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Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer

Tomohiro Kaku [a,*], Takenori Hitaka [b], Akio Ojida [b,†], Nobuyuki Matsunaga [c], Mari Adachi [b], Toshimasa Tanaka [d], Takahito Hara [e], Masuo Yamaoka [e], Masami Kusaka [f], Teruaki Okuda [g], Satoru Asahi [h], Shuichi Furuya [i], Akihiro Tasaka [j]

[a] CNS Drug Discovery Unit, Takeda Pharmaceutical Company, Ltd., Shonan Research Center, 26-1, Muraoka-Higashi 2-Chome, Fujisawa, Kanagawa 251-0012, Japan
[b] Medicinal Chemistry Research Laboratories, Takeda Pharmaceutical Company, Ltd., Yodogawa-ku, Osaka, Japan
[c] MD Drug Discovery Unit, Takeda Pharmaceutical Company, Ltd., Yodogawa-ku, Osaka, Japan
[d] Discovery Research Laboratories, Takeda Pharmaceutical Company, Ltd., Shonan Research Center, Fujisawa, Kanagawa, Japan
[e] Oncology Drug Discovery Unit, Takeda Pharmaceutical Company, Ltd., Shonan Research Center, Fujisawa, Kanagawa, Japan
[f] Strategic CMC Planning Department, Takeda Pharmaceutical Company, Ltd., Yodogawa-ku, Osaka, Japan
[g] Discovery Research Laboratories, Takeda Pharmaceutical Company, Ltd., Yodogawa-ku, Osaka, Japan
[h] Drug Metabolism & Pharmacokinetics Research Laboratories, Takeda Pharmaceutical Company, Ltd., Yodogawa-ku, Osaka, Japan
[i] Research Alliance Office, Takeda Pharmaceutical Company, Ltd., Tsukuba Research Center, Tsukuba, Ibaraki, Japan
[j] Environment & Safety Department, Takeda Pharmaceutical Company, Ltd., Yodogawa-ku, Osaka, Japan

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ABSTRACT

A novel naphthylmethylimidazole derivative **1** and its related compounds were identified as 17,20-lyase inhibitors. Based on the structure–activity relationship around the naphthalene scaffold and the results of a docking study of **1a** in the homology model of 17,20-lyase, the 6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazole derivative (+)-**3c** was synthesized and identified as a potent and highly selective 17,20-lyase inhibitor. Biological evaluation of (+)-**3c** at a dose of 1 mg/kg in a male monkey model revealed marked reductions in both serum testosterone and dehydroepiandrosterone concentrations. Therefore, (+)-**3c** (termed orteronel [TAK-700]) was selected as a candidate for clinical evaluation and is currently in phase III clinical trials for the treatment of castration-resistant prostate cancer.

1. Introduction

The role of endogenous androgens in the development and progression of prostate cancer has long been recognized,[1,2] and patients with advanced prostate cancer have been efficiently treated with hormonal therapy such as surgical castration or the administration of a luteinizing-hormone-releasing-hormone (LH-RH) agonist. However, most of these patients eventually relapse during medical treatment and develop castration-resistant prostate cancer (CRPC). Sadly, management of later stages of this disease is often unsuccessful. The molecular mechanisms leading to the progression of CRPC are not yet fully defined, but recent studies[3–5] have suggested that residual adrenal androgens that remain after castration could be responsible for CRPC evolution. Therefore, pharmacological methods to reduce adrenal androgen production can be considered as a new therapeutic option to delay CRPC progression.

One possible approach to decrease androgens levels in tumor and plasma is to inhibit the 17,20-lyase activity of CYP17A1, which is an enzyme known to be responsible for androgen biosynthesis in both testes and adrenal glands. Indeed, over the past decade, steroidal and nonsteroidal inhibitors of 17,20-lyase[6–40] such as YM-116[23] and abiraterone acetate[7] (Fig. 1) have been evaluated in clinical studies and have been found to effectively reduce circulating androgen levels. More recently we have also developed a novel series of 17,20-lyase inhibitors such as compound **1** (Fig. 1) which was observed to possess human 17,20-lyase inhibition with an IC_{50} of 16 nM.[41] However, our studies also found that **1** showed potent CYP3A4 inhibition with an IC_{50} value of 3600 nM.

Abbreviations: CRPC, castration-resistant prostate cancer; CYP, cytochrome P450; DHEA, dehydroepiandrosterone; LH-RH, luteinizing-hormone-releasing-hormone.

* Corresponding author. Tel.: +81 466 32 1114.
E-mail address: Kaku_Tomohiro@takeda.co.jp (T. Kaku).
† Present address: Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan.

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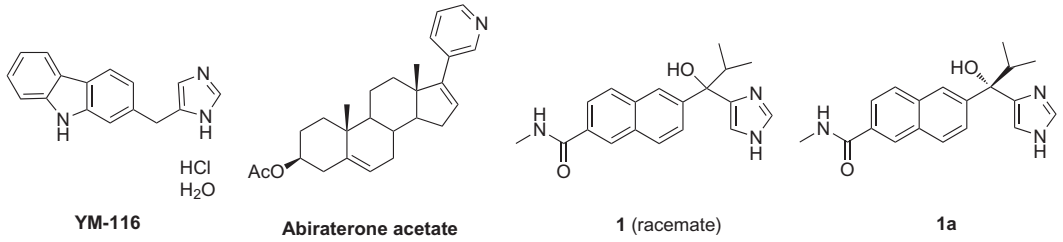


Figure 1. Structures of 17,20-lyase inhibitors.

CYP3A4 is one of the key metabolizing enzymes of the cytochrome P450 (CYP) superfamily of heme-containing monooxygenases. As the CYP family of enzymes plays important roles not only in the xenobiotic metabolism and detoxification of a large number of drugs, but also in the biosynthesis of endogenous steroids, CYP inhibitors have potential risk to induce drug–drug interactions or significant systemic side effects. Therefore, high specificity and selectivity for 17,20-lyase over the other CYPs is an important requirement for clinical use. Here, we report the design, synthesis and biological activities of the novel 6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazole derivatives and related compounds as highly selective 17,20-lyase inhibitors. In addition, we discuss our observations on achieving high selectivity over other CYP enzymes for (+)-**3c**.

2. Results and discussion

2.1. Molecular design

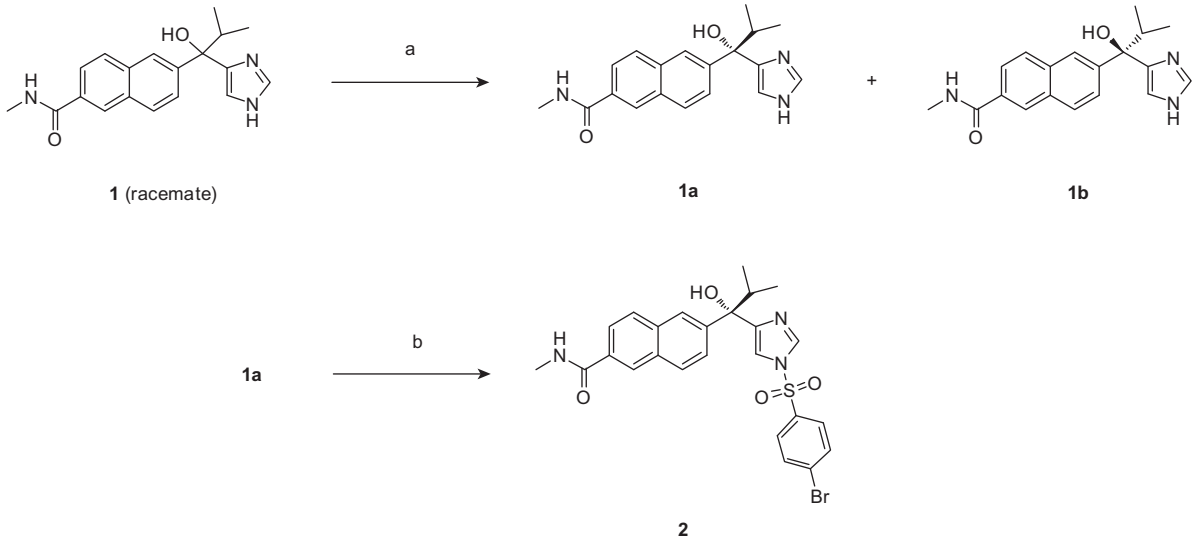
As described above, racemic **1** showed promising profiles for further development, therefore, optical resolution of compound **1** was conducted using preparative HPLC on a Chiralpak AD column, as shown in Scheme 1. The absolute configuration of the enantiomer **1a** was determined to be *S* by the X-ray crystallographic analysis of the sulfonyl derivative **2**, as shown in Figure 2. The biological evaluation revealed that **1a** showed potent human 17,20-lyase inhibition with an IC_{50} value of 5.5 nM, along with relatively potent CYP3A4 inhibition of an IC_{50} value of 7800 nM. Therefore, we continued to further modify this series of compounds, with the aim of improving selectivity to 17,20-lyase over other CYP enzymes.

To understand the binding mode of **1a**, we conducted a docking study of the compound using a homology model of the catalytic domain of 17,20-lyase, which was constructed based on the mammalian CYP2C5 X-ray crystal structure.[42] In the docking experiment it was postulated that **1a** should be coordinated to the heme iron via the nitrogen of the imidazole ring moiety; the results of the docking study of **1a** are illustrated in Figure 3. The docking study suggested that: (a) **1a** formed two hydrogen bonds with the enzyme (the carbamoyl moiety of **1a** with Thr101, and the hydroxyl group of **1a** with Thr306; (b) the naphthalene ring occupied a hydrophobic region surrounded by Ala113, Phe114, Ile205, Ala302 and Ile371; and (c) the isopropyl group was located in a small hydrophobic pocket created by Val483, Phe484 and Leu485.

In the docking experiment, both the hydrogen bonds with Thr101 and Thr306 and the coordinated bond to the heme iron seem to contribute to the in vitro inhibitory activity of **1a**. Therefore, further modifications were mainly focused on the conversion of the isopropyl moiety of **1a**. Due to the limited space around the isopropyl group, we developed a new class of 17,20-lyase inhibitors with a fused imidazole ring system, as shown in Figure 4.

2.2. Chemistry

The preparations of key intermediates **6**, **7** and **9a–e** are shown in Scheme 2. The synthesis of cyclic ketone **6** from compound **4** was achieved by a modification of the procedure reported by Christine et al.[43] Compound **4** was converted to **5**, using hydrogen bromide (HBr) in acetic acid (AcOH), and isolated as HBr salt.



Scheme 1. Reagents and conditions: (a) optical resolution using preparative HPLC on a Chiralpak AD column, **1a** (>99.9% ee) and **1b** (99.8% ee), respectively; (b) 4-bromobenzenesulfonyl chloride, Et_3N, DMF, room temperature, 79%.

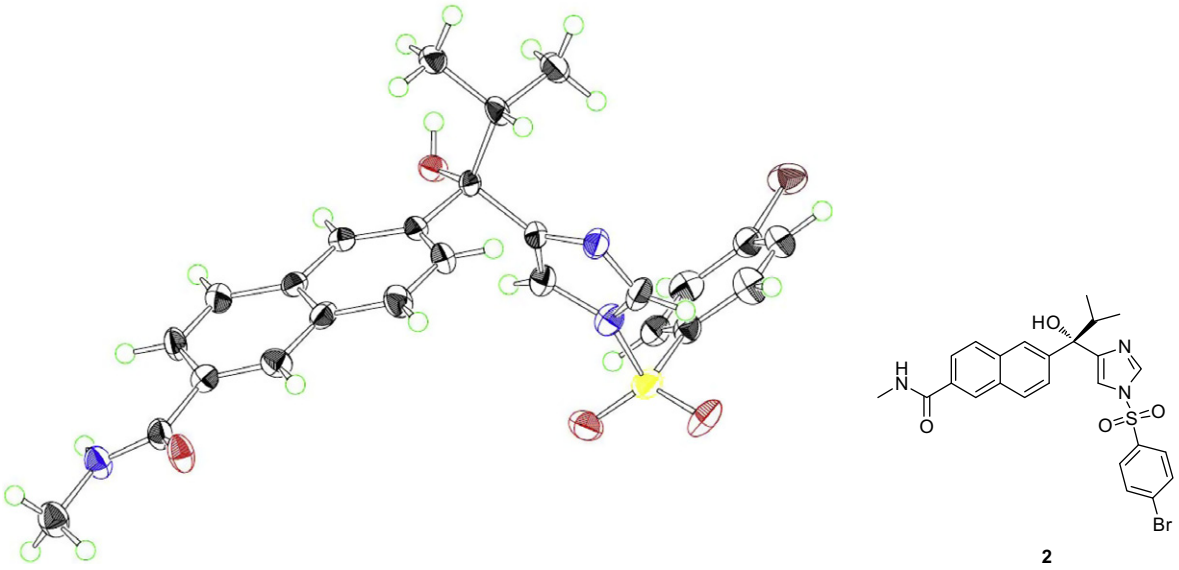


Figure 2. ORTEP drawing of **2**.

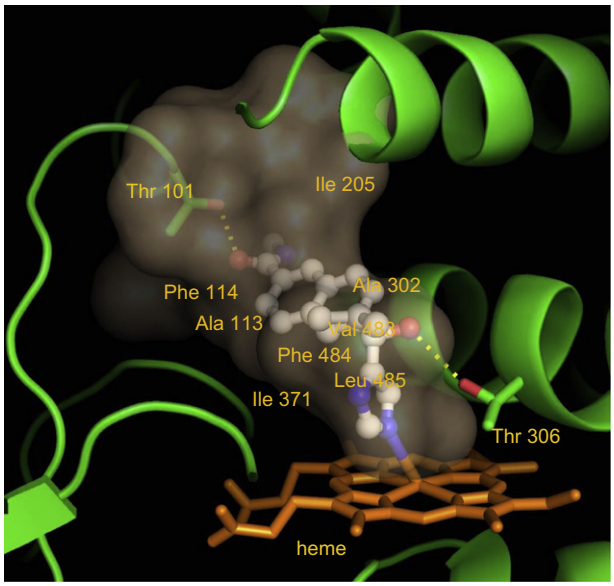


Figure 3. Docking study of **1a** in a homology model of 17,20-lyase.

