacetate, followed by hydrolysis of ester, produced compound **24**, and condensation of **24** and ammonia produced compound **25**. Nucleophilic aromatic substitution of either 2-chloro-pyrazine or 2-chloro-pyrimidines with compound **21** produced target compounds **26**−**30**. A racemic alcohol was synthesized by oxidation of alcohol **21** with (*R*)-configuration, followed by reduction of the resulting ketone **42**. Alcohol **31** with (*S*)-configuration was separated using chiral chromatography of the racemic alcohol. Compound **32** was synthesized by nucleophilic aromatic substitution of 2-chloro-5-fluoro-pyrimidine with alcohol **31**.

Scheme 3. Synthesis of aldosterone synthase inhibitors **21**−**32**.



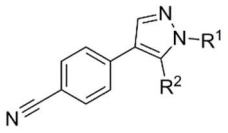
Reagents and conditions: (a) TFA, $CHCl_3$, rt, 16 h; (b) $NaNO_2$, AcOH, rt, 15 h; (c) TFAA, THF, rt, 15 h, 55% in 3 steps; (d) trimethyl-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethynyl]silane, 1,2-dichlorobenzene, 185 °C, 21 h, 53%; (e) 4-bromo-3-methyl-benzonitrile, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,4-dioxane, H_2O, MW, 150 °C, 15 min; (f) TBAF in THF, THF, rt to 70 °C, 3 days, 71% in 2 steps; (g) H_2, $Pd(OH)_2/C$, EtOH, rt, 16 h, 76%; (h) 4-bromo-3-methyl-benzonitrile, $PdCl_2(PPh_3)_2$, Na_2CO_3, DME, H_2O, 80 °C, 15 h, 56%; (i) TBAF in THF, THF, 80 °C, 17 h, 76%; (j) MeI, NaH, DMF, rt, 4 h, 45%; (k) ethyl chloro acetate, NaH, THF, 0 °C to rt, 2 h, 50%; (l) NaOH aq., THF, EtOH, rt, 1.5 h, 100%; (m) NH_3 aq., WSC, N-hydroxylsuccinimide, chloroform, rt, 40 min, 70%; (n) ArCl, NaH, DMF, and/or THF, rt, 1–16 h, 29%−91%; (o) 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one, $CHCl_3$, rt, 4 h, 73%; (p) $NaBH_4$, MeOH, rt, 4 h, then Chiralpak ID column (n-hexane/propan-2-ol/THF/diethyl amine, 70/15/15/0.1), 45%; (q) 2-chloro-5-fluoro-pyrimidine, NaH, THF, rt, 2.5 h,100%.

Results and Discussion

To begin our investigation of new CYP11B2 inhibitors featuring a pyrazole template to coordinate the heme-iron motif, we replaced the pyridine moiety of compound **4** with various *N*-alkyl pyrazoles while keeping the benzonitrile ring structure common to imidazole-based

CYP11B2 inhibitors, such as FAD286 and LCI-699. *In vitro* hCYP11B2 and hCYP11B1 inhibitory activity and selectivity factor (the ratio between the IC_{50} values against CYP11B2 and CYP11B1) are shown in Table 1. Unfortunately, 1-methylpyrazole (**6**) had no CYP11B2 inhibitory activity. However, compound **7**, obtained by introduction of a methyl group at the C-5 position of the pyrazole moiety of compound **6**, showed a potency of CYP11B2 IC_{50} = 290 nM. Improvements in potency were observed with the ethyl (**8**) group and trifluoromethyl (**9**) group at the C-5 position, while introduction of hydrophilic substituents, such as the hydroxymethyl (**10**) group and dimethylaminomethyl (**11**) group, resulted in the loss of CYP11B2 inhibitory activity. Moreover, further enlargement of the methyl group at the N-1 position of the pyrazole ring to an ethyl group produced compound **12** (CYP11B2 IC_{50} = 72 nM), which demonstrated improved potency. These increases in CYP11B2 inhibitory activities following the introduction of alkyl groups at the N-1 and C-5 positions were due to hydrophobic interactions between the alkyl groups and the active site cavity of CYP11B2, and previous reports that state that the active site cavity of CYP11B2 is lined with nonpolar and aromatic residues favorable for binding steroid substrates support this observation.[4] However, compounds **7**−**10** and **12** showed no CYP11B1 inhibitory activities. We anticipated that incorporation of a cyclic ring structure at the 1,5-position of the pyrazole ring would introduce favorable conformational constraint for interaction with the CYP11B2 cavity. In fact, the cyclized compound, 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (**13**), showed dramatically improved potency (CYP11B2 IC_{50} = 13 nM) and high selectivity over CYP11B1 (Selectivity Factor = 440).

Table 1. Structure-Activity Relationship (SAR) Data for Pyrazole Derivatives **6**–**13**.



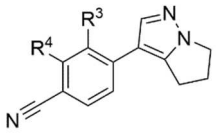
6-13

compd	R^1	R^2	hCYP11B2 IC_{50} (nM)	hCYP11B1 IC_{50} (nM)	Selectivity Factor	Microsomal metabolic stability (h)/(mky) (%)[a]
6	Me	H	>1000	NT		NT
7	Me	Me	290	>10000	>35	62/42
8	Me	Et	150	>10000	>67	73/21
9	Me	CF_3	280	>10000	>36	NT/0.9
10	Me	CH_2OH	>1000	>10000		104/90
11	Me	CH_2NMe_2	>1000	NT		90/54
12	Et	Me	72	>10000	>140	43/10
13	-$CH_2CH_2CH_2$-		13	5700	440	12/3
LCI-699 **(2)**			1.2	22	18	NT

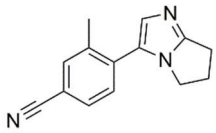
[a]The percentage of the remaining substrate after 20 min incubation with NADPH is calculated from its concentration after 20 min divided by that at incubation time zero. h = human, mky = cynomolgus monkey.

The substituent effects of the benzene ring are listed in Table 2. Introducing a halogen group, such as fluoro (**14**) or chloro (**15**), to the benzene ring at the meta position with respect to the nitrile group enhanced the CYP11B2 inhibitory activities. Furthermore, introduction of a methyl group at the meta position with respect to the nitrile group led to the most potent compound **16** (CYP11B2 IC_{50} = 3.7 nM), whereas a methoxy (**17**) group decreased the CYP11B2 inhibitory activities. Introduction of a methyl (**18**) or fluoro (**19**) group at the ortho position with respect to the nitrile group led to decreased CYP11B2 inhibitory activities in comparison with substituents at the meta position.

Table 2. Structure-Activity Relationship (SAR) Data for Pyrazole Derivatives **13**−**19** and Imidazole Derivative **20**.



13-19



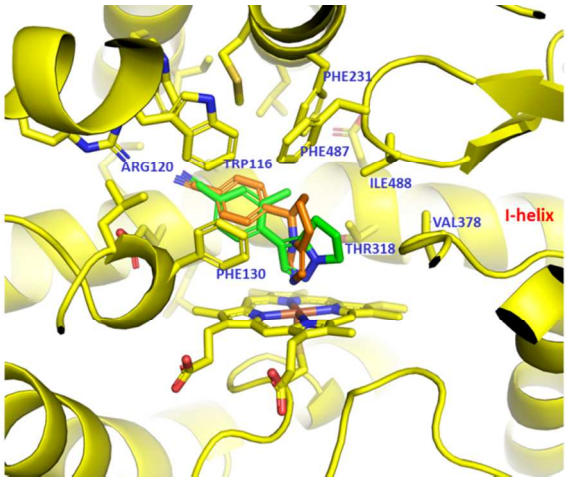
20

compd	R^3	R^4	hCYP11B2 IC_{50} (nM)	hCYP11B1 IC_{50} (nM)	Selectivity Factor	Microsomal metabolic stability (h)/(mky) (%)[a]
13	H	H	13	5700	440	12/3
14	F	H	9.2	1200	130	9/0.4
15	Cl	H	5.4	480	89	10/0.3
16	Me	H	3.7	350	95	22/10
17	OMe	H	34	2200	65	4/0.4
18	H	Me	6.7	590	88	0.4/0.3
19	H	F	26	2100	81	1/0.4
20			2.5	58	23	75/75

[a]The percentage of the remaining substrate after 20 min incubation with NADPH is calculated from its concentration after 20 min divided by that at incubation time zero. h = human, mky = cynomolgus monkey.

To understand the structure-activity relationships (SAR) of these inhibitors, compound **16** was docked into human CYP11B2 using GOLD[18] (Figure 3). The docking study revealed that the nitrogen of the 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole moiety was directly coordinated to the heme iron and that the benzonitrile ring engaged in aromatic interaction with the Trp116 side chain, occupying a similar position to that of FAD286. The methyl group at the meta position with respect to the nitrile group contributed to the stabilization of the twisted form between the pyrazole ring and the benzene ring, and interacted with the side chains of Phe231 and Thr318.These interactions would explain the reason for which compound **16** was 3.5 times as potent as compound **13**. The hydrophobic interaction of the dihydropyrrole moiety with the Ile488 and Thr318 side chains may also contribute to potency improvement.

Figure 3. Overlay of the compound **16** docking model (in green) and the crystal structure of CYP11B2 (in yellow) in complex with FAD286 (in orange) (PDB ID: 4FDH).

