ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

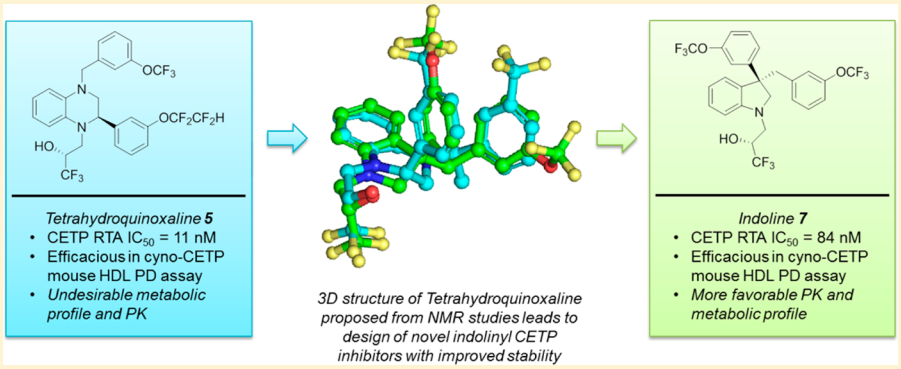
Discovery of Novel Indoline Cholesterol Ester Transfer Protein Inhibitors (CETP) through a Structure-Guided Approach

Jonathan E. Wilson,*[,†] Ravi Kurukulasuriya,[†] Mikhail Reibarkh,[‡] Maud Reiter, Aaron Zwicker, Kake Zhao,[†] Fengqi Zhang,[†] Rajan Anand,[†] Vincent J. Colandrea, Anne-Marie Cumiskey,[§] Alejandro Crespo,[‡] Ruth A. Duffy,[§] Beth Ann Murphy,[§] Kaushik Mitra,[∥] Douglas G. Johns,[⊥] Joseph L. Duffy,[†] and Petr Vachal[†]

[†]Department of Medicinal Chemistry and [‡]Department of Structural Chemistry, Merck Research Laboratories, Merck & Co, Inc., P.O. Box 2000, Rahway, New Jersey 07065, United States

[§]Department of Pharmacology, [∥]Department of Drug Metabolism and Pharmacokinetics, and [⊥]Department of Biology, Merck Research Laboratories, Merck & Co, Inc., P.O. Box 2000, Kenilworth, New Jersey 07033, United States

Supporting Information



ABSTRACT: Using the collective body of known (CETP) inhibitors as inspiration for design, a structurally novel series of tetrahydroquinoxaline CETP inhibitors were discovered. An exemplar from this series, compound **5**, displayed potent in vitro CETP inhibition and was efficacious in a transgenic cynomologus-CETP mouse HDL PD (pharmacodynamic) assay. However, an undesirable metabolic profile and chemical instability hampered further development of the series. A three-dimensional structure of tetrahydroquinoxaline inhibitor **6** was proposed from ^{1}H NMR structural studies, and this model was then used in silico for the design of a new class of compounds based upon an indoline scaffold. This work resulted in the discovery of compound **7**, which displayed potent in vitro CETP inhibition, a favorable PK–PD profile relative to tetrahydroquinoxaline **5**, and dose-dependent efficacy in the transgenic cynomologus-CETP mouse HDL PD assay.

KEYWORDS: *CETP inhibition, cholesterol ester transfer protein, HDL, indoline, tetrahydroquinoxaline*

High levels of plasma low-density lipoprotein (LDL) are clinically associated with atherosclerosis and its consequences, which can include coronary heart disease, stroke, and peripheral vascular disease.[1,2] Despite the successful development of therapies targeting cholesterol absorption, biosynthesis, and clearance, atherosclerosis remains a serious public health issue.[3] As such the development of additional, novel therapies for cardiovascular disease is needed.