Subsequent cyclization of **5** was performed effectively in the presence of triethylamine (Et_3N), to produce **6** in moderate yield. Dimethylation of **6** using methyl iodide (MeI) afforded **7** with moderate yield. Coupling of commercially available carboxylic acid **8** with alkylamine was performed by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) and 1-hydroxybenzotriazole (HOBt) or by conversion of carboxylic acid **8** into the corresponding acid chloride to give good to moderate yields of **9a–e**, respectively. The designed fused imidazole derivatives and related compounds were prepared as shown in Scheme 3. The addition of lithium species, generated from **8** and **9a–e** with *n*-butyllithium (*n*-BuLi), to ketone **6** or **7** gave moderate to low yields of the desired alcohols **3a**, **3c**, **3e–h**, respectively. Coupling of **3a** with ammonium salt of HOBt was performed in the same manner as described for **9a–d** to afford **3b** in low yield. Removal of hydroxyl group of **3c** by using Pd/C under a hydrogen atmosphere was carried out to give **3d** in good yield. Diisopropylcarbamoyl derivative **3h** was treated with lithium amides, generated from methylamine with *n*-BuLi, to give the desired **3i** in low yield. Compound **9a** was subjected to coupling reaction with **10**[44] to form **3j** in moderate yield.

We next focused on the preparation and asymmetric synthesis of (+)-**3c** as a candidate for further development. The synthetic strategy toward racemic **3c** and (+)-**3c** is outlined in Scheme 4 as a retrosynthetic analysis. Initially, we adopted two approaches, routes A and B, to prepare racemic **3c**, which could be efficiently resolved by high-performance liquid chromatography (HPLC) or diastereomeric salt formation to obtain optically pure (+)-**3c**. Route A is a convergent synthetic method via cyclic ketone I,[43] whereas route B represents a linear synthetic method via diol III and ketone IV. Otherwise, we developed an asymmetric synthesis of (+)-**3c** using a newly developed stereocontrolled Reformatsky reaction (route C).[45]

2.3. Improved method for the synthesis of racemic 3c and preparation of (+)-3c via optical resolution of racemic 3c

The improved method for the synthesis of racemic **3c** is illustrated in Scheme 5 (route A). As described above, the coupling reaction of lithium species, generated from **9a** with *n*-BuLi to ketone **6** resulted in 26% yield, therefore, the reaction condition using

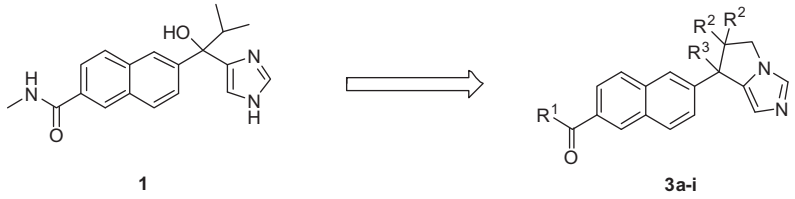
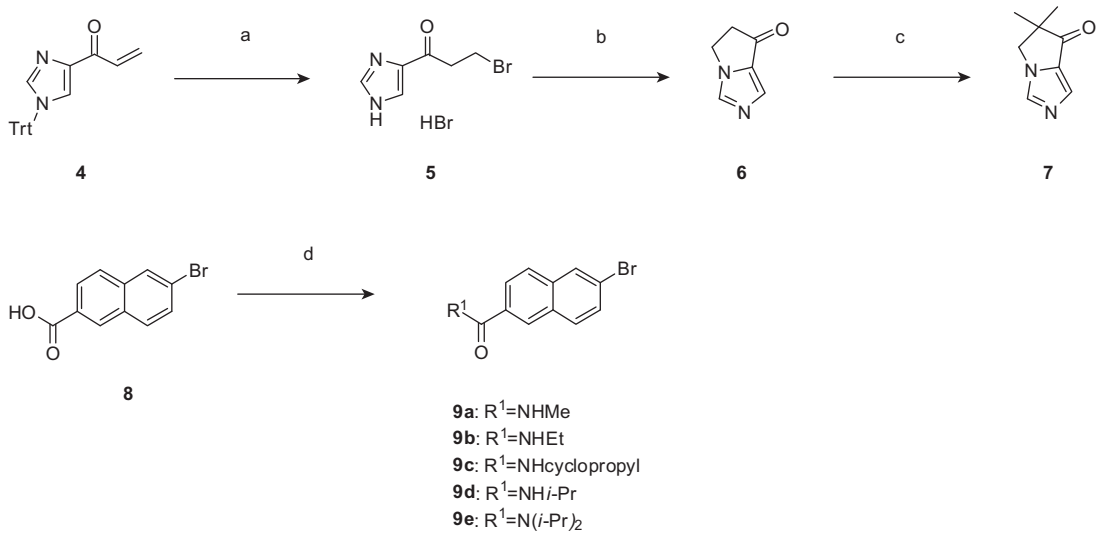
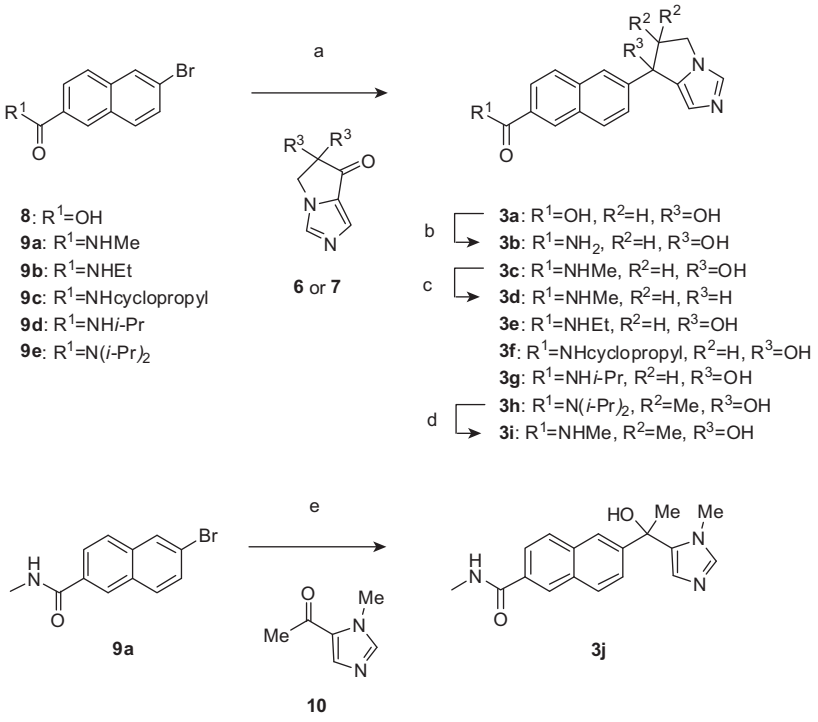


Figure 4. Molecular design of novel 17,20-lyase inhibitors.



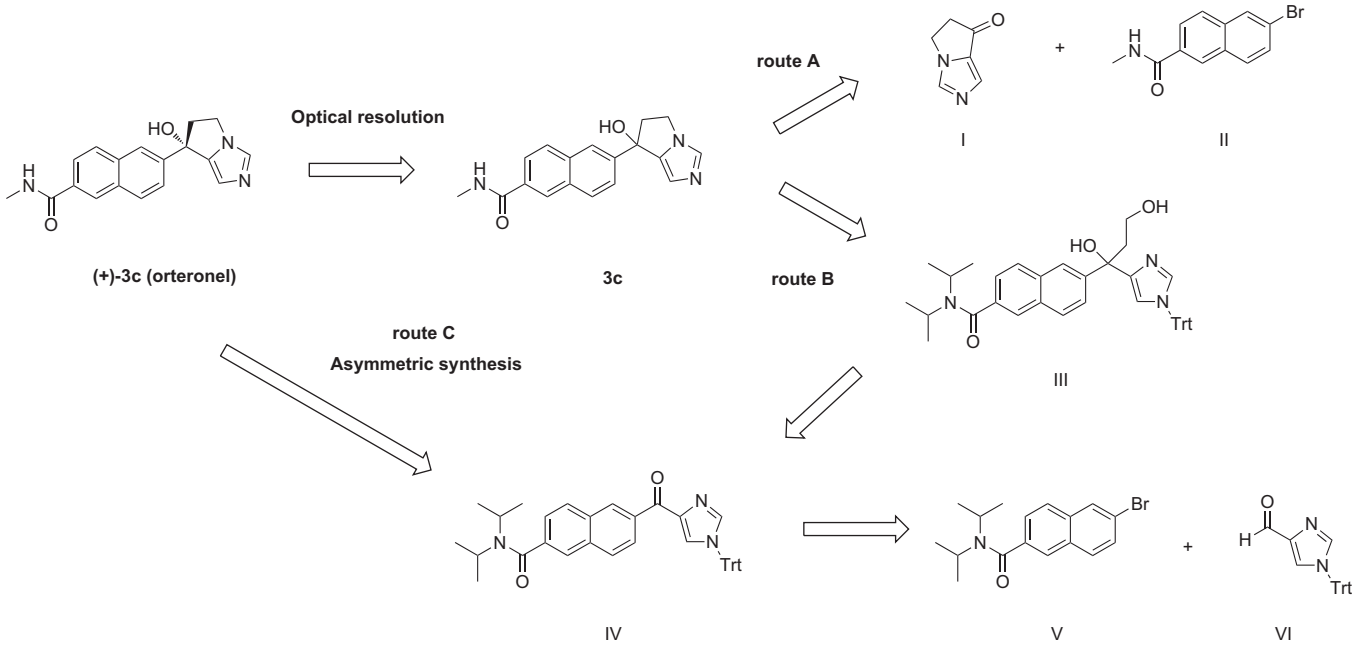
Scheme 2. Reagents and conditions: (a) 25% HBr, AcOH, 0 °C to room temperature, quant.; (b) Et_3N, CH_3CN, 70 °C, 61%; (c) MeI, *t*-BuOK, THF, 43%; (d) for **9a**–**d**, EDCI·HCl, HOBt, $(i\text{-Pr})_2NEt$, R_1H, DMF, 0 °C to room temperature, 60–82%; for **9e**, (i) $SOCl_2$, DMF, THF, 60 °C, (ii) *N*,*N*-diisopropylamine, Et_3N, THF, 0 °C to room temperature, 88%.



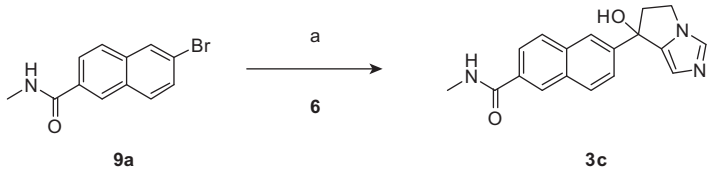
Scheme 3. Reagents and conditions: (a) for **3a**, (i) **8**, *n*-BuLi, THF, −100 to −80 °C, (ii) **6**, THF, −70 °C, 12%, for **3c**, **3e**–**h**, (i) **9a**–**e**, *n*-BuLi, THF, −70 °C, (ii) **6** or **7**, THF, −70 °C, 26–37%; (b) EDCI·HCl, ammonium salt of HOBt, $(i\text{-Pr})_2NEt$, DMF, 0 °C to room temperature, 12%; (c) H_2 (3–4 atom), Pd/C, 1 N HCl, MeOH, room temperature, 72%; (d) *n*-BuLi, $MeNH_2$, THF, −70 °C to room temperature, 33%; (e) *n*-BuLi, THF, −65 to 60 °C, 38%.

lithium species from **9a** and cyclic ketone **6** was optimized. As a result, the use of 1 equiv of *o*-trifluoromethylphenyl lithium gave improved yield of desired product **3c**. That is, the addition of lithium species, generated from 2-bromobenzotrifluoride with *n*-BuLi to **9a** gave lithium salt, which was allowed to react with *n*-BuLi to afford dilithium salt in the reaction vessel. The obtained dilithium salt was then subjected to a coupling reaction with cyclic ketone **6** to produce in 45% yield of racemic **3c**.

An alternative method to synthesize racemic **3c** is shown in Scheme 6 (route B). Coupling of lithium species, generated from amide **9e** and *n*-BuLi, to aldehyde **11** gave alcohol **12**. Oxidation of the obtained alcohol **12** was conducted using manganese (IV) oxide (MnO_2) to give ketone **13** with moderate yield in two steps. Addition of lithium enolates, formed by ethyl acetate (AcOEt) in the presence of lithium diisopropylamide (LDA), to **13** gave **14**. The alcohol **14** was then reduced using Red-Al (sodium bis(2-methoxyethoxy)aluminum hydride) to provide the diol **15** with good yield in two steps. The diol **15** was allowed to react with methanesulfonyl chloride (MsCl) and the resulting mesylate underwent cyclization under basic conditions to produce the cyclized compound **16** in good yield. Subsequent coupling of **16** with lithium amides, generated from methylamine ($MeNH_2$) with *n*-BuLi, gave the desired racemic **3c** in moderate yield.



Scheme 4. Retrosynthesis of compounds (+)-**3c** and **3c**.



Scheme 5. Improved method for the synthesis of racemic **3c**. Reagents and conditions: (a) (i) 2-bromobenzotrifluoride, *n*-BuLi, THF, −65 °C, (ii) **9a**, THF, −55 °C, then (iii) *n*-BuLi, THF, −65 °C, (iv) **6**, THF, −65 °C, 45%.

Successively, optical resolution of racemic **3c** obtained above was performed by preparative HPLC using a Chiralpack AD column to yield (+)-**3c** and (−)-**3c** with 99.8% ee, respectively, as shown in Scheme 7.