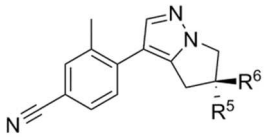


Replacement of the pyrazole moiety with an imidazole moiety (**20**) led to an increase in CYP11B2 inhibitory activity but decreased selectivity over CYP11B1 (Selectivity Factor = 23, Table 2). Compound **16** has a fused structure similar to that of imidazole-based inhibitor LCI-699, with a 6,7-dihydro-5H-pyrrolo[1,2-c]imidazole moiety, and pyridine-based inhibitor **5**, with a 5,6,7,8-tetrahydroisoquinoline moiety. Compared with these inhibitors, pyrazole-based inhibitor **16** had high selectivity over CYP11B1. From the above, it is presumed that the electronic character and steric effect of 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole could attenuate the magnitude of the interaction between pyrazole and the heme-iron motif. It is thought that CYP11B2 inhibitory activity is improved through the increased magnitude of the CYP11B2-specific hydrophobic interactions, which may also lead to improved selectivity over CYP11B1. And our results may be consistent with studies of fugal CYP51 inhibitors suggesting that the magnitude of the Fe-N interaction could be tuned by modulating the heterocycles' electronic character and that weaker coordination may help minimizing off-target inhibitory activity,[19] and

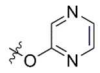
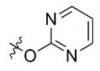
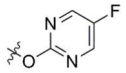
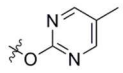
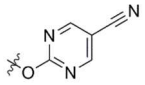
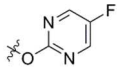
with another study suggesting that introducing a substituent ortho to the nitrogen to coordinate the heme-iron motif would weaken the magnitude of the Fe-N interaction.[16]

We found that compound **16** had inhibitory activity toward CYP11B2, with high potency and selectivity over CYP11B1; however, the metabolic stability of a series of *N*-alkyl pyrazole derivatives **13**−**19** was unsatisfactory for oral dosing. To improve metabolic stability, we attempted to introduce a hydrophilic substituent at the C-5 position of the 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole ring based on the results of the docking study (Figure 3). The introduction of a hydroxyl group with (*R*)-configuration at the C-5 position of 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole produced **21**, with significantly improved metabolic stability, but slightly reduced inhibitory activity (CYP11B2 IC_{50} = 12 nM). Moreover, compound **21** displayed increased selectivity over CYP11B1 (Selectivity Factor = 160) compared with unsubstituted compound **16**.

Table 3. Structure-Activity Relationship (SAR) Data for Pyrazole Derivatives **16**, **21**−**32**.



21-32

compd	R^5	R^6	hCYP11B2 IC_{50} (nM)	hCYP11B1 IC_{50} (nM)	Selectivity Factor	Microsomal metabolic stability (h)/(mky) (%)[a]
16	H	H	3.7	350	95	22/10
21	OH	H	12	1900	160	103/86
22	OMe	H	3.2	<100	<31	28/18
23	OCH_2Ph	H	2.3	<100	<44	56/32
24	OCH_2COOH	H	>1000	NT		101/105
25	OCH_2CONH_2	H	14	410	29	98/94
26		H	2.2	130	59	86/73
27		H	4.0	470	120	96/70
28		H	4.7	1100	230	96/90
29		H	4.2	180	43	85/71
30		H	2.2	<100	<45	100/98
31	H	OH	29	2400	83	NT
32	H		71	>10000	>140	100/91

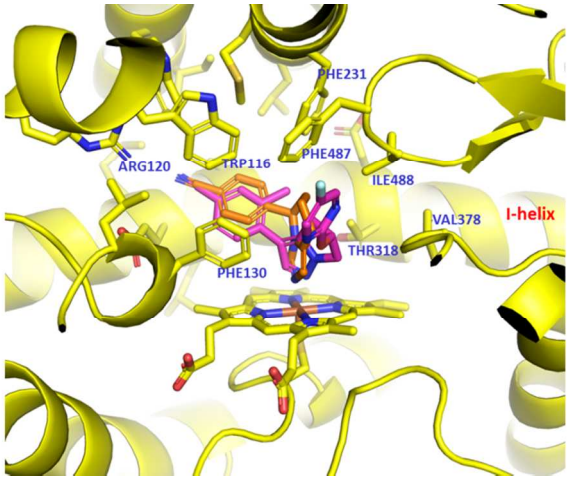
[a]The percentage of the remaining substrate after 20 min incubation with NADPH is calculated from its concentration after 20 min divided by that at incubation time zero. h = human, mky = cynomolgus monkey.

Next, we optimized the hydroxyl group to enhance inhibitory activity and selectivity over CYP11B1. Substitution of the hydroxyl group with a methyl group or benzyl group produced compounds **22** and **23**, respectively, each having improved potency; however, both compounds

showed greatly reduced selectivity over CYP11B1 and reduced metabolic stability in comparison with compound **21**. The introduction of a carboxyl acid moiety (**24**) destroyed the CYP11B2 inhibitory activities, while introduction of carboxamide moiety (**25**) slightly reduced the inhibitory activity. Investigation of heteroaryl ethers, such as pyrazine ether and pyrimidine ether, resulted in compounds **26** and **27** with improved potency. In particular, pyrimidine ether **27** showed more than 100-fold selectivity over CYP11B1 as well as high metabolic stability. Then, we investigated the substituent effect on pyrimidine. The introduction of a fluoro group at the 5-position of the pyrimidine ring (**28**) resulted in a two-fold increase in selectivity over CYP11B1 while maintaining potency compared with the unsubstituted pyrimidine of compound **27**; however, the introduction of methyl and nitrile groups at the 5-position of the pyrimidine ring produced compounds **29** and **30**, respectively, which had high potency but decreased selectivity over CYP11B1. SAR studies demonstrated that 5-fluoro pyrimidine **28** is a well-balanced compound, having good potency and high selectivity. We investigated alcohol **31** and pyrimidine ether **32** with (S)-configuration. However, compounds **31** and **32** showed reduced CYP11B2 inhibitory activities in comparison with corresponding enantiomers **21** and **28**.

Figure 4 shows the docking model of compound **28** in hCYP11B2. The docking study suggested that a substituent with (*R*)-configuration at the C-5 position of the 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole ring was pointed to a large, solvent-exposed binding site. In addition, fluoro pyrimidine moiety forms an additional interaction with Phe487 and Phe130; however, the docking study also suggested that a substituent with the opposite (*S*)-configuration was pointed to a small, limited binding site because of the I-helix, which would explain the reason for which compounds **3**1 and **32** showed reduced CYP1B2 inhibitory activities.

Figure 4. Overlay of the compound **28** docking model (in magenta) and the crystal structure of CYP11B2 (in yellow) in complex with FAD286 (in orange) (PDB ID: 4FDH).



To confirm that this series of pyrazole compounds did not interfere with steroidogenic cytochromes, we investigated the hCYP19A1 inhibitory activities of selected compounds.[20] As shown in Table 4, compounds **21** and **28** exhibited almost no hCYP19A1 inhibitory activity at a concentration of 1 μM.

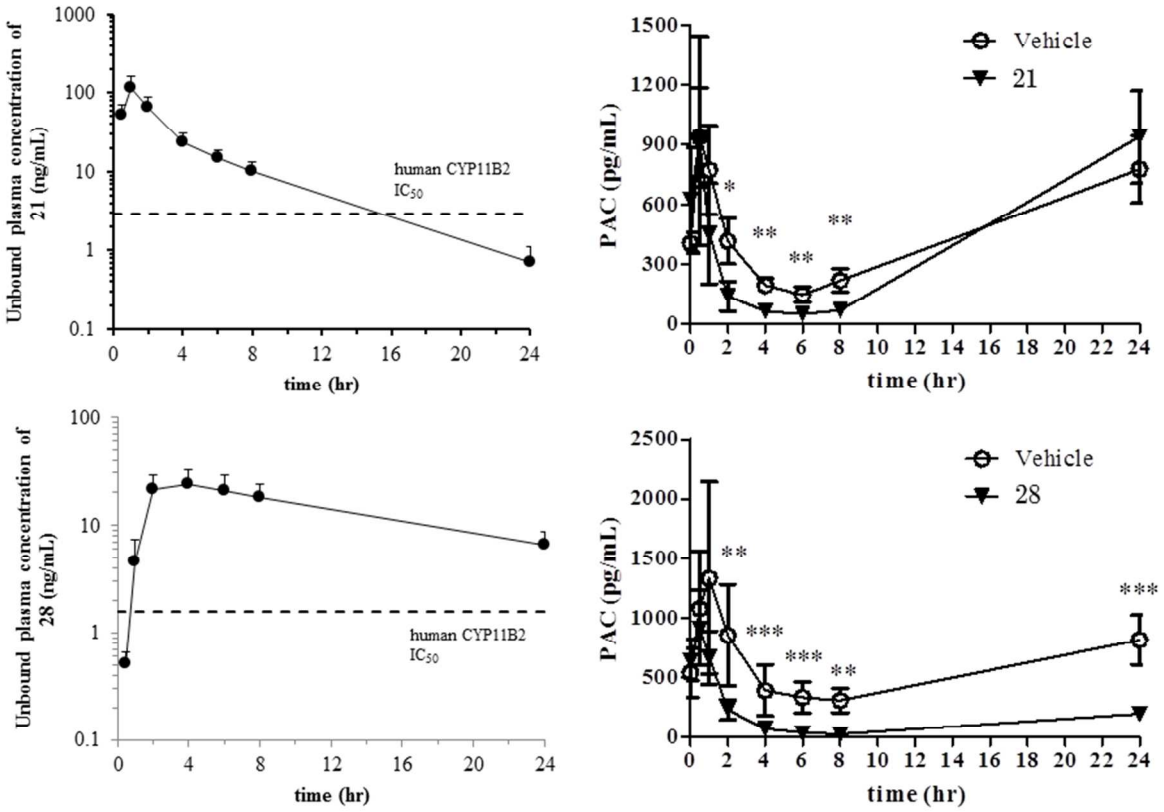
Table 4. hCYP19A1 inhibitory activities of selected compounds.