In contrast to LDL, high plasma levels of high-density lipoprotein (HDL) are correlated with a cardioprotective effect.[4] This may be attributed to higher hepatic clearance of cholesterol via HDL relative to LDL through a process known as reverse cholesterol transport.[5−7] However, HDL has antioxidant and anti-inflammatory properties that may also contribute to its antiatherogenic effects.[8,9] The balance and interplay between LDL and HDL is influenced by cholesterol ester transfer protein (CETP), a plasma protein that facilitates the exchange of cholesterol esters from HDL particles to LDL particles and triglycerides from LDL to HDL. Thus, inhibition of CETP would augment cholesterol excretion by increasing the HDL to LDL ratio and could hypothetically provide an opportunity for the treatment of cardiovascular disease via increased cholesterol efflux. Because of the prevalence of health conditions caused by cardiovascular disease this hypothesis is currently being tested in the clinic by multiple organizations.[10−13]

Due to our continuing interest in new therapies for cardiovascular disease, including CETP inhibition, we initiated

Received: October 15, 2015
Accepted: January 4, 2016

DOI: 10.1021/acsmedchemlett.5b00404
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

an effort to identify structurally novel CETP inhibitors. We were inspired by the structural homology of the CETP inhibitors **1**, **2**, and **3** that had been described by Pfizer, Pharmacia, and Johnson and Johnson for two reasons.[14−16] First, these three classes of compounds had all demonstrated efficacy in vivo. Second, we sought to investigate diverse chemical matter that would be complementary to Merck's established class of oxazolidinone CETP inhibitors, exemplified by the clinical compound, **4**, anacetrapib (Figure 1).[17] From

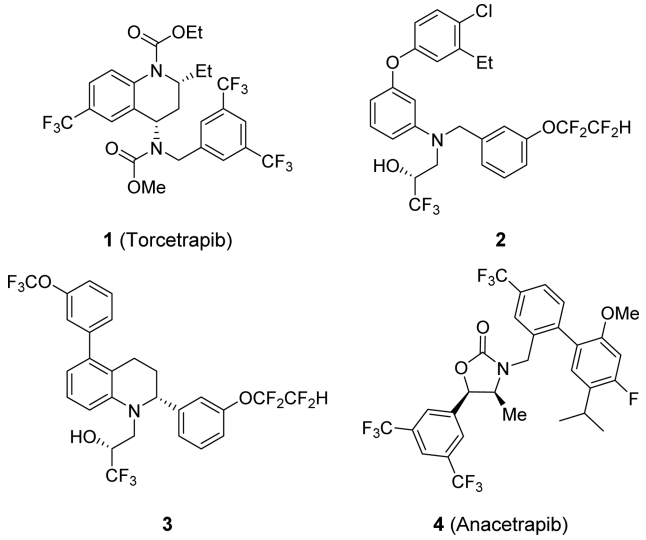


Figure 1. Structures used as inspiration for design of structurally novel CETP inhibitors.

this exercise we designed a novel series of substituted tetrahydroquinoxalines that bear elements of the four aforementioned classes of CETP inhibitors, while at the same time being distinct from each of them. This investigation led to the discovery of compound **5**, which displayed promising in vitro CETP inhibition and reasonable rat PK (Figure 2).[18] Interestingly, the optimal stereochemistry at C-2 of the tetrahydroquinoxaline is opposite to that found in tetrahydroquinoline **3**.[19]

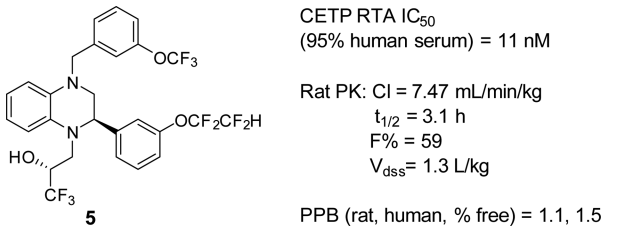


Figure 2. In vitro and PK properties of tetrahydroquinoxaline CETP inhibitor **5**.

