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Discovery of a Novel Piperidine-Based Inhibitor of Cholesteryl Ester Transfer Protein (CETP) That Retains Activity in Hypertriglyceridemic Plasma

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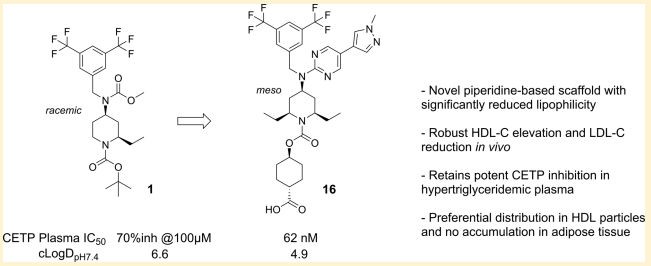
Supporting Information

ABSTRACT: Herein we describe the discovery and characterization of a novel, piperidine-based inhibitor of cholesteryl ester transfer protein (CETP) with a core structure distinct from other reported CETP inhibitors. A versatile synthesis starting from 4-methoxypyridine enabled an efficient exploration of the SAR, giving a lead molecule with potent CETP inhibition in human plasma. The subsequent optimization focused on improvement of pharmacokinetics and mitigation of off-target liabilities, such as CYP inhibition, whose improvement correlated with increased lipophilic efficiency. The effort led to the identification of an achiral, carboxylic acid-bearing compound **16** (TAP311) with excellent pharmacokinetics in rats and robust efficacy in hamsters. Compared to anacetrapib, the compound showed substantially reduced lipophilicity, had only modest distribution into adipose tissue, and retained potency in hypertriglyceridemic plasma in vitro and in vivo. Furthermore, in contrast to torcetrapib, the compound did not increase aldosterone secretion in human adrenocortical carcinoma cells nor in chronically cannulated rats. On the basis of its preclinical efficacy and safety profile, the compound was advanced into clinical trials.



INTRODUCTION

Coronary heart disease (CHD) is the leading cause of mortality in the industrialized world. It has long been recognized that plasma low density lipoprotein (LDL) cholesterol levels directly correlate with CHD risk. Pharmacological reduction of LDL cholesterol via 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase inhibitors (statins) reduces CHD risk by approximately 30%. However, considerable residual CHD risk remains.[1] Several epidemiological studies have also shown that plasma high density lipoprotein (HDL) cholesterol levels inversely correlate with CHD risk although the clinical benefit of increased HDL cholesterol has yet to be demonstrated.[2,3]

Cholesteryl ester transfer protein (CETP) is a 74 kD circulating glycoprotein secreted mainly by the liver. CETP mediates the exchange of cholesteryl ester and triglyceride (TG) between plasma lipoproteins, including LDL, very low density lipoprotein (VLDL), and HDL.[4] CETP activity has been correlated with CHD risk in several epidemiological studies.[4] Moreover, CETP polymorphisms that are associated with reduced CETP activity are associated with reduced CHD risk.[4−6]

Owing to the promise of its therapeutic potential, multiple CETP inhibitors have been evaluated in clinical trials. Three CETP inhibitors torcetrapib, dalcetrapib, and evacetrapib, have failed in phase 3 cardiovascular outcome trials. In 2006, development of torcetrapib was abruptly terminated based on increased cardiac events and mortality observed in the treatment group of the ILLUMINATE trial.[7] It is now generally accepted that the increased mortality associated with torcetrapib was due to adverse off-target effects, including increased blood pressure and corticosteroid concentrations.[8−11] In contrast, dalcetrapib, which has modest effects on HDL and LDL cholesterol levels, was terminated due to futility.[12] The ACCELERATE trial for evacetrapib[13] was also terminated upon futility analysis despite robust increases in HDL cholesterol and reductions in LDL cholesterol. The last of

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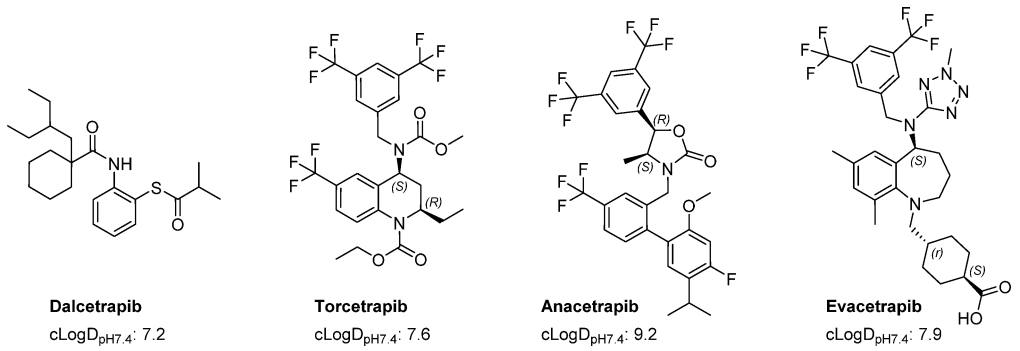


Figure 1. Structures and cLogD$_{pH7.4}$ of CETP inhibitors that have reached phase 3 clinical trials.

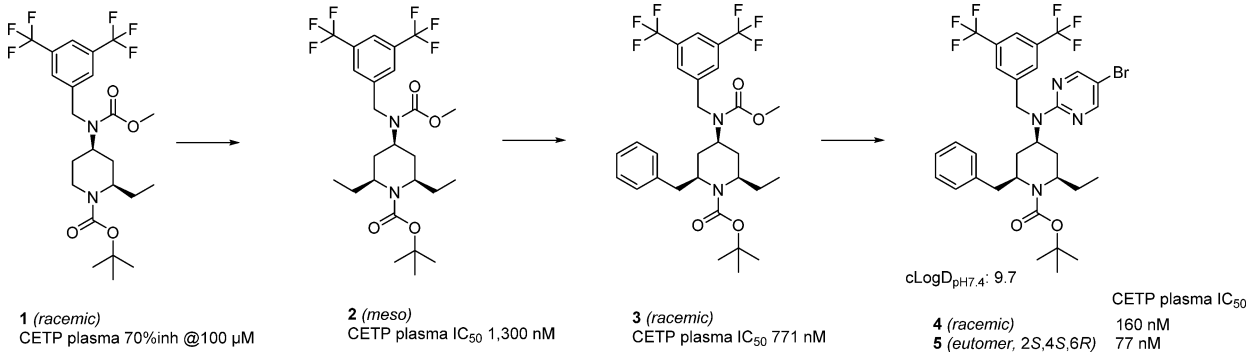
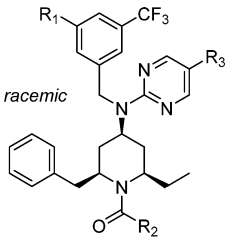


Figure 2. Initial optimization.



	R_1	R_2	R_3	CETP plasma IC_{50}	cLogD$_{pH7.4}$	LipE$_{cLogD}$
4	CF_3	O*t*-Bu	Br	160 nM	9.7	–2.9
6	OMe	O*t*-Bu	Br	>10,000 nM	9.0	< –4.0
7	Cl	O*t*-Bu	Br	240 nM	9.5	–2.9
8	CF_3	NHEt	Br	4,800 nM	8.1	–2.8
9	CF_3	*n*-Pr	Br	510 nM	7.9	–1.6
10	CF_3	O*i*-Pr	Br	200 nM	9.3	–2.6
11	CF_3	O*t*-Bu	OMe	71 nM	9.4	–2.2
12	CF_3	O*t*-Bu		164 nM	9.0	–2.2

Figure 3. SAR of the pyrimidine analogues.

these trials REVEAL (anacetrapib) was expected to provide the best evidence (i.e., >30000 patients, 5 year follow-up) as to the relevance of pharmacological CETP inhibition for CHD risk reduction.[14] It was very recently reported that anacetrapib reduces CHD risk by 9%, while overall mortality was not affected.[15] The reduction in CHD risk is consistent with the benefit predicted from a 17% reduction in LDL cholesterol, which suggests a limited role of increased HDL, if any, in CHD risk reduction.

In our efforts to identify a best-in-class CETP inhibitor, we noted that most clinical CETP inhibitors suffer from high lipophilicity (Figure 1), arguably owing to the nature of the binding sites that accommodate highly lipophilic substrates cholesteryl ester (CE) and TG.[16] The most striking example is anacetrapib, with cLogD$_{pH7.4}$ (abbreviated hereafter as cLogD for brevity) of 9.2, which has high distribution to adipose tissue and an exceptionally long terminal elimination half-life in humans.[17] As for the pressor effects and corticosteroid effects associated with torcetrapib, we hypothesized that structural deviation from the tetrahydroquinoline core that has been associated with the aldosterone risk[10] should mitigate these off-target liabilities. Thus, we set out to identify a structurally distinct, novel CETP inhibitor scaffold with reduced lipophilicity and without the off-target liabilities of torcetrapib.

RESULTS AND DISCUSSION

In our search for a novel CETP inhibitor that is structurally distinct from torcetrapib, we explored a number of simpler scaffolds to understand the most essential features of various CETP inhibitors known at the time. Among many prototypes prepared, we identified **1** as a hit (Figure 2). Although weak in potency (70% inhibition at 100 μM compound concentration), we were intrigued by the unique piperidine scaffold of **1**, presenting a relatively simple and modular structure with high sp^3 content, which also seemed amenable to an efficient structure–activity relationship (SAR) exploration.

We initially explored the synthetically accessible 6-position of the piperidine. Fortuitously, the mere introduction of an ethyl

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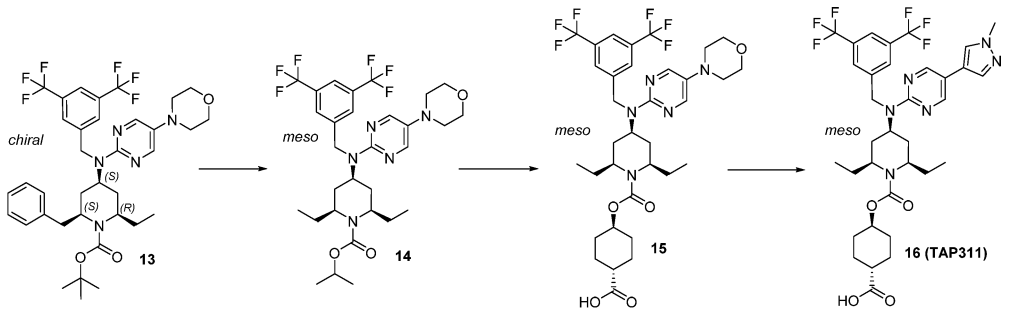

	13	14	15	16 (TAP311)
CETP Plasma IC_{50}	60 nM	60 nM	150 nM	62 nM
$cLogD_{pH7.4}$ / $LipE_{cLogD}$	9.0 / −1.8	7.6 / −0.3	5.0 / 1.9	4.9 / 2.4
CYP inhibition				
3A4 Midazolam/Testosterone	>50 μM / >50 μM	2.2 μM / >50 μM	25 μM / >50 μM	10.6 μM / >50 μM
2D6	>50 μM	>30 μM	>50 μM	31 μM
2C9	>50 μM	6.6 μM	>50 μM	19 μM
Rat PK (1 mg/kg *i.v.*, 3 mg/kg *p.o.*)				
$T_{1/2}$	7.4 h	4.6 h	5.6 h	5.8 h
CL_p	0.1 L/h/kg	0.6 L/h/kg	0.09 L/h/kg	0.09 L/h/kg
V_d	0.7 L/kg	2.35 L/kg	0.67 L/kg	0.57 L/kg
AUC_{inf}	456 nM h	2,750 nM h	28,500 nM h	27,200 nM h
F	3%	35%	58%	43%

Figure 4. Final optimization.

group at this position to give compound **2** (Figure 2) resulted in dramatically improved CETP inhibition, with half-maximum inhibitory concentration (IC_{50}) of 1300 nM in human plasma. Further improvement was made through introduction of a benzyl group in place of one of the ethyl groups to give compound **3** (IC_{50} 771 nM). Another boost in potency was achieved via replacement of the methyl carbamate with a pyrimidine to give compound **4** (IC_{50} 160 nM). The active enantiomer (eutomer) of racemic **4** was determined to be **5**, whose absolute stereochemistry was confirmed by single compound X-ray crystallography to be 2*S*,4*S*,6*R* (Supporting Information, Figure 3). As expected, compound **5** was approximately 2-fold more potent than the racemate **4**, while the inactive enantiomer (distomer) showed no CETP inhibition.