2.4. Optical resolution of racemic 3c by diastereomeric salt formation with (2*S*,3*S*)-(−)-tartranilic acid

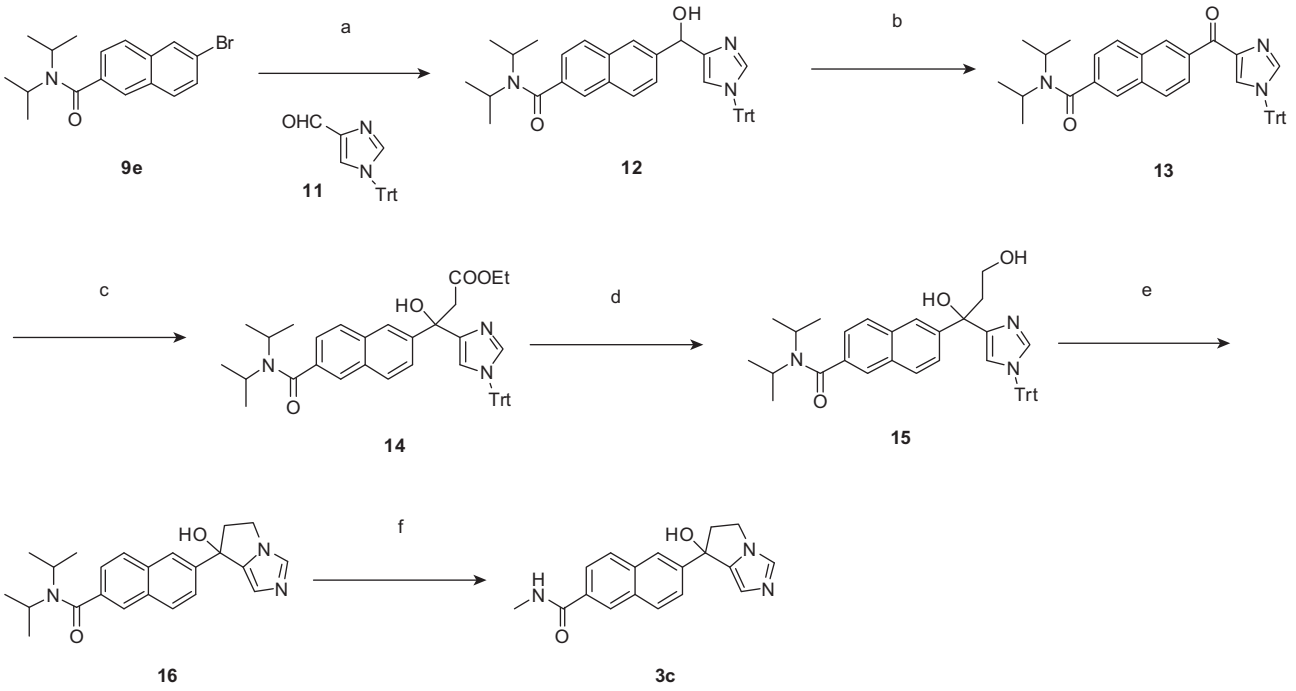
To prepare optically active (+)-**3c** by an alternative method using diastereomeric salt formation, we screened a number of optically active acid derivatives. As a result, it was found that 1 equiv of (+)-**3c** efficiently formed poorly soluble crystals with 2 equiv of (2*S*,3*S*)-(−)-tartranilic acid in ethanol (EtOH) and subsequent recrystallization of the obtained diastereomeric salts gave optically purified (99% de) salts, which were subjected to neutralization with 1 *N* NaOH, followed by recrystallization to yield free (+)-**3c** with >99% ee.

2.5. X-ray crystallographic analysis

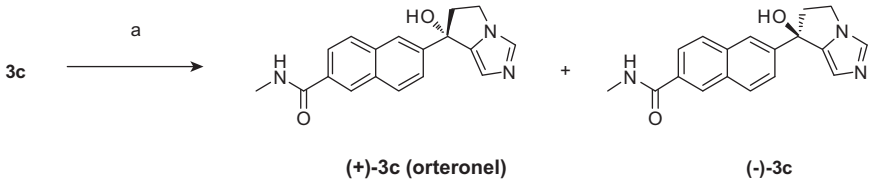
To determine the absolute configuration of (+)-**3c**, the crystal structure of the diastereomeric salt of (+)-**3c** with (2*S*,3*S*)-(−)-tartranilic acid was analyzed. The crystal contained one molecule of (+)-**3c** and two molecules of (2*S*,3*S*)-(−)-tartranilic acid in the asymmetric unit. The absolute configuration of (+)-**3c** was determined to be *S* using the absolute stereochemistry of (2*S*,3*S*)-(−)-tartranilic acid as an internal reference (Fig. 5).

2.6. Asymmetric synthesis of (+)-3c

To establish an efficient and practical asymmetric synthesis of (+)-**3c**, we focused on modification of the coupling reaction utilizing ketone **13** and lithium enolates, as shown in Scheme 6, for the application of a chelation-assisted enantioselective Reformatsky reaction.[45] Asymmetric Reformatsky reactions are versatile and effective methods for providing chiral β-hydroxy esters and, to date, several asymmetric Reformatsky reactions have been reported by several groups.[46–58] In most of the reported cases, 1,2-aminoalcohols were used as chiral ligands to give high enantioselectivity in the asymmetric Reformatsky reactions. More recently, our group reported chelation-assisted enantioselective Reformatsky reactions of ketones, using cinchona alkaloids as chiral ligands.[45] We have previously analyzed a selection of chiral ligands and the effect of additives such as pyridine, as well as the effect of reaction temperature on the enantioselectivity in the Reformatsky reaction. Table 1 summarizes the selected results for the asymmetric Reformatsky reaction with ketone **13** using zinc reagents (**17a** and **17b**) generated from ethyl or *tert*-butyl bromoacetate and activated zinc. The enantioselectivity of the obtained tertiary alcohol **18a** was improved by reduction of the reaction temperature (entry 1 vs 3), the addition of an excess amount of pyridine (entry 1 vs 4) and the addition of a slight excess of cinchonine with an excess amount of pyridine (entry 4 vs 5), respectively in this reaction. Additionally, the use of Reformatsky reagent with *tert*-butyl ester (**17b**, entries 2 and 6) gave a slightly improved enantioselectivity, up to 95% ee, when



Scheme 6. Reagents and conditions: (a) (i) *n*-BuLi, toluene/THF, −70 °C, (ii) **11**, THF, −70 °C; (b) MnO_2, CH_2Cl_2, room temperature, 60% in 2 steps; (c) AcOEt, LDA, THF, −70 °C to −30 °C; (d) Red-Al, toluene, −15 °C to 0 °C, 98% in 2 steps; (e) (i) MsCl, $(i\text{-Pr})_2$NEt, THF, 0–10 °C, (ii) CH_3CN, 70 °C, (iii) $(i\text{-Pr})_2$NEt, MeOH, CH_3CN, 70 °C, 87%; (g) *n*-BuLi, $MeNH_2$, THF, −78 °C to room temperature, 79%.



Scheme 7. Optical resolution of racemic **3c**. Reagents and conditions: (a) optical resolution using preparative HPLC on a Chiralpak AD column, (+)-**3c** (99.8% ee) and (−)-**3c** (99.8% ee), respectively.

compared with the corresponding reagent with ethyl ester (**17a**, entries 1 and 5) under the same reaction conditions. The chiral tertiary alcohol of **18b** with 71% ee obtained in entry 2 was successfully converted to (+)-**3c** with 69% ee as a similar manner described in Scheme 6, and the configuration of **18b** obtained in this reaction using cinchonine as a chiral ligand was determined to be *S*.

2.7. In vitro inhibition of 17,20-lyase, CYP3A4 and 11-hydroxylase

All compounds synthesized as racemates were tested in vitro for inhibition of rat and human 17,20-lyase, human CYP3A4. Selected compounds were resolved by HPLC and the resulting optical isomers were tested using the same in vitro assay. The impact of

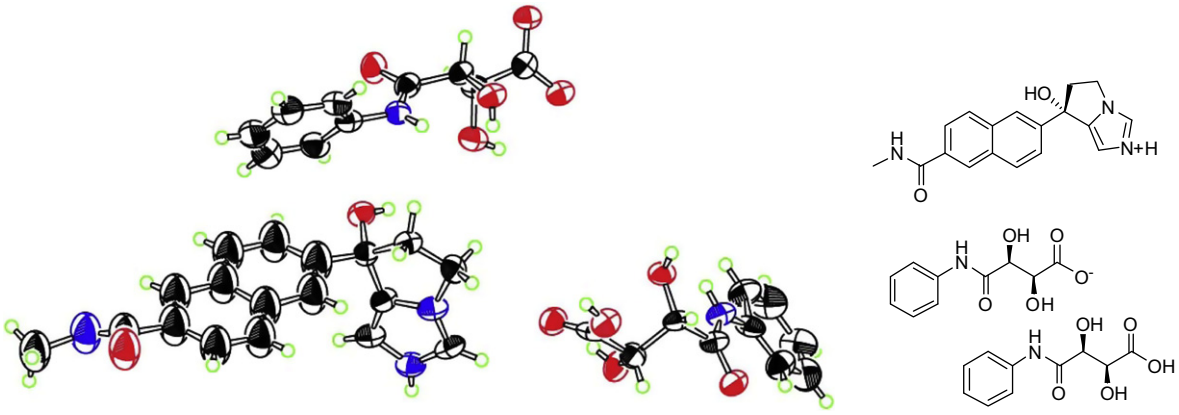
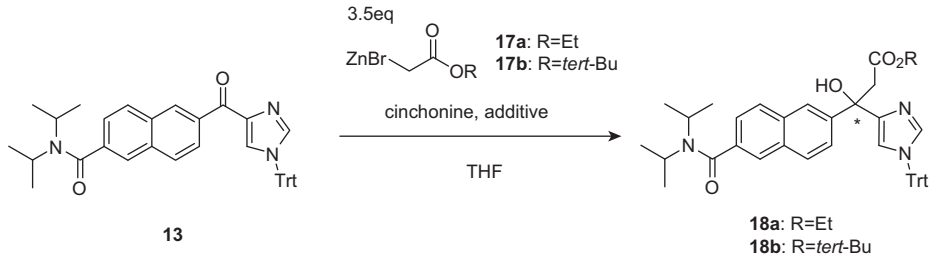


Figure 5. ORTEP drawing of (+)-**3c** with (2*S*,3*S*)-(−)-tartranilic acid.

Table 1
Reaction conditions of newly developed asymmetric Reformatsky reaction using ketone **13** and zinc reagents **17a** and **17b**



Entry	R	Temp (°C)	Additive (equiv)	Cinchonine (equiv)	ee (%)	Yield[a] (%)
1	Et	0	None	1	67	Quant.
2	*tert*-Bu	0	None	1	71[b]	Quant.
3	Et	−42	None	1	76	97
4	Et	−42	Pyridine (3)	1	85	98
5[c]	Et	−42	Pyridine (4)	1.25	90	99
6[d]	*tert*-Bu	−42	Pyridine (4)	1.25	95 (92)[e]	Quant. (97)[e]

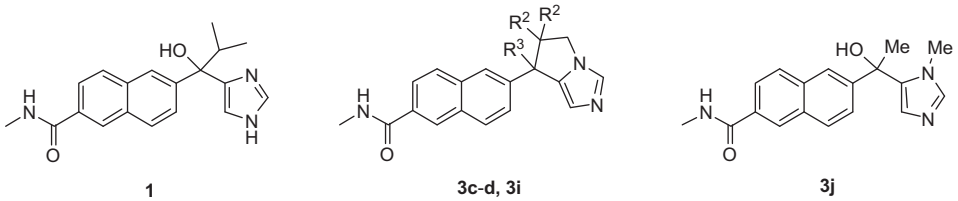
[a] Determined by HPLC.
[b] The absolute configuration of **18b** was determined to be *S* after the conversion to (+)-**3c**.
[c] 4 equiv of Reformatsky reagent **17a** was used.
[d] 4 equiv of Reformatsky reagent **17b** was used.
[e] Large scale preparation (2.50 g of **13** was used).

fused imidazole derivatives and related compound **3j** on inhibition of rat and human 17,20-lyase, 11-hydroxylase and CYP3A4 are shown in Tables 2 and 3. The cyclized compound **3c** demonstrated both promising inhibitory activity against human 17,20-lyase with IC_{50} value of 38 nM and selectivity between human 17,20-lyase and the other CYPs (vs 11-hydroxylase and CYP3A4). On the contrary, *gem*-dimethyl derivatives **3i** with fused imidazole ring exhibited decreased inhibitory activity against human 17,20-lyase with lower selectivity between human 17,20-lyase and the other CYPs, compared with **3c**. The results were consistent with our previous docking experiment of **1a**, and they suggested that the bulkyness near the coordinated site should give some negative impact on inhibitory activity against 17,20-lyase. Additionally, for better understanding of excellent selectivity between human 17,20-lyase and the other CYPs of **3c**, a few analogues of **3c** were also investigated.

As a result, the removal of hydroxyl group (**3d**) of **3c** led to considerably decreased selectivity between human 17,20-lyase and the other CYPs while keeping the potent inhibitory activity against human 17,20-lyase. The cleaved derivatives (**3j**) of fused imidazole ring on **3c** was also investigated to result in both decreased inhibitory activity against 17,20-lyase and selectivity between 17,20-lyase and CYP3A4. These results indicated that both hydroxyl group and fused ring structure of **3c** may have the responsibility for its selectivity between 17,20-lyase and CYP3A4.

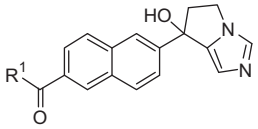
The effect of substitution of carbamoyl group on inhibition of rat and human 17,20-lyase, 11-hydroxylase and CYP3A4 was summarized in Table 3. Replacement of methylcarbamoyl group of **3c** with carbamoyl group (**3b**) had little effect on inhibitory activity against human 17,20-lyase and selectivity between 17,20-lyase and CYP3A4, but led to decreased selectivity between 17,20-lyase and 11-hydroxylase. Replacing the methyl group of **3c** with small

Table 2
Inhibitory effect of compounds **1**, **3c–d**, **3i–j** on rat and human 17,20-lyase, 11-hydroxylase and CYP3A4



Compound no.	R^2	R^3	Enzyme inhibition IC_{50} (nM)			
			17, 20-Lyase		11-hydroxylase	CYP3A4
			Rat	Human		
1	—		6.1	16	>1000	3600
3c	H	OH	54	38	>1000	>10,000
3i	Me	OH	110	88	520	3200
3d	H	H	15	13	290	<1000
3j	—		>1000	410	>1000	6900

Table 3
Inhibitory effect of compounds **3b–c**, **3e–g** on rat and human 17,20-lyase, 11-hydroxylase and CYP3A4



3b-c, 3e-g

Compound no.	R^1	Enzyme inhibition IC_{50} (nM)			
		17, 20-lyase		11-hydroxylase	CYP3A4
		Rat	Human		
3c	NHMe	54	38	>1000	>10,000
3b	NH_2	24	29	100	>10,000
3e	NHEt	110	190	>1000	>10,000
3f	NHcyclopropyl	100	290	>1000	>10,000
3g	NH*i*-Pr	160	400	>1000	>10,000

alkyl groups such as ethyl (**3e**), cyclopropyl (**3f**) and *i*-propyl (**3g**) groups resulted in 5–11-fold weaker inhibitory activity against human 17,20-lyase. These results suggested that the methylcarbamoyl group (**3c**) was optimal in terms of both inhibitory activity against human 17,20-lyase and selectivity between 17,20-lyase and the other CYPs.