compd	hCYP19A1 %inhibition at 1 μM
21	1
28	12

From the set of compounds which exhibited potent inhibition of hCYP11B2, compounds **21** and **28** were chosen for the assessment of aldosterone-lowering effects in cynomolgus monkeys because of their good metabolic stability, moderate plasma protein binding (monkey, **21**: 59%, **28**: 87%), and high passive permeability (**21**: $P_{app} = 68.4 \times 10^{-6}$ cm/sec, **28**: $P_{app} = 62.3 \times 10^{-6}$

cm/sec).[21] Time course data of the tested compounds' unbound plasma concentrations and plasma aldosterone concentrations (PAC) after oral administration of a single dose (**21**: 3 mg/kg, **28**: 1 mg/kg) are shown in Figure 5. Good passive permeability and solubility allowed compound **21** to reach a maximum concentration (C_{max}) rapidly after dosing. These concentrations remained higher than the hCYP11B2 IC_{50} for 8 h and dropped below the IC_{50} after 24 h. However, the time to reach C_{max} of compound **28** was 4 h as a median value (ranging 2−24 h), and plasma concentrations of **28** remained higher than hCYP11B2 IC_{50} for 24 h. Consistent with the observed plasma concentrations, the reduction of PAC was observed for 2−8 h after dosing with compound **21** and for 24 h after dosing with compound **28**. Investigation of pyrazole-based CYP11B2 inhibitors following imidazole-based and pyridine-based inhibitors led to compound **28** having good and sustainable in vivo potency. Furthermore, compound **28** achieved improved selectivity over CYP11B1 in humans (Selectivity Factor = 230) in comparison with LCI-699 (Selectivity Factor = 18, Table 1).

Figure 5. Time courses of unbound plasma concentrations of compounds **21** and **28**, and plasma aldosterone concentration (PAC) after oral administration of 3 mg/kg of compound **21** and 1 mg/kg of compound **28**. Data are expressed as mean + SD (unbound plasma concentration of compound) and mean ± S.E.M. (PAC) of six monkeys. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. 0.5% CMC treatment (paired t-test).



The results of the present study demonstrate that pyrazole is a suitable CYP11B2 inhibitor template; it coordinates the CYP11B2 heme-iron motif, similar to inhibitors having imidazole and pyridine templates. Previous studies have improved the activity of selective antifungal drugs by replacing the heme-binding moiety to balance the magnitude of the Fe-N interaction at the

enzyme active site. A similar approach may be applicable to designing potent and selective CYP11B2 inhibitors and other CYP-targeted inhibitors.

Conclusion

A series of pyrazole derivatives was developed and explored as novel CYP11B2 inhibitors following imidazole-based and pyridine-based inhibitors. Optimization of pyrazole substituents produced compounds **16**, which have a 5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole moiety with high potency and selectivity over CYP11B1. Improvement of metabolic stability was achieved by introduction of pyrimidine ether group, and compound **28** exhibited aldosterone-lowering effect at 1 mg/kg dosing in cynomolgus monkeys. The approach to balance the magnitude of the Fe-N interaction may be effective to design selective CYP11B2 inhibitors.

Experimental Section

Materials and reagents. Reagents, starting materials, and solvents were purchased from commercial suppliers and were used as received. Purification of intermediates and final products was carried out using a Purif-α purification system (Shoko Scientific) with prepacked normal-phase SiO_2 or NH-SiO_2 cartridges eluted with optimized gradients of either n-hexane-ethyl acetate or chloroform-methanol as described. The purity was determined by HPLC, measured by an Agilent 1100 system or Shimadzu Nexera system (see Supporting Information), and was >95% for all final compounds. ^{1}H and ^{13}C NMR spectra were measured using a Bruker DPX-300 or AVANCE 400. The NMR signals were expressed in ppm downfield from tetramethylsilane as the internal standard ($\delta = 0$). Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet. The high-resolution mass spectrometry (HRMS)

experiments were performed using a LTQ Orbitrap XL™ mass spectrometer (Thermo Fisher Scientific Inc.). Optical rotations were measured on a SEPA200 (HORIBA).

Synthesis of Inhibitor Compounds

Synthesis of 4-(1-methylpyrazol-4-yl)benzonitrile (6). Tetrakis(triphenylphosphine)palladium (0) (83 mg, 0.0721 mmol), 4-bromobenzonitrile (310 mg, 1.73 mmol), and saturated aqueous $NaHCO_3$ solution (1.5 mL) were added to a solution of 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (300 mg, 1.44 mmol) in 1,2-dimethoxyethane (3.0 mL) at room temperature. After being stirred for 5 h under reflux, the reaction mixture was poured into water and extracted with ethyl acetate. The combined organic phase was washed with saturated brine solution, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 90:10 to 80:20) to yield compound **6** (205 mg, 1.12 mmol, 78%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.80 (s, 1H), 7.68 (s, 1H), 7.60–7.65 (m, 2H), 7.51–7.57 (m, 2H), 3.96 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 137.4, 137.1, 132.8, 127.7, 125.7, 121.6, 119.0, 109.6, 39.3. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{11}H_{10}N_3$, 184.08692; found 184.08700.

Synthesis of 4-(1,5-dimethylpyrazol-4-yl)benzonitrile (7). Potassium carbonate (477 mg, 3.45 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (62 mg, 0.150 mmol), and palladium acetate (17 mg, 0.075 mmol) were added to a solution of 4-bromo-1,5-dimethyl-pyrazole (262 mg, 1.50 mmol) and 4-cyanophenylboronic acid (441 mg, 3.0 mmol) in acetonitrile (2.3 mL) and water (1.5 mL) at room temperature. The mixture was subjected to microwave irradiation for 30 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate,

65:35 to 35:65) to yield the crude product. Trituration with isopropyl ether, followed by filtration, yielded compound **7** (203 mg, 1.03 mmol, 69%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.64−7.70 (m, 2H), 7.59 (s, 1H), 7.42−7.48 (m, 2H), 3.86 (s, 3H), 2.41 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 138.9, 137.3, 135.8, 132.5, 127.8, 119.3, 119.1, 109.5, 36.7, 10.6. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{12}H_{12}N_3$, 198.10257; found 198.10270.

Synthesis of 4-(5-ethyl-1-methyl-pyrazol-4-yl)benzonitrile (8). Potassium carbonate (477 mg, 3.45 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (62 mg, 0.150 mmol), and palladium acetate (17 mg, 0.075 mmol) were added to a solution of 4-bromo-5-ethyl-1-methyl-pyrazole (284 mg, 1.50 mmol) and 4-cyanophenylboronic acid (441 mg, 3.0 mmol) in acetonitrile (2.3 mL) and water (1.5 mL) at room temperature. The mixture was subjected to microwave irradiation for 30 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 75:25 to 50:50) to yield the crude product. Trituration with isopropyl ether, followed by filtration, yielded compound **8** (187 mg, 1.01 mmol, 69%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.64−7.68 (m, 2H), 7.58 (s, 1H), 7.42−7.46 (m, 2H), 3.88 (s, 3H), 2.79 (q, J = 7.4 Hz, 2H), 1.26 (t, J = 7.4 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 141.4, 139.0, 137.5, 132.6, 127.8, 119.1, 118.7, 109.6, 36.5, 17.8, 13.5. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{13}H_{14}N_3$, 212.11822; found 212.11822.

Synthesis of 4-[1-methyl-5-(trifluoromethyl)pyrazol-4-yl]benzonitrile (9). Potassium carbonate (477 mg, 3.45 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (62 mg, 0.150 mmol), and palladium acetate (17 mg, 0.075 mmol) were added to a solution of 4-bromo-1-methyl-5-(trifluoromethyl)pyrazole (344 mg, 1.50 mmol) and 4-cyanophenylboronic acid (441

mg, 3.0 mmol) in acetonitrile (2.3 mL) and water (1.5 mL) at room temperature. The mixture was subjected to microwave irradiation for 30 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by NH-silica gel chromatography (n-hexane/ethyl acetate, 90:10) to yield compound **9** (211 mg, 0.84 mmol, 57%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65–7.70 (m, 2H), 7.53 (s, 1H), 7.42–7.48 (m, 2H), 4.08 (q, J = 1.4 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 138.6, 135.8, 132.2, 129.9, 128.1 ($J_{C\text{-}F}$ = 38 Hz), 123.4, 121.7 ($J_{C\text{-}F}$ = 268 Hz), 118.6, 111.8, 39.2 ($J_{C\text{-}F}$ = 2.2 Hz). HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{12}H_9N_3F_3$, 252.07431; found 252.07452.