During the course of this work we discovered that compound **5**, analogues of **5**, and tetrahydroquinoxaline intermediates en route to **5** would gradually oxidize to the monocationic and aromatic dicationic quinoxalinium species when stored under ambient conditions over a timespan of days. These degradation products could be observed by LCMS. Additionally, metabolite identification studies following incubation of **5** in human liver microsomes showed extensive oxidative metabolism leading to dealkylation and the formation of possible reactive intermediates (Figure 3).[20] Although **5** was found to be efficacious in

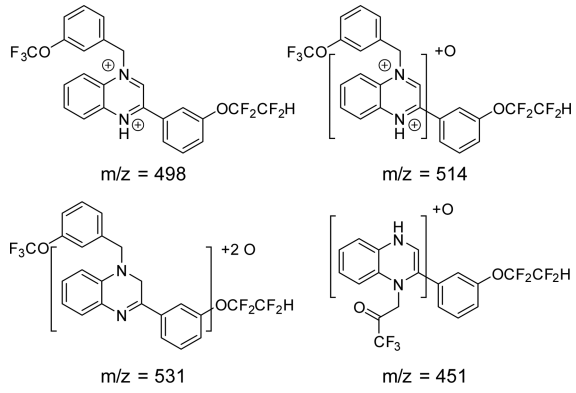


Figure 3. Proposed structures of oxidative metabolites observed after incubation of compound **5** with human liver microsomes.

vivo in a transgenic cynomologus-CETP mouse pharmacodynamic assay, it displayed highly nonlinear PK.[21,22] These observations lead us to view the pharmacodynamic effects of **5** with skepticism.

We first sought to address the oxidative liabilities of the core by stabilizing the tetrahydroquinoxaline ring system through introduction of a heteroatom or an electron withdrawing substituent in the aromatic portion of the core. Unfortunately these strategies generally led to a reduction in potency and little or no change in the metabolic or chemical stability of the compounds.[23] Due to concerns about the generation of reactive intermediates, the potential difficulties of progressing a chemically unstable compound, and our inability to stabilize the tetrahydroquinoxaline core without a substantial loss of potency we chose to re-engineer this series.

Faced with this challenge, we turned our attention to elucidation of the three-dimensional structure of compounds like **5** with the hope that structural insight would spur the design of novel scaffolds that would mimic the three-dimensional chemical shape of the tetrahydroquinoxalines but would be devoid of the metabolic and chemical instability inherent to this core. Stereochemical analysis by ^{1}H NMR revealed a dependence of the molecular conformation on the relative stereochemistry of C-2 and the 1,1,1-trifluoropropanol side chain that allowed for the assignment of the absolute stereochemistry of compound **6**. This led to the proposal of a U-shaped three-dimensional structure in which the C-2 aromatic group is in a pseudoaxial position and the *N*-benzyl group lies parallel to the C-2 group on the same face of the tetrahydroquinoxaline core (Figure 4).[24]

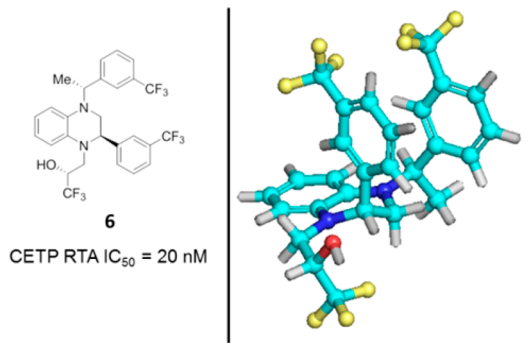


Figure 4. Proposed three-dimensional structure of **6**.

DOI: 10.1021/acsmedchemlett.5b00404
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Using the proposed solution-phase conformation of the tetrahydroquinoxalines as inspiration, we designed molecules that could adopt the U-shape that we speculated was critical for CETP inhibition and that would lack the metabolic and chemical instability of the tetrahydroquinoxaline core. Although we were well aware that the solution-phase conformation may not be representative of the active conformation of the tetrahydroquinoxaline inhibitors when bound to CETP, we nevertheless chose to pursue this strategy in the hope that we would uncover novel CETP inhibitor chemotypes. Using this strategy, we designed the 3,3-disubstituted indoline **7**, which overlaid well with the tetrahydroquinoxaline inhibitor **6** fixed in the proposed U-shaped three-dimensional structure arrived at from NMR structural studies (Figure 5).[25] Compounds of this