The enantiomers, (+)-**3c** and (−)-**3c**, both exhibited potent inhibitory activity against human 17,20-lyase and promising selectivity between human 17,20-lyase and CYP3A4, as shown in Table 4. In particular, the inhibitory effect on CYP3A4 of (+)-**3c** was hardly detectable at 10,000 nM. Therefore, (+)-**3c** was identified as a highly selective inhibitor with a greater than 500-fold selectivity between 17,20-lyase and CYP3A4. The enantiomers were also assessed for the inhibition of 11-hydroxylase, and both (+)-**3c** and (−)-**3c** exhibited low probability of inhibition against 11-hydroxylase below 1000 nM.

2.8. A preliminary study in cynomolgus monkeys

In addition to the in vitro studies, the ability of (+)-**3c** and (−)-**3c** to reduce serum androgen concentrations were examined in a preliminary study using cynomolgus monkeys, and in these studies both testicular androgen testosterone and adrenal androgen dehydroepiandrosterone (DHEA) levels were measured. As a result, (+)-**3c** administered orally at a dose of 1 mg/kg reduced serum testosterone and DHEA levels 8 h after administration (Table 5). In contrast, (−)-**3c** had a smaller effect on these parameters. This difference in in vivo efficacy between (+)-**3c** and (−)-**3c** may reflect differences in in vitro inhibitory activities and the pharmacokinetic profiles of the two compounds, but additional investigations would be required to examine these possibilities. In summary, these biological evaluations revealed that (+)-**3c** had a promising profile for further development in terms of both in vitro inhibitory activity and in vivo potency with exploitable selectivity between 17,20-lyase and CYP3A4.

Table 4
Inhibitory effect of (+)-**3c** and (−)-**3c** on rat and human 17,20-lyase, 11-hydroxylase and CYP3A4

Compound no.	Enzyme inhibition IC_{50} (nM)			
	17, 20-lyase		11-hydroxylase	CYP3A4
	Rat	Human		
(+)-**3c**	48	19	>1000	>10,000
(−)-**3c**	180	48	>1000	>10,000

2.9. In vivo efficacy

In a monkey model, both testosterone and DHEA levels were investigated at different time points (2, 5 and 10 h) after dosing to evaluate in vivo potency of (+)-**3c**, as shown in Table 6. When given orally to monkeys at a dose of 1 mg/kg, (+)-**3c** markedly reduced serum testosterone and DHEA at 5 h after administration. This finding suggested that (+)-**3c** should be useful for the clinical treatment of both hormone-dependent prostate cancer and CRPC.

2.10. Inhibitory effect of (+)-3c on CYP isoform-specific activities

In a series of in vitro studies, the possible inhibitory effect of the selected compound (+)-**3c** on CYP-specific activities was investigated using microsomes expressing human CYP isoforms. As shown in Table 7, (+)-**3c** inhibited the specific activities of CYP2C19; however, the IC_{50} values for all the CYP-specific activities were greater than 10,000 nM. Inhibitory effects on CYP2A6, CYP2B6 and CYP3A4 were hardly detectable with IC_{50} values of up to 30,000 nM. Compound (+)-**3c** was shown to exhibit greater inhibitory effects on 17,20-lyase compared with the other CYP isoforms, thus indicating that (+)-**3c** is a selective 17,20-lyase inhibitor.

2.11. Docking experiments and enzyme selectivity

To determine the binding mode of (+)-**3c**, a docking study was conducted using a homology model for the catalytic domain of 17,20-lyase. In the docking experiment, it was postulated that (+)-**3c** was coordinated to the catalytic heme iron via the nitrogen of the imidazole ring moiety. The results of the docking study are shown in Figure 6, and suggest that the naphthalene ring of

Table 5
In vivo effects of (+)-**3c** and (−)-**3c** on serum testosterone and DHEA levels after single oral dosing (1 mg/kg) in cynomolgus monkeys

Compound	% of average 0 h values, mean ± SD	
	Serum testosterone	Serum DHEA
	8 h	8 h
Vehicle vs (+)-**3c**	101 ± 54	54 ± 25
(+)-**3c** (1 mg/kg)	30 ± 23	12 ± 8.0
Vehicle vs (−)-**3c**	296 ± 277	25 ± 6.2
(−)-**3c** (1 mg/kg)	258 ± 302	91 ± 126

Table 6
In vivo effects of (+)-**3c** on serum testosterone and DHEA levels after single oral dosing (1 mg/kg) in cynomolgus monkeys

Compound	% of pre-treatment average[a], mean ± SD					
	Serum testosterone			Serum DHEA		
	2 h	5 h	10 h	2 h	5 h	10 h
Vehicle	90.0 ± 16.6	63.0 ± 19.2	269.8 ± 94.5	97.9 ± 22.3	88.6 ± 4.4	7.2 ± 0.99
(+)-**3c** (1 mg/kg)	75.9 ± 50.0	14.2 ± 3.2	55.6 ± 18.7	61.1 ± 15.1	32.8 ± 11.1	7.6 ± 1.8

[a] Mean serum testosterone and DHEA levels on three consecutive days prior to treatment.

Table 7
Inhibitory effect of (+)-**3c** on human 17,20-lyase (CYP17A1) and marker enzyme activities of microsomes expressing human CYP isoforms

Enzyme inhibition IC_{50} (nM) CYP isoforms										
17A1 (17,20-lyase)	1A2	2A6	2B6	2C8	2C9 (Arg)	2C9 (Cys)	2C19	2D6	2E1	3A4
19	28,000	>30,000	>30,000	>30,000	>30,000	>30,000	14,000	>30,000	>30,000	>30,000

Marker enzyme activities are as follows; CYP1A2: 7-ethoxyresorufin *O*-deethylation; CYP2A6: coumarin 7-hydroxylation; CYP2B6: ethoxycoumarin *O*-deethylation; CYP2C8, 2C9(Arg), 2C9(Cys): tolbutamide hydroxylation; CYP2C19: (*S*)-mephenytoin 4′-hydroxylation; CYP2D6: (+)-bufuralol 1′-hydroxylation; CYP2E1: 4-nitrophenol hydroxylation; CYP3A4: testosterone 6β-hydroxylation.

(+)-**3c** is contained in a hydrophobic pocket surrounded by Ala 113, Phe 114, Ile 205, Ala 302 and Ile 371. In addition, the carbamoyl moiety of (+)-**3c** was shown to form a hydrogen bond with Thr 101, while the hydroxy group formed a hydrogen bond with Thr 306. These results were consistent with our previous experiments on **1a**. Moreover, the superimposition of (+)-**3c** with 17α-hydroxypregnenolone, a known substrate of 17,20-lyase, demonstrated that the naphthalene ring of (+)-**3c** and the B and C rings of the steroidal skeleton of 17α-hydroxypregnenolone are located in a similar position (Fig. 7).

Using the same techniques, a homology model for the catalytic domain of CYP3A4 was constructed to rationalize the characteristic properties of (+)-**3c** identified as a highly selective 17,20-lyase inhibitor. First, the impact of conformational change as a factor contributing to enzyme selectivity was examined for two different inhibitors, compounds **1a** (CYP3A4 IC_{50} = 7800 nM) and (+)-**3c** (CYP3A4 IC_{50} = >30,000 nM) (Fig. 8). In the homology model, it was shown that when **1a** is bound to the active site of CYP3A4, the naphthyl group is located in the pocket, as shown in Figure 8. However, in the case of (+)-**3c**, owing to the high rigidity of the molecule, it was difficult to superimpose (+)-**3c** with **1a** for this model. Furthermore, we determined that when (+)-**3c** was coordinated to the heme iron via the nitrogen of the fused imidazole ring moiety, a part of the naphthyl group appeared to be out of the pocket, shown as a red dotted circle in Figure 8. Based on these observations and previously discussed SAR study in Table 2, the conformational rigidity of the fused imidazole ring moiety of (+)-**3c** might contribute to its selectivity of 17,20-lyase over CYP3A4.

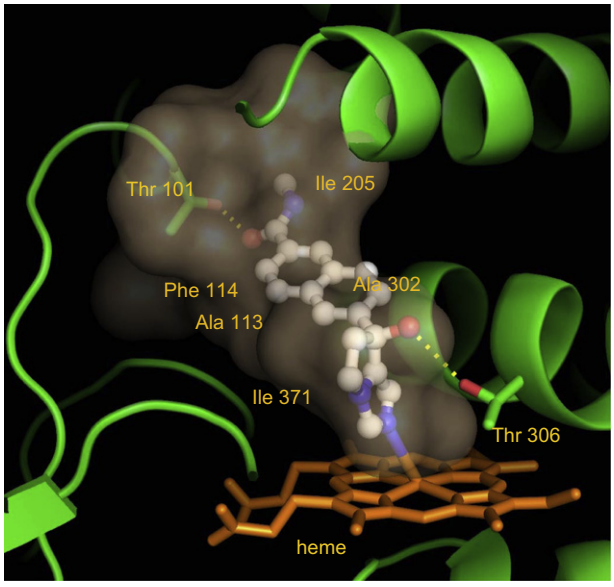


Figure 6. Docking study of (+)-**3c** in the homology model of 17,20-lyase.

Another potential factor that may affect enzyme selectivity is the lipophilicity of the inhibitors. As described above, CYP family of enzymes is known to prefer lipophilic molecules as substrates or inhibitors. Therefore, an index such as *C*log*P*, which represents the lipophilicity of a molecule, may, at least in part, provide helpful information on enzyme selectivity. The *C*log*P* values of compounds **1a** and (+)-**3c** were 1.5 and 0.3, respectively, and the correlation between the potency of inhibition for CYP3A4 and *C*log*P* values of these two inhibitors were as expected; (+)-**3c** with a lower *C*log*P* value showed less potent inhibition against CYP3A4 than **1a** with a higher *C*log*P* value. Based on these data, the decreased inhibition of CYP3A4 by (+)-**3c** seemed to be partly due to a decrease in lipophilicity of the molecule. However, regarding the inhibition of 17,20-lyase, (+)-**3c** shows potent inhibition with a lower *C*log*P* value; therefore, additional contributions such as hydrogen bond interactions proposed by docking experiments should be considered for further discussion. In summary, these observations suggested that two main factors, namely conformational rigidity of the fused imidazole ring moiety and the low *C*log*P* value of (+)-**3c**, may account for the observed selectivity for 17,20-lyase versus CYP3A4.

3. Conclusions

We have successfully synthesized a new class of 17,20-lyase inhibitor. As a result of incorporating the fused imidazole ring system, (+)-**3c** was identified as a potent and highly selective inhibitor of 17,20-lyase. Further biological evaluation of (+)-**3c** revealed it to be associated with potent reductions in both serum testosterone and DHEA concentrations in monkeys administered a 1 mg/kg dose of (+)-**3c**. Furthermore, molecular modeling and general consideration for CYP enzymes suggests that the structural and physicochemical properties of (+)-**3c**, such as conformational rigidity and low *C*log*P* value, may account for the observed selectivity for 17,20-lyase over other CYP enzymes. In general, ring systems such

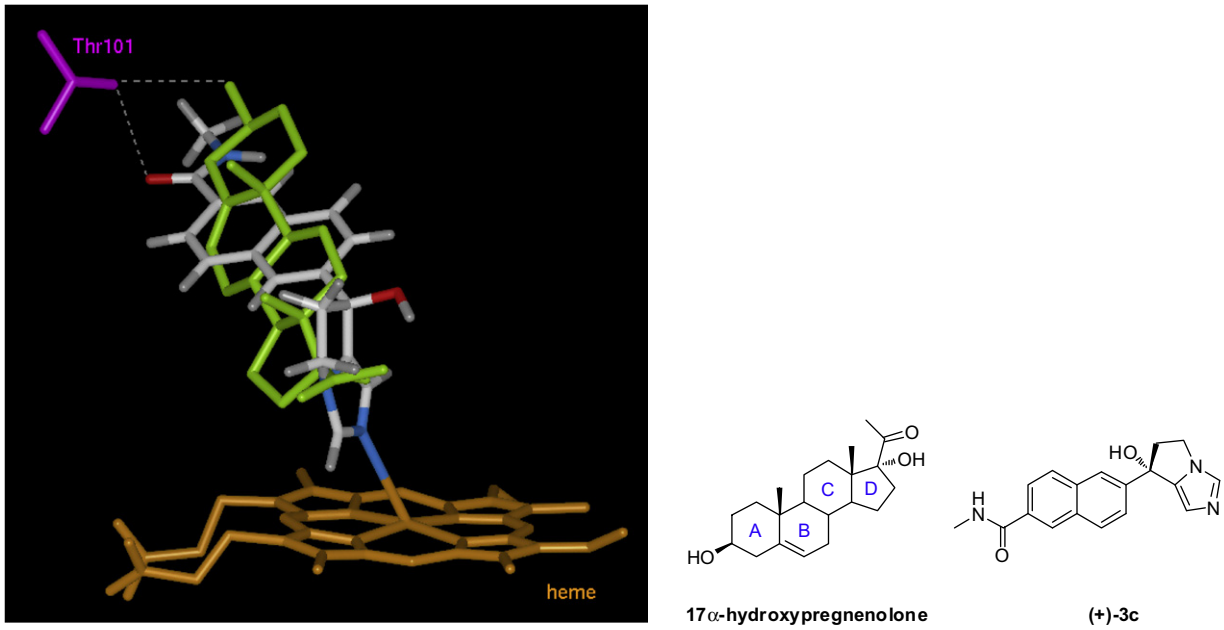


Figure 7. Overlay of (+)-**3c** (white) with 17α-hydroxypregnenolone (green) in the homology model of 17,20-lyase.

as imidazole and pyridine rings are known to be good ligands for heme iron, which is found in the CYP enzymes. Therefore, difficulties are often encountered when developing CYP inhibitors with these rings owing to their ability to bind nonselectively to heme iron found in the CYP enzymes. We believe that these findings regarding the selectivity of (+)-**3c** over the CYPs could be applied to other series of CYP inhibitors and our approach to (+)-**3c** may provide a new methodology in this field. Finally, (+)-**3c** (orteronel [TAK-700]) was selected as a candidate for further development and is currently being evaluated in patients in phase III clinical trials for the potential treatment of prostate cancer.

4. Experimental

4.1. General

Melting points were determined using a BUCHI Melting Point B-545 apparatus and are uncorrected. Infrared (IR) spectra were taken using a Shimadzu FT-IR-8200PC spectrometer. ^{1}H NMR spectra were recorded using a Varian Gemini-200 or Varian Mercury-300 spectrometer; chemical shifts are given in ppm with tetramethylsilane as an internal standard, and coupling constants (*J*) are measured in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br s = broad singlet. Reactions were followed by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} precoated TLC plates (E. Merck). Column chromatography was carried out using Silica Gel 60 (E. Merck, Darmstadt, Germany).