Synthesis of 4-[5-(hydroxymethyl)-1-methyl-pyrazol-4-yl]benzonitrile (10). Potassium carbonate (477 mg, 3.45 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (62 mg, 0.150 mmol), and palladium acetate (17 mg, 0.075 mmol) were added to a solution of (4-bromo-2-methyl-pyrazol-3-yl)methanol (287 mg, 1.50 mmol) and 4-cyanophenylboronic acid (441 mg, 3.0 mmol) in acetonitrile (2.3 mL) and water (1.5 mL) at room temperature. The mixture was subjected to microwave irradiation for 30 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 97:3) to yield the crude product. Trituration with isopropyl ether, followed by filtration, yielded compound **10** (184 mg, 0.86 mmol, 59%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.65–7.70 (m, 2H), 7.60 (s, 1H), 7.50–7.54 (m, 2H), 4.76 (d, J = 4.4 Hz, 2H), 4.00 (s, 3H), 2.06 (t, J = 4.4 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 137.9, 137.7, 137.3, 132.6, 128.4, 121.1, 118.9, 110.2, 53.6, 37.0. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{12}H_{12}ON_3$, 214.09749; found 214.09747.

Synthesis of 4-(5-formyl-1-methyl-pyrazol-4-yl)benzonitrile (11a). Potassium carbonate (477 mg, 3.45 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (62 mg, 0.150 mmol), and palladium acetate (17 mg, 0.075 mmol) were added to a solution of 4-bromo-2-methyl-pyrazole-3-carbaldehyde (284 mg, 1.50 mmol) and 4-cyanophenylboronic acid (441 mg, 3.0 mmol) in acetonitrile (2.3 mL) and water (1.5 mL) at room temperature. The mixture was subjected to microwave irradiation for 30 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 85:15 to 75:25) to yield the crude product. Trituration with isopropyl ether, followed by filtration, yielded compound **11a** (235 mg, 1.11 mmol, 75%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.90 (s, 1H), 7.71–7.81 (m, 2H), 7.66 (s, 1H), 7.51–7.57 (m, 2H), 4.26 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 180.0, 137.7, 135.4, 134.8, 132.7, 129.7, 129.2, 118.4, 112.0, 40.3. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{12}H_{10}ON_3$, 212.08184; found 212.08199.

Synthesis of 4-[5-[(dimethylamino)methyl]-1-methyl-pyrazol-4-yl]benzonitrile (11). Sodium triacetoxyborohydride (90 mg, 0.426 mmol) was added to a solution of compound **11a** (30 mg, 0.142 mmol) and dimethylamine (2 mol/L THF solution, 0.21 mL, 0.426 mmol) in 1,2-dichloroethane (1.0 mL) at 0 °C. After stirring at room temperature for 18 h, the reaction mixture was then poured into saturated aqueous $NaHCO_3$ solution, extracted with ethyl acetate, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate) to yield compound **11** (23 mg, 0.100 mmol, 67%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63–7.70 (m, 2H), 7.60 (s, 1H), 7.55–7.59 (m, 2H), 3.97 (s, 3H), 3.50 (s, 2H), 2.21 (s, 6H).

^{13}C-NMR (100 MHz, $CDCl_3$): δ 138.6, 137.3, 136.8, 132.4, 128.7, 121.3, 119.1, 109.8, 52.2, 45.0, 37.4. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{17}N_4$, 241.14477; found 241.14519.

Synthesis of 4-(1-ethyl-5-methyl-pyrazol-4-yl)benzonitrile (12). Potassium carbonate (477 mg, 3.45 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (62 mg, 0.150 mmol), and palladium acetate (17 mg, 0.075 mmol) were added to a solution of 4-bromo-1-ethyl-5-methyl-pyrazole (284 mg, 1.50 mmol) and 4-cyanophenylboronic acid (441 mg, 3.0 mmol) in acetonitrile (2.3 mL) and water (1.5 mL) at room temperature. The mixture was subjected to microwave irradiation for 30 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 75:25 to 55:45) to yield the crude product. Trituration with isopropyl ether, followed by filtration, yielded compound **12** (187 mg, 0.863 mmol, 59%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.64−7.70 (m, 2H), 7.62 (s, 1H), 7.44−7.48 (m, 2H), 4.17 (q, J = 7.3 Hz, 2H), 2.42 (s, 3H), 1.47 (t, J = 7.3 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 139.0, 137.5, 134.9, 132.5, 127.8, 119.2, 119.1, 109.4, 44.4, 15.3, 10.4. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{13}H_{14}N_3$, 212.11822; found 212.11845.

Synthesis of 4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)benzonitrile (13). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (56 mg, 0.300 mmol) and 4-cyanophenylboronic acid (88 mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was

concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 75:25 to 45:55) to yield compound **13** (36 mg, 0.172 mmol, 57%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 1H), 7.58−7.65 (m. 2H), 7.47−7.53 (m, 2H), 4.19 (t, J = 7.3 Hz, 2H), 3.12 (t, J = 7.3 Hz, 2H), 2.73 (quint, J = 7.4 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.7, 141.3, 138.2, 132.8, 125.1, 119.2, 114.0, 108.7, 47.8, 26.4, 24.1. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{13}H_{12}N_3$, 210.10257; found 210.10270.

Synthesis of 4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)-3-fluoro-benzonitrile (14). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (56 mg, 0.300 mmol) and (4-cyano-2-fluoro-phenyl)boronic acid (99 mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 80:20 to 50:50) to yield compound **14** (55 mg, 0.243 mmol, 81%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.89 (d, J = 2.2 Hz, 1H), 7.52 (t, J = 7.7 Hz, 1H), 7.43 (dd, J = 8.0, 1.4 Hz, 1H), 7.38 (dd, J = 10.5, 1.7 Hz, 1H), 4.22 (t, J = 7.2 Hz, 2H), 3.11 (t, J = 6.9 Hz, 2H), 2.70 (quint, J = 7.2 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 158.3 ($J_{C\text{-}F}$ = 250 Hz), 144.9, 143.1 ($J_{C\text{-}F}$ = 6.6 Hz), 128.4 ($J_{C\text{-}F}$ = 3.8 Hz), 128.2 ($J_{C\text{-}F}$ = 5.9 Hz), 126.9 ($J_{C\text{-}F}$ = 15 Hz), 119.8 ($J_{C\text{-}F}$ = 26 Hz), 117.9 ($J_{C\text{-}F}$ = 2.9 Hz), 109.7 ($J_{C\text{-}F}$ = 9.5 Hz), 108.4 ($J_{C\text{-}F}$ = 1.5 Hz), 48.0, 26.1, 24.7 ($J_{C\text{-}F}$ = 5.9 Hz). HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{13}H_{11}N_3F$, 228.09315; found 228.09326.

Synthesis of 3-chloro-4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)benzonitrile (15). Bis(triphenylphosphine)palladium chloride (18.7 mg, 0.027 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.54 mL, 1.07 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (51 mg, 0.273 mmol) and (2-chloro-4-cyano-phenyl)boronic acid (47 mg, 0.260 mmol) in 1,4-dioxane (1.4 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 100 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 67:33 to 33:67) to yield compound **15** (19 mg, 0.078 mmol, 28%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.85 (s, 1H), 7.72 (d, J = 1.7 Hz, 1H), 7.53 (dd, J = 8.0, 1.7 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 4.23 (t, J = 7.2 Hz, 2H), 3.03 (t, J = 7.7 Hz, 2H), 2.69 (quint, J = 7.2 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 145.0, 143.6, 137.6, 133.7, 132.7, 130.5, 130.3, 117.7, 112.1, 110.9, 48.0, 26.4, 24.7. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{13}H_{11}N_3Cl$, 244.06360; found 244.06372.

Synthesis of 4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)-3-methyl-benzonitrile (16). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (56 mg, 0.300 mmol) and (4-cyano-2-methyl-phenyl)boronic acid (97 mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 75:25 to 45:55) to yield compound **16** (43 mg, 0.192

mmol, 64%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.51−7.53 (m. 1H), 7.46 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 4.22 (t, *J* = 7.2 Hz, 2H), 2.95 (t, *J* = 7.3 Hz, 2H), 2.68 (quint, *J* = 7.3 Hz, 2H), 2.41 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 144.2, 143.2, 138.1, 136.6, 134.2, 129.6, 129.5, 119.2, 113.8, 109.8, 47.9, 26.5, 23.9, 21.0. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{14}N_3$, 224.11822; found 224.11839.

Synthesis of 4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)-3-methoxy-benzonitrile (17). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (56 mg, 0.300 mmol) and (4-cyano-2-methoxy-phenyl)boronic acid (106 mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 80:20 to 20:80) to yield compound **17** (45 mg, 0.188 mmol, 63%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.92 (s, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.27 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.14 (d, *J* = 1.4 Hz, 1H), 4.20 (t, *J* = 7.4 Hz, 2H), 3.92 (s, 3H), 3.06 (t, *J* = 7.4 Hz, 2H), 2.66 (quint, *J* = 7.2 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 155.8, 144.8, 143.7, 128.2, 128.1, 125.0, 119.2, 114.1, 110.6, 109.7, 55.6, 47.9, 26.2, 25.0. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{14}ON_3$, 240.11314; found 240.11337.

Synthesis of 4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)-2-methyl-benzonitrile (18). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (56 mg, 0.300 mmol) and (4-cyano-3-methyl-phenyl)boronic acid (97

mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 80:20 to 30:70) to yield compound **18** (35 mg, 0.157 mmol, 52%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.34−7.37 (m, 1H), 7.30 (dd, J = 8.0, 1.1 Hz, 1H), 4.20 (t, J = 7.2 Hz, 2H), 3.12 (t, J = 7.7 Hz, 2H), 2.72 (quint, J = 7.2 Hz, 2H), 2.55 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.6, 142.4, 141.4, 138.0, 133.1, 126.2, 122.5, 118.5, 114.0, 109.3, 47.7, 26.4, 24.2, 21.7. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{14}N_3$, 224.11822; found 224.11819.