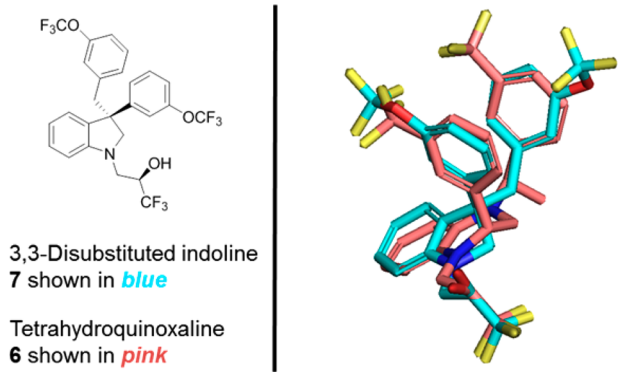


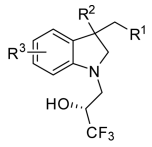
Figure 5. Overlay of tetrahydroquinoxaline **6** with 3,3-disubstituted indoline **7** (hydrogens removed for clarity).

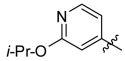
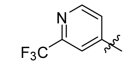
type should not inherently be prone to the oxidative chemical instability associated with the tetrahydroquinoxaline core because the aromatization process that results from oxidation of **7** is not possible on an indoline scaffold in which the 3-position is a fully substituted quaternary carbon atom.

In order to rapidly survey a broad range of substituents at the C-3 position of the indoline core, compounds were evaluated for CETP inhibition first as a mixture of diastereomers at C-3, and the results are presented in Table 1. To our delight, indoline **8** afforded significant intrinsic CETP inhibitory potency with an IC_{50} of 69 nM. Truncation of the arene at R1 or R2 completely ablated CETP activity (compounds **9** and **10**). Substitution at the 3-position of the R1 arene was preferred over unsubstituted, 2-substituted, and 4-substituted benzenes (compounds **11**, **12**, and **13**). Multiple 3-substituted benzenes and heteroarenes were explored at R1. However, none of these substituents were found to be superior to the 3-trifluoromethoxy group (compounds **14** through **19**). Attempts to transpose the 3,5-bis-trifluoromethylbenzene group of anacetrapib and torcetrapib onto the indoline core were unsuccessful (compounds **20** and **21**). The 3-trifluoromethoxybenzene was again found to be optimal at R2 despite attempts to find suitable replacements (compounds **22** through **27**). Finally, 4-, 5-, 6-, and 7-substituted indolines were explored, but the unsubstituted core was found to be superior to those that were surveyed with respect to CETP inhibition (compounds **28** through **34**).

The diastereomers and enantiomers of compound **8** were synthesized and resolved to determine the impact of stereochemistry on CETP inhibitory potency (Table 2). The C-3 diastereomers, **7** and **35**, show a substantial difference in CETP

Table 1. 3,3-Disubstituted Indoline CETP Inhibitor SAR



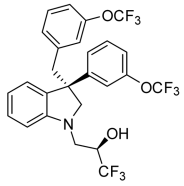
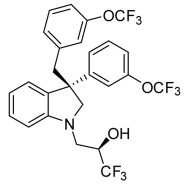
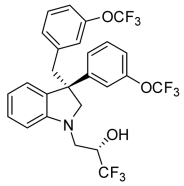
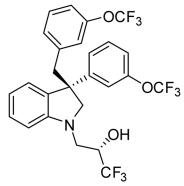
compound[a]	R^1 =	R^2 =	R^3 =	CETP RTA IC_{50} (nM)[b]
8	3-OCF_3-C_6H_4	3-OCF_3-C_6H_4	H	69
9	3-OCF_3-C_6H_4	CH_3	H	>10,000
10	H	3-OCF_3-C_6H_4	H	>10,000
11	C_6H_4	3-OCF_3-C_6H_4	H	746
12	2-OCF_3-C_6H_4	3-OCF_3-C_6H_4	H	3,374
13	4-OMe-C_6H_4	3-OCF_3-C_6H_4	H	9,683
14	3-CN-C_6H_4	3-OCF_3-C_6H_4	H	917
15	3-CO_2Me-C_6H_4	3-OCF_3-C_6H_4	H	937
16	3-CO_2H-C_6H_4	3-OCF_3-C_6H_4	H	>10,000
17	3-SO_2Me-C_6H_4	3-OCF_3-C_6H_4	H	8,958
18	3-Br-C_6H_4	3-OCF_3-C_6H_4	H	308
19		3-OCF_3-C_6H_4	7-F	1996
20	3,5-bis-CF_3-C_6H_4	3-OCF_3-C_6H_4	H	>10,000
21	3-OCF_3-C_6H_4	3,5-bis-CF_3-C_6H_4	H	>10,000
22	3-OCF_3-C_6H_4	3-CF_3-C_6H_4	H	1,218
23	3-OCF_3-C_6H_4	3-OCF_2CF_2H-C_6H_4	H	94
24	3-OCF_3-C_6H_4	3-Cl	H	1,375
25	3-OCF_3-C_6H_4	4-OCF_3-C_6H_4	H	232
26	3-$OCF_3C_6H_4$		H	101
27	3-$OCF_3C_6H_4$		H	2,602
28	3-OCF_3-C_6H_4	3-OCF_3-C_6H_4	4-CH_3	>10,000
29	3-OCF_3-C_6H_4	3-OCF_3-C_6H_4	5-F	763
30	C_6H_5	3-OCF_3-C_6H_4	5-OCF_3	>10,000
31	3-OCF_3-C_6H_4	C_6H_5	5-OCF_3	>10,000
32	3-OCF_3-C_6H_4	3-OCF_3-C_6H_4	6-CF_3	>10,000
33	3-OCF_3-C_6H_4	3-OCF_3-C_6H_4	7-Me	491
34	3-OCF_3-C_6H_4	3-OCF_3-C_6H_4	7-F	222