Although potent, compound **4** is highly lipophilic, with a cLogD value exceeding that of anacetrapib (9.7 vs 9.2). Thus, the next phase of our optimization was focused on reducing lipophilicity while maintaining or improving potency, using racemic **4** as the lead (Figure 3). While many attempts were made to replace the bis-trifluoromethyl groups on the *N*-benzyl substituent, they all resulted in substantial loss in potency, as exemplified by the methoxy group in compound **6**. The only exception was chlorine (compound **7**, IC_{50} 240 nM), which offered minimal improvement in lipophilicity. Exploration of the piperidine nitrogen capping group was also accompanied by reduced potency, as seen with ethyl urea **8** and *n*-butyl amide **9**. Isopropyl carbamate **10** (IC_{50} 200 nM) was equipotent but, again, with minimal improvement in lipophilicity. The only point of SAR that showed concomitant improvement in potency and reduced lipophilicity was at the 5-position of the pyrimidine. Both 5-methoxypyrimidine compound **11** and 5-morpholinopyrimidine compound **12** were potent and slightly less lipophilic (cLogD 9.4 and 9.0, respectively) than compound **4** (cLogD 9.7).

While we did not explicitly use the parameter of lipophilic efficiency (LipE = pIC_{50} − cLogP)[18,19] at the time of our research, it provides a parameter that, in retrospect, illustrates a main aspect of our compound optimization. Because cLogP does not take ionization into account and overestimates the partitioning of ionized molecules to the octanol phase,[20] we calculated a modified LipE based on cLogD at pH 7.4 instead of cLogP and refer to the values as $LipE_{cLogD}$. By this analysis, compound **4** has a relatively poor $LipE_{cLogD}$ value of −2.9 and none of the transformations tested in Figure 3 resulted in substantial improvement in lipophilic efficiency, perhaps with the marginal exception of the *n*-butyl amide **9**. It was only in the final optimization that the issue of lipophilic efficiency was addressed, which indeed resulted in improved pharmacokinetic profiles, as described below.

Compound **13**, the active enantiomer of racemic compound **12**, showed robust inhibition of CETP activity (IC_{50} 60 nM) (Figure 4). However, pharmacokinetic (PK) studies in Sprague−Dawley (SD) rats showed that compound **13** had very poor oral availability (3%). Reasoning that we needed a less lipophilic start point, we turned our attention back to the symmetric 2,6-diethylpiperidine compound **2**, which showed only marginally weaker CETP inhibition than the substantially more lipophilic 2-benzyl-6-ethylpiperidine analogue **3** (Figure 2). We therefore made the 2,6-diethyl analogue of compound **13** (Figure 4). Gratifyingly, compound **14** retained potent inhibition of CETP (IC_{50} 60 nM), while for the first time substantially improving lipophilic efficiency relative to 2-benzyl-6-ethylpiperidines such as compound **13** ($LipE_{cLogD}$ −0.3 vs −1.8). In addition, compound **14** presented a symmetric, achiral molecule, eliminating the need for chiral resolution or asymmetric synthesis. Compound **14** showed a much improved PK profile in SD rats when dosed at 1 mg/kg iv and 3 mg/kg po, with an area under the curve (AUC) of 2750 nM·h and oral availability of 35%. However, compound **14** was an inhibitor of CYP3A4 (2.2 μM) and CYP2C9 (6.6 μM), thereby suggesting the risk of drug−drug interaction. This is a particular concern as a CETP inhibitor would likely be coadministered with a statin (i.e., current standard-of-care), many of which are metabolized primarily by either CYP3A4 or CYP2C9.[21]

As CYP enzymes often have affinity for lipophilic xenobiotics,[22] we hypothesized that further reduction in lipophilicity by introduction of a carboxylic acid motif might mitigate this liability. Among several carboxylic acid bearing analogues prepared, we were gratified to find that compound **15** retained

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good CETP inhibition (IC_{50} 150 nM) and showed a much improved CYP inhibition profile compared to compound **14** (CYP3A4 midazolam 25 vs 2.2 μM, CYP2C9 > 50 vs 6.6 μM). In addition, the introduction of a carboxylic acid resulted in a dramatically improved rat pharmacokinetic profile with reduced clearance (0.09 L/h/kg), volume of distribution (0.67 L/kg), and increased exposure (AUC 28,500 nM·h) from a 3 mg/kg po dose.

Lastly, we screened a variety of capping groups for the pyrimidine, as we were concerned with the potential generation of reactive metabolites from the morpholine motif and wanted a further boost in potency. We found that the methylpyrazole derivative, (1*r*,4*r*)-4-(((2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)-benzyl)(5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carbonyl)oxy)cyclohexane-1-carboxylic acid **16** (TAP311),[23] offered the best balance of CETP inhibition (IC_{50} 62 nM), selectivity (>170-fold vs CYPs), lipophilicity (cLogD 4.9), and rat pharmacokinetic profile (CL 0.09 L/h/kg, V_d 0.57 L/kg, AUC 27200 nM·h, *F* 43%) from 1 mg/kg iv and 3 mg/kg po doses. The compound also had the best lipophilic efficiency ($LipE_{cLogD}$ 2.4) among all compounds described herein. The protein binding of compound **16** was determined to be 99.4% in human plasma.

The improvement in the pharmacokinetic profile with carboxylic acid bearing carbamates was generally consistent across many analogues. It should be noted that all compounds were tested as an amorphous form and dosed as a simple supension in 0.5% methylcellulose, including compound **16**. The high bioavailability observed with these compounds makes a sharp contrast to other CETP inhibitors such as torcetrapib and anacetrapib, which in our hands showed bioavailability of approximately 8−9% in the same suspension formulation.

Experimental assessment of octanol−water distribution at pH 7.6 revealed a significantly lower distribution coefficient (Log $D_{pH7.6}$ 5.2) for **16** compared to the neutral CETP inhibitors dalcetrapib, torcetrapib, and anacetrapib (Table 1).

Table 1. Measured Log $D_{pH7.6}$, $cLogD_{pH7.4}$, CETP Plasma IC_{50}, and $LipE_{cLogD}$ of Clinical CETP Inhibitors and Compound 16

	dalcetrapib	torcetrapib	anacetrapib	evacetrapib	compd **16**
Log $D_{pH7.6}$	>6.0[a]	>4[a]	>5.9[a]	5.7	5.2
$cLogD_{pH7.4}$	7.2	7.6	9.2	5.2	4.9
CETP plasma IC_{50}	4100 nM	18 nM	18 nM	110 nM	62 nM
$LipE_{cLogD}$	−1.7	0.2	−1.5	1.8	2.4

[a]Concentration in water phase below detection limit.

In fact, for neutral CETP inhibitors, an accurate experimental log *D* value could not be determined because compound concentrations in the aqueous phase were below the detection limit in our protocol. On the other hand, the calculated $cLogD_{pH7.4}$ for carboxylic acid bearing evacetrapib and **16** closely matched their experimental Log $D_{pH7.6}$ values.

When administered once daily for 2 weeks to chow-fed hamsters, compound **16** (0.3−10 mg/kg po) showed comparable exposure to anacetrapib at all doses and statistically significant decreases in CETP activity at all doses compared to vehicle, while anacetrapib showed statistically significant decreases in CETP activity at 3 and 10 mg/kg doses compared to vehicle (Figure 5A,B). Furthermore, robust, dose-dependent increases in HDL cholesterol were observed for **16** and anacetrapib (Figure 5C), along with dose-dependent reductions in LDL cholesterol with maximum reductions of 41% and 33% for **16** and anacetrapib, respectively (Figure 5D).

Consistent with a previous report,[24] we observed markedly high adipose and adrenal tissue versus plasma drug concentration ratios in chow-fed hamsters treated once daily with anacetrapib (10 mg/kg po) for 14 days (Figure 6). In sharp contrast, hamsters treated with **16** (10 mg/kg po) for the same time period had substantially lower adipose and adrenal tissue versus plasma drug concentration ratios. Liver to plasma drug concentration ratios were similar for **16** and anacetrapib. These results suggest that, unlike anacetrapib, **16** does not preferentially distribute to adipose tissue. This could be an important differentiating factor from anacetrapib, which has been detected in the plasma of patients 2−4 years after cessation of dosing.[25] Others have also reported similar improvements in tissue distribution profile by reducing overall lipophilicity of the CETP inhibitors.[24,26]

We next evaluated the efficacy of **16** and anacetrapib in vitro using serum samples from patients having a wide range of TG concentrations. With increasing TG content in this assay, anacetrapib lost efficacy, while **16** retained efficacy (Figure 7A). This trend was recapitulated ex vivo when the compounds were dosed orally in hamsters treated with 4-(1,1,3,3-tetramethylbutyl)phenol polymer with formaldehyde and oxirane (Triton WR-1339),[27] an inhibitor of lipoprotein lipase activity that results in elevated plasma TG levels.[28] The inhibitory effect of **16** on CETP activity in the plasma of the treated hamsters was largely maintained, while this was not the case for anacetrapib (Figure 7B). The retained efficacy of **16** in the setting of high TG is expected to be clinically relevant, as hypertriglyceridemia is observed in a significant portion of dyslipidemic patients. Overall, 31% of the adult US population has an elevated plasma TG level (≥150 mg/dL) and high (≥200 mg/dL) or very high (≥500 mg/dL) fasting TG levels are found in 16.2% and 1.1% of adults, respectively.[29] Combined with the fact that TG concentrations can increase by more than 100 mg/dL following a high-fat meal,[30,31] a substantial portion of patients taking a CETP inhibitor may have a TG level where the observed difference between **16** and anacetrapib may be relevant. Interestingly, anacetrapib showed a trend for greater CHD risk reduction in patients with higher TG concentrations despite a potential decrease in efficacy,[15] suggesting that compound **16** has an opportunity to further impact this patient subpopulation.

Given this observation, we hypothesized that the differential efficacy of **16** versus anacetrapib in the hypertriglyceridemic setting may be related to the compound distribution profile across different lipoprotein fractions, owing to the marked differences in their lipophilicity. Indeed, when the compounds were spiked into serum from human donors with normal TG levels (TG < 150 mg/dL), **16** was found predominantly in the HDL and lipoprotein deficient serum (LPDS) fractions (Figure 8A), whereas anacetrapib was more evenly distributed among HDL, LDL, and triglyceride rich lipoproteins (TRL) (Figure 9A). In serum with higher TG content (300−500 and >750 mg/dL), **16** showed similar distribution among lipoprotein fractions (Figure 8B,C), while anacetrapib was predominantly found in TRL fractions (Figure 9B,C). Because CETP is mainly associated with HDL in the circulation, these observations corroborate well with the retained potency of **16** in hypertriglyceridemic plasma.

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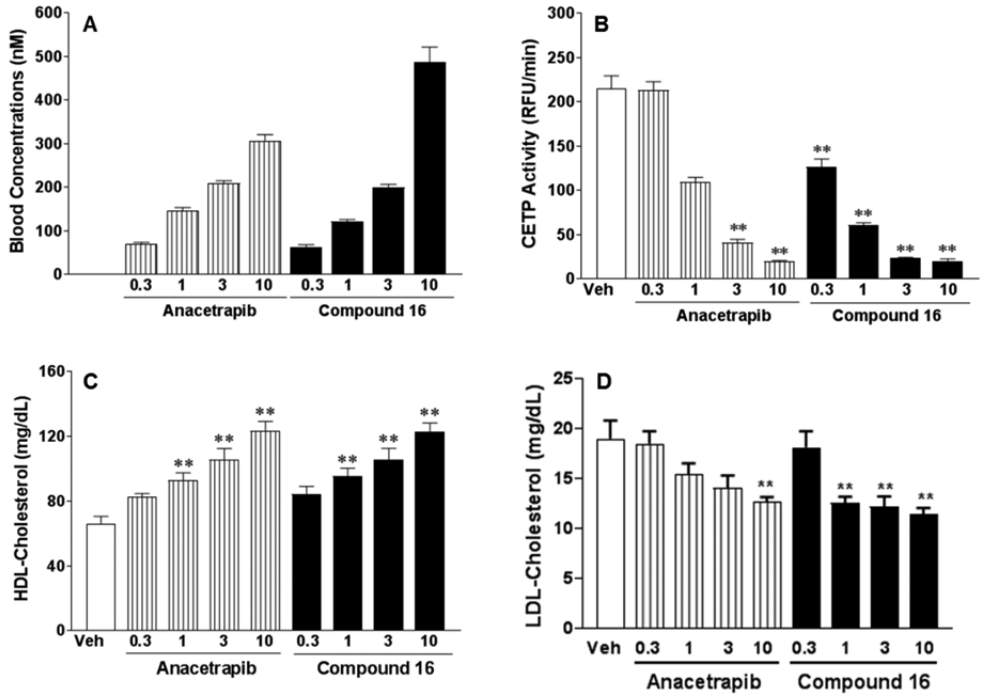

Figure 5. Pharmacokinetics and pharmacodynamics of anacetrapib and **16** in hamsters. Compound (0.3, 1, 3, and 10 mg/kg po) or vehicle (Veh) was dosed once a day for 14 days to hamsters fed a standard chow diet. Blood was withdrawn 24 h post last dose and analyzed for total compound concentrations (A), CETP inhibition (B), HDL-cholesterol levels (C), and LDL-cholesterol levels (D). Values are mean ± SEM of six animals. ** $P < 0.01$, significant versus the vehicle group (Dunnett's multiple comparisons test following one-way ANOVA).