Synthesis of 4-(5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl)-2-fluoro-benzonitrile (19). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazole (56 mg, 0.300 mmol) and (4-cyano-3-fluoro-phenyl)boronic acid (99 mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 70:30 to 40:60) to yield compound **19** (65 mg, 0.286 mmol, 95%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.84 (s, 1H), 7.57 (dd, J = 8.3, 6.9 Hz, 1H), 7.29 (dd, J = 8.0, 1.4 Hz, 1H), 7.21 (dd, J = 10.7, 1.7 Hz, 1H), 4.21 (t, J = 7.4 Hz, 2H), 3.13 (t, J = 7.7 Hz, 2H), 2.75 (quint, J = 7.7 Hz, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 163.7 ($J_{C\text{-}F}$ = 257 Hz), 144.2, 141.4, 141.1 ($J_{C\text{-}F}$ = 8.8 Hz), 133.8, 120.8 ($J_{C\text{-}F}$ = 2.9 Hz), 114.4, 113.2

($J_{C\text{-}F}$ = 2.2 Hz), 111.8 ($J_{C\text{-}F}$ = 21Hz), 97.3 ($J_{C\text{-}F}$ = 16 Hz), 47.8, 26.3, 24.1. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{13}H_{11}N_3F$, 228.09315; found 228.09326.

Synthesis of 4-(6,7-dihydro-5H-pyrrolo[1,2-a]imidazol-3-yl)-3-methyl-benzonitrile (20). Bis(triphenylphosphine)palladium chloride (10.5 mg, 0.020 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.60 mL, 1.20 mmol) were added to a solution of 3-bromo-6,7-dihydro-5H-pyrrolo[1,2-a]imidazole (56 mg, 0.300 mmol) and (4-cyano-2-methyl-phenyl)boronic acid (97 mg, 0.600 mmol) in 1,4-dioxane (2.0 mL) at room temperature. The mixture was subjected to microwave irradiation for 10 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by NH-silica gel chromatography (chloroform/ethyl acetate, 80:20 to 50:50) to yield compound **20** (49 mg, 0.220 mmol, 73%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.55−7.57 (m. 1H), 7.51 (dd, J = 8.0, 1.1 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.12 (s, 1H), 3.94 (t, J = 7.0 Hz, 2H), 2.97 (t, J = 7.6 Hz, 2H), 2.68 (quint, J = 7.4 Hz, 2H), 2.43 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 155.9, 137.5, 134.7, 134.3, 133.9, 129.5, 128.9, 126.6, 118.8, 111.0, 44.9, 26.7, 23.6, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{14}N_3$, 224.11822; found 224.11836.

Synthesis of 4-[(5*R*)-5-hydroxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (21). Tetrabutylammonium fluoride (1 mol/L THF solution, 104 mL, 104 mmol) was added to a solution of compound **41** (6.47 g, 20.8 mmol) in THF (20 mL) at room temperature. After stirring at 80 °C for 17 h, the reaction mixture was poured into water, extracted with ethyl acetate, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate/methanol, 100:0 to 95:5) to yield the crude product. Trituration with n-hexane and

ethyl acetate, followed by filtration, yielded compound **21** (3.80 g, 15.9 mmol, 76%) as a gray solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.50−7.53 (m. 1H), 7.46 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 5.11−5.20 (m, 1H), 4.44 (dd, *J* = 11.8, 6.1 Hz, 1H), 4.17 (dd, *J* = 11.8, 3.0 Hz, 1H), 3.30 (dd, *J* = 16.5, 6.3 Hz, 1H), 2.92 (dd, *J* = 16.5, 2.8 Hz, 1H), 2.50 (d, *J* = 5.2 Hz, 1H), 2.40 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.1, 141.7, 137.7, 136.7, 134.2, 129.6, 129.6, 119.1, 114.7, 110.0, 73.5, 59.9, 35.0, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{14}ON_3$, 240.11314; found 240.11339. $[\alpha]^{20}_D$ +53.15 (*c* 1.11, $CHCl_3$).

Synthesis of 4-[(5*R*)-5-methoxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (22). Sodium hydride (60w/w% in mineral oil, 16 mg, 0.486 mmol) was added to a solution of compound **21** (97 mg, 0.405 mmol) in DMF (35 mL) at 0 °C. After stirring at room temperature for 20 min, iodomethane (69 mg, 0.486 mmol) was added to the reaction mixture and stirred at room temperature for 4 h. The reaction mixture was then poured into water, extracted with ethyl acetate, and the combined organic phase was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 95:5) to yield the crude product. Trituration with diethyl ether, followed by filtration, yielded compound **22** (46 mg, 0.182 mmol, 45%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.50−7.53 (m. 1H), 7.47 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 4.63−4.70 (m, 1H), 4.40 (dd, *J* = 11.8, 6.1 Hz, 1H), 4.22 (dd, *J* = 11.6, 3.3 Hz, 1H), 3.42 (s, 3H), 3.22 (dd, *J* = 16.5, 6.6 Hz, 1H), 2.96 (dd, *J* = 16.5, 3.3 Hz, 1H), 2.40 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.0, 141.7, 137.8, 136.7, 131.2, 129.6, 129.6, 119.1, 114.5, 110.0, 82.0, 57.1, 53.9, 31.7, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{15}H_{16}ON_3$, 254.12879; found 254.12926. $[\alpha]^{20}_D$ +37.08 (*c* 0.70, $CHCl_3$).

Synthesis of 4-[(5*R*)-5-benzyloxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (23). Bis(triphenylphosphine)palladium chloride (17 mg, 0.024 mmol) and sodium carbonate (2 mol/L aqueous solution, 0.485 mL, 0.970 mmol) were added to a solution of compound **39** (100 mg, 0.364 mmol) and 4-bromo-3-methyl-benzonitrile (71 mg, 0.364 mmol) in 1,4-dioxane (1.2 mL) at room temperature. The mixture was subjected to microwave irradiation for 15 min at 150 °C and allowed to cool to room temperature. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. Tetrabutylammonium fluoride (1 mol/L THF solution, 0.290 mL, 0.290 mmol) was added to a solution of the residue in THF (1.2 mL) at room temperature. After stirring at room temperature for 2 days, additional tetrabutylammonium fluoride (1 mol/L THF solution, 0.290 mL, 0.290 mmol) was added to the reaction mixture and heated at 70 °C for 24 h. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 60:40) to yield compound **23** (57 mg, 0.173 mmol, 71%) as a pale brown amorphous solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.50−7.53 (m. 1H), 7.46 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.29−7.40 (m, 5H), 7.29 (d, *J* = 8.0 Hz, 1H), 4.81−4.90 (m, 1H), 4.58−4.65 (m, 2H), 4.40 (dd, *J* = 11.8, 6.3 Hz, 1H), 4.25 (dd, *J* = 11.6, 3.6 Hz, 1H), 3.23 (dd, *J* = 16.2, 6.9 Hz, 1H), 3.03 (dd, *J* = 16.2, 3.6 Hz, 1H), 2.40 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.0, 141.6, 137.8, 137.1, 136.7, 134.2, 129.6, 129.6, 128.7, 128.2, 127.8, 119.1, 114.5, 110.0, 79.7, 71.8, 54.2, 32.1, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{21}H_{20}ON_3$, 330.16009; found 330.16025. $[\alpha]^{20}_D$ +67.50 (*c* 0.40, $CHCl_3$).

Synthesis of ethyl 2-[[(5*R*)-3-(4-cyano-2-methyl-phenyl)-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-5-yl]oxy]acetate (24a). Sodium hydride (60w/w% in mineral oil, 37 mg, 0.920

mmol) was added to a solution of compound **21** (200 mg, 0.836 mmol) and ethyl chloro acetate (205 mg, 0.836 mmol) in THF (10 mL) at 0 °C. After stirring at room temperature for 1 h, additional sodium hydride (60w/w% in mineral oil, 37 mg, 0.920 mmol) and ethyl chloro acetate (205 mg, 0.836 mmol) was added to the reaction mixture. After stirring at the same temperature for 30 min, additional sodium hydride (60w/w% in mineral oil, 37 mg, 0.920 mmol) was added to the reaction mixture. After stirring at the same temperature for 30 min, the reaction mixture was poured into saturated aqueous ammonium chloride solution at 0 °C, extracted with ethyl acetate, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexahne/ethyl acetate, 60:40 to 30/70) to yield the crude product. Trituration with isopropyl ether, followed by filtration, yielded compound **24a** (137 mg, 0.418 mmol, 50%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.53 (s, 1H), 7.47 (d, *J* = 8.0, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 4.85−4.93 (m, 1H), 4.46 (dd, *J* = 11.8, 6.3 Hz, 1H), 4.31 (dd, *J* = 11.8, 3.3 Hz, 1H), 4.24 (q, *J* = 7.2 Hz, 2H), 4.18 (s, 2H), 3.28 (dd, *J* = 16.5, 6.6 Hz, 1H), 3.08 (dd, *J* = 16.5, 3.0 Hz, 1H), 2.41 (s, 3H), 1.30 (t, *J* = 7.2 Hz, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 169.7, 143.0, 141.4, 137.6, 136.7, 134.2, 129.6, 129.5, 119.1, 114.5, 110.0, 81.2, 67.0, 61.3, 54.1, 32.0, 20.9, 14.2. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{18}H_{20}O_3N_3$, 326.14992; found 326.15054. $[\alpha]^{20}_D$ +21.43 (*c* 0.42, $CHCl_3$).