[a]The compounds are a 1:1 mixture of diastereomers (C-2 stereocenter is racemic). [b]This in vitro assay measures the ability of the compounds to inhibit the CETP mediated transfer of [3*H*]-cholesterol or [3*H*]-triolein from LDL to HDL. The assay was performed using endogenous CETP in 95% human serum (HS), $n = 1$. Data for the same in vitro assay run in 2% human serum are available in the Supporting Information.

inhibition as would be expected from consideration of overlays with compound **6**. The remaining two diastereomers, **36** and **37**, were also considerably less potent. From this data it appears that the quaternary stereocenter is the primary driver for potency (**7** and **36** versus **35** and **37**), while the alcohol stereochemistry has a less of an impact on potency (**7** versus **36**). In the CETP RTA in vitro assay in 95% human serum compound **7** is superior to compounds **2** and **3** (**2**, IC_{50} = 197 nM; **3**, IC_{50} = 295 nM) and comparable with the clincical

DOI: 10.1021/acsmedchemlett.5b00404
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Table 2. Effect of Stereochemistry on CETP Potency

compound / CETP RTA IC_{50} value[a]	
 **7** / 84 nM	 **35** / 3,827 nM
 **36** / 325 nM	 **37** / >10,000 nM

[a]This in vitro assay measures the ability of the compounds to inhibit the CETP mediated transfer of [3*H*]-cholesterol oleate or [3*H*]-triolein from LDL to HDL. The assay was performed using endogenous CETP in 95% human serum (n = 1). Data for the same assay run in 2% human serum are available in the Supporting Information.

CETP inhibitors **1** and **4** (**1**, IC_{50} = 45 nM; **4**, IC_{50} = 53 nM).[15,16]

The CETP inhibitory action of the indoline series was further confirmed by the ability of **7** to increase HDL-cholesterol in an exposure-dependent manner when orally administered to cynomologus-CETP transgenic mice (Figure 6).[21,26] This

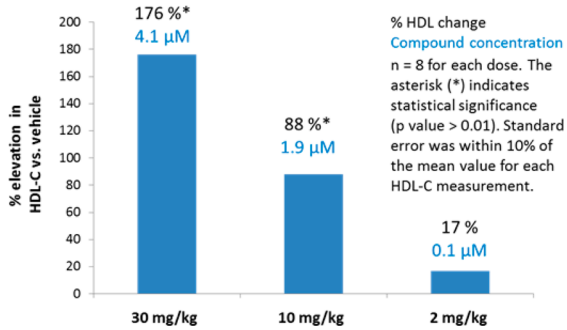


Figure 6. Elevation of HDL-C 4 h postdose in cynomologus-CETP transgenic mice orally administered compound **7**.