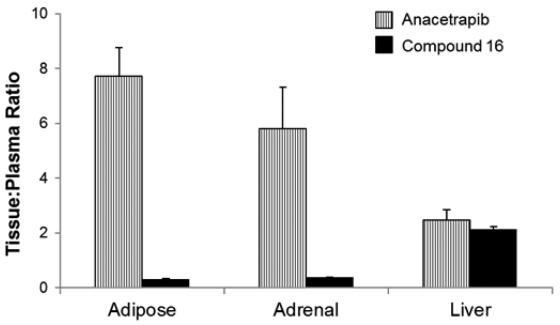

Figure 6. Distribution profiles of anacetrapib and compound **16** in various tissues of hamsters administered anacetrapib or compound **16**. Anacetrapib and compound **16** were dosed orally once each day for 14 days to hamsters fed a standard chow diet. Blood and tissues were collected 24 h after the final dose and analyzed for compound concentrations. Data represent mean total concentration ± SEM for seven animals per group.

Lastly, given the pressor and corticosteroid effects of torcetrapib, we evaluated the effects of **16** and torcetrapib on aldosterone production in H295R cells (Supporting Information, Figure 1) and blood pressure and plasma aldosterone levels in chronically cannulated SD rats (Supporting Information, Figure 2). We observed a marked increase in aldosterone secretion upon treatment of H295R cells with torcetrapib, whereas increases in aldosterone were not observed with **16** or anacetrapib. In contrast to torcetrapib, **16** did not increase mean arterial pressure or plasma aldosterone levels in conscious, cannulated SD rats.

CHEMISTRY

While our literature survey for the preparation of various 2,4- and 2,4,6-substituted piperidines revealed several possible synthetic routes, we opted for the sequential functionalization of acylated 4-methoxypyridine that provided a modular synthetic route for a rapid SAR exploration and compound optimization. Thus, a variety of 2,4,6-trisubstituted piperidines were synthesized by 1,4-addition of a Grignard reagent to the acylated or carbamoylated species of 4-methoxypyridine, followed by 1,4-addition of alkyl cuprates to the resultant dihydropiperidones **17b,c** to give 2,6-disubstituted piperidine-4-ones **18b,c** (Scheme 1).[32] We found that, generally, the *cis*-addition of the cuprate was favored when R_2 was benzyloxy compared to *tert*-butoxy. Subsequent reductive amination gave the all-*cis* isomer as the major product. Capping the secondary amines **19a−c** with methyl chloroformate gave **1** and **2**, while further deprotection of **20** and capping with *tert*-butoxycarbonyl (BoC) group gave **3**.

Pyrimidine capped compounds in Figure 3 were accessed in a similar manner via ketone **18d** (Scheme 2). We initially investigated the SAR with racemic compounds as shown in Figure 3, resolving selected compounds of interest at the end such as with racemic **4** and **12** into eutomers **5** and **13**, respectively. Reductive amination of the ketone **18d** gave all-*cis* 2,4,6-trisubstituted piperidine **21**, which was deprotected to give **22**. Capping the primary amine of **22** with 5-bromo-2-chloropyrimidine gave **23**, followed by alkylation with 3,5-disubstituted benzyl bromide to give **4**, **6−7**. Deprotection of the Boc group to **24** was followed by acylation to give **8−10**. The bromopyrimidine **4** was converted to the methoxy analogue **11** and morpholinyl analogues **12** and **13** through copper and palladium catalyzed couplings, respectively.

The compounds for final optimization (Figure 4) were prepared from the aforementioned ketone intermediate **18b** or the isopropyl analogue **18e** (Scheme 3). The same sequence of transformation as in Scheme 2 gave the all-*cis* intermediates **27a,b**, which were then elaborated into compounds **14** and **29a**

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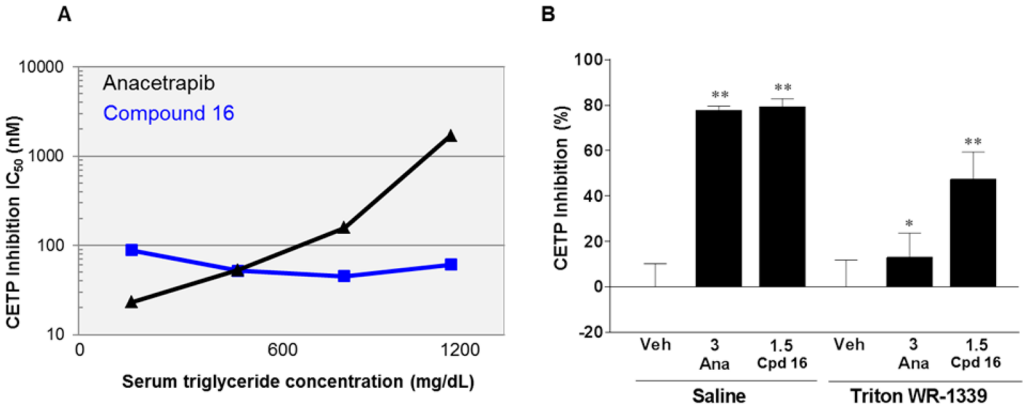

Figure 7. Effect of triglyceride level on efficacy of anacetrapib and **16**. (A) In vitro CETP activity IC_{50} values of anacetrapib and **16** in serum pooled from donors with varying TG levels. Values are the mean of two replicates for each serum pool. (B) Ex vivo inhibition of plasma CETP activity by anacetrapib (Ana) and **16** dosed orally in hamsters treated with saline or Triton WR-1339 (Veh). Doses of Ana and **16** were 3 and 1.5 mg/kg, respectively. Plasma samples were collected 24 h post dose. Values are mean ± SEM of five animals. $*P < 0.05$, $**P < 0.01$, significant difference from each Veh group (Dunnett's multiple comparisons test following one-way ANOVA).

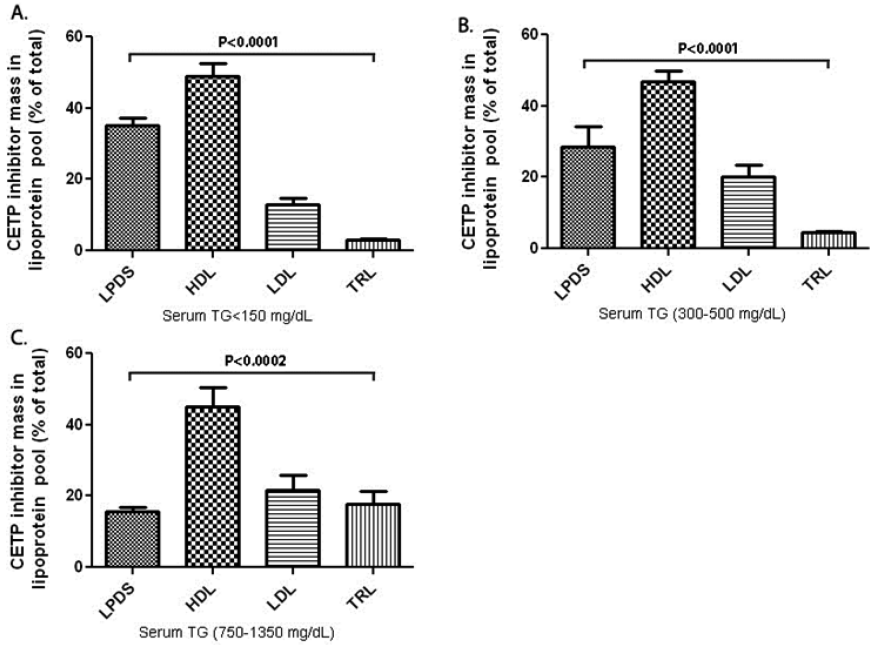

Figure 8. In vitro distribution profile of compound **16** among serum lipoproteins when spiked into serum from healthy donors with normal (A), high (B), or very high (C) triglyceride levels. The extraction of compound **16** was done in duplicate for each sample. The absolute mass of **16** was calculated in each fraction and, as shown in the *Y* axis, was expressed relative to the total sum of **16** in all lipoprotein pools (mean ± SEM). One-way ANOVA was performed to compare the percent distribution of **16** among serum lipoproteins.

through palladium catalyzed coupling with morpholine. In a similar manner, **29b** was prepared by palladium catalyzed coupling with the pinacol boronate of the methylpyrazole. Deprotection of the Boc group was followed by acylation with chloroformate **26**, which was prepared by esterification of the acid **25** followed by treatment with triphosgene and base to form the chloroformate. Finally, hydrolysis of the methyl ester gave **15** and **16**. The all-*cis* configuration of the piperidine substituents of **16** was confirmed by single compound X-ray crystallography (Supporting Information, Figure 4).

CONCLUSION

We have identified **16** as a novel, piperidine-based CETP inhibitor with excellent pharmacokinetic and efficacy profiles. The compound has a substantially lower log $D_{pH6.8}$ compared to CETP inhibitors that have entered phase 3 clinical trials to date. On the basis of our preclinical data package, we propose that the lower lipophilicity of compound **16** contributes to its lack of accumulation in adipose tissue and retained efficacy versus anacetrapib in the setting of high plasma triglycerides. Unlike torcetrapib, compound **16** does not increase blood pressure or plasma aldosterone levels in vivo. Taken together, our results indicate that compound **16** is a promising candidate for targeting residual cardiovascular disease risk. On the basis of its preclinical efficacy and safety profile, compound **16** was advanced into clinical trials.[33]

EXPERIMENTAL SECTION

Methods. *Software.* Figures 5−9 were generated with and statistical analyses were performed using GraphPad Prism version 7.

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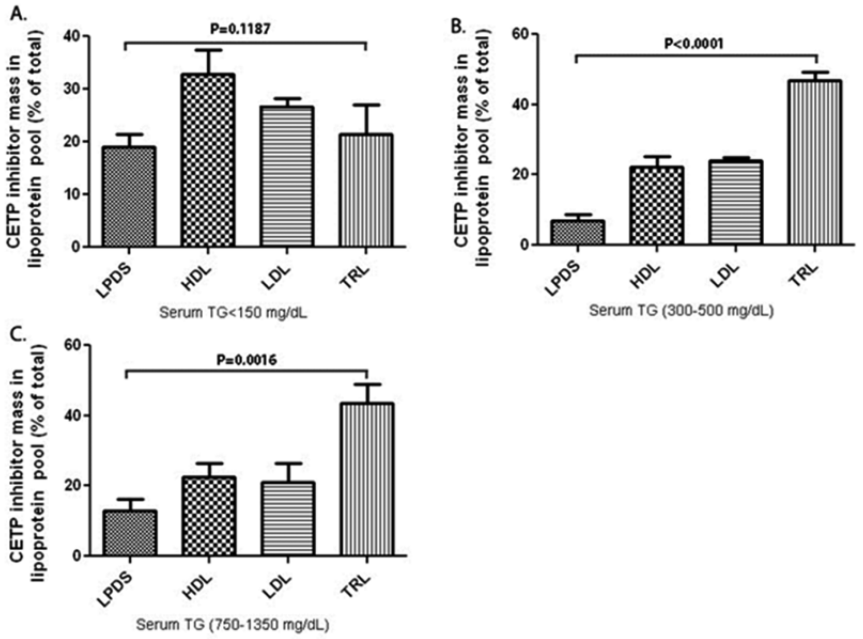
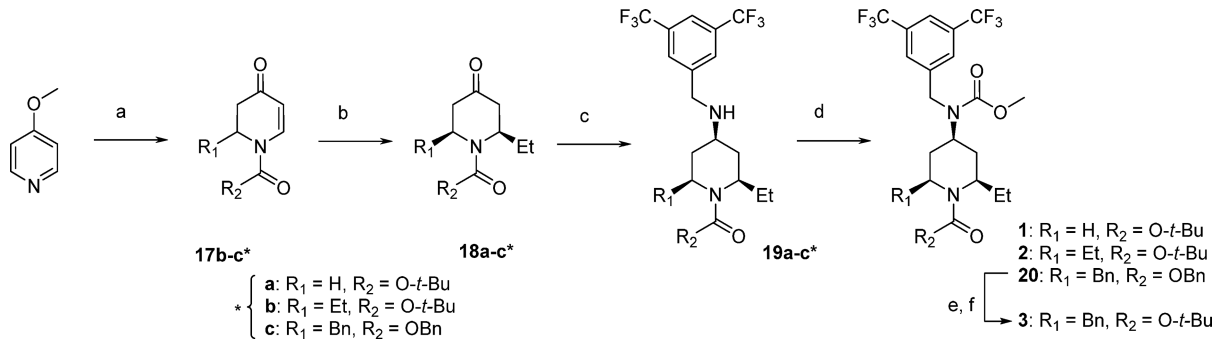


Figure 9. In vitro distribution of anacetrapib among serum lipoproteins when spiked into serum from healthy donors with normal (A), high (B), or very high (C) triglyceride levels. Anacetrapib extraction was done in duplicate for each sample. The absolute mass of anacetrapib was calculated in each fraction and, as shown in the *Y* axis, was expressed relative to the total sum of anacetrapib in all lipoprotein pools (mean ± SEM). One-way ANOVA was performed to compare the percent distribution of anacetrapib among serum lipoproteins.