Synthesis of ethyl 2-[[(5*R*)-3-(4-cyano-2-methyl-phenyl)-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-5-yl]oxy]acetate (24). Sodium hydroxide (2 mol/L aqueous solution, 0.31 mL, 0.627 mmol) was added to a solution of the compound **24a** (136 mg, 0.418 mmol) in EtOH (5.0 mL) and THF (6.0 mL) at room temperature. After stirring at room temperature for 1.5 h, the reaction mixture was acidified with hydrochloric acid. The resulting precipitation was collected by filtration, washed with ethanol, and dried under reduced pressure to yield compound **24** (124 mg,

0.417 mmol, 100%) as a colorless solid. ^{1}H-NMR (400 MHz, DMSO−d_6): δ 12.77 (s, 1H), 7.75 (s, 1H), 7.74 (d, J = 1.4 Hz, 1H), 7.64 (dd, J = 8.0, 1.4 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 4.81−4.90 (m, 1H), 4.37 (dd, J = 12.1, 5.8 Hz, 1H), 4.14−4.21 (m, 3H), 3.26−3.36 (m, 1H), 3.03 (dd, J = 16.8, 1.9 Hz, 1H), 2.40 (s, 3H). ^{13}C-NMR (100 MHz, DMSO−d_6): δ 171.3, 142.3, 142.2, 137.6, 136.1, 134.0, 129.6, 129.1, 119.0, 113.3, 108.3, 80.6, 65.7, 53.9, 31.4, 20.5. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{16}H_{16}O_3N_3$, 298.11862; found 298.11906. $[\alpha]^{20}_D$ +33.33 (*c* 0.60, MeOH).

Synthesis of 2-[[(5*R*)-3-(4-cyano-2-methyl-phenyl)-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-5-yl]oxy]acetamide (25). Ethyl-3-(3-dimethylaminopropyl)carbodimide hydrogen chloride (43 mg, 0.225 mmol) was added to a suspension of the compound **24** (50 mg, 0.169 mmol) and N-hydroxysuccinimide (24 mg, 0.210 mmol) in chloroform at room temperature. After stirring at the same temperature for 3 h, aqueous ammonia (0.20 mL, 2.97 mmol) was added to the reaction mixture. After stirring at the same temperature for 40 min, the reaction mixture was poured into water, extracted with chloroform, and the combined organic phase was concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 90:10) to yield compound **25** (35 mg, 0.118 mmol, 70%) as a colorless solid. ^{1}H-NMR (400 MHz, DMSO−d_6): δ 7.76 (s, 1H), 7.74 (d, J = 1.4 Hz, 1H), 7.65 (dd, J = 8.0, 1.4 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.26−7.33 (m, 2H), 4.77−4.84 (m, 1H), 4.36 (dd, J = 12.1, 5.5 Hz, 1H), 3.92 (s, 2H), 3.30 (d, J = 12.1, 5.5 Hz, 1H), 3.03 (dd, J = 16.8, 6.3 Hz, 1H), 3.09 (dd, J = 16.8, 1.9 Hz, 1H), 2.40 (s, 3H). ^{13}C-NMR (100 MHz, DMSO−d_6): δ 171.0, 142.3, 142.3, 137.6, 136.1, 134.0, 129.6, 129.1, 119.0, 113.3, 108.3, 80.9, 67.8, 53.7, 31.2, 20.5. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{16}H_{17}O_2N_4$, 297.13460; found 297.13496. $[\alpha]^{20}_D$ +62,50 (*c* 0.16, MeOH).

Synthesis of 3-methyl-4-[(5*R*)-5-pyrazin-2-yloxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]benzonitrile (26). Sodium hydride (60w/w% in mineral oil, 27 mg, 0.669 mmol) was added to a solution of compound **21** (80 mg, 0.334 mmol) in THF (3.0 mL) at room temperature. After stirring at the same temperature for 30 min, 2-chloropyrazine (50 mg, 0.401 mmol) was added to the reaction mixture and stirred for 3 h. The reaction mixture was poured into saturated aqueous ammonium chloride, extracted with chloroform, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexnane/ethyl acetate, 50:50 to 0:100) to yield compound **26** (72 mg, 0.226 mmol, 68%) as a pale yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.27 (d, J = 2.2 Hz, 1H), 8.22 (d, J = 2.8 Hz, 1H), 8.11 (dd, J = 2.8, 1.4 Hz, 1H), 7.69 (s, 1H), 7.52−7.55 (m, 1H), 7.48 (dd, J = 8.0, 1.4 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 6.09−6.15 (m, 1H), 4.69 (dd, J = 12.7, 6.1 Hz, 1H), 4.38 (dd, J = 12.7, 2.8 Hz, 1H), 3.52 (dd, J = 17.0, 6.9 Hz, 1H), 3.17 (dd, J = 17.0, 2.5 Hz, 1H), 2.43 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 158.7, 143.4, 141.5, 140.4, 137.6, 137.5, 136.7, 136.3, 134.2, 129.6, 129.5, 109.1, 114.6, 110.7, 76.6, 54.5, 32.0, 21.0. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{18}H_{16}ON_5$, 318.13494; found 318.13511. $[\alpha]^{20}_D$ +90.00 (*c* 0.55, $CHCl_3$).

Synthesis of 3-methyl-4-[(5*R*)-5-pyrimidin-2-yloxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]benzonitrile (27). Sodium hydride (60w/w% in mineral oil, 21 mg, 0.522 mmol) and 2-chloropyrimidine (36 mg, 0.313 mmol) were added to a solution of compound **21** (50 mg, 0.209 mmol) in DMF (27 mL) at room temperature. After stirring at room temperature for 4 h, the reaction mixture was poured into water, extracted with ethyl acetate, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by NH-silica gel chromatography (n-hexane/ethyl acetate, 60:40 to 30:70) to yield compound **27** (37 mg, 0.112 mmol, 56%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$):

δ 8.56 (d, *J* = 4.7 Hz, 2H), 7.65 (s, 1H), 7.52−7.54 (m. 1H), 7.47 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.03 (t, *J* = 4.7 Hz, 1H), 6.09−6.15 (m, 1H), 4.70 (dd, *J* = 12.4, 6.1 Hz, 1H), 4.42 (dd, *J* = 12.4, 2.8 Hz, 1H), 3.51 (dd, *J* = 17.1, 6.9 Hz, 1H), 3.22 (dd, *J* = 17.1, 2.8 Hz, 1H), 2.42 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 164.1, 159.6, 143.3, 141.5, 137.7, 136.8, 134.2, 129.6, 129.6, 119.1, 115.9, 114.6, 110.1, 77.6, 54.5, 32.0, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{18}H_{16}ON_5$, 318.13494; found 318.13467. $[\alpha]^{20}_D$ +115.33 (*c* 0.71, $CHCl_3$).

Synthesis of 4-[(5*R*)-5-(5-fluoropyrimidin-2-yl)oxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (28). Sodium hydride (60w/w% in mineral oil, 277 mg, 6.94 mmol) and 2-chloro-5-fluoro-pyrimidine (552 mg, 4.16 mmol) were added to a solution of compound **21** (830 mg, 3.47 mmol) in THF (35 mL) at 0 °C. After stirring at room temperature for 4 h, the reaction mixture was poured into water, extracted with ethyl acetate, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 95:5) to yield the crude product. Trituration with ethyl acetate and n-hexane, followed by filtration yielded compound **28** (799 mg, 2.38 mmol, 69%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.42 (s, 2H), 7.65 (s, 1H), 7.51−7.54 (m. 1H), 7.47 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 6.00−6.10 (m, 1H), 4.68 (dd, *J* = 12.4, 6.1 Hz, 1H), 4.41 (dd, *J* = 12.7, 2.8 Hz, 1H), 3.51 (dd, *J* = 17.1, 6.9 Hz, 1H), 3.20 (dd, *J* = 17.1, 2.8 Hz, 1H), 2.42 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 160.2 ($J_{C\text{-}F}$ = 1.5 Hz), 154.8 ($J_{C\text{-}F}$ = 256.0 Hz), 147.0 ($J_{C\text{-}F}$ = 22.7 Hz), 143.3, 141.4, 137.6, 136.8, 134.2, 129.6, 129.6, 119.1, 114.6, 110.1, 78.4, 54.4, 32.0, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{18}H_{15}ON_5F$, 336.12551; found 336.12531. $[\alpha]^{20}_D$ +111.00 (*c* 1.04, $CHCl_3$).

Synthesis of 3-methyl-4-[(5*R*)-5-(5-methylpyrimidin-2-yl)oxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]benzonitrile (29). Sodium hydride (60w/w/% in mineral oil, 17 mg, 0.418 mmol) and 2-chloro-5-methyl-pyrimidine (35 mg, 0.272 mmol) were added to a solution of compound **21** (50 mg, 0.210 mmol) in THF (1.0 mL) and DMF (1.0 mL) at room temperature. After stirring at room temperature for 16 h, the reaction mixture was poured into water, extracted with chloroform, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 90:10) to yield **29** (20 mg, 0.060 mmol, 29%) as a brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.37 (s, 2H), 7.65 (s, 1H), 7.51−7.53 (m. 1H), 7.46 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 6.04−6.11 (m, 1H), 4.68 (dd, *J* = 12.4, 6.1 Hz, 1H), 4.40 (dd, *J* = 12.7, 3.0 Hz, 1H), 3.49 (dd, *J* = 16.8, 6.9 Hz, 1H), 3.20 (dd, *J* = 16.8, 2.8 Hz, 1H), 2.42 (s, 3H), 2.27 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 162.7, 159.4, 143.3, 141.6, 137.7, 136.7, 134.1, 129.6, 129.6, 124.8, 119.1, 114.5, 110.1, 77.5, 54.5, 32.0, 20.9, 14.6. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{19}H_{18}ON_5$, 332.15059; found 332.15059. $[\alpha]^{20}_D$ +90.56 (*c* 0.27, $CHCl_3$).