exposure-dependent PD effect of **7**, which displays good pharmacokinetic properties in a rat (dose 0.15 mg/kg: Cl = 36.0 mL/min/kg; $t_{1/2}$ = 4.76 h; V_{dss} = 6.70 L/kg) is in stark contrast to **5**, which exhibited an unusual exposure−PD relationship. This difference is most likely due to the increased chemical and metabolic stability of **7** relative to **5**. Unfortunately, the in vivo efficacy of **7** can not be directly compared with compounds **1**−**4** because HDL measurements from the cyno-CETP mouse PD assay were recorded at different time points for the respective sets of compounds.[27] However, the PK profile, promising in vivo efficacy, and structural novelty of the indoline series render them an attractive starting point for the development of future CETP inhibitors.

In summary, a novel class of CETP inhibitors based on an indoline scaffold has been discovered.[28,29] The indoline series was designed from in silico overlays with tetrahydroquinoxaline CETP inhibitor **6**, of which a three-dimensional structure was proposed from structural information derived from ^{1}H NMR studies. Indoline **7** is a potent inhibitor of CETP in vitro, displays improved metabolic and chemical stability relative to its progenitor, tetrahydroquinoxaline **5**, and shows dose-dependent efficacy in the cynomologus-CETP mouse HDL pharmacodynamic assay. Furthermore, the three-dimensional structures of **6** and **7** proposed herein may be a useful template from which to design additional novel classes of CETP inhibitors.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.5b00404.

Experimental procedures and characterization for compounds **5** through **37**, a description of the in vitro assay, and data for compounds **5**−**37** in the CETP RTA assay run in 2% human serum (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jonathan_wilson@merck.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Christopher Sinz, Dr. Christopher Plummer, and Eric Meade for reviewing the article and providing comments.

■ ABBREVIATIONS

CETP, cholesterol ester transfer protein; HDL, high density lipoprotein; PPB, plasma protein binding; HS, human serum; LDL, low density lipoprotein; PK, pharmacokinetic; PD, pharmacodynamic

■ REFERENCES

(1) Kearney, P. M.; Blackwell, L.; Collins, R.; Keech, A.; Simes, J.; Peto, R.; Armitage, J.; Baigent, C. Efficacy of cholesterol-lowering therapy in 18686 people with diabetes in 14 randomised trials of statins: A meta-analysis. *Lancet* **2008**, *371*, 117−125.

(2) Baigent, C.; Keech, A.; Kearney, P. M.; Blackwell, L.; Buck, G.; Pollicino, C.; Kirby, A.; Sourjina, T.; Peto, R.; Collins, R.; Simes, J. Efficacy and safety of cholesterol-lowering treatment: Prospective metaanalysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* **2005**, *366*, 1267−1278.

(3) World Health Organization Cardiovascular Diseases—Fact Sheet No. 317. http://www.who.int/en/ (January 2015).

(4) Singh, I. M.; Shishehbor, M. H.; Ansell, B. J. High density lipoprotein as a therapeutic target. *J. Am. Med. Assoc.* **2007**, *298*, 786.

(5) Bruce, C.; Chouinard, R. A., Jr.; Tall, A. R. Plasma lipid transfer proteins, high-density lipoproteins, and reverse cholesterol transport. *Annu. Rev. Nutr.* **1998**, *18*, 297−330.

(6) Pieters, M. N.; Schouten, D.; Berkel, T. J. C. Van. In Vitro and In Vivo Evidence for the Role of HDL in Reverse Cholesterol Transport. *Biochim. Biophys. Acta, Mol. Basis Dis.* **1994**, *1225*, 125−134.