Scheme 1[a]



[a]Reagents and conditions: (a) PhOCOCl, EtMgBr, tetrahydrofuran, −40 °C; then *t*-BuOK (for **17b**) or BnOCOCl, BnMgBr (for **17c**); (b) BF_3·Et_2O, EtMgBr, CuI, tetrahydrofuran, −78 °C to rt; (c) 3,5-bis(trifluoromethyl)benzylamine, Ti(O*i*Pr)$_4$, MeOH, then $NaBH_4$, 0 °C to rt (**19a**), 3,5-bis(trifluoromethyl)benzylamine, $NaBH(OAc)_3$, acetic acid, dichloroethane, 0 °C to rt (**19b,c**); (d) MeOCOCl, pyridine, 0 °C to rt (for **1**), or MeOCOCl, 4-dimethylaminopyridine, CH_2Cl_2, rt (for **2**, **20**); (e) $PdCl_2$, Et_3SiH, Et_3N, CH_2Cl_2, rt; (f) Boc_2O, 60 °C, no solvent.

Calculated Octanol–Water Distribution Coefficient. Calculated cLogD at pH 7.4 was determined using the following approximation:

$$\text{cLogD}_{\text{pH}} = \text{cLogP} - \text{Log}(1 + 10^{\text{pH}-\text{cp}K_a})$$

where cpK_a was determined using FOCUS software[34] with built-in MoKa algorithm.[35]

Lipophilic Efficiency.[18,19] $\text{LipE}_{\text{cLogD}} = \text{p}[\text{CETP human plasma IC}_{50}] - \text{cLogD}_{\text{pH7.4}}$.

CETP IC_{50} Determination in Human Plasma. A pool of ethylenediaminetetraacetic acid (EDTA)–plasma from healthy male donors was obtained from New Drug Development Research Center, Inc. (Hokkaido, Japan). Human dyslipidemic serum (1 lot, lot no. N83376) was obtained from Uniglobe Research Co. (Reseda, CA). To evaluate the effect of compounds, 50 μL of human plasma (final 50% plasma), 35 μL of the assay buffer, and 1 μL of compound dissolved in dimethyl sulfoxide (DMSO, final 1%, Sigma, St. Louis, MO, catalogue no. D2650) were added to each well of a 96-well half-area black, flat bottom polystyrene NBS microplate (Corning, Corning, NY, catalogue no. 3686). The reaction (final volume of 100 μL) was started by the addition of 14 μL of donor solution diluted with the assay buffer. Fluorescence intensities (relative fluorescence unit, RFU) were measured every 30 min at 485 nm (excitation) and 535 nm (emission) for 120 min at 37 °C using ARVO SX+L (PerkinElmer, Wellesley, MA). CETP activity (RFU/min) was determined from the changes in fluorescence intensity from 30 to 90 min. The inhibitory activity of the compound was calculated as % inhibition of enzyme activity using 1 or 10 μM torcetrapib (as positive control) and DMSO as 100% and 0% inhibition, respectively. The IC_{50} value of duplicate wells was obtained by logistic equation (Y = bottom + (top − bottom)/(1 + (x/IC_{50})Hill slope) using Origin software, version 7.5 SR3 (OriginLab Co., Northampton, MA).

Log D (pH 7.6) Determination. Phosphate buffered saline (pH 7.4) saturated with octanol and 1-octanol saturated with buffer were prepared prior to the start of the log *D* assay. Aliquots of 10 mM DMSO compound solution along with an internal reference compound were dispensed in triplicate in 96-well polypropylene 2 mL plates. The DMSO was removed using a GeneVac HT4X evaporator for approximately 1 h.

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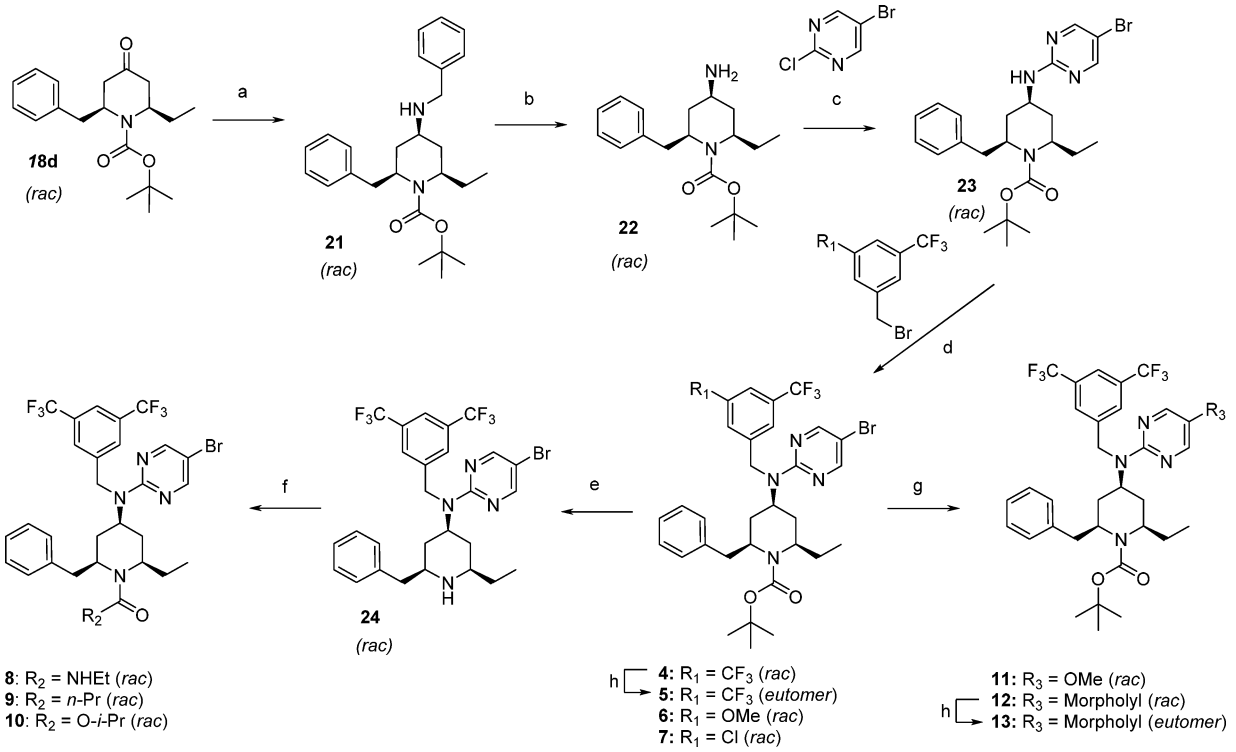
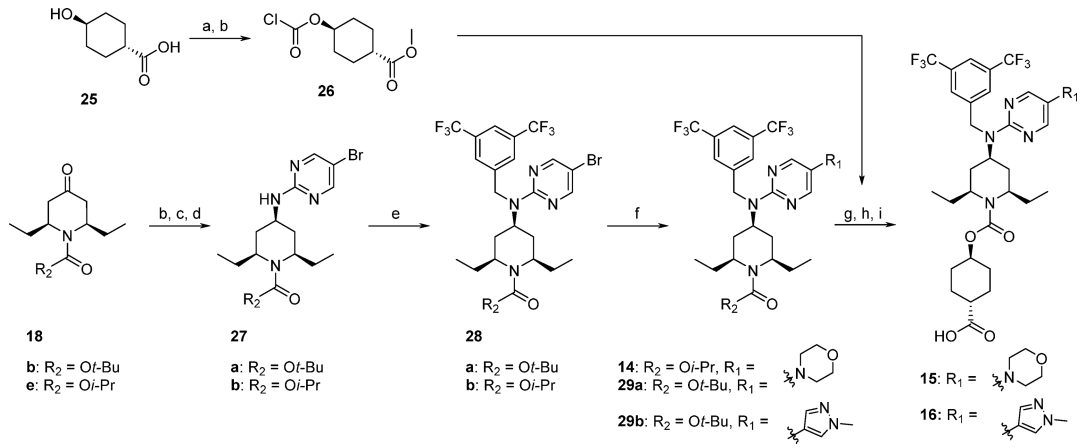
Scheme 2[a]



[a]Reagents and conditions: (a) $BnNH_2$, $BF_3{\cdot}Et_2O$, toluene, reflux, $NaBH_4$, MeOH, rt; (b) Pd/C, H_2, EtOH, 55 °C; (c) *i*-Pr_2NEt, DMF, 120 °C; (d) NaH, DMF, 0 °C to rt; (e) 4 N HCl in EtOAc, rt; (f) EtNCO, iPr_2NEt, DMF, rt (for **8**), or *n*-PrCOCl, iPr_2NEt, DMF, rt (for **9**), or iPrOCOCl, iPr_2NEt, DMF, rt (for **10**); (g) NaOMe, CuI, DMF, 85 °C (for **11**), or $Pd_2(dba)_3$, (2-biphenyl)di*tert*-butylphosphine, NaOtBu, morpholine, toluene, 100 °C (for **12**); (h) chiral HPLC.

Scheme 3[a]



[a]Reagents and conditions: (a) MeI, K_2CO_3, DMF, 0 °C to rt; (b) triphosgene, pyridine, CH_2Cl_2, 0 °C to rt; (c) benzylamine, $Ti(O\text{-}i\text{-}Pr)_4$, MeOH, rt, then $NaBH_4$, 0 °C to rt; (d) Pd/C, H_2, EtOH, 60 °C; (e) 2-chloro-5-bromopyrimidine, *i*-Pr_2NEt, DMF, 120 °C; (f) 3,5-bis(trifluoromethyl)-benzylamine, NaH, DMF, 0 °C to rt; (g) morpholine, $Pd_2(dba)_3$, (2-biphenyl)di-*tert*-butylphosphine, *t*-BuONa, toluene, 100 °C (**14** and **29a**), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole, $Pd(PPh_3)_4$, Na_2CO_3, DME-H_2O, 90 °C (**29b**); (h) 4 N HCl in EtOAc, rt, (i) **26**, *i*-Pr_2NEt, DMF, rt; (j) NaOH, tetrahydrofuran−H_2O, rt.

Dried samples were incubated with 300 μL of octanol and 300 μL of buffer and shaken for at least 4 h. Plates were centrifuged for 15 min at 4000 rpm to separate the octanol and buffer layers. Sample preparation and phase separation were automated using liquid handling workstations. Both octanol and buffer phases were quantified using a tandem mass spectrometer. Log *D* was derived from the ratio of compound peak area responses in each phase, adjusted to the internal standard peak areas. Log *P* was equivalent to log *D* when the compound was un-ionized at pH 7.4.

Statement on Animal Welfare. All procedures were conducted in accordance with approved Novartis Animal Care and Use Committee protocols and the Guide for the Care and Use of Laboratory Animals.

Pharmacokinetic Studies. The test article was administered intravenously (1 mg/mL/kg bolus, in *N*-methyl pyrrolidin-2-one/PEG 200) or orally (3 mg/5 mL/kg, suspended in 0.5% aqueous methylcellulose solution) to conscious 7-week old male Sprague−Dawley rats (Charles River, Yokohama, Japan). Blood samples were taken at various time points after administration. The samples were

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treated with methanol for protein precipitation, and the supernatants were analyzed by liquid chromatography followed by electrospray/tandem mass spectrometric detection. Pharmacokinetic variables were estimated using noncompartmental methods. Animals were fed a standard diet and had free access to tap water throughout the experiment. This work was conducted with the permission of the Novartis Tsukuba Research Institute Animal Welfare Committee (permission no.: AWCJ06054).

Measurement of Human Plasma Protein Binding. Binding of compound **16** to human plasma proteins was determined by ultracentrifugation method. Approximately 0.5 mL of human plasma was prepared in Beckman ultracentrifuge tube for each sample after spiking with [^{14}C]compound **16** at multiple concentrations (50−10000 ng/mL). The remaining samples were centrifuged for 3 h at 37 °C at 306000*g* using a Sorvall RCM120GX microultracentrifuge and 80K rotor. The centrifuge was allowed to stop without using the brake. Aliquots (0.05 mL) of the supernatant were carefully removed without disturbing the separate layers and then transferred to scintillation vials for radioactivity analysis. The fraction of drug bound to plasma proteins equals $(T_t - T_s)/T_t$, where T_t is the total radioactivity in the uncentrifuged sample and T_s is the radioactivity in the supernatant after ultracentrifugation.