Synthesis of 2-[[(5*R*)-3-(4-cyano-2-methyl-phenyl)-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-5-yl]oxy]pyrimidine-5-carbonitrile (30). Sodium hydride (60w/w% in mineral oil, 17 mg, 0.418 mmol) was added to a solution of compound **21** (50 mg, 0.210 mmol) in THF (2.0 mL) at room temperature. After stirring at room temperature for 20 min, 2-chloro-pyrimidine-5-carbonitrile (44 mg, 0.284 mmol) was added to the reaction mixture and stirred at room temperature for 1 h. The reaction mixture was then poured into water, extracted with chloroform, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 50:50 to 0:100) to yield compound **30** (65 mg, 0.190 mmol, 91%) as a pale yellow amorphous solid. ^{1}H-NMR (400

MHz, $CDCl_3$): δ 8.84 (s, 2H), 7.67 (s, 1H), 7.52−7.54 (m. 1H), 7.47 (dd, *J* = 8.0, 1.1 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 6.14−6.22 (m, 1H), 4.72 (dd, *J* = 12.7, 6.1 Hz, 1H), 4.43 (dd, *J* = 12.7, 2.8 Hz, 1H), 3.55 (dd, *J* = 17.1, 6.9 Hz, 1H), 3.23 (dd, *J* = 17.1, 2.5 Hz, 1H), 2.42 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 164.6, 162.7, 143.6, 140.9, 137.3, 136.8, 134.2, 129.6, 129.6, 119.0, 114.8, 114.1, 110.3, 104.1, 79.3, 54.2, 31.9, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{19}H_{15}ON_6$, 343.13019; found 343.13001. $[\alpha]^{20}_D$ +104.47 (*c* 0.60, $CHCl_3$).

Synthesis of 4-[(5*S*)-5-hydroxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (31). Sodium borohydride (6.0 mg, 0.160 mmol) was added to a solution of compound **42** (30 mg, 0.126 mmol) in methanol (1.0 mL) and THF (0.50 mL) at room temperature. After stirring at room temperature for 4 h, the reaction mixture was poured into 10% aqueous ammonium chloride solution, extracted with chloroform, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (ethyl acetate) to yield (*rac*)-4-[5-hydroxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (25 mg, 0.102 mmol). The two enantiomers were separated on a Chiralpak ID column (n-hexane/propan-2-ol/THF/diethyl amine, 70/15/15/0.1) to yield compound **21** (9.8 mg, 0.041 mmol, 45%) and **31** (9.9 mg, 0.041 mmol, 45%), respectively, as colorless solids. Enantiomer **31** ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63 (s, 1H), 7.50−7.53 (m. 1H), 7.46 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 5.11−5.20 (m, 1H), 4.44 (dd, *J* = 11.8, 6.1 Hz, 1H), 4.17 (dd, *J* = 11.8, 3.0 Hz, 1H), 3.30 (dd, *J* = 16.5, 6.3 Hz, 1H), 2.92 (dd, *J* = 16.5, 2.8 Hz, 1H), 2.30−2.65 (br s, 1H), 2.40 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 143.1, 141.7, 137.7, 136.7, 134.2, 129.6, 129.6, 119.1, 114.7, 110.0, 73.5, 59.9, 35.0, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd for $C_{14}H_{14}ON_3$, 240.11314; found 240.11354. $[\alpha]^{20}_D$ −49.58 (*c* 0.12, $CHCl_3$).

Synthesis of 4-[(5*S*)-5-(5-fluoropyrimidin-2-yl)oxy-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (32). Sodium hydride (60w/w% in mineral oil, 3.3 mg, 0.084 mmol) and 2-chloro-5-fluoro-pyrimidine (8.3 mg, 0.063 mmol) were added to a solution of compound **31** (10 mg, 0.042 mmol) in THF (0.4 mL) at room temperature. After stirring at the same temperature for 2.5 h, the reaction mixture was poured into water, extracted with ethyl acetate, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 95:5) to yield compound **32** (14 mg, 0.042 mmol, 100%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 2H), 7.66 (s, 1H), 7.51−7.54 (m. 1H), 7.47 (dd, J = 7.7, 1.4 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 6.00−6.10 (m, 1H), 4.69 (dd, J = 12.4, 6.1 Hz, 1H), 4.41 (dd, J = 12.4, 2.8 Hz, 1H), 3.51 (dd, J = 17.1, 7.2 Hz, 1H), 3.21 (dd, J = 17.1, 2.8 Hz, 1H), 2.42 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 160.2 ($J_{C\text{-}F}$ = 1.5 Hz), 154.8 ($J_{C\text{-}F}$ = 256.0 Hz), 147.0 ($J_{C\text{-}F}$ = 22.7 Hz), 143.4, 141.4, 137.5, 136.7, 134.2, 129.6, 129.6, 119.1, 114.6, 110.0, 78.4, 54.4, 32.0, 20.9. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{18}H_{15}ON_5F$, 336.12551; found 336.12601. $[\alpha]^{20}_D$ −114.71 (*c* 0.17, $CHCl_3$).

Synthesis of (5*R*)-5-benzyloxy-5,6-dihydro-4H-pyrrolo[1,2-c]oxadiazol-7-ium-3-olate (38). TFA (100 mL, 1.31 mol) was added to a solution of (2*S*,4*R*)-4-benzyloxy-1-tert-butoxycarbonyl-pyrrolidine-2-carboxylic acid (50.0 g, 156 mmol) in chloroform (400 mL) at 0 °C. After being stirred at room temperature for 16 h, the reaction mixture was concentrated under reduced pressure, and the residue was used for the next reaction without further purification. Sodium nitrite (11.8 g, 171 mmol) was added to a solution of the residue in acetic acid (400 mL) at room temperature. After stirring at room temperature for 15 h, the reaction mixture was concentrated under reduced pressure, and the residue was used for the next reaction without further

purification. This residue dissolved in THF (300 mL) was added to a solution of trifluoroacetic anhydride (87.9 mL, 622 mmol) in THF (600 mL) at 0 °C. After being stirred at room temperature for 15 h, the reaction mixture was concentrated under reduced pressure and azeotropically dried with toluene. The residue was purified by NH-silica gel chromatography (n-hexane/chloroform, 70:30 to 30:70) and silica gel chromatography (chloroform/methanol, 100:0 to 90:10) to yield compound **38** (19.9 g, 85.8 mmol, 55%) as a pale beige solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30−7.42 (m, 5H), 4.80−4.89 (m, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.56 (d, *J* = 11.8 Hz, 1H), 4.48 (dd, *J* = 13.8, 6.1 Hz, 1H), 4.36 (dd, *J* = 13.8, 3.3 Hz, 1H), 3.13 (dd, *J* = 16.5, 6.6 Hz, 1H), 2.97 (dd, *J* = 16.5, 3.0 Hz, 1H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 165.3, 136.3, 128.8, 128.6, 128.0, 108.4, 78.8, 72.1, 58.5, 29.2. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{12}H_{13}O_3N_2$, 233.09207; found 233.09240. $[\alpha]^{20}_D$ +5.67 (*c* 1.06, $CHCl_3$).

Synthesis of [(5*R*)-5-benzyloxy-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-2-yl]-trimethyl-silane (39). Trimethyl-[2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)ethynyl]silane (64.9 g, 290 mmol) was added to a solution of compound **38** (19.2 g, 82.7 mmol) in 1,2-dichlorobenzene (414 mL) at room temperature. After stirring at 185 °C for 21 h, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/chloroform, 70:30 to 30:70) to yield compound **39** (18.0 g, 43.6 mmol, 53%) as a brown oil. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.30−7.38 (m, 5H), 4.75−4.83 (m, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.56 (d, *J* = 11.8 Hz, 1H), 4.33 (dd, *J* = 11.8, 6.3 Hz, 1H), 4.17 (dd, *J* = 11.8, 4.1 Hz, 1H), 3.29 (dd, *J* = 16.8, 6.9 Hz, 1H), 3.05 (dd, *J* = 16.8, 3.9 Hz, 1H), 1.28 (s, 12H), 0.32(9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 165.2, 153.1, 137.5, 128.5, 128.0, 127.8, 82.9, 80.4, 71.5, 53.6, 31.5, 25.0, 24.9,

−0.82. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{22}H_{34}O_3N_2BSi$, 413.24263; found 413.24305. $[\alpha]^{20}_D$ +19.49 (*c* 1.03, $CHCl_3$).

Synthesis of (5*R*)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-trimethylsilyl-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-5-ol (40). Palladium hydroxide on carbon (6.29 g) was added to a solution of compound **39** (18.0 g, 43.6 mmol) in ethanol (396 mL) at 0 °C, and the reaction vessel was charged with hydrogen gas. After stirring at room temperature for 16 h, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. Trituration with ethyl acetate and n-hexane, followed by filtration, yielded compound **40** (10.7 g, 33.1 mmol, 76%) as a colorless solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.01−5.09 (m, 1H), 4.35 (dd, *J* = 12.1, 6.1 Hz, 1H), 4.09 (dd, *J* = 11.8, 2.8 Hz, 1H), 3.29 (dd, *J* = 17.1, 6.6 Hz, 1H), 2.94 (dd, *J* = 17.1, 3.0 Hz, 1H), 1.28 (s, 12H), 0.32(9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 165.5, 153.2, 83.0, 74.2, 56.5, 34.5, 24.9, 24.9, −0.82. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{15}H_{28}O_3N_2BSi$, 323.19568; found 323.19590. $[\alpha]^{20}_D$ +30.27 (*c* 1.02, $CHCl_3$).