4.2. Optical resolution of 1 using preparative HPLC

Compound **1** (12.7 g, 39.3 mmol) was subjected to optical resolution by preparative HPLC using a Chiralpak AD column (50 mm × 500 mm) using hexane/ethanol (85/15) as an eluent, detected at 254 nm, to afford **1a** (6.09 g, 18.8 mmol, 48%) as a first elution and **1b** (6.02 g, 18.6 mmol, 47%) as a second elution. **1a**: $[\alpha]_D^{25}$ −55.7 (*c* 0.908, MeOH); t_R = 17.1 min (Chiralpak AD 4.6 mmID × 250 mmL, hexane/ethanol = 85/15, 0.8 mL/min, at 254 nm); >99.9% ee. Compound **1b**: $[\alpha]_D^{25}$ +56.1 (*c* 0.924, MeOH); t_R = 29.0 min (Chiralpak AD 4.6 mmID × 250mmL, hexane/ethanol = 85/15, 0.8 mL/min, at 254 nm); 99.8% ee.

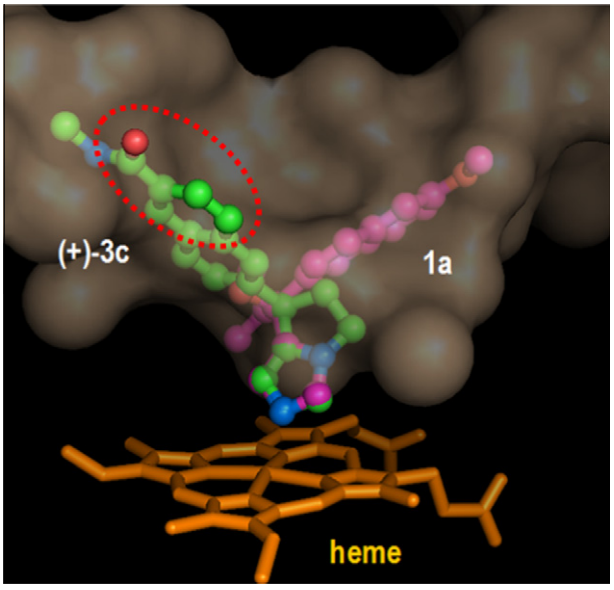


Figure 8. Docking study of **1a** (magenta) with (+)-**3c** (green) in the homology model of CYP3A4.

4.3. 6-[(1*S*)-1-{1-[(4-Bromophenyl)sulfonyl]-1*H*-imidazol-4-yl}-1-hydroxy-2-ethylpropyl]-*N*-methylnaphthalene-2-carboxamide (2)

To a solution of **1a** (210 mg, 0.65 mmol) and Et_3N (0.226 mL, 1.62 mmol) in DMF (5 mL) was added dropwise 4-bromobenzenesulfonyl chloride (166 mg, 0.65 mmol) and the mixture was stirred at room temperature for 16 h. After being diluted with aqueous $NaHCO_3$ solution, the appeared solid was filtered off, washed with H_2O and dried in air. The obtained solid was recrystallized from AcOEt and tetrahydrofuran (THF) to give **2** (278 mg,

0.512 mmol, 79%) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ: 0.62 (3H, d, J = 6.8 Hz), 0.73 (3H, d, J = 6.8 Hz), 2.64–2.78 (1H, m), 2.82 (3H, d, J = 4.5 Hz), 5.55 (1H, s), 7.49 (1H, d, J = 1.5 Hz), 7.80–7.94 (6H, m), 7.96–8.04 (2H, m), 8.09 (1H, s), 8.33 (1H, s), 8.37 (1H, d, J = 1.5 Hz), 8.54 (1H, q, J = 4.6 Hz). $[\alpha]_D^{25}$ +57.6 (c 0.187, MeOH).

4.4. 3-Bromo-1-(1*H*-imidazol-4-yl)-1-propanone hydrogen bromide (5)

25% hydrogen bromide in acetic acid (100 mL) was added dropwise to a cooled (10 °C) solution of **4** (29.0 g, 80.0 mmol) in acetic acid (130 mL) and the mixture was stirred for 2 h at room temperature. After being diluted with diisopropylether (*i*-Pr_2O) the precipitate was collected by filtration and washed with *i*-Pr_2O to give **5** (22.3 g, quant.) as a pale yellow powder. ^{1}H NMR (CD_3OD) δ: 3.54–3.81 (4H, m), 8.50 (1H, d, J = 1.2 Hz), 9.15 (1H, d, J = 1.2 Hz).

4.5. 5,6-Dihydro-7*H*-pyrrolo[1,2-*c*]imidazol-7-one (6)

A solution of Et_3N (15.3 mL, 110 mmol) in CH_3CN (25 mL) was added dropwise to a hot (70 °C) suspension of **5** (28.5 g, 100 mmol) in CH_3CN (1100 mL) and the mixture was stirred at 70 °C for 2 h. Triethylamine (25 mL, 179 mmol) was then added to the mixture, and the solution was stirred at 70 °C for a further 30 min. After cooling to room temperature, the insoluble material was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the insoluble material was again filtered off. The filtrate was concentrated under reduced pressure and the resulting residue was separated by chromatography on silica gel (CH_2Cl_2/5% ammonia in MeOH = 10:1) to give **6** (6.67 g, 61%) as a colorless powder. ^{1}H NMR($CDCl_3$) δ: 3.24 (2H, t, J = 6.5 Hz), 4.41 (2H, t, J = 6.5 Hz), 7.60 (1H, s), 7.74 (1H, s). IR(KBr): 3121, 1713, 1537, 1489, 1412, 1319, 1204, 1109 cm^{-1}.

4.6. 6,6-Dimethyl-5,6-dihydro-7*H*-pyrrolo[1,2-*c*]imidazol-7-one (7)

Potassium *tert*-butoxide (*t*-BuOK) (3.14 g, 28.0 mmol) was added dropwise to a cooled (0 °C) solution of **6** (1.71 g, 14.0 mmol) in anhydrous tetrahydrofuran (THF) (60 mL), and the solution was stirred for 15 min at 0 °C. To the mixture, MeI (1.74 ml, 28.0 mmol) was added dropwise, and the whole was stirred for 2 h at 0 °C. After dilution with 20% aqueous ammonium chloride solution and addition of sodium chloride, the aqueous phase was extracted with THF and the extracts were dried over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by column chromatography on silica gel (AcOEt) to give **7** (0.90 g, 43%) as a colorless powder. Mp 87 °C (*i*-Pr_2O). IR (KBr): 3112, 2971, 1713, 1535, 1211, 1113, 841, 654 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 1.37 (6H, s), 4.15 (2H, s), 7.63 (1H, s), 7.71 (1H, s). Anal. Calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 64.09; H, 6.98; N, 18.81.

4.7. 6-Bromo-*N*-methyl-2-naphthamide (9a)

Under an argon atmosphere, to a cooled (0 °C) solution of **8** (60.26 g, 240 mmol), EDCI·HCl (55.21 g, 288 mmol), HOBt·H_2O (44.1 g, 288 mmol) and *N*,*N*-diisopropylethylamine ((*i*-Pr)$_2$NEt) (37.23 g, 288 mmol) in anhydrous *N*,*N*-dimethylformamide (DMF) (960 mL) was added dropwise to a solution of $MeNH_2$ (2 M solution in THF; 192 mL, 384 mmol) and the whole was stirred at room temperature for 18 h. After dilution with water, the precipitate was filtered off, washed with H_2O and *i*-Pr_2O and dried under the reduced pressure to give **9a** (60.6 g, 82%) as a colorless powder. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 3.04 (3H, s), 7.60 (1H, dd, J = 1.8 Hz, 8.6 Hz), 7.78 (2H, d, J = 8.6 Hz), 7.85 (1H, dd, J = 1.8 Hz, 8.6 Hz), 8.03 (1H, d, J = 1.8 Hz), 8.25 (1H, s). IR (KBr): 3274, 1638, 1622, 1559, 1495, 1408, 1316, 1159 cm^{-1}. Anal. Calcd for $C_{12}H_{10}NOBr.0.1H_2O$: C, 54.20; H, 3.87; N, 5.27; Br, 30.25. Found: C, 54.03; H, 3.72; N, 5.24. Compounds **9b–d** were prepared in the same manner as described for the preparation of **9a**.

4.8. 6-Bromo-*N*-ethyl-2-naphthamide (9b)

Yield 60%. ^{1}H NMR ($CDCl_3$) δ:1.30 (3H, t, J = 7.3 Hz), 3.56 (2H, dq, J = 5.6 Hz, 7.3 Hz), 6.29 (1H, br s), 7.60 (1H, dd, J = 2.0 Hz, 8.8 Hz), 7.77 (2H, d, J = 8.8 Hz), 7.85 (1H, dd, J = 2.0 Hz, 8.8 Hz), 8.03 (1H, d, J = 2.0 Hz), 8.24 (1H, s). IR (KBr): 3275, 2976, 1638, 1620, 1555, 1460, 1314, 1186 cm^{-1}. Anal. Calcd for $C_{13}H_{12}NOBr.0.25H_2O$: C, 55.24; H, 4.46; N, 4.96; Br, 28.73. Found: C, 55.54; H, 4.16; N, 4.80.

4.9. 6-Bromo-*N*-cyclopropyl-2-naphthamide (9c)

Yield 70%. ^{1}H NMR ($CDCl_3$) δ: 0.64–0.73 (2H, m), 0.87–0.97 (2H, m), 2.90–3.02 (1H, m), 6.42 (1H, br s), 7.60 (1H, dd, J = 2.0 Hz, 8.8 Hz), 7.77 (2H, d, J = 8.8 Hz), 7.82 (1H, dd, J = 2.0 Hz, 8.8 Hz), 8.03 (1H, d, J = 2.0 Hz), 8.21 (1H, d, J = 2.0 Hz). IR (KBr): 3254, 3061, 1632, 1618, 1541, 1491, 1318, 1138 cm^{-1}. Anal. Calcd for $C_{14}H_{12}NOBr$: C, 57.95; H, 4.17; N, 4.83; Br, 27.54. Found: C, 57.71; H, 4.12; N, 4.79.

4.10. 6-Bromo-*N*-isopropyl-2-naphthamide (9d)

Yield 70%. ^{1}H NMR ($CDCl_3$) δ:1.31 (6H, d, J = 6.6 Hz), 4.27–4.44 (1H, m), 6.09 (1H, d, J = 7.8 Hz), 7.60 (1H, dd, J = 1.8 Hz, 8.8 Hz), 7.78 (2H, d, J = 8.8 Hz), 7.84 (1H, dd, J = 1.8 Hz, 8.8 Hz), 8.03 (1H, d, J = 1.8 Hz), 8.22 (1H, s). IR (KBr):3262, 2973, 1634, 1620, 1557, 1468, 1352, 1186 cm^{-1}. Anal. Calcd for $C_{14}H_{14}NOBr$: C, 57.55; H, 4.83; N, 4.79; Br, 27.35. Found: C, 57.40; H, 4.65; N, 4.83.

4.11. 6-Bromo-*N*,*N*-diisopropyl-2-naphthamide (9e)

A solution of 6-bromo-2-naphthoic acid **8** (100 g, 398 mmol), thionylchloride ($SOCl_2$) (37.7 mL, 517 mmol) and DMF (0.5 mL) in THF (1000 mL) was stirred at 60 °C for 90 min. After cooling to room temperature, the solvent and excess thionylchloride were evaporated. The resulting residue was dissolved in dry THF (400 mL) and the solution was added dropwise to a cooled (0 °C) solution of diisopropylamine (112 mL, 799 mmol) and Et_3N (112 mL, 804 mmol) in THF (800 mL). After stirring at room temperature for 1 h, about half the amount of solvent was evaporated. The mixture was then diluted with AcOEt and the organic phase was washed with H_2O, 1 *N* NaOH solution and brine, dried over $MgSO_4$ and evaporated. The residue was washed with *i*-Pr_2O to give **9e** (117 g, 88%) as a colorless crystal. ^{1}H NMR ($CDCl_3$) δ: 1.36 (12H, br s), 3.71 (2H, br s), 7.44 (1H, dd, J = 1.2 Hz, 8.6 Hz), 7.58 (1H, dd, J = 2.2 Hz, 8.8 Hz), 7.70–7.79 (3H, m), 8.01 (1H, d, J = 1.2 Hz). IR (KBr) : 2968, 1620, 1435, 1369, 1333, 895, 814 cm^{-1}. Anal. Calcd for $C_{17}H_{20}NOBr$: C, 61.09; H, 6.03; N, 4.19; Br, 23.91. Found: C, 61.08; H, 5.88; N, 4.11.

4.12. 6-(7-Hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl)-2-naphthoic acid (3a)

Under an argon atmosphere, **8** (1.51 g, 6.0 mmol) was dissolved in dry THF (50 mL) and the solution was cooled to −100 °C in a liquid N_2 and diethylether bath. A *n*-BuLi hexane solution (1.6 M: 7.88 mL, 12.6 mmol) was added dropwise over 5 min with stirring and the mixture was stirred at the same temperature for 30 min and at −80 °C for 10 min. After cooling to −100 °C, a solution of **6** (610 mg, 6.0 mmol) in dry THF (11 mL) was added dropwise over 5 min and the whole was stirred at the same temperature for

30 min. After being warmed up to −70 °C over 30 min, the reaction was quenched with saturated aqueous ammonium chloride solution. The mixture was diluted with AcOEt and the organic layer was separated. The aqueous phase was concentrated to dryness under reduced pressure. The resulting residue was chromatographed on silica gel ($CHCl_3$/MeOH/H_2O = 65:25:4) and concentrated under the reduced pressure. The residue was dissolved in MeOH and concentrated again under the reduced pressure. The appeared precipitates were washed with diethylether to give **3a** (180 mg, 12%) as a colorless powder. Mp >250 °C decomp (Et_2O–MeOH). ^{1}H NMR (CD_3OD) δ: 2.87–3.13 (2H, m), 4.28–4.50 (2H, m), 6.94 (1H, s), 7.65 (1H, dd, J = 1.6 Hz, 8.6 Hz), 7.90 (1H, d, J = 8.4 Hz), 7.99 (1H, d, J = 8.6 Hz), 8.01 (1H, s), 8.06 (1H, dd, J = 1.4 Hz, 8.4 Hz), 8.09 (1H, s), 8.57 (1H, s). IR (KBr): 3500–3000, 1698, 1609, 1551, 1480, 1397, 1325, 1086 cm^{-1}. FAB-Ms MH+ 295.