Hamster Efficacy Study. Ten-week-old, male golden Syrian hamsters were purchased from Japan SLC Inc. (Shizuoka, Japan). Animals underwent a one-week acclimation period. Compounds were prepared in a 0.5% methyl cellulose (MC, methyl cellulose 400 cP, catalogue no. 138-05072, Wako Pure Chemical Industries, Osaka, Japan) suspension. Compound **16** (0.3, 1, 3, and 10 mg/kg), anacetrapib (0.3, 1, 3, and 10 mg/kg), or vehicle (10 mL/kg) was administrated once a day in the morning for 2 weeks by oral gavage. Then 24 h after administration of the last dose (16 h fast), whole blood samples were collected from the abdominal vein by venipuncture under isoflurane (Forane, Abbott Japan Co. Ltd., Tokyo, Japan) inhalant anesthesia. After centrifugation at 15000 rpm (rotor no. AF-2536A, Kubota Co., Tokyo, Japan) for 10 min at 4 °C, heparinized plasma (final concentration about 28.6 U/mL, heparin sodium injection "Ajinomoto"; Ajinomoto, Tokyo, Japan) or EDTA−plasma was prepared and stored at −80 °C until use. It should be noted that some animals in the 0.3 mg/kg anacetrapib group were mistakenly administered a dose of 1 mg/kg (one animal on day 5 and two animals on day 7). Because each of the three animals only received one incorrect dose during the two-week study period, we concluded that the results would not be significantly altered by this error. Therefore, all animals were included in the data analysis. This study was performed according to the approval of the Novartis Tsukuba Research Institute Animal Welfare Committee (permission no. AWCJ06077).

Lipoprotein cholesterol content in the hamster efficacy study was assessed using an HPLC-size exclusion chromatography system with an online enzymatic dual detection system for lipids. Plasma samples were diluted (5-fold) with saline after filtration (0.45 μm, Millipore Co, catalogue no. UFC30HV00) and injected into the HPLC system at a volume of 100 μL (as 20 μL of plasma), every 95 min, using an autosampler. Plasma lipoproteins were separated using a single Superose 6 column and filtered phosphate buffered saline (0.45 μm, Millipore Co., Bedford, MA, catalogue no. SJHVM4710; PBS, Dainippon Pharmaceutical, Osaka, Japan catalogue no. 28-103-05 FN) at a flow rate of 0.5 mL/min. Each enzymatic reagent was pumped at a flow rate of 0.25 mL/min. Both enzymatic reactions proceeded at 37 °C in a reactor coil (Teflon tube, 15 m × 0.4 mm id) in the column oven. The color developed after the enzyme reaction was measured at 580 nm, and the electric signal was monitored (every 0.5 s). A control pooled EDTA-plasma sample was used to standardize lipoprotein cholesterol levels. Cholesterol levels in control hamster plasma were measured manually (cholesterol E-test, catalogue no. 439-17501), and the level was 122 mg/dL. Lipoprotein cholesterol concentrations were calculated using chromatogram areas observed with control hamster plasma samples.

Drug Distribution among Serum Lipoprotein Fractions. Human serum samples were obtained from Uniglobe Research Corporation (Reseda, CA), Bioreclamation (Hicksville, NY) or from the in-house donor program at the Novartis Institute for BioMedical Research. Donors were not fasted before blood collection. Serum was isolated by centrifugation of samples at 2500 rpm for 20 min and stored at −80 °C until use. Three pools of human serum samples were prepared for this study. The mean TG concentration (mg/dL) of each pool were as follows: 122, 347, and 958, respectively, to represent normal, high, and very high levels. Once thawed, serum samples were gently mixed prior to incubation with compound. Two μL of each CETP inhibitor (**16**, anacetrapib) in dimethyl sulfoxide (2 mg/mL) were added to 4 mL of serum, mixed well, and incubated in a 37 °C water bath for 1 h.

Lipoprotein fractions were isolated from serum by density gradient ultracentrifugation, according to Wasan et al.,[36] with minor modifications. NaBr (0.36 g/mL) was added to serum containing 500 ng/mL of CETP inhibitor. Samples were mixed by gentle inversion to facilitate the dissolution of salt. Density gradient layers were carefully generated from the bottom of tube as follows: 3 mL serum/CETPi/NaBr (d = 1.28), 2.8 mL NaBr/H_2O (d = 1.21), 2.8 mL NaBr/H_2O (d = 1.063), and 2.8 mL NaBr/H_2O (d = 1.006). Tubes were kept in an ice-bath before centrifugation for 18.5 h at 15 °C, using an SW41 Rotor in an L80 ultracentrifuge (Beckman Coulter, Brea, CA). A fraction recovery system (FRS, Beckman Coulter) was used to collect fractions (∼0.3 mL) from the bottom of each centrifuge tube. The density of fractions, relative to water, was measured to determine fractions for pooling. 2-Propanol was used to recover TRL adhering to the inner wall of the centrifuge tube. Fractions were pooled as follows: Lipoprotein-deficient serum ($1.21 < d < 1.30$), HDL ($1.065 < d \leq 1.21$), LDL ($1.019 < d \leq 1.065$), and TRL ($d < 1.019$).

For the measurement of lipoprotein drug concentrations, serum and LPDS were diluted 1:3 using saline. Duplicate 50 μL aliquots of undiluted TRL, LDL, or HDL and diluted serum or LPDS were transferred into a 96-well plate (catalogue no. 17P687, Thermo Fisher Scientific) for the determination of CETP inhibitor concentration by LC-MS/MS. All samples were frozen and stored at −20 °C freezer prior to use. Extraction of CETP inhibitor from lipoprotein pools was carried out using an acetonitrile protein precipitation method. A Freedom EVO 150 and a Freedom EVO (Tecan, San Jose, CA) were used in this procedure. Calibration standards, composed of a CETP inhibitor (0−10,000 ng/mL) in 50% acetonitrile, and quality control (QC) samples of a single concentration (50 ng/mL) were prepared. Then 25 μL of each calibration standard or QC or test sample were added to a 1 mL 96-well plate. Then 150 μL of acetonitrile with 50 ng/mL glyburide (ISTD) was added to each well. The mixture was vortexed vigorously, followed by centrifugation at ∼4000 rpm for 5 min. Next, 125 μL of supernatant/well was transferred to a new 1 mL 96-well plate, followed by the addition of 50 μL of water. For each mixed sample, a 10 μL aliquot was injected into the LC-MS/MS system.

Tissue Drug Exposure Analysis. A ∼100 mg sample of each tissue type (adipose, adrenal, liver) was harvested and placed in a 1.5 mL microcentrifuge tube. All samples were flash frozen and placed at −80 °C for storage. Drug concentrations were determined by LC-MS/MS.

Triton WR-1339 Treated Hamster Study. The effect of acute hypertriglyceridemia in vivo on the extent of CETP inhibition caused by anacetrapib and **16** were investigated. Compound or vehicle was administered once by gavage to hamsters fed a standard chow diet. Triton WR-1339 (100 mg/kg) or saline was injected intravenously 5 h after administration of compound. Plasma parameters were measured 3 h after injection of Triton WR-1339. Two doses for each inhibitor were selected: around 55−75% (lower dose) or 75−80% (higher dose) inhibition in CETP activity in the saline group. Plasma CETP activity ex vivo was determined as follows: 50 μL of hamster plasma (final 80% plasma) was mixed with 10 μL of donor solution diluted with assay buffer composed of 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and 2 mM EDTA in a 96-well half-area black flat-bottom plate (catalogue no. 3686, Corning, Corning, NY). Fluorescence intensity was measured every 15 min at an excitation wavelength of 485 nm and an emission wavelength of 535 nm for 120 min at 37 °C using ARVO SX+L (PerkinElmer, Wellesley, MA).

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CETP activity (RFU/min) was defined as the change in fluorescence intensity from 30 to 90 min.

Chemical Syntheses. Unless otherwise specified, all solvents and reagents were obtained from commercial sources and used without further purification, including intermediates **17c**, **18a**, and **25**. All reactions were performed under nitrogen atmosphere unless otherwise noted. Normal-phase flash chromatography was performed using Merck silica gel 60 (230−400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX or a Bruker AV400 (400 MHz for ^{1}H and 100 MHz for ^{13}C). Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (0.00 ppm) or residual peaks from the corresponding solvent as an internal standard and coupling constants (J) in Hz. Multiplicity abbreviation are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app = apparent, br = broad. LC-MS analyses were performed on an HPLC system with a C18 column coupled to a single quad mass spectrometer with electrospray ionization (ESI). High resolution mass spectra were obtained with Acquity Xevo G2 QToF system with UPLC (Acquity UPLC BEH C18 column, 2−98% acetonitrile in water both with 0.1% formic acid) coupled to time-of-flight detection after electrospray ionization. Chemical purity of the compounds were assessed by LCMS and confirmed to have at least 95% purity by UV/ELSD, except compound **7**, which showed 90% purity by UV.

*rac-tert-Butyl (2R,4R)-4-((3,5-Bis(trifluoromethyl)benzyl)-(methoxycarbonyl)amino)-2-ethylpiperidine-1-carboxylate (**1**).* To a mixture of *rac-tert*-butyl 2-ethyl-4-oxopiperidine-1-carboxylate (**18a**) (1 g, 4.4 mmol) and 3,5-bis(trifluoromethyl)benzylamine (technical grade ∼85%, 1.38 g, 4.84 mmol) in MeOH (7.5 mL) and 1,2-dichloroethane (7.5 mL) was added titanium tetraisopropoxide (∼0.15 mL) at rt under nitrogen. The reaction mixture was allowed to stir at the same temperature for 6 h and quenched with saturated aqueous NH_4Cl. The organic products were extracted twice with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated to give a crude oil (**19a**). The crude product was dissolved in pyridine (10 mL), and methyl chloroformate (2.0 g, 22 mmol) was added at 0 °C. The mixture was gradually warmed up to rt and stirred for 13.5 h. The reaction was quenched with 1 M aqueous HCl, and the products were extracted with EtOAc, washed with brine, dried, filtered, and concentrated to a crude product which was purified by silica gel column chromatography to afford *rac-tert*-butyl (2*R*,4*R*)-4-((3,5-bis(trifluoromethyl)benzyl)(methoxycarbonyl)amino)-2-ethylpiperidine-1-carboxylate (**1**) (1.3 g, 58% in 2 steps). ESI-MS m/z: 513 $(M + H)^+$.

*tert-Butyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)-(methoxycarbonyl)amino)-2,6-diethylpiperidine-1-carboxylate (**2**).* To a mixture of *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)-benzyl)amino)-2,6-diethylpiperidine-1-carboxylate (**19b**) (20.8 mg, 0.043 mmol) and DMAP (10.6 mg, 0.086 mmol) in DCM (0.3 mL) was added methyl chloroformate (6.1 μL, 0.086 mmol) at rt under nitrogen. The mixture was stirred overnight and purified by preparative TLC (hexane/EtOAc = 2:1) to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(methoxycarbonyl)-amino)-2,6-diethylpiperidine-1-carboxylate (**2**) (21.2 mg, 91%). ESI-MS m/z: 541 $(M + H)^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(methoxycarbonyl)amino)-6-ethylpiperidine-1-carboxylate (**3**).* To a mixture of *rac*-benzyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis-(trifluoromethyl)benzyl)(methoxycarbonyl)amino)-6-ethylpiperidine-1-carboxylate (**20**) (710 mg, 1.12 mmol) and $PdCl_2$ (39 mg, 0.22 mmol) in DCM (11 mL) were added Et_3N (61.3 μL, 0.44 mmol) and Et_3SiH (358 μL, 2.24 mmol) at rt under nitrogen. The mixture was allowed to stir for 11.5 h. Additional Et_3SiH (1.4 mL, 8.76 mmol) was added portionwise over 4.5 h. After stirring for a further 3 h, the reaction mixture was concentrated and purified by silica gel column chromatography (DCM/MeOH/NH_3(28% in H_2O) = 300:10:1) to afford *rac*-methyl ((2*S*,4*S*,6*R*)-2-benzyl-6-ethylpiperidin-4-yl)(3,5-bis-(trifluoromethyl)benzyl)carbamate (560 mg, 99%). ESI-MS m/z: 503 $(M + H)^+$.

A mixture of *rac*-methyl ((2*S*,4*S*,6*R*)-2-benzyl-6-ethylpiperidin-4-yl)(3,5-bis(trifluoromethyl)benzyl)carbamate (560 mg, 1.11 mmol) and Boc_2O (909 mg, 4.17 mmol) was allowed to stir at 60 °C for 19 h. The mixture was cooled and purified by silica gel column chromatography (EtOAc/heptane = 6:94 to 60:40) to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)-(methoxycarbonyl)amino)-6-ethylpiperidine-1-carboxylate (**3**) (410 mg, 61%). MS (ESI^+) m/z: 603 $(M + H)^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**4**).* To a suspension of *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**23**) (0.952 g, 2.00 mmol) in DMF (20 mL) was added NaH (60% dispersion in oil, 160 mg, 4.00 mmol) at 0 °C under nitrogen. The mixture was warmed up to rt and stirred for 20 min. To the mixture was added 3,5-bis(trifluoromethyl)benzyl bromide (921 mg, 3.00 mmol) at 0 °C. The reaction mixture was allowed to stir from 0 °C to rt for 4 h and then quenched with H_2O. The products were extracted with EtOAc, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The obtained residue was purified by silica gel column chromatography to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**4**) (962 mg, 68%). ESI-MS m/z: 701 $[M + H]^+$.