Synthesis of 4-[(5*R*)-5-hydroxy-2-trimethylsilyl-5,6-dihydro-4H-pyrrolo[1,2-b]pyrazol-3-yl]-3-methyl-benzonitrile (41). Bis(triphenylphosphine)palladium chloride (1230 mg, 1.75 mmol), 4-bromo-3-methyl-benzonitrile (10.3 g, 52.6 mmol), and sodium carbonate (2 mol/L aqueous solution, 88 mL, 175 mmol) were added to a solution of compound **40** (11.3 g, 35.1 mmol) in 1,2-dimethoxyethane (351 mL) at room temperature. After stirring at 80 °C for 15 h, the reaction mixture was poured into water and extracted with ethyl acetate. The combined organic phase was washed with saturated brine solution, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (n-hexane/ethyl acetate, 50:50 to 20:80) to yield compound **41** (6.16 g, 19.8 mmol, 56%) as a pale yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.50−7.53 (m, 1H), 7.46 (dd, *J* = 7.7, 1.1 Hz, 1H),

7.26 (d, J = 7.7 Hz, 1H), 5.10−5.16 (m, 1H), 4.47 (dd, J = 12.1, 6.1 Hz, 1H), 4.17 (dd, J = 12.1, 3.3 Hz, 1H), 3.05 (dd, J = 16.5, 6.6 Hz, 1H), 2.66 (dd, J = 16.2, 3.0 Hz, 1H), 2.46 (d, J = 5.5 Hz, 1H), 2.17 (s, 3H), 0.06 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.2, 142.0, 139.9, 138.8, 133.1, 132.1, 128.9, 120.8, 119.0, 111.2, 74.0, 56.7, 33.5, 20.1, −0.61. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{17}H_{22}ON_3Si$, 312.15267; found 312.15283. $[\alpha]^{20}_D$ +17.35 (*c* 1.04, $CHCl_3$).

Synthesis of 3-methyl-4-(5-oxo-4,6-dihydropyrrolo[1,2-b]pyrazol-3-yl)benzonitrile (42). A solution of compound **21** (500 mg, 2.04 mmol) in chloroform (30 mL) was combined with 1,1,1-triacetoxy-1,1-dihydro-1,2-benziodoxol-3(1H)-one (1.33 g, 3.14 mmol) at room temperature. After stirring at room temperature for 4 h, the reaction mixture was poured into water, extracted with chloroform, and the combined organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography (chloroform/methanol, 100:0 to 97:3) to yield the crude product. Trituration with diethyl ether, followed by filtration yielded compound **42** (355 mg, 1.50 mmol, 73%) as a pale brown solid. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.70 (s, 1H), 7.54−7.57 (m. 1H), 7.50 (dd, J = 8.0, 1.4 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 4.68 (s, 2H), 3.64 (s, 2H), 2.41 (s, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 204.4, 141.9, 138.5, 137.0, 136.8, 134.3, 129.8, 129.7, 118.8, 115.7, 110.8, 56.1, 38.3, 20.8. HRMS-ESI m/z $[M+H]^+$ calcd. for $C_{14}H_{12}ON_3$, 238.09749; found 238.09789.

Docking Simulation. The 3D structures of the compounds used were generated using LigPrep 2.6[22] before docking. Subsequently, these compounds were docked into hCYP11B2 (PDB code 4DVQ) using GOLD 5.2[18] with the standard set of parameters, where the positions of the atoms of the protein were fixed. The docking procedures were validated by docking FAD286 and by comparing with X-ray crystal structure of FAD286 (PDB code 4FDH). Figures were rendered using PyMol.[23]

In Vitro Enzyme Assays

Enzyme preparation. The pcDNA3.1-human CYP11B2 and pcDNA3.1-human CYP11B1 plasmids were transfected into a Chinese hamster lung fibroblast V79 cell line to produce a stable cell line expressing human CYP11B2 and CYP11B1, respectively. The cells were cultured and grown in Dulbecco's modified Eagle's/Ham's medium supplemented with 10% calf serum and 1% G418 disulfate solution at 37 °C, 95% air, and 5% CO_2. The cells were homogenized with a 5 mmol/L Tris-HCl buffer (pH 7.4) containing 250 mmol/L sucrose in a Teflon (Registered Trademark) Potter Elvehjem homogenizer and centrifuged (800 × g, 15 min). The supernatant was separated and centrifuged again (10000 × g, 15 min) to obtain the mitochondrial fraction as a pellet.

Human CYP11B2 Assay. The mitochondrial fraction was diluted with buffer composed of 10 mmol/L KH_2PO_4, 10 mmol/L Tris, 20 mmol/L KCl, 25 mmol/L sucrose, 5 mmol/L $MgCl_2$, and 0.05 % bovine serum albumin, and was dispensed to a 96-well plate. Deoxycorticosterone (0.5 μmol/L) and NADPH (150 μmol/L) were added to each well and incubated for 1.5 h at room temperature with or without the test compound. The aldosterone concentration in the incubated solution was determined by the Homogeneous Time-Resolved Fluorescence (HTRF) method, and percent inhibition of aldosterone production by test compounds was calculated. The concentration-response curves were generated by plotting the percent inhibition against the logarithmic concentration of the test compound. The IC_{50} value was calculated from the concentration-response curves using non-linear regression (GraphPad Prism5; GraphPad Software, Inc.).

Human CYP11B1 Assay. The human CYP11B1 assay was performed using the same method as the human CYP11B2 assay, with slight variation as follows; deoxycorticosterone concentration:

2.0 μmol/L, incubation time: 1.5–2 h. Corticosterone concentration in the incubated solution was determined by HTRF method.

***In Vivo* Cynomolgus Monkey Experiments.** This study was approved by the Experimental Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation (Saitama, Japan). Six male cynomolgus monkeys from HAMRI CO., LTD. (Ibaraki, Japan) of approximately 4–5 years of age were used. Monkeys were maintained at room temperature on a 12 h light/dark cycle and allowed access to a diet of standard laboratory chow (PS-A; Oriental Yeast, Tokyo, Japan) and tap water *ad libitum*. Monkeys were divided into two groups of three animals. Pairwise oral administration of 0.5% CMC or test compound was conducted, and at least 1 week of recovery was allowed between sequential experiments. Following the placebo or compound administration, blood samples were collected at 0.5, 1, 2, 4, 6, and 24 h using heparin-treated syringes. Blood was centrifuged at 21,600 g for 5 min at 4 °C to separate the plasma. Plasma aldosterone concentration was measured by radioimmunoassay (SPAC-S aldosterone kit, FUJIREBIO INC.). Plasma concentrations of compounds **21** and **28** were measured by liquid chromatography separation coupled with tandem mass spectrometric detection (LC/MS/MS). The lower limit of quantification (LLOQ) of compounds **21** and **28** are 3 ng/mL and 0.4 ng/mL as total (bound and unbound) concentration, respectively. All plasma samples from monkeys dosed with compound **21** showed that the compound was below the LLOQ at 24 h after dosing, and its concentrations were extrapolated from the calibration curve as reference values. The data were expressed as the mean + SD or mean ± standard error of the mean of six monkeys. A Student's paired t-test was used to evaluate pairwise comparisons of responses after dosing with the test compound or a vehicle at each time point. Statistical analyses were performed using SAS

software, version 8.0.0 (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Stability in Liver Microsomes. Stability in liver microsomes is determined using the substrate depletion method. The incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), 0.25 mg/mL liver microsomes, 5 mM $MgCl_2$, 1 mM NADPH, and test compounds (1 μM). Prior to the addition of NADPH, the mixture was incubated at 37 °C for 10 min. The reaction was initiated by the addition of NADPH and incubated at 37 °C for 0 and 20 min and was terminated by the addition of acetonitrile/methanol (v/v; 1/1) containing an internal standard for quantification. The resulting mixture was centrifuged at ca. 15,000 × g for 10 min at 4 °C, and an aliquot of the supernatant was sampled for LC/MS/MS analysis.

Plasma Protein Binding (PPB) Assay. The plasma protein binding assay was performed using a rapid equilibrium dialysis (RED) device. Test compounds or reference materials were spiked in plasma to a final concentration of 10 μM, and the mixture was vortexed at ambient temperature. An aliquot of the mixture was transferred to the sample chamber of a RED device insert, and PBS was added to the buffer chamber. After dialysis at 37 °C with shaking in a CO_2 incubator, plasma and buffer samples were prepared for analysis. Concentrations of the test compounds in both plasma and buffer were determined by LC/MS/MS.

ASSOCIATED CONTENT

Supporting Information

The HPLC conditions for determinations of the the purity, ^{1}H and ^{13}C NMR spectrum and Molecular Formula Strings. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

PAC, plasma aldosterone concentration; MW, microwave.

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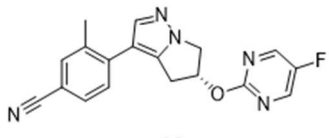
Table of Contents Graphic



28

hCYP11B2 IC_{50} = 4.7 nM

hCYP11B1 IC_{50} = 1100 nM

Selectivity(B1/B2) 230