4.13. 6-(7-Hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-c]imidazol-7-yl)-2-naphthamide (3b)

To a cooled (0 °C) solution of **3a** (449 mg, 1.53 mmol), EDCI·HCl (321 mg, 1.67 mmol), ammonium salt of HOBt (301 mg, 1.98 mmol) in anhydrous DMF (7.6 mL) was added dropwise (i-Pr)$_2$NEt (216 mg, 1.67 mmol) and the mixture was stirred at room temperature for 18 h. After being concentrated under the reduced pressure with silica gel (3 g), the residue was purified by column chromatography on silica gel ($CHCl_3$/7%NH_3 in MeOH = 19:1) and recrystallized from EtOH to give **3b** (53 mg, 12%). Mp >186 °C decomp ($CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 2.94–3.00 (2H, m), 4.15–4.40 (2H, m), 6.82 (1H, s), 7.58 (1H, s), 7.66 (1H, dd, J = 1.8 Hz, 8.6 Hz), 7.90 (2H, s), 7.95 (1H, d, J = 8.6 Hz), 8.07 (1H, s), 8.40 (1H, s). IR (KBr): 3345, 1663, 1618, 1599, 1493, 1414, 1080 cm^{-1}. Anal. Calcd for $C_{17}H_{15}N_3O_2$·1.0H_2O: C, 65.58; H, 5.50; N, 13.50. Found: C, 65.63; H, 5.50; N, 13.73.

4.14. 6-(7-Hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-c]imidazol-7-yl)-*N*-methyl-2-naphthamide (3c)

Under an argon atmosphere, **9a** (436 mg, 1.65 mmol) was dissolved in dry THF (30 mL) and the mixture was cooled to −65 °C in a dry ice acetone bath. A n-BuLi hexane solution (1.6 M: 2.28 mL, 3.63 mmol) was added with stirring and the mixture was stirred at the same temperature for 1.5 h. After stirring, a solution of **6** (183 mg, 1.5 mmol) in dry THF (3 mL) was added dropwise and the whole was stirred at the same temperature for 1.5 h. After dilution with saturated aqueous ammonium chloride solution, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in ethanol and the insoluble material was filtered off. The filtrate was concentrated under reduced pressure and the resulting residue was chromatographed on silica gel ($CHCl_3$/7% ammonia in MeOH = 19:1) and crystallized from $CHCl_3$ and diethylether to give **3c** (121 mg, 26%) as a colorless powder. Mp >185 °C decomp ($CHCl_3$ + diethylether). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 2.89–3.02 (2H, m), 3.04 (3H, s), 4.12–4.25 (1H, m), 4.27–4.43 (1H, m), 6.79 (1H, s), 7.20 (1H, q, J = 4.6 Hz), 7.54 (1H, s), 7.63 (1H, dd, J = 1.8 Hz, 8.6 Hz), 7.83 (2H, s), 7.89 (1H, d, J = 8.6 Hz), 8.03 (1H, s), 8.28 (1H, s). IR (KBr): 3500–3000, 1644, 1605, 1559, 1497, 1464, 1318, 1082 cm^{-1}. Anal. Calcd for $C_{18}H_{17}N_3O_2$·0.1$CHCl_3$: C, 68.09; H, 5.40; N, 13.16. Found: C, 68.14; H, 5.20; N, 12.94. Compounds **3e**–**h** and **3j** were prepared in the same manner as described for the preparation of **3c**.

4.15. *N*-Ethyl-6-(7-hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]-imidazol-7-yl)-2-naphthamide (3e)

Yield 29%. Mp 157–8 °C ($CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 1.29 (3H, t, J = 7.2 Hz), 2.85–3.06 (2H, m), 3.46–3.60 (2H, m), 4.10–4.24 (1H, m), 4.27–4.41 (1H, m), 6.88 (1H, s), 6.89 (1H, t, J = 5.2 Hz), 7.50 (1H, s), 7.62 (1H, dd, J = 1.6 Hz, 8.6 Hz), 7.81 (2H, s), 7.87 (1H, d, J = 8.6 Hz), 8.02 (1H, s), 8.26(1H, s). IR (KBr): 3283, 1642, 1605, 1557, 1495, 1447, 1316, 1080 cm^{-1}. Anal. Calcd for $C_{19}H_{19}N_3O_2$·0.25H_2O: C, 70.03; H, 6.03; N, 12.89. Found: C, 69.94; H, 6.12; N, 12.74.

4.16. *N*-Cyclopropyl-6-(7-hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-c]imidazol-7-yl)-2-naphthamide (3f)

Yield 30%. Mp 146–7 °C ($CHCl_3$–MeOH). ^{1}H NMR (DMSO-d_6) δ: 0.58–0.79 (4H, m), 2.75–3.00 (3H, m), 4.12–4.32 (2H, m), 6.17 (1H, s), 6.66 (1H, s), 7.63 (1H, dd, J = 1.4 Hz, 8.8 Hz), 7.64 (1H, s), 7.90 (1H, dd, J = 1.4 Hz, 8.6 Hz), 7.98 (2H, d, J = 8.6 Hz), 8.05 (1H, s), 8.39 (1H, s), 8.61 (1H, d, J = 4.4 Hz). IR (KBr): 3258, 1644, 1630, 1603, 1541, 1495, 1316, 1080 cm^{-1}. Anal. Calcd for $C_{20}H_{19}N_3O_2$·0.75H_2O: C, 69.25; H, 5.96; N, 12.11. Found: C, 69.35; H, 6.22; N, 12.15.

4.17. 6-(7-Hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-c]imidazol-7-yl)-*N*-isopropyl-2-naphthamide (3g)

Yield 37%. Mp >165 °C decomp ($CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 1.31 (6H, d, J = 6.4 Hz), 2.87–3.06 (2H, m), 4.12–4.26 (1H, m), 4.27–4.44 (1H, m), 6.56 (1H, d, J = 7.8 Hz), 6.78 (1H, s), 7.52 (1H, s), 7.64 (1H, dd, J = 1.8 Hz, 8.8 Hz), 7.82 (2H, s), 7.89 (1H, d, J = 8.8 Hz), 8.03 (1H, s), 8.25 (1H, s). IR (KBr): 3277, 1640, 1628, 1603, 1557, 1493, 1350, 1080 cm^{-1}. Anal. Calcd for $C_{20}H_{21}N_3O_2$·0.75H_2O: C, 68.85; H, 6.50; N, 12.04. Found: C, 68.75; H, 6.43; N, 12.09.

4.18. *N*,*N*-Diisopropyl-6-(7-hydroxy-6,7-dihydro-6,6-dimethyl-5*H*-pyrrolo[1,2-*c*] imidazol-7-yl)-2-naphthamide (3h)

Yield 29%. Mp 250 °C decomp (AcOEt). ^{1}H NMR ($CDCl_3$) δ: 0.8 (3H, s), 1.34 (3H, s), 1.00–1.80 (6H, br), 3.50–4.00 (2H, br), 3.79 (1H, d, J = 10.2 Hz), 4.14 (1H, d, J = 10.2 Hz), 6.89 (1H, s), 7.41–7.46 (2H, m), 7.72–7.86 (4H, m), 8.05 (1H, s). IR (KBr): 2965, 1630, 1335 cm^{-1}. Anal. Calcd for $C_{23}H_{27}N_3O_2$·0.25H_2O: C,72.32; H, 7.26; N, 11.00. Found: C, 72.56; H, 7.32; N, 10.72.

4.19. 6-[1-Hydroxy-1-(1-methyl-1*H*-imidazol-5-yl)ethyl]-*N*-methylnaphthalene-2- carboxamide (3j)

Yield 38%. ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 1.95 (3H, s), 3.04 (3H, s), 3.25 (3H, s), 7.14 (1H, s), 7.35 (1H, s), 7.41 (1H, dd, J = 1.8, 8.8 Hz), 7.80–7.90 (3H, m), 7.93 (1H, s), 8.28 (1H, s). IR (KBr): 3241, 3048, 2975, 1640, 1622, 1603, 1553, 1505, 1410, 1321, 1271, 1248, 1167, 1117, 1073 cm^{-1}. Anal. Calcd. for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58. Found: C, 69.75; H, 6.19; N, 13.49.

4.20. 6-(6,7-Dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl)-*N*-methyl-2-naphthamide (3d)

Under a hydrogen atmosphere (3–4 atom), a mixture of **3c** (77 mg, 0.25 mmol), 1 N HCl (0.5 ml) and 10% palladium carbon (Pd/C) (39 mg) was dissolved in MeOH (5 mL) and the mixture was vigorously stirred at room temperature for 12 h. After the filtration of the catalyst, the filtrate was neutralized with aqueous potassium carbonate solution (0.25 M, 1 mL, 0.25 mmol), the mixture was concentrated under reduced pressure. The resulting residue was chromatographed on silica gel ($CHCl_3$/7% ammonia in MeOH = 19:1) and recrystallized from $CHCl_3$–Et_2O to give **3d** (53 mg, 72%) as a colorless powder. Mp 174–5 °C ($CHCl_3$–Et_2O). ^{1}H NMR ($CDCl_3$) δ: 2.55–2.74 (1H, m), 3.07 (3H, d, J = 5.0 Hz), 3.04–3.26 (1H, m), 4.01–4.27 (2H, m), 4.57 (1H, t, J = 7.6 Hz), 6.62

(1H, q, J = 5.0 Hz), 6.79 (1H, s), 7.39 (1H, dd, J = 1.6 Hz, 8.4 Hz), 7.55 (1H, s), 7.70 (1H, s), 7.77–7.95 (3H, m), 8.29 (1H, s). IR (KBr): 3210, 1644, 1605, 1553, 1489, 1410, 1321 cm^{-1}. Anal. Calcd for $C_{18}H_{17}N_3O{\cdot}0.25H_2O$: C, 73.08; H, 5.96; N, 14.20. Found: C, 72.99; H, 6.07; N, 13.99.

4.21. 6-(7-Hydroxy-6,7-dihydro-6,6-dimethyl-5*H*-pyrrolo[1,2-c]imidazol-7-yl)-*N*-methyl-2-naphthamide (3i)

n-BuLi (1.6 M in hexane; 3.3 mL, 5.28 mmol) was added dropwise to a cooled (−70 °C) solution of methylamine (2 M in THF; 2.65 mL, 5.30 mmol) in anhydrous THF (4 mL) and the solution was stirred for 20 min at −70 °C. This solution was transferred to a cooled (0 °C) suspension of **3h** (500 mg, 1.23 mmol) in anhydrous THF (10 mL) and the reaction mixture was stirred for 16 h at room temperature. After dilution with brine (200 mL) and water (100 mL), the mixture was extracted with AcOEt and THF (1:1). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was recrystallized from AcOEt to give **3i** (137 mg, 33%) as a colorless powder. Mp 250 °C decomp (AcOEt). ^{1}H NMR ($CDCl_3$) δ: 0.65 (3H, s), 1.33 (3H, s), 3.08 (3H, d, J = 4.8 Hz), 3.78 (1H, d, J = 10.2 Hz), 4.14 (1H, d, J = 10.2 Hz), 6.55 (1H, br), 6.72 (1H, s), 7.45 (1H, s), 7.68–7.82 (4H, m), 8.00 (1H, s), 8.26 (1H, s). IR (KBr): 3241, 1640, 810 cm^{-1}. Anal. Calcd for $C_{20}H_{21}N_3O_2$: C, 71.18; H, 6.38; N, 12.21. Found: C, 71.08; H, 6.28; N, 12.23.

4.22. Synthesis of 6-(7-hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl)-*N*-methyl-2-naphthamide (3c) by improved method of route A

Under an argon atmosphere, 2-bromobenzotrifluoride (33.05 g, 147 mmol) was dissolved in dry THF (600 mL) and the mixture was cooled to −65 °C in a dry ice acetone bath. A *n*-BuLi hexane solution (1.6 M: 93.7 mL, 150 mmol) was added with stirring and the mixture was stirred at the same temperature for 30 min. After stirring, a cooled (10 °C) solution of **9a** (38.03 g, 144 mmol) in dry THF (2.88 L) was added at not more than −55 °C and the mixture was stirred for 20 min. An additional *n*-BuLi hexane solution (1.6 M: 94.5 mL, 151 mmol) was added at not more than −65 °C and the mixture was further stirred for 30 min. To the mixture, a solution of **6** (14.66 g, 120 mmol) in a dry THF solution (240 mL) was added dropwise and the resulting solution was stirred at the same temperature for 1.5 h. After dilution with saturated aqueous ammonium chloride solution, the mixture was concentrated under reduced pressure. The resulting residue was dissolved in ethanol and the insoluble material was filtered off. The filtrate was concentrated under reduced pressure and the resulting residue was chromatographed on silica gel ($CHCl_3$/7% ammonia in MeOH = 19:1 to 9:1) and recrystallized from MeOH to give **3c** (16.44 g, 45%) as a colorless powder. The spectral data were identical to those of the authentic sample.

4.23. *N*,*N*-Diisopropyl-6-[hydroxy(1-trityl-1*H*-imidazol-4-yl)-methyl]-2-naphthamide (12)

A solution of **9e** (50.0 g, 150 mmol) in anhydrous THF (250 mL) was added dropwise to a cooled (−70 °C) solution of *n*-BuLi (1.6 M in hexane; 98.3 mL, 158 mmol) in anhydrous toluene (1000 mL) over a 30 min period while the temperature of the reaction mixture was maintained at under −60 °C. After stirring for 20 min at −70 °C, a solution of **11** (38.9 g, 115 mmol) in anhydrous THF (200 mL) was added dropwise over a 20-min period, before stirring for 20 min at −70 °C. After dilution with water, the organic layer was separated and the aqueous layer was extracted with AcOEt (×2). The extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude mixture of **12** as a yellow viscous oil. This material was used for the next reaction without further purification. The analytical sample was obtained by recrystallization from MeOH (colorless powder). ^{1}H NMR ($CDCl_3$) δ: 1.28 (12H, br s), 3.73 (2H, br s), 4.73 (1H, s), 5.93 (1H, s), 6.59 (1H, d, J = 0.8 Hz), 7.06–7.13 (6H, m), 7.27–7.33 (9H, m), 7.36 (1H, dd, J = 1.5, 8.5 Hz), 7.42 (1H, d, J = 1.4 Hz), 7.48 (1H, dd, J = 1.7, 8.7 Hz), 7.71–7.79 (3H, m), 7.88 (1H, s); IR (KBr) 3177, 2964, 1630, 1445, 1337, 1126, 710, 702 cm^{-1}. Anal. Calcd for $C_{40}H_{39}N_3O_2{\cdot}0.3$MeOH: C, 80.22; H, 6.72; N, 6.96. Found: C, 80.38; H, 6.53; N, 7.05.