*Chiral tert-Butyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**5**).* *rac*-Benzyl (2*S*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (*cis*-racemate) (***rac*-18c**) (45 g) was purified by two consecutive chiral chromatography runs. The first elution with supercritical carbon dioxide with 5% 2-propanol (isocratic) in a Daicel Chiralcel OD-H column (30 mm × 250 mm) resulted in good separation of the enantiomers but with undesired *trans*-isomer which coeluted. The second elution with supercritical carbon dioxide with 5% 2-propanol (isocratic) with a Daicel Chiralpak AD-H (30 mm × 250 mm) gave diastereomerically pure enantiomers. The enantiomeric excess of the separated enantiomers were determined by chiral HPLC (Daicel Chiralpak AD-H 0.46 cm × 25 cm, *n*-heptane:EtOH = 80:20, 1 mL/min, 210 nM). T_r = 6.90 min (***pro-distomer*-18c**, 98.3% ee); 8.53 min (***pro-eutomer*-18c**, 95.9% ee).

***Pro-eutomer*-18c** (absolute configuration unknown at this stage) was converted to compound **5** and ***pro-distomer*-18c** to its enantiomer using the same sequence of reactions as with racemic **4** (Scheme 2, conditions a, b, c, and d). Compound **5** was shown to be the active enantiomer by inhibition of CETP activity in human plasma. Compound **5** was then crystallized in tetrahydrofuran−methanol co-solvent and the absolute configuration was determined by X-ray crystallography (Supporting Information, Figure 3) to be 2*S*,4*S*,6*R*.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((5-bromopyrimidin-2-yl)(3-methoxy-5-(trifluoromethyl)benzyl)amino)-6-ethylpiperidine-1-carboxylate (**6**).* Compound **6** was synthesized according to a procedure similar to that described for compound **4**, using 3-methoxy-5-(trifluoromethyl)benzyl bromide instead of 3,5-bis(trifluoromethyl)-benzyl bromide. Yield: 16.4 mg (25%). ESI-MS m/z: 663 $[M + H]^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((5-bromopyrimidin-2-yl)(3-chloro-5-(trifluoromethyl)benzyl)amino)-6-ethylpiperidine-1-carboxylate (**7**).* Compound **7** was synthesized according to a procedure similar to that described for compound **4**, using 3-chloro-5-(trifluoromethyl)benzyl bromide instead of 3,5-bis(trifluoromethyl)-benzyl bromide. Yield: 49 mg (73%). ESI-MS m/z: 667 $[M + H]^+$.

*rac-(2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-N,6-diethylpiperidine-1-carboxamide (**8**).* To a mixture of *rac-N*-((2*S*,4*S*,6*R*)-2-benzyl-6-ethylpiperidin-4-yl)-*N*-(3,5-bis(trifluoromethyl)benzyl)-5-bromopyrimidin-2-amine hydrochloride (**24**) (50 mg, 0.078 mmol) in DMF (1 mL) were added ethyl isocyanate (13.7 μL, 0.157 mmol) and DIPEA (41 μL, 0.235 mmol) at rt. The mixture was stirred at rt for 18 h, added to saturated aqueous $NaHCO_3$, and extracted with dichloromethane. The combined organic layers were passed through a phase separator and concentrated in vacuo. The residue was purified by preparative HPLC to give *rac*-(2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-*N*,6-diethylpiperidine-1-carboxamide (**8**) (20.5 mg, 39%). ESI-MS m/z: 672 $[M + H]^+$.

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*rac-1-((2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidin-1-yl)butan-1-one (**9**).* Compound **9** was synthesized according to a procedure similar to that described for compound **8**, using butyryl chloride instead of ethyl isocyanate. Yield: 21 mg (40%). ESI-MS *m/z*: 671 $[M + H]^+$.

*rac-Isopropyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**10**).* Compound **10** was synthesized according to a procedure similar to that described for compound **8**, using isopropyl chloroformate instead of ethyl isocyanate. Yield: 20 mg (37%). ESI-MS *m/z*: 687 $[M + H]^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-methoxypyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**11**).* A mixture of *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**4**) (114 mg, 0.16 mmol), sodium methoxide (25 wt % in methanol, 111 μL, 0.48 mmol), and copper iodide (I) (62 mg, 0.32 mmol) in DMF (1.5 mL) was stirred at 85 °C for 2.5 h and then allowed to cool to rt. The mixture was diluted with water and ethyl acetate and then passed through a Celite pad. The filtrate was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was purified by preparative HPLC to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-methoxypyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**11**) (26 mg, 25%). ESI-MS *m/z*: 653 $[M + H]^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-morpholinopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**12**).* To *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**4**) (0.038 mmol, 27 mg) in a flask purged with N_2 were added $Pd_2(dba)_3$ (0.0077 mmol, 7 mg), 2-(di-*tert*-butylphosphino)biphenyl (0.0154 mmol, 4.6 mg), sodium *tert*-butoxide (0.154 mmol, 14.8 mg), and morpholine (0.077 mmol, 7 μL). The flask was purged again with N_2, toluene (0.4 mL) was added, and the mixture was heated to 100 °C for 4 h. The mixture was cooled, quenched with silica gel, and the slurry subjected to silica gel column chromatography to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**12**) (13.37 mg, 50%). ESI-MS *m/z*: 708 $[M + H]^+$.

*Chiral tert-Butyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(5-morpholinopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**13**).* The chiral separation of *rac*-**12** was carried out by chiral HPLC (Daicel Chiralpak AD-H 5 cm × 50 cm, *n*-hexane:*i*-PrOH = 95:5, 1 mL/min, 220 nm) to afford compound **13** and the distomer. The enantiomeric excess was determined by chiral HPLC (Daicel Chiralpak AD-H 0.46 cm × 25 cm, *n*-hexane:*i*-PrOH = 95:5, 1 mL/min, 220 nm). T_r = 8.22 min (compound **13**, >99% ee); 11.00 min (the distomer, >99% ee)

*Isopropyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**14**).* To a solution of isopropyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**28b**) (250 mg, 0.40 mmol) and morpholine (39 μL, 0.44 mmol) in toluene (5 mL) were added $Pd_2(dba)_3$ (36.6 mg, 0.04 mmol), 2-(di-*tert*-butylphosphino)biphenyl (12 mg, 0.04 mmol), and sodium *tert*-butoxide (42 mg, 0.44 mmol). The reaction mixture was allowed to stir at 70 °C for 1.5 h under nitrogen and then allowed to cool to rt. The mixture was diluted with brine and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was purified by silica gel column chromatography eluting with ethyl acetate/heptane to afford isopropyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**14**) (126 mg, 0.20 mmol. 50%) as a white powder. ESI-MS *m/z*: 632.3 $[M + H]^+$.

*(1r,4r)-4-(((2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carbonyl)-oxy)cyclohexane-1-carboxylic Acid (**15**).* A solution of *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**29a**) (250 mg, 0.38 mmol) in 4 M HCl in ethyl acetate (3 mL) was allowed to stir from 0 °C to rt for 3 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford *N*-(3,5-bis(trifluoromethyl)benzyl)-*N*-((2*R*,4*r*,6*S*)-2,6-diethylpiperidin-4-yl)-5-morpholinopyrimidin-2-amine (195 mg). The obtained product was used in the next step without further purification.

To a solution of *N*-(3,5-bis(trifluoromethyl)benzyl)-*N*-((2*R*,4*r*,6*S*)-2,6-diethylpiperidin-4-yl)-5-morpholinopyrimidin-2-amine (95 mg, 0.17 mmol) in DMF (0.1 mL) were added methyl (1*r*,4*r*)-4-((chlorocarbonyl)oxy)cyclohexane-1-carboxylate (**26**) (76 mg, 0.35 mmol) and diisopropylethylamine (68 μL, 0.52 mmol) at rt. The reaction mixture was allowed to stir at rt for 3 h and then diluted with DCM and water. The product was extracted with DCM, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with EtOAc/heptane to afford (1*r*,4*r*)-4-(methoxycarbonyl)cyclohexyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (53 mg).

To a solution of (1*r*,4*r*)-4-(methoxycarbonyl)cyclohexyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)-amino)-2,6-diethylpiperidine-1-carboxylate (53 mg, 0.073 mmol) in THF (0.7 mL) and MeOH (0.3 mL) was added 1 M aqueous LiOH (0.37 mL, 0.37 mL) at rt. The reaction mixture was allowed to stir at rt for 17 h and then quenched with 1 M aqueous HCl. The mixture was further diluted with water. The product was extracted with DCM. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained crude product was purified by silica gel column chromatography eluting with MeOH/DCM to afford (1*r*,4*r*)-4-(((2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carbonyl)oxy)-cyclohexane-1-carboxylic acid (**15**) (33 mg, 0.046 mmol, 63%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 0. 84 (t, *J* = 7.45 Hz, 6H), 1.35−1.53 (m, 6H), 1.55−1.67 (m, 2H) 1.74−1.83 (m, 2H) 2.03−2.18 (m, 6H), 2.30−2.40 (m, 1H), 2.99−3.05 (m, 4H), 3.83−3.87 (m, 4H), 4.10−4.19 (m, 2H), 4.62−4.76 (m, 2H), 4.79 (s, 2H), 7.69 (s, 2H), 7.73 (s, 1H), 8.09 (s, 2H). ESI-MS *m/z*: 716.4 $[M + H]^+$.

*(1r,4r)-4-(((2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-(1-methyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carbonyl)oxy)cyclohexane-1-carboxylic Acid (**16**).* To a mixture of *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)-(5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**29b**) (24.6 g) in ethyl acetate (60 mL) was added dropwise 4 M HCl in ethyl acetate (200 mL) over 15 min, and the mixture was stirred at rt for 2 h. Precipitates were collected by filtration and then dissolved in a mixture of saturated $NaHCO_3$ and EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford *N*-(3,5-bis(trifluoromethyl)benzyl)-*N*-((2*R*,4*r*,6*S*)-2,6-diethylpiperidin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-amine (18.5 g) as a colorless solid. ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.87 (t, 6H), 1.18−1.28 (m, 2H), 1.37−1.45 (m, 4H), 1.63 (bs, 2H), 1.79 (d, 2H), 2.61−2.67 (m, 2H), 3.95 (s, 3H), 4.88 (s, 3H), 7.52 (s, 1H), 7.65 (s, 1H), 7.68 (s, 2H), 7.72 (s, 1H), 8.43 (s, 2H).

To a mixture of *N*-(3,5-bis(trifluoromethyl)benzyl)-*N*-((2*R*,4*r*,6*S*)-2,6-diethylpiperidin-4-yl)-5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-amine (15.0 g, 27.7 mmol) and *N*,*N*-diisopropylethylamine (38.0 mL, 218 mmol) in DMF (19 mL) was added a solution of methyl (1*r*,4*r*)-4-((chlorocarbonyl)oxy)cyclohexane-1-carboxylate (**26**) (12.2 g, 55.1 mmol) in DMF (1 mL). The mixture was stirred for 1 h at room temperature, then another portion of **26** (2.94 g, 13.3 mmol) was added. The mixture was stirred for 1 h at rt, and then another portion of **26** (3.13 g, 14.2 mmol) was added. After the mixture was stirred for 30 min, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (gradient from 12% to 80% ethyl acetate in hexane) to afford (1*r*,4*r*)-4-(methoxycarbonyl)-cyclohexyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-(1-meth-

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yl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (21.3 g). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.85 (t, 6H), 1.24−1.27 (m, 1H), 1.34−1.46 (m, 4H), 1.47−1.65 (m, 9H), 1.74−1.84 (m, 2H), 2.01−2.19 (m, 7H), 2.26−2.33 (m, 1H), 3.67 (s, 3H), 3.95 (s, 3H), 4.09−4.20 (m, 2H), 4.61−4.67 (m, 1H), 4.76−4.82 (m, 1H), 4.86 (s, 2H), 7.53 (s, 1H), 7.66 (s, 1H), 7.70 (s, 2H), 7.75 (s, 1H), 8.43 (s, 2H).