(7) The association between HDL and reverse cholesterol transport with cardiovascular outcomes is disputed. For a discussion of this, see:

DOI: 10.1021/acsmedchemlett.5b00404
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

Levinson, S. S.; Wagner, S. G. Implications of reverse cholesterol transport: Recent studies. *Clin. Chim. Acta* **2015**, *15*, 154−161.
(8) Barter, P. J.; Nicholls, S.; Rye, K.-A.; Anantharamaiah, G. M.; Navab, M.; Fogelman, A. M. Antiinflammatory properties of HDL. *Circ. Res.* **2004**, *95*, 764−772.
(9) Salman Bandeali, S.; Farmer, J. High-density lipoprotein and atherosclerosis: The role of antioxidant activity. *Curr. Atheroscler. Rep.* **2012**, *14*, 101−107.
(10) Rader, D. J.; deGoma, E. M. Future of cholesteryl ester transfer protein inhibitors. *Annu. Rev. Med.* **2014**, *65*, 385−403.
(11) Mantlo, N. B.; Escribano, A. Update on the discovery and development of cholesteryl ester transfer protein inhibitors for reducing residual cardiovascular risk. *J. Med. Chem.* **2014**, *57*, 1−17.
(12) Sikorski, J. A. Oral cholesteryl ester transfer protein (CETP) inhibitors: A potential new approach for treating coronary artery disease. *J. Med. Chem.* **2006**, *49*, 1.
(13) Johns, D. G.; Duffy, J. L.; Fisher, T.; Hubbard, B. K.; Forrest, M. J. On- and off-Target pharmacology of torcetrapib: Current understanding and implications for the structure activity relationships (SAR), discovery and development of cholesteryl ester-transfer protein (CETP) inhibitors. *Drugs* **2012**, *72*, 491−507.
(14) DeNinno, M. P.; Magnus-Aryitey, G. T.; Ruggeri, R. B.; Wester, R. T. 4-Carboxy-amino-2-substituted-1,2,3,4-tetrahydro-quinolines as CETP inhibitors. Patent Application WO2000/17164, March 30, 2000.
(15) Reinhard, E. J.; Wang, J. L.; Durley, R. C.; Fobian, Y. M.; Grapperhaus, M. L.; Hickory, B. S.; Massa, M. A.; Norton, M. B.; Promo, M. A.; Tollefson, M. B.; Vernier, W. F.; Connolly, D. T.; Witherbee, B. J.; Melton, M. A.; Regina, K. J.; Smith, M. E.; Sikorski, J. A. Discovery of a simple picomolar inhibitor of cholesteryl ester transfer protein. *J. Med. Chem.* **2003**, *46*, 2152−2168.
(16) Kuo, G.-H.; Rano, T.; Pelton, P.; Demarest, K. T.; Gibbs, A. C.; Murray, W. V.; Damiano, B. P.; Connelly, M. A. Design, synthesis, and biological evaluation of (2R,αS)-3,4-Dihydro-2-[3-(1,1,2,2-tetrafluoroethoxy)phenyl]-5-[3-(trifluoromethoxy)-phenyl]-α-(trifluoromethyl)-1(2H)-quinoline-ethanol as a potent and orally active cholesteryl ester transfer protein inhibitor. *J. Med. Chem.* **2009**, *52*, 1768−1772.
(17) Smith, C. J.; Ali, A.; Hammond, M. L.; Li, H.; Lu, Z.; Napolitano, J.; Taylor, G. E.; Thompson, C. F.; Anderson, M. S.; Chen, Y.; Eveland, S. S.; Guo, Q.; Hyland, S. A.; Milot, D. P.; Sparrow, C. P.; Wright, S. D.; Cumiskey, A.-M.; Latham, M.; Peterson, L. B.; Rosa, R.; Pivnichny, J. V.; Tong, X.; Xu, S. S.; Sinclair, P. J. Biphenyl-substituted oxazolidinones as cholesteryl ester transfer protein inhibitors: Modifications of the oxazolidinone ring leading to the discovery of anacetrapib. *J. Med. Chem.* **2011**, *54*, 4880−4895.
(18) Eveland, S. S.; Milot, D. P.; Guo, Q.; Chen, Y.; Hyland, S. A.; Peterson, L. B.; Jezequel-Sur, S.; O'Donnell, G. T.; Zuck, P. D.; Ferrer, M.; Strulovici, B.; Wagner, J. A.; Tanaka, W. K.; Hilliard, D. A.; Laterza, O.; Wright, S. D.; Sparrow, C. P.; Anderson, M. S. A high-precision fluorogenic cholesteryl ester transfer protein assay compatible with animal serum and 3456-well assay technology. *Anal. Biochem.* **2007**, *368*, 239−249.
(19) The remaining three diastereomers were prepared and the CETP RTA IC_{50} values for those compounds are as follows (alcohol stereochemistry is listed first followed by the C-2 stereochemistry): (*R*,*S*)-isomer = 3273 nM; (*S*,*S*)-isomer = 716 nM; (*S*,*R*)-isomer = 60 nM.