4.24. *N*,*N*-Diisopropyl-6-[(1-trityl-1*H*-imidazol-4-yl)carbonyl]-2-naphthamide (13)

A suspension of a crude mixture of **12** and MnO_2 (150 g) in CH_2Cl_2 (300 mL) was stirred for 90 min at room temperature. The mixture was filtered through Celite, and the solid was washed with THF. The filtrate was concentrated, and the residue was recrystallized from EtOH to give **13** (41.0 g, 60% two steps) as a colorless powder. ^{1}H NMR ($CDCl_3$) δ: 1.26–1.82 (12H, br d), 3.72 (2H, br s), 7.13–7.22 (6H, m), 7.34–7.42 (9H, m), 7.45 (1H, dd, J = 1.4, 8.4 Hz), 7.58 (1H, d, J = 1.4 Hz), 7.79–7.80 (2H, m), 7.90 (1H, d, J = 8.8 Hz), 7.98 (1H, d, J = 8.4 Hz), 8.29 (1H, dd, J = 1.6, 8.8 Hz), 8.98 (1H, s); IR (KBr) 2972, 1643, 1624, 1520, 1443, 1371, 1333, 1175, 756, 704 cm^{-1}. Anal. Calcd for $C_{40}H_{37}N_3O_2$: C, 81.19; H, 6.30; N, 7.10. Found: C, 81.04; H, 6.26; N, 7.11.

4.25. Ethyl 2-{6-[(diisopropylamino)carbonyl]-2-naphthyl}-2-hydroxy-2-(1-trityl-1*H*-imidazol-4-yl) acetate (14)

Ethyl acetate (14.9 mL, 152 mmol) was added dropwise to a cooled (−70 °C) solution of LDA (freshly prepared from diisopropylamine [21.3 mL, 152 mmol] and *n*-BuLi [1.6 M in hexane, 95.0 mL, 152 mmol]) in anhydrous THF (600 mL). After stirring for 30 min, a solution of **13** (60.0 g, 101 mmol) in anhydrous THF (150 mL) was added dropwise. The reaction mixture was stirred for 30 min at −70 °C and then allowed to warm to −30 °C. After cooling to −50 °C, the reaction mixture was diluted with water and the organic phase was separated. The aqueous phase was extracted with THF–toluene (1:1). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **14** as a pale yellow oil. This material was used for the next reaction without further purification. The analytical sample was obtained by recrystallization from toluene (colorless powder): ^{1}H NMR ($CDCl_3$) δ: 1.14 (3H, t, J = 7.0 Hz), 1.34 (12H, br s), 3.17 (1H, d, J = 16.2 Hz), 3.50 (1H, d, J = 16.2 Hz), 3.72 (2H, br s), 3.08 (2H, q, J = 7.0 Hz), 5.15 (1H, s), 6.84 (1H, d, J = 1.4 Hz), 7.07–7.14 (6H, m), 7.26–7.34 (9H, m), 7.37 (1H, d, J = 1.4 Hz), 7.38 (1H, dd, J = 1.7, 8.3 Hz), 7.68 (1H, dd, J = 1.8, 8.8 Hz), 7.74–7.84 (3H, m), 8.03 (1H, d, J = 1.0 Hz); IR (KBr) 3454, 2968, 1705, 1636, 1371, 1337, 1213, 746, 704 cm^{-1}. Anal. Calcd for $C_{44}H_{45}N_3O_4$: C, 77.73; H, 6.67; N, 6.18. Found: C, 77.69; H, 6.67; N, 6.12.

4.26. 6-[1,3-Dihydroxy-1-(1-trityl-1*H*-imidazol-4-yl)propyl]-*N*,*N*-diisopropyl-2-naphthamide (15)

Red-Al (65% in toluene; 110 mL, 355 mL) was added dropwise to a cooled (−15 °C) solution of **14** in anhydrous toluene (600 mL) over a 30-min period. The reaction mixture was stirred for 2.5 h at −10 to −0 °C. The reaction was quenched with the addition of water (12 mL). After dilution with THF (300 mL), aqueous 15% NaOH solution (12 mL) and water (36 mL) were added, and the mixture was stirred until the suspension turned to a clear solution. After the addition of Celite (120 g), the suspension was stirred for 10 min, and the solid was then filtered and washed with THF. The filtrate was concentrated (ca. 60% of the solvent was

evaporated), and the solution was washed with 10% citric acid solution, water, saturated $NaHCO_3$, and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was recrystallized from hexane–AcOEt to give **15** (63.7 g, 98%) as a colorless powder: ^{1}H NMR ($CDCl_3$) δ: 1.34 (12H, br s), 2.27–2.40 (1H, m), 2.48–2.61 (1H, m), 3.70 (2H, t, J = 5.0 Hz), 3.83 (3H, br s), 4.54 (1H, s), 6.78 (1H, d, J = 1.6 Hz), 7.08–7.17 (6H, m), 7.28–7.40 (11H, m), 7.51 (1H, dd, J = 1.8, 8.4 Hz), 7.71–7.81 (3H, m), 7.97 (1H, s); IR (KBr) 3497, 3200, 2964, 1634, 1445, 1335, 748, 702 cm^{-1}. Anal. Calcd for $C_{42}H_{43}N_3O_3$: C, 79.09; H, 6.80; N, 6.59. Found: C, 78.83; H, 6.71; N, 6.59.

4.27. 6-(7-Hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl)-*N*,*N*-diisopropyl-2-naphthamide (16)

Methanesulfonyl chloride (9.21 mL, 119 mmol) was added dropwise to a cooled (0 °C) solution of **15** (63.0 g, 98.8 mmol) and (i-Pr)$_2$NEt (34.5 mL, 198 mmol) in anhydrous THF (400 mL) while keeping the temperature of the solution below 10 °C. After stirring for 30 min, water was added and the resulting mixture was extracted with AcOEt (×2). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give a crude mixture of the mesylate as a pink amorphous powder. The mesylate was dissolved in CH_3CN (300 mL), and the solution was heated at 70 °C for 20 min, during which time the spot of the mesylate disappeared on TLC. After the addition of MeOH (100 mL) and (i-Pr)$_2$NEt (34.5 mL, 198 mmol), the mixture was heated at 70 °C for 6 h. The mixture was concentrated in vacuo (about half of the solvent was evaporated), diluted with water, and extracted with AcOEt (×3). The combined organic layers were washed with brine (×2) and concentrated in vacuo. The remaining solid was filtered and washed with AcOEt to give **16** (32.5 g, 87%) as a colorless powder. The analytical sample was obtained by recrystallization from EtOH and AcOEt (colorless powder): ^{1}H NMR ($CDCl_3$) δ: 1.18–1.50 (12H, br d), 2.78–2.97 (2H, m), 3.69–3.77 (2H, br d), 4.01–4.09 (1H, m), 4.18–4.28 (1H, m), 6.58 (1H, s), 7.26 (1H, s), 7.36 (1H, dd, J = 1.2, 5.6 Hz), 7.59 (1H, dd, J = 1.2, 5.8 Hz), 7.72–7.78 (3H, m), 7.99 (1H, s); IR (KBr) 3275, 2964, 1611, 1487, 1450, 1371, 1342, 800 cm^{-1}. Anal. Calcd for $C_{23}H_{27}N_3O_2$·0.1EtOH: C, 72.93; H, 7.28; N, 11.00. Found: C, 72.73; H, 7.10; N, 11.05.

4.28. 6-(7-Hydroxy-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]imidazol-7-yl)-*N*-methyl-2-naphthamide (3c) by route B

n-BuLi (1.6 M in hexane; 199 mL, 318 mmol) was added dropwise over 30 min to a cooled (−70 °C) solution of methylamine (2 M in THF; 159 mL, 318 mmol) in anhydrous THF (300 mL) and the solution was stirred for 20 min at −70 °C. This solution was transferred to a cooled (0 °C) suspension of **16** (30 g, 79.5 mmol) in anhydrous THF (450 mL) via a Teflon needle, and **16** was completely dissolved. The reaction mixture was stirred for 16 h at room temperature. After dilution with brine (200 mL) and water (100 mL), the mixture was stirred for 10 min, and the precipitated compound **3c** was separated by filtration. The organic layers of the filtrate were separated, and the aqueous layer was extracted with AcOEt and THF (1:1, ×2). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was filtered and washed with AcOEt to give an additional amount of compound **3c**. The two separately obtained portions of compound **3c** were combined and dissolved in hot EtOH (150 mL) and MeOH (10 mL). The insoluble material was filtered, and the filtrate was concentrated, followed by recrystallization from EtOH and AcOEt to give **3c** (19.2 g, 79%) as a colorless powder. The spectral data were identical to those of the authentic sample.

4.29. Optical resolution of 3c using preparative HPLC

Compound **3c** (12.8 g, 41.6 mmol) was subjected to optical resolution by preparative HPLC using a Chiralpak AD column (50 mm × 500 mm) using hexane/ethanol (1/1) as an eluent, detected at 254 nm, to afford (−)-**3c** (6.40 g, 20.8 mmol, 50%) as a first elution and (+)-**3c** (6.32 g, 20.6 mmol, 49%) as a second elution. The spectral data were identical to those of the racemic **3c** except for optical rotation.

Compound (−)-**3c**: $[\alpha]_D^{25}$ −82.7 (c 0.526, MeOH); t_R = 9.9 min (Chiralpak AD 4.6 mmID × 250mmL, hexane/ethanol = 1/1, 0.5 mL/min, at 254 nm); 99.8% ee. Compound (+)-**3c**: $[\alpha]_D^{25}$ +83.8 (c 0.474, MeOH); t_R = 19.1 min (Chiralpak AD 4.6 mmID × 250 mmL, hexane/ethanol = 1/1, 0.5 mL/min, at 254 nm); 99.8% ee.

4.30. Preparation of Reformatsky reagent (17a)

A catalytic amount of chlorotrimethylsilane (ca. 0.1 mL) was added to a suspension of zinc powder (1.04 g, 16.0 mmol) in anhydrous THF (8 mL), and the suspension was stirred for 20 min at room temperature, in which time the zinc powder a formed sponge-like precipitate. Ethyl bromoacetate (1.77 mL, 16.0 mmol) in anhydrous THF (20 mL) was added to the solution dropwise over a 20 min period while ensuring that the temperature of the solution remained below 30 °C. The solution was stirred for a further 20 min to give **17a** as a yellow solution. This solution was immediately used for the next reaction after acid-alkaline titration (0.50 M).

4.31. Preparation of Reformatsky reagent (17b)

A catalytic amount of chlorotrimethylsilane (ca. 0.1 mL) was added to a suspension of zinc powder (1.04 g, 16.0 mmol) in anhydrous THF (8 mL) and the suspension was stirred for 20 min at room temperature, in which time the zinc powder formed a spongy precipitate. After heating at 50 °C, *tert*-butyl bromoacetate (2.36 mL, 16.0 mmol) in anhydrous THF (20 mL) was added to the solution dropwise over a 20 min period while ensuring that the temperature of the solution was kept below 60 °C. The solution was stirred for a further 20 min and then allowed to cool to room temperature to give **17b** as a yellow solution. This solution was used for the next reaction after acid-alkaline titration (0.35–0.50 M).

4.32. Titration of Reformatsky reagent

A THF solution of Reformatsky reagent (1.0 mL), 0.10 *N* HCl (20 mL), together with a few drops of aqueous Methyl Orange solution was added to a distilled water (10 mL). After stirring for 10 min at room temperature, the solution was titrated with 0.10 *N* NaOH solution. The concentration of Reformatsky reagent was calculated with the following equation: Reformatsky reagent (M) = (20 − X) × 0.1 (where X is a amount of 0.10 *N* NaOH consumed).

4.33. General procedure for asymmetric Reformatsky reaction

Synthesis of *tert*-Butyl 2-{6-[(diisopropylamino)carbonyl]-2-naphthyl}-2(S)-hydroxy-2-(1-trityl-*1H*-imidazol-4-yl)acetate (18b, Entry 2 in Table 1).

Cinchonine (142 mg, 0.42 mmol) was added to a cooled (0 °C) solution of **17b** (1.47 mmol), and the solution was stirred for 20 min at 0 °C. After cooling to the appropriate temperature, a solution of **13** (250 mg, 0.42 mmol) in anhydrous THF (2.5 mL) was added dropwise over a 5 min period, and the mixture was stirred until completion of the reaction (checked by HPLC). The optical

purity of **18b** was determined by HPLC using a chiral column (Chiralpak AD, 4.6 mmID × 250 mmL). The analytical conditions were as follows: mobile phase; hexane/EtOH = 85/15, flow rate; 0.8 mL/min, temperature; 25 to 35 °C, detection; UV (254 nm), retention time (t/min); (*R*)-**18b** (13.0 min), (*S*)-**18b** (18.4 min), **13** (21.2 min). The yield of **18b** was determined by the rate of the peak intensity of **18b** (ε at 254 nm in EtOH = 12,700) and **13** (ε at 254 nm in EtOH = 32,000).

The reaction of **13** with **17a** to produce **18a** was conducted in a similar manner as described above.

4.34. Large scale preparation of 18b (entry 6 in Table 1)

A solution of **17b** (0.35 M; 48.2 mL, 16.9 mmol) and pyridine (1.37 mL, 17.0 mmol) was added dropwise to a cooled (0 °C) solution of cinchonine (1.55 g, 5.28 mmol) in anhydrous THF (10 mL), and the solution was stirred for 20 min at 0 °C. After cooling to −42 °C, a solution of **13** (2.50 g, 4.22 mmol) in anhydrous THF (20 mL) was added dropwise over 10 min, and the mixture was stirred for 4 h at −42 °C. The mixture was diluted with 1 *N* HCl, and then diluted with AcOEt. The resulting solution was successively washed with 1 *N* HCl (×2), water, saturated $NaHCO_3$ solution, and brine followed by drying over $MgSO_4$. After removal of the solvent in vacuo, the residue was purified by flash column chromatography on silica gel (hexane/AcOEt = 3:1 to 2:1) to give **18b** (2.9 g, 97%, 92% ee) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ: 1.31 (9H, s), 1.0–1.6 (12H, br d), 3.12 (1H, d, J = 16.0 Hz), 3.40 (1H, d, J = 16.0 Hz), 3.69 (2H, br s), 5.26 (1H, s), 6.86 (1H, d, J = 1.8 Hz), 7.07–7.12 (6H, m), 7.25–7.32 (9H, m), 7.36–7.39 (2H, m), 7.70 (1H, dd, J = 1.8, 8.7 Hz), 7.73–7.78 (2H, m), 7.82 (1H, d, J = 8.4 Hz), 8.03 (1H, s); IR (KBr) 3462, 2972, 1732, 1705, 1634, 1445, 1369, 1337, 1159 cm^{-1}.

4.35. Alignment of amino acid sequences

The amino acid sequences of CYP3A4 and 17,20-lyase (CYP17) were taken from the ExPASy web site (http://tw.expasy.org/). The amino acid sequences for which three-dimensional (3D) structures were available at the time (*Pseudomonas putida* P450CAM, *Pseudomonas* sp. P450TERP, *Saccharopolyspora erythraea* P450eryF, *Fusarium oxysporum* P450NOR, *Bacillus megaterium* P450BM3, and mammalian CYP2C5) were aligned according to their 3D structures using the homology module of Insight II (ver. 2000, Accelrys Inc.). Subsequently, amino acid sequences of human CYPs were aligned by sequence homology using ClustalW. The combined alignments were manually modified so there were no insertions or deletions in the portions corresponding to probable secondary structures. The following four characteristic motifs of CYPs were aligned: WXXXR (where W is tryptophan, R is arginine, and X is any amino acid) in the alpha-helix C; EXXR (where E is glutamate, R is arginine, and X is any amino acid) in the alpha-helix K; ZXXPXXZXPXXZ (where P is proline, Z is an aromatic amino acid, and X is any amino acid) after the alpha-helix K; and FXXGXXXCXG (where F is phenylalanine, G is glycine, C is cysteine, and X is any amino acid) before the alpha-helix L.

4.36. Homology modeling

According to the alignment, homology models of CYP3A4 and 17,20-lyase (CYP17) were constructed based on the crystal structure of mammalian CYP2C5 (PDB code: 1DT6) using the homology module of Insight II. Using the search/generate loops function of Insight II, conformations of the insertion and deletion sections in the alignment were obtained according to the known 3D structures. After making some manual adjustments to remove large steric hindrances, the whole structure was subjected to energy minimization for 1000 steps with the steepest descent minimizer, and subsequently 5000 steps with the conjugate gradient minimizer, to a maximum gradient of 0.1 kcal/mol^{-1} Å^{-1}, using the Discover-ESFF force field (ver 980, Accelrys Inc.). During the minimization procedure, the dielectric constant was set to $4 * r$, where r is the distance between two interacting atoms. The force constant of tethering constraints for the backbone of structurally conserved regions and heme was set to 40 kcal/Å^2 to prevent large movement from the initial positions.

4.37. Docking of Inhibitors

Covalent bonding models of imidazole were constructed by full energy minimization using the Discover-ESFF force field (v980, Accelrys Inc.). The residual parts of compounds were then connected to the imidazole. After connecting the compounds to the heme Fe in the 17,20-lyase model, its binding modes were explored by systematic analysis around the rotatable bonds within the ligands and protein side chains within 4 Å of the ligand (torsion driving). During this procedure, energy values were estimated based on the Discover-ESFF force field. The most stable binding mode of compounds was energy minimized with the 17,20-lyase model using the Discover-ESFF force field. In the minimization procedure, heme and the backbone of the protein was fixed. All computational procedures were carried out on O2/R10000 workstations of Silicon Graphics Inc.

4.38. Determination of the Absolute Configuration of 2

A colorless platelet (0.15 × 0.15 × 0.05 mm^3) of **2** was obtained by re-crystallization from AcOEt and THF. A diffractometer Rigaku RAXIS RAPID was used with graphite monochromated Cu-Kα radiation to obtain the following crystal data: $C_{25}H_{24}BrN_3O_4S$, $0.5C_4H_8O$, crystal system monoclinic, space group $P2_1$ (#4), lattice parameters a = 14.3835(3) Å, b = 10.6353(2) Å, c = 17.6165(3) Å, α = 90°, β = 110.3646(7)°, γ = 90°, V = 2526.41(8) Å^3, Z = 4, T = 100 K. Of the 25,453 reflections collected, 8673 were unique (R_{int} = 0.054). The refinement converged with R_1 = 0.059 and wR_2 = 0.138 for 7131 reflections with $I > 2\sigma(I)$. The absolute configuration of **2** was established as *S* based on the Flack parameter,[59] 0.03(2), refined using 3806 Friedel pairs. The crystal contained two molecules of **2** and a molecule of THF in the asymmetric unit. Further details of the X-ray structure data are available on request from the Cambridge Crystallographic Data Centre (CCDC deposition number: 794848).

4.39. Determination of the absolute configuration of (+)-3c (orteronel)

A colorless platelet of (+)-**3c** with (2S,3S)-(-)-tartranilic acid (0.35 × 0.10 × 0.01 mm3) was obtained by re-crystallization of MeOH/*i*-Pr_2O/THF. A diffractometer Rigaku RAXIS RAPID was used with graphite monochromated Cu-Kα radiation to obtain the following crystal data: C18H17N3O2, 2C10H11NO5, crystal system monoclinic, space group C2 (#5), lattice parameters a = 28.9799(6) Å, b = 5.4377(1) Å, c = 26.0080(6) Å, α = 90°, β = 103.338(1)°, γ = 90°, V = 3987.9(2) Å3, Z = 4, T = 173 K. Of the 22,522 reflections collected, 7029 were unique (Rint = 0.065). The refinement converged with R1=0.127 and wR2 = 0.388 for 2903 reflections with I > 2σ(I). The absolute configuration of (+)-3c was established as S using the absolute stereochemistry of (2S,3S)-(-)-tartranilic acid as an internal reference. The crystal contained one molecule of (+)-3c and two molecules of tartranilic acid in the asymmetric unit. There were two tunnel-shaped solvent domains along the b-axis in a unit cell. Although several peaks originating from the crystallization solvent were observed in these

solvent domains, they were disordered. Therefore, the solvent peaks were excluded from the structure refinement process. Further details of the X-ray structure data are available on request from the Cambridge Crystallographic Data Centre (CCDC deposition number: 781831).

4.40. Inhibitory activity on rat steroid 17,20-lyase in vitro

Inhibitory activity against rat enzymes was determined according to a method described previously[60] with some modifications. Testes excised from 13-week-old male SD rats were homogenized, and testicular microsomes were prepared by centrifugation. The reaction mixture contained 75 mM phosphate buffer (pH 7.4), 7 μg of the microsome protein, 10 nM [1,2-^{3}H]-17α-hydroxyprogesterone (NEN), 5 mM NADPH (Oriental Yeast), and 1–1000 nM test compounds in a total volume of 20 μL at room temperature. The concentration of reagents was expressed as the final concentration in the reaction mixture. The test compounds were serially diluted with dimethylformamide, and then diluted fivefold with distilled water before 5 μL of the diluted solution was added to the reaction mixture. After incubating for 15 min at 37 °C the reaction was terminated by the addition of 40 μL of ethyl acetate, then vortexed for 30 s and briefly centrifuged. Thirty microliters of the organic phases were applied to silica gel TLC plates (Whatman, LHPK). The substrate and the products, androstenedione and testosterone, were separated using the toluene–acetone (7:2) solvent system. Detection of the spots and measurement of the radioactivity as PSL were performed with a BAS2000 Bio-image analyzer (FUJIX). The concentration of the test compounds needed to reduce the concentration of the products by 50% (the concentration of the control group in which no test compound is added is taken as 100%) was calculated.

4.41. Inhibitory activity on human steroid 17,20-lyase in vitro

Inhibitory activity on human enzymes was determined as described above. The reaction mixture contained 75 mM phosphate buffer (pH 7.4), 1 mM magnesium chloride, 0.5 pmol of recombinant P450c17 (Biotechnology Laboratories, Takeda), 0.5 pmol of recombinant cytochrome b5 (Pan Vera), 20.8 ng of recombinant NADPH-cytochrome P450 reductase (Pan Vera), 10 nM [1,2-^{3}H]-17α-hydroxypregnenolone (Amersham), 5 mM NADPH (Oriental Yeast), and 1–1000 nM test compound in a total volume of 20 μL. The concentration of reagents was expressed as the final concentration in the reaction mixture. The test compounds were serially diluted with dimethylformamide, and then diluted fivefold with distilled water before 5 μL of the diluted solution was added to the reaction mixture. After incubating for 15 min incubation at 37 °C the reaction was terminated by the addition of 40 μL of ethyl acetate, then vortexed for 30 s and briefly centrifuged. Thirty microliters of the organic phases were applied to silica gel TLC plates (Whatman, LHPK). The substrate and the product DHEA were separated using the cyclohexane–ethyl acetate (3:2) solvent system.

4.42. Inhibitory activity on CYP3A4 in vitro for screening (Table 2)

The reaction mixture contained 50 mM phosphate buffer (pH 7.4), 10 pmol/mL recombinant CYP3A4 (Gentest), 100 μM testosterone, NADPH regenerating system (0.5 mM NADP (Oriental Yeast)), 5 mM glucose-6-phosphate (Oriental Yeast), 1 mM $MgCl_2$, 1.5 unit/mL G-6-P dehydrogenase (Oriental Yeast), and 1 or 10 μM test compounds in a total volume of 200 μL. The total microsome protein content was adjusted by control microsome protein (Gentest). The concentration of reagents was expressed as the final concentration in the reaction mixture. The reaction mixture was incubated for 30 min at 37 °C and terminated by the addition of 200 μL of acetonitrile. After addition of 400 μL of water, the reaction mixture was centrifuged at 14,000 rpm for 10 min. The 6-hydroxytestosterone contents in the supernatants were determined using a HPLC system (Shimadzu LC-10, column: Inertsil ODS-3 (4.6 × 150 mm), GL Sciences).

4.43. Inhibitory activity on rat 11-hydroxylase in vitro

Adrenal glands excised from SD rats were homogenized, and the mitochondrial fraction was prepared at room temperature by serial centrifugation. Rat 11-hydroxylase activity was measured according to a method described for side-chain cleavage activity previously[61] with some modifications. The reaction mixture contained 200 mM mannitol, 4.5 mM HEPES, 2.3 mM potassium phosphate (pH 7.4), 0.1 mM EDTA·2 K, 0.03% BSA (crystallized, Miles), 4.5 mM NADPH (Oriental Yeast), 11 mM calcium chloride, 4 μg of mitochondria protein, 10 nM [1,2-^{3}H]-hydroxy-11-deoxycorticosterone (11-deoxycortisol) (NEN, dissolved in 0.02% Tween-80), and 1–1000 nM test compounds in a total volume of 150 μL. The concentrations of reagents were expressed as the final concentration in the reaction mixture. The test compounds were serially diluted with dimethylformamide, and 1.5 μL was added directly to the reaction mixture. After 30 min incubation at 37 °C the reaction was terminated by addition of 400 μL of ethyl acetate and 100 μL of distilled water, then vortexed for 30 s and briefly centrifuged. Three hundred microliters of the organic phase was transferred to a new tube and evaporated until dry using nitrogen gas. The steroids were dissolved with 30 μL of ethyl acetate and the whole volume was applied to silica gel TLC plates (Whatman, LHPK). The substrate and the products (11-deoxycortisol and cortisol) were separated in the toluene–acetone (7:2) solvent system.

4.44. Effect of (+)-3c on metabolic activities of CYP-expressing microsomes

The effect of (+)-**3c** on CYP isoforms was assessed by incubating a marker substrate with the microsomes expressing each human CYP isoform in the presence of 3, 10, 30 and 100 μmol/L of (+)-**3c**. The incubation mixture contained 10 mmol/L $MgCl_2$, 3.2 mg/mL glucose-6-phosphate, 0.8 mg/mL β-NADP+ and 4 units/mL glucose-6-phosphate dehydrogenase in 80 mmol/L phosphate buffer (pH 7.4). In the case of CYP2A6, 2B6 and 2C9, 80 mmol/L Tris–HCl buffer (pH 7.4) was used instead of the phosphate buffer. After preincubation of the (+)-**3c** and CYP isoform-specific substrate mixture at 37 °C for 5 min, the reaction was initiated by adding microsomes derived from specific CYP-expressing human B-lymphoblastoid cells. The concentration of the substrate was referenced from the data sheet from GENTEST Corp. (7-ethoxyresorufin *O*-deethylation for CYP1A2, coumarin 7-hydroxylation for CYP2A6, *S*-(+)-mephenytoin 4′-hydroxylation for CYP2C19, (±)-bufuralol 1′-hydroxylation for CYP2D6, 4-nitrophenol hydroxylation for CYP2E1 and testosterone 6β-hydroxylation activity for CYP3A4) or the published reports (ethoxycoumarin *O*-deethylation for CYP2B6 and tolbutamide hydroxylation for CYP2C8 and 2C9) [62,63] with slight modifications.

The marker reactions specific for CYP isoforms other than CYP3A4 were measured according to published analytical methods[62,64–68] with slight modifications. Testosterone 6β-hydroxylation activity for CYP3A4 was analyzed using the following HPLC conditions: column, Inertsil ODS-2 (150 × 4.6 mm I.D.; GL Science, Tokyo, Japan); detection wavelength, 254 nm; flow rate, 1.0 mL/min; column temperature, 50 °C; mobile phase, 10 mmol/L acetate buffer (pH 4.3)/acetonitrile = 7/3. Control marker enzymatic activities were measured for the preincubation samples in the presence of methanol alone without (+)-**3c**.

4.45. Effect of (+)-3c and (−)-3c in male cynomolgus monkeys

Adult male cynomolgus monkeys (Keari, Wakayama, Japan) housed in a temperature-controlled room (23 ± 2 °C) with a 12:12 h light/dark cycle (illumination from 7:00 am to 7:00 pm) were used for the single dosing experiments. The care and use of animals and the experimental protocol used in this study were approved by the Experimental Animal Care and Use Committee of Takeda Pharmaceutical Company Ltd. The test compounds (+)-**3c** and (−)-**3c** were suspended in 0.5% methylcellulose and administered orally at a dose of 1 mg/kg. Blood samples were collected just before dosing and 8 h (in a preliminary study) or 2, 5 and 10 h after dosing. Serum was stored at −30 °C until assayed by radioimmunoassay (RIA). Concentrations of testosterone and DHEA were determined using a Testosterone I-125 kit (Dia Sorin s.r.l., Italy) and a DHEA RIA kit (Diagnostics System Laboratories, USA), respectively, according to the manufacturer's instructions.

Serum steroid concentrations are highly variable among individuals, show circadian variation, and are affected by stress. Thus, serum steroid concentrations were expressed as percentages of the mean pretreatment values (the mean concentrations in sera collected 48 and 24 h and just before treatment) in the single administration experiments.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.066. These data include MOL files and InChiKeys of the most important compounds described in this article.

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