To a mixture (1*r*,4*r*)-4-(methoxycarbonyl)cyclohexyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-(1-methyl-1*H*-pyrazol-4-yl)-pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (15.4 g, 21.2 mmol) in THF (100 mL) and MeOH (100 mL) was added aqueous 2 M NaOH (45 mL) at 0 °C. The mixture was stirred at rt for 24 h, cooled to 0 °C, and aqueous 1 M HCl (90 mL) was added dropwise. The mixture was concentrated and extracted with EtOAc. The organic layer was washed with water followed by brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained solid was dissolved in a mixture of ethyl acetate (15 mL) and diisopropyl ether (135 mL) at 80 °C, then gradually cooled to rt. The crystalline solid was collected by filtration, washed with diisopropyl ether, and then dried under vacuum at 50 °C to afford (1*r*,4*r*)-4-(((2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-(1-methyl-1*H*-pyrazol-4-yl)pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carbonyl)oxy)cyclohexane-1-carboxylic acid (**16**) (10.9 g). HRMS calculated exact mass 711.30880, observed 711.30908 $[M + H]^+$ (Δ0.4 ppm). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.85 (t, 6 H) 1.36−1.70 (m, 7 H) 1.75−1.85 (m, 3H) 2.03−2.20 (m, 6 H) 2.31−2.41 (m, 1 H) 3.95 (s, 3 H) 4.11−4.22 (m, 2 H) 4.61−4.71 (m, 1 H) 4.76−4.88 (m, 1 H) 4.86 (s, 2 H) 7.53 (s, 1 H) 7.66 (s, 1 H) 7.70 (s, 2 H) 7.75 (s, 1 H) 8.43 (s, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 10.7, 12.14, 26.3, 30.4, 30.6, 31.1, 38.7, 40.9, 45.6, 49.3, 52.7, 72.2, 116, 116.3, 120.3, 123.3, 126.8, 127.5, 130.1, 135.4, 144.5, 154.5, 155.1, 159.7, 176.2. ^{19}F NMR (376 MHz, DMSO-d_6), δ (ppm): −61.3.

*rac-tert-Butyl 2-Ethyl-4-oxo-3,4-dihydropyridine-1(2H)-carboxylate (**17b**).* To a solution of 4-methoxypyridine (15.6 g, 143 mmol) in dry THF (1 L) cooled to −35 °C was added phenyl chloroformate (22.7 g, 144 mmol). After stirring for 1 h, 1 M ethyl magnesium bromide in THF (150 mL, 150 mmol) was added slowly over 30 min. The mixture was warmed to 10 °C over 2 h and then quenched with water. The reaction mixture was extracted twice with Et_2O (1 L). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. To a solution of the resulting colorless oil in dry THF (500 mL) at −78 °C was added potassium *tert*-butoxide (64 g, 572 mmol). The reaction mixture was stirred from −78 °C to rt overnight. The reaction mixture was diluted with Et_2O and then quenched with ice. The organic layer was separated, washed three times with 1.5 M aqueous NaOH, and then with brine, dried over $MgSO_4$, and concentrated in vacuo to afford *rac-tert*-butyl 2-ethyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (**17b**) as a pale-yellow oil (27.8 g, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.92 (t, 3H), 1.55 (s, 9H), 1.57−1.75 (m, 3H), 2.44 (d, 1H), 2.80 (dd, 1H), 4.55 (d, 1H), 5.26 (d, 1H), 7.74 (d, 1H). ESI-MS *m*/*z*: 226 $[M + H]^+$.

*tert-Butyl (2R,6S)-2,6-Diethyl-4-oxopiperidine-1-carboxylate (**18b**).* To copper(I) iodide (0.82 mmol, 156 mg) in a flask purged with nitrogen was added 1 M EtMgBr in THF (0.82 mmol, 0.82 mL) at −78 °C. After stirring for 30 min, $BF_3{\cdot}Et_2O$ (0.41 mmol, 57.9 mg) was added, and the mixture was stirred for 10 min at the same temperature. To the suspension was added a solution of *rac-tert*-butyl 2-ethyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (**17b**) (0.41 mmol, 92.7 mg) in tetrahydrofuran (3.3 mL) at −78 °C. The mixture was allowed to stir at the same temperature for 1.5 h and then at −40 °C for 2 h. This mixture was warmed to rt, quenched with saturated aqueous NH_4Cl, and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and purified by silica gel column chromatography (hexane/EtOAc = 10:1) to afford *tert*-butyl (2*R*,6*S*)-2,6-diethyl-4-oxopiperidine-1-carboxylate (**18b**) (50 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.91 (t, 6H), 1.44−1.53 (m, 11H), 1.65−1.70 (m, 2H), 2.35 (dd, 2H), 2.64−2.68 (m, 2H), 4.48 (bs, 2H). ESI-MS *m*/*z*: 200 (M^+ − *t*Bu + 2).

*rac-Benzyl (2S,6R)-2-Benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (**rac-18c**).* To copper(I) iodide (2.29 g, 12 mmol) in a flask was added 1 M EtMgBr in THF (14 mL, 14 mmol) at −78 °C under nitrogen followed by addition of $BF_3{\cdot}Et_2O$ (0.75 mL, 6 mmol). After stirring for 20 min at the same temperature, a solution of *rac*-benzyl 2-benzyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (**17c**) (1.93 g, 6 mmol) in THF (40 mL) was added portionwise at the same temperature. The mixture was gradually warmed up to rt, stirred for 12 h, and then quenched with saturated aqueous NH_4Cl. The products were extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography to afford a mixture of *rac*-benzyl (2*S*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (*cis*-racemate) (***rac*-18c**) and the corresponding *trans*-racemate in a 10:1 ratio (1.60 g, 76%).

*rac-tert-Butyl (2S,6R)-2-Benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (**rac-18d**).* A suspension of a 10:1 mixture of *rac*-benzyl (2*S*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (***rac*-18c**) (*cis*-racemate) and the *trans*-racemate, and 10% Pd/C (850 mg) in MeOH (170 mL) was allowed to stir at rt under hydrogen until complete consumption of the substrate (monitored by TLC). The reaction mixture was filtered and concentrated to give a crude product. The crude product was purified by silica gel column chromatography (hexane/EtOAc) to afford *rac*-(2*S*,6*R*)-2-benzyl-6-ethylpiperidin-4-one (3.88 g, 74%) as a yellow oil. ESI-MS *m*/*z*: 218 $(M + H)^+$.

A mixture of *rac*-(2*S*,6*R*)-2-benzyl-6-ethylpiperidin-4-one (3.86 g, 17.8 mmol) and Boc_2O (7.84 g, 35.9 mmol) was allowed to stir at 60 °C for 2 h. The mixture was cooled to rt and purified by silica gel column chromatography (hexane/EtOAc) to afford *rac-tert*-butyl (2*S*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (***rac*-18d**) (5.37 g, 95%) as a pale-yellow oil. ESI-MS *m*/*z*: 262 $(M - t\text{Bu} + 2H)^+$.

*Isopropyl (2R,6S)-2,6-Diethyl-4-oxopiperidine-1-carboxylate (**18e**).* Into a solution of 4-methoxypyridine (53.7 g, 0.492 mol) in THF (1150 mL) were added isopropyl chloroformate (62 mL, 0.542 mol) and then EtMgBr (1 M in THF, 517 mL, 517 mmol) at −50 °C. This reaction mixture was stirred for 1 h, warming toward rt. The reaction mixture was diluted with water (100 mL) at 5 °C and then filtered. The filter cake was washed with EtOAc, and the filtrate was extracted with EtOAc. The combined organic layers were washed with 1 M aqueous HCl and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to afford isopropyl 2-ethyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (102.1 g), which was used in the next step without further purification.

To a suspension of copper(I) iodide (37 g, 195.3 mmol) in THF (100 mL) cooled to −65 °C were added EtMgBr (195.3 mL, 195.3 mmol), $BF_3{\cdot}Et_2O$ (12.2 mL, 97.65 mmol), and isopropyl 2-ethyl-4-oxo-3,4-dihydropyridine-1(2*H*)-carboxylate (22 g, 97.65 mmol) in THF (100 mL) at −65 °C in this order. This reaction mixture was stirred for 16 h, warming to rt. The reaction mixture was diluted with half-saturated aqueous NH_4Cl and EtOAc and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with ethyl acetate/hexane to afford isopropyl (2*R*,6*S*)-2,6-diethyl-4-oxopiperidine-1-carboxylate (**18e**) (10 g, 30.65 mmol, 40%).

*tert-Butyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)amino)-2,6-diethylpiperidine-1-carboxylate (**19b**).* To a mixture of *tert*-butyl (2*R*,6*S*)-2,6-diethyl-4-oxopiperidine-1-carboxylate (**18b**) (53 mg, 0.21 mmol), 3,5-bis(trifluoromethyl)benzylamine (technical grade ∼85%, 61.3 mg, 0.25 mmol), and acetic acid (14 μL, 0.25 mmol) in 1,2-dichloroethane (0.5 mL) was added $NaBH(OAc)_3$ (89 mg, 0.42 mmol) at rt under nitrogen. The mixture was stirred for 20 h and then quenched with 1 M aqueous NaOH. The organic products were extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by preparative TLC (hexane/EtOAc = 2:1) to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)amino)-2,6-diethylpiperidine-1-carboxylate (**19b**) (21.2 mg, 20%). ESI-MS *m*/*z*: 483 $(M + H)^+$.

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*rac-Benzyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)amino)-6-ethylpiperidine-1-carboxylate (**19c**).* To a 10:1 mixture of *rac*-benzyl (2*S*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (*cis*-racemate) (***rac*-18c**) and *rac*-benzyl (2*R*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (*trans*-racemate) (1.60 g, 4.55 mmol), 3,5-bis(trifluoromethyl)benzylamine (technical grade ~85%, 1.55 g, 6.37 mmol), and acetic acid (348 μL, 6.37 mmol) in 1,2-dichloroethane (10 mL) was added $NaBH(OAc)_3$ (1.93 g, 9.1 mmol) at rt. The mixture was stirred for 13 h and then quenched with 1 M aqueous NaOH. The organic products were extracted with DCM. The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (DCM/EtOAc) to afford *rac*-benzyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)amino)-6-ethylpiperidine-1-carboxylate (**19c**) (990 mg, 38%). ESI-MS *m*/*z*: 579 $(M + H)^+$.

*rac-Benzyl (2S,4S,6R)-2-Benzyl-4-((3,5-bis(trifluoromethyl)-benzyl)(methoxycarbonyl)amino)-6-ethylpiperidine-1-carboxylate (**20**).* To a mixture of *rac*-benzyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis-(trifluoromethyl)benzyl)amino)-6-ethylpiperidine-1-carboxylate (**19c**) (990 mg, 1.71 mmol) and DMAP (420 mg, 3.42 mmol) in DCM (20 mL) was added methyl chloroformate (304 μL, 4.28 mmol) at rt under nitrogen, The mixture was stirred for 3 h and quenched with brine. The organic layer was separated and purified by silica gel column chromatography (DCM/EtOAc) to afford *rac*-benzyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis(trifluoromethyl)benzyl)(methoxycarbonyl)amino)-6-ethylpiperidine-1-carboxylate (**20**) (710 mg, 65%). ESI-MS *m*/*z*: 637.0 $(M + H)^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-(benzylamino)-6-ethylpiperidine-1-carboxylate (**21**).* A mixture of *rac-tert*-butyl (2*S*,6*R*)-2-benzyl-6-ethyl-4-oxopiperidine-1-carboxylate (***rac*-18d**) (5.36 g, 16.8 mmol), benzylamine (2.00 mL, 18.3 mmol) and $BF_3{\cdot}Et_2O$ (10 μL) in toluene (200 mL) was refluxed in a Dean−Stark apparatus for 20 min. The reaction mixture was cooled to rt and concentrated to give a crude oil. The crude product was dissolved in MeOH (200 mL), and then $NaBH_4$ (393 mg, 10.4 mmol) was added portionwise at rt. The reaction mixture was quenched with saturated aqueous NH_4Cl and then concentrated. The residue was diluted with water and extracted with dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by silica gel column chromatography (DCM/MeOH) to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-(benzylamino)-6-ethylpiperidine-1-carboxylate (**21**) (4.18 g, 61%) as a pale-yellow oil. ESI-MS *m*/*z* 409: $(M + H)^+$.

*rac-tert-Butyl (2S,4S,6R)-4-Amino-2-benzyl-6-ethylpiperidine-1-carboxylate (**22**).* A mixture of *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-(benzylamino)-6-ethylpiperidine-1-carboxylate (**21**) (2.89 g, 7 mmol) and 10% Pd/C (100 mg, 0.09 mmol) in EtOH (80 mL) was allowed to stir at 40 °C under hydrogen until complete consumption of the substrate. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (DCM/MeOH) to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-4-amino-2-benzyl-6-ethylpiperidine-1-carboxylate (**22**) (1.85 g, 83%). ESI-MS *m*/*z*: 319 $(M + H)^+$.

*rac-tert-Butyl (2S,4S,6R)-2-Benzyl-4-((5-bromopyrimidin-2-yl)-amino)-6-ethylpiperidine-1-carboxylate (**23**).* A mixture of *rac-tert*-butyl (2*S*,4*S*,6*R*)-4-amino-2-benzyl-6-ethylpiperidine-1-carboxylate (**22**) (1.75 g, 5.5 mmol), 5-bromo-2-chloropyridine (1.6 g, 8.25 mmol), and DIPEA (1.92 mL, 11 mmol) in DMF (16.5 mL) was heated at 120 °C for 4 h under nitrogen. The mixture was cooled to rt and diluted with brine. The products were extracted with EtOAc. The organic layer was washed with saturated aqueous NH_4Cl and water and then concentrated to give a brown solid. The solid was triturated with diisopropyl ether to afford *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate as a colorless solid (**23**) (1.52 g, 58%).

*rac-N-((2S,4S,6R)-2-Benzyl-6-ethylpiperidin-4-yl)-N-(3,5-bis-(trifluoromethyl)benzyl)-5-bromopyrimidin-2-amine Hydrochloride (**24**).* A mixture of *rac-tert*-butyl (2*S*,4*S*,6*R*)-2-benzyl-4-((3,5-bis-(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-6-ethylpiperidine-1-carboxylate (**4**) (1.29 mmol, 900 mg) and a solution of 4 M HCl in EtOAc was stirred for 4 h at rt, then concentrated in vacuo. To the obtained residue was added Et_2O, and the solid was collected by filtration to give *rac-N*-((2*S*,4*S*,6*R*)-2-benzyl-6-ethylpiperidin-4-yl)-*N*-(3,5-bis(trifluoromethyl)benzyl)-5-bromopyrimidin-2-amine hydrochloride (**24**) (1.2 mmol, 771 mg, 94% as HCl salt). ESI-MS *m*/*z*: 601 $[M + H]^+$.

*Methyl (1r,4r)-4-Hydroxycyclohexane-1-carboxylate (**25**).* To a solution of (1*r*,4*r*)-4-hydroxycyclohexane-1-carboxylic acid (2.12 g, 14.7 mmol) in DMF (15 mL) were added potassium carbonate (2.44 g, 17.6 mmol) and methyl iodide (1.1 mL, 17.6 mmol) at 0 °C. The reaction mixture was allowed to stir from 0 °C to rt for 2 h. The reaction mixture was diluted with water and EtOAc. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford methyl (1*r*,4*r*)-4-hydroxycyclohexane-1-carboxylate (**25**) (1.62 g, 10.29 mmol, 70%). ^1H NMR (400 MHz, CDCl3), δ (ppm): 1.23−1.33 (m, 2H), 1.48−1.56 (m, 2H), 1.99−2.05 (m, 4H), 2.22−2.30 (m, 1H), 3.58−3.64 (m, 1H), 3.67 (s, 3H).

*Methyl (1r,4r)-4-((Chlorocarbonyl)oxy)cyclohexane-1-carboxylate (**26**).* To a solution of triphosgene (1.52 g, 5.12 mmol) in DCM (15 mL) were added a solution of pyridine (870 μL, 10.8 mmol) in DCM (5 mL) and methyl (1*r*,4*r*)-4-hydroxycyclohexane-1-carboxylate (**25**) (1.62 g, 10.29 mmol) at 0 °C. The reaction mixture was allowed to stir from 0 °C to rt for 3 h. The reaction mixture was diluted with aqueous NH_4Cl. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated in vacuo to afford methyl (1*r*,4*r*)-4-((chlorocarbonyl)oxy)cyclohexane-1-carboxylate (**26**) (1.77 g, 3.63 mmol, 78%). ^1H NMR (400 MHz, CDCl3), δ (ppm): 1.53−1.64 (m, 4H), 2.05−2.18 (m, 4H), 2.30−2.37 (m, 1H), 3.68 (m, 3H), 4.77−4.83 (m, 1H).

*tert-Butyl (2R,4r,6S)-4-((5-Bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**27a**).* To a mixture of *tert*-butyl (2*R*,6*S*)-2,6-diethyl-4-oxopiperidine-1-carboxylate (**18b**) (11.1 g, 44 mmol) in MeOH (150 mL) were added benzylamine (7.1 mL, 65 mmol) and titanium tetraisopropoxide (26 mL, 87 mmol) at 0 °C, and the mixture was stirred overnight at rt. After addition of sodium borohydride (2.5 g, 65 mmol), the mixture was stirred for an additional 1 h at rt. H_2O and EtOAc were added to the mixture, and the resulting precipitate was removed by filtration. The filtrate was washed sequentially with saturated aqueous $NaHCO_3$ and brine. The product was extracted from the aqueous layer with EtOAc, and the combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to obtain *tert*-butyl (2*R*,4*r*,6*S*)-4-(benzylamino)-2,6-diethylpiperidine-1-carboxylate as a clear oil (13.9 g, 92%). The diastereomeric ratio was typically 10:1 (*cis*:*trans*). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.87 (t, 6H), 1.17−1.23 (m, 2H), 1.45 (s, 9H), 1.46−1.50 (m, 3H), 1.73−1.80 (m, 2H), 2.22−2.28 (m, 2H), 2.67−2.71 (m, 1H), 3.78 (s, 2H), 3.98−4.03 (m, 2H).

A mixture of *tert*-butyl (2*R*,4*r*,6*S*)-4-(benzylamino)-2,6-diethylpiperidine-1-carboxylate (4.0 g, 11.4 mmol) and 10% Pd/C (400 mg) in EtOH (80 mL) was stirred for 5 h at 55 °C under hydrogen. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated in vacuo to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-amino-2,6-diethylpiperidine-1-carboxylate as clear oil (2.9 g, 99%), which was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.90 (t, 6H), 1.06−1.12 (m, 3H), 1.38−1.50 (m, 12H), 1.71−1.77 (m, 2H), 2.15−2.21 (m, 2H), 2.84 (m, 1H), 4.00−4.04 (m, 2H).

A mixture of *tert*-butyl (2*R*,4*r*,6*S*)-4-amino-2,6-diethylpiperidine-1-carboxylate (21.1 g, 82.3 mmol), 5-bromo-2-chloropyrimidine (17.1 g, 88.5 mmol), and diisopropylethylamine (28.0 mL, 161 mmol) in DMF (220 mL) was stirred at 120 °C for 3.5 h. After cooling to rt, the mixture was diluted with water (300 mL) and extracted with EtOAc (200 mL × 2). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. To the obtained solid was added diisopropyl ether (65 mL), and the solid was dissolved under reflux. The resulting solution was cooled to rt, then *n*-hexane (65 mL) was added. Precipitates were collected by filtration and washed with diisopropyl ether to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-((5-bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**27a**) (12.4 g, 36%). ^1H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.89 (t, 6H), 1.19−1.27 (m, 2H), 1.39−1.44 (m, 3H), 1.45 (s,

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9H), 1.73−1.84 (m, 2H), 2.39−2.45 (m, 2H), 3.83−3.92 (m, 1H), 4.09−4.17 (m, 2H), 4.94 (d, 1H), 8.27 (s, 2H).

*Isopropyl (2R,4r,6S)-4-((5-Bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**27b**).* Compound **27b** was synthesized according to a procedure similar to that described for compound **23**, using compound **18e** in 30% yield in three steps.

*tert-Butyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**28a**).* To a solution of *tert*-butyl (2*R*,4*r*,6*S*)-4-((5-bromopyrimidin-2-yl)-amino)-2,6-diethylpiperidine-1-carboxylate (**27a**) (6.05 g, 14.6 mmol) in DMF (60 mL) was added NaH (60% in oil, 0.70 g, 17.6 mmol) at 0 °C. After the solution was stirred for 1 h, 3,5-bis(trifluoromethyl)-benzyl bromide (3.23 mL, 17.6 mmol) was added, and the reaction mixture was warmed to rt and stirred for 20 min. After the reaction was quenched with H_2O at 0 °C, the mixture was extracted with ethyl acetate, washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The obtained residue was purified by silica gel column chromatography to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis-(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-2,6-diethyl-piperidine-1-carboxylate (**28a**) (8.02 g, 86%) as yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.84 (t, 6H), 1.37−1.56 (m, 5H), 1.47 (s, 9H), 1.73−1.84 (m, 2H), 2.09−2.15 (m, 2H), 4.10 (p, 2H), 4.63−4.74 (m, 1H), 4.81 (s, 2H), 7.66 (s, 2H), 7.75 (s, 1H), 8.32 (s, 2H). ESI-MS *m*/*z*: 639.2 $[M + H]^+$.

*Isopropyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**28b**).* Compound **28b** was synthesized according to a procedure similar to that described for compound **28a**, using compound **27b** in 74% yield.

*tert-Butyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-morpholinopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**29a**).* To a solution of *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis-(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-2,6-diethyl-piperidine-1-carboxylate **28a** (300 mg, 0.47 mmol) and morpholine (82 μL, 0.94 mmol) in toluene (5 mL) were added $Pd_2(dba)_3$ (86 mg, 0.09 mmol), 2-(di-*t*-butylphosphiphino)biphenyl (56 mg, 0.19 mmol), and *t*-BuONa (180 mg, 1.88 mmol). The reaction mixture was allowed to stir at 100 °C for 3 h under nitrogen and then allowed to cool to rt. The mixture was diluted with brine and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The obtained residue was purified by silica gel column chromatography eluting with EtOAc/heptane to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-morpholino-pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**29a**) (250 mg, 0.38 mmol. 81%) as a white powder. ESI-MS *m*/*z*: 646 $[M + H]^+$.

*tert-Butyl (2R,4r,6S)-4-((3,5-Bis(trifluoromethyl)benzyl)(5-(1-methyl-1H-pyrazol-4-yl)pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**29b**).* A mixture of *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-bromopyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**28a**) (28.2 g, 44.0 mmol), 1-methylpyrazole-4-boronic acid pinacol ester (11.0 g, 53.0 mmol), and Na_2CO_3 (6.99 g, 66.0 mmol) in dimethoxyethane (340 mL) and H_2O (33 mL) was purged with N_2, then $Pd(PPh_3)_4$ (2.54 g, 2.2 mmol) was added. The mixture was stirred at 90 °C for 4 h, and then additional amounts of $Pd(PPh_3)_4$ (2.00 g, 1.7 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1*H*-pyrazole (3.68 g, 18.0 mmol), Na_2CO_3 (2.39 g, 23.0 mmol), and H_2O (11 mL) were added. The mixture was stirred at 90 °C for a further 16 h, then cooled to rt, and H_2O (400 mL) was added. The product was extracted with EtOAc (200 mL × 2), and the combined organic layers were washed with water followed by brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (gradient from 12% to 60% EtOAc in hexane) to afford *tert*-butyl (2*R*,4*r*,6*S*)-4-((3,5-bis(trifluoromethyl)benzyl)(5-(1-methyl-1*H*-pyrazol-4-yl)-pyrimidin-2-yl)amino)-2,6-diethylpiperidine-1-carboxylate (**29b**) (ca. 25 g), which was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.84 (t, 6H), 1.38−1.55 (m, 5H), 1.46 (s, 9H), 1.74−1.85 (m, 2H), 2.11−2.19 (m, 2H), 3.90 (s, 3H), 4.08−4.16 (m, 2H), 4.75−4.87 (m, 3H), 7.52 (s, 1H), 7.65 (s, 1H), 7.71 (s, 2H), 7.76 (s, 1H), 8.42 (s, 2H). ESI-MS *m*/*z*: 641.6 $[M + H]^+$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.7b00900.

Aldosterone and pressor effects of torcetrapib, anacetrapib, and compound **16**; single compound X-ray crystallography for the confirmation of relative stereochemistry of compound **16** (PDF)

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K. Yamada and M.B. prepared the manuscript. M.M., K. Yamada, O.O., K. Yasoshima, T.K., I.U., Y.I., and H.I. designed and synthesized the compounds, K.N. and G.Z. developed the human plasma CETP assay, A.I. and H.M. developed and performed hamster PD studies, W.H. developed and performed CYP assays, M.P. and D.L. developed and performed aldosterone secretion assay in H295R cells, M.P. performed plasma lipoprotein distribution studies, G.L. performed pharmacokinetic and tissue distribution studies, D.F.R. developed and supervised cannulated SD rat studies, and M.M., H.M., and M.B. directed the discovery program.

Notes

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■ ABBREVIATIONS USED

CETP, cholesteryl ester transfer protein; LDL, low density lipoprotein; HDL, high density lipoprotein; CYP, cytochrome P450; CHD, coronary heart disease; HMGCoA, 3-hydroxy-3-methylglutaryl-coenzyme A; TG, triglyceride; VLDL, very low density lipoprotein; TG, triglycerides; LipE, lipophilic efficiency; LPDS, lipoprotein deficient serum; TRL, triglyceride rich lipoproteins; SD rats, Sprague−Dawley rats; H295R cells, human adrenal corticocarcinoma cells

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