(20) Compound **5** (10 μmol) was incubated with C57 mouse liver microsomes (1 mg/mL protein) for 60 min at 37 °C. Structure assignments are tentative, based on LC−MS/MSE data. The elemental compositions of the metabolites were confirmed by high-resolution mass analysis (QTOF).
(21) Compounds or noncompound-containing vehicle were orally administered to transgenic mice that overexpress the cynomolgus cholesteryl ester transfer protein. HDL-cholesterol was measured from plasma collected at 4 h postdose using a commercially available kit (Wako Diagnostics). The effect of the test compound on HDL-cholesterol is expressed as the percent elevation compared to that of the vehicle group. See ref 17 and the following reference for further details of the pharmacodynamic assay. Marotti, K. R.; Castle, C. K.; Murray, R. W.; Rehberg, E. F.; Polites, H. G.; Melchior, G. W. The role of cholesteryl ester transfer protein in primate apolipoprotein A-I metabolism. Insights from studies with transgenic mice. *Arterioscler., Thromb., Vasc. Biol.* **1992**, *12*, 736−44.
(22) Compound **5** did not show the expected dose−exposure relationship when dosed orally at 1, 3, and 10 mg/kg in the transgenic cynomolgus-mouse PD assay. The exposures were 1.3, 1.8, and 1.9 μM, respectively.
(23) Both isomers of the 1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazine core were prepared in addition to one isomer of the 1,2,3,4-tetrahydropyrido[3,4-*b*]pyrazine core. Only one isomer of 1,2,3,4-tetrahydropyrido[2,3-*b*]pyrazine showed CETP activity, but none of the compounds showed improved chemical or metabolic stability. Fluoro- and trifluoromethyl-quinoxalines were also explored but were not found to have improved stability.
(24) The details of the NMR studies can be found in the Supporting Information.
(25) Ensemble conformations for both compounds **6** and **7** were generated using omega (openeye scientific software); Hawkins, P. C. D.; Skillman, A. G.; Warren, G. L.; Ellingson, B. A.; Stahl, M. T. *J. Chem. Inf. Model.* **2010**, *50*, 572−584. and overlaid using rocs (openeye scientific software); Hawkins, P. C. D.; Skillman, A. G.; Nicholls, A. *J. Med. Chem.* **2007**, *50*, 74. The best overlaid pair was then refined with the flexible alignment module of the MOE package (Molecular Operating Environment, 2014.09; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015). Figures 4 and 5 were rendered using PyMOL (The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC)..
(26) The data in Figure 6 for the 2 mg/kg dose and the 10 and 30 mg/kg doses were collected on different days. A positive control, structurally related to anacetrapib (CETP RTA IC50 in 95% human serum = 43 nM) was run in both instances. The positive control increased HDL by 67% on the day the 2 mg/kg dose was administered. On the day of the 10 and 30 mg/kg dosing, the positive control increased HDL by 191%.
(27) HDL measurements in the cyno-CETP mouse PD assay conducted at Merck for compound **7** were measured at 4 h postdose, while measurements for compounds **2** and **3** were taken at 18 h postdose (compound **2**, 30 mpk PO dose, −20% change in HDL 18 h postdose relative to vehicle; compound **3**, 30 mpk PO dose, +4.2% change in HDL 18 h postdose relative to vehicle).
(28) Wilson, J. E.; Vachal, P.; Kurukulasuriya, R. 3,3′-Disubstituted Indolines as Inhibitors of Cholesterol Ester Transfer Protein. PCT Int. Appl., WO2015054088, April 16, 2015.
(29) Anand, R.; Colandrea, V. J.; Reiter, M.; Vachal, P.; Zwicker, A.; Wilson, J. E.; Zhang, F.; Zhao, K. Benzopiperazine Derivatives As CETP Inhibitors. PCT Int. Appl. WO2013028382 A1, December 4, 2014.

DOI: 10.1021/acsmedchemlett.5b00404
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX