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Design and synthesis of new tetrahydroquinolines derivatives as CETP inhibitors

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ABSTRACT

This letter describes the discovery and SAR optimization of tetrazoyl tetrahydroquinoline derivatives as potent CETP inhibitors. Compound **6m** exhibited robust HDL-c increase in hCETP/hApoA1 double transgenic model and favorable pharmacokinetic properties.

Plasma cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein that is secreted mainly from the liver and that circulates in plasma, bound mainly to HDL.[1] It facilitates the transfer of cholesteryl esters from the atheroprotective high density lipoprotein (HDL) to the proatherogenic low density lipoprotein cholesterol (LDL) and very low density lipoprotein cholesterol (VLDL), leading to lower levels of HDL but raising the levels of proatherogenic LDL and VLDL. Epidemiologic data supports an inverse relation between HDL-c and coronary heart disease.[2] Therefore, there has been a great interest in developing CETP inhibitors[3] to increase HDL-c plasma levels. Many studies in animals support the hypothesis that inhibition of CETP activity is beneficial. For example, the inhibition of CETP can reduce the progression of atherosclerosis in rabbits.[4] In humans, subjects with heterozygous CETP deficiency and an HDL cholesterol level >60 mg/ml have a reduced risk of coronary heart disease.[5] Also clinical trials have confirmed that pharmacological inhibition of CETP produces an increment of serum HDL levels in humans.[6] However, the efficacy of CETP inhibitors on reducing cardiovascular events remains uncertain. The phase III trials in patients treated with torcetrapib (**1**) were discontinued, following an excess of deaths and cardiac adverse effects.[6a,7] Most likely, these undesirable effects arise from some compound-specific and off-target effects, including increases in blood pressure, sodium, bicarbonate and aldosterone levels, as well as a decrease in potassium levels.[8] These untoward effects have not been observed with anacetrapib (**2**), dalcetrapib (**3**) or evacetrapib (**4**).[9] (Fig. 1)

Our research program is based on identifying orally active CETP inhibitors. Along with other groups,[10] we focused our attention on exploring the tetrahydroquinoline scaffold. This communication describes structure–activity relationship studies of tetrazoyl derivatives **6**. Subsequent optimization was undertaken, leading to the discovery of several analogues that significantly raised HDL-c in a double transgenic mouse model expressing human CETP and apolipoprotein AI (apo AI) assay.

We began by investigating the best replacement of the methylcarbamate at the nitrogen at C-4 in compound **1**. As with our previous approach toward the 1,5-tetrahydronaphthyridine series,[11] we explored different heterocycles. The synthesis of tetrazoyl **6**, pyrazole **7** and isoxazole **8** derivatives is outlined in Scheme 1.

Commercially available 4-(trifluoromethyl)aniline was converted to (+/−)-**13** by reaction with the appropriate aldehyde followed by treatment with vinyl acetamide in the presence of *p*-toluenesulfonic acid. Subsequent reaction of (+/−)-**13** with the required chloroformate followed by acetamide hydrolysis, gave rise to 4-amino derivatives (+/−)-**14**. The enantioselective synthesis of (2*R*,4*S*)-**14** was performed by coupling (*R*)-3-aminopentanenitrile with 1-chloro-4-(trifluoromethyl)benzene, nitrile followed by hydrolysis, then treatment with benzyl chloroformate in the presence of lithium *tert*-butoxide. Subsequent reduction of the imide (2*R*,4*S*)-**15** and final diastereoselective cyclization,[12] provided the tetrahydroquinoline (2*R*,4*S*)-**16**. Acylation of the tetrahydroquinoline nitrogen with isopropyl chloroformate and benzyl carbamate cleavage afforded (2*R*,4*S*)-**14**. Intermediates **14** were

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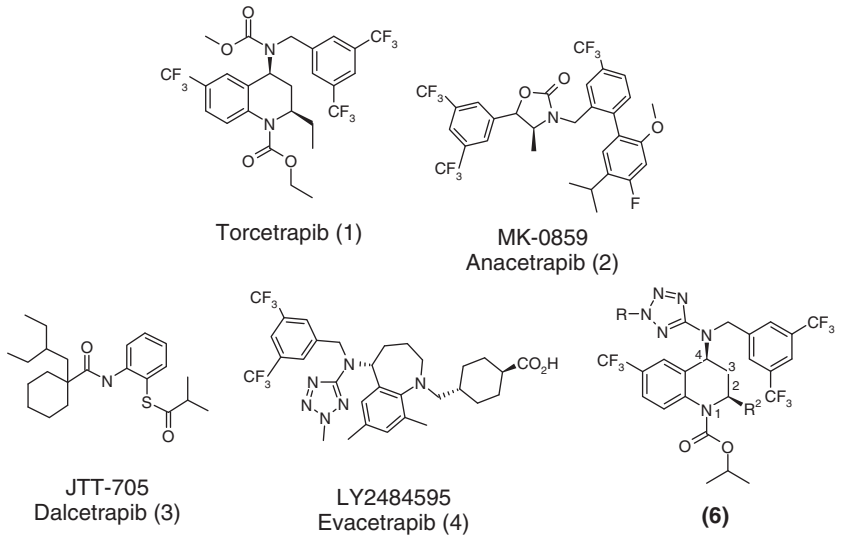
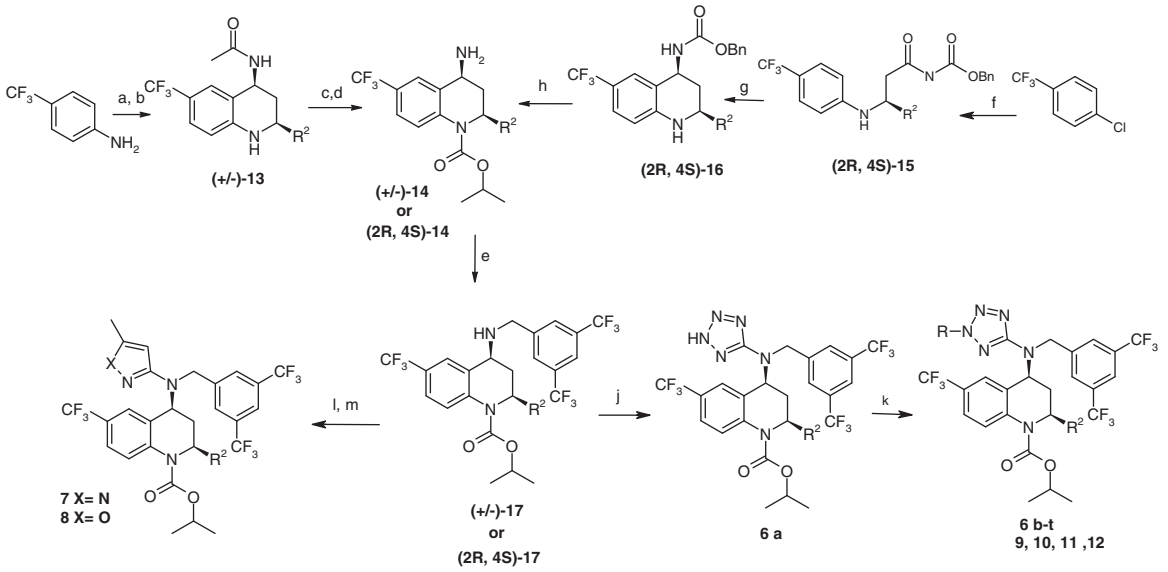


Figure 1. CETP inhibitors.



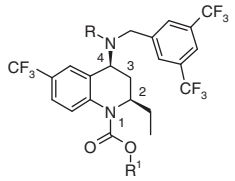
Scheme 1. Reagents and conditions: (a) benzotriazole, R_2CHO, toluene; (b) vinyl acetamide, $pTsOH{\cdot}H_2O$, toluene, RT −70 °C; (c) R_1OCOCl, Py, 0 °C-rt; (d) 5 N HCl, 100 °C; (e) (3,5-bis-CF_3)PhCHO, $NaBH(OAc)_3$, AcOH, DCE, rt; (f) (i) (*R*)-3-aminopentanenitrile methanesulfonic acid salt, CH_2Cl_2, $CsCO_3$, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, phenylboronic acid, palladium acetate, toluene, 80 °C, (ii) conc H_2SO_4, toluene, 35 °C, (iii) benzyl chloroformate, 1 M lithium *t*-butoxide in THF, Et_2O, −10–0 °C; (g) sodium borohydride, EtOH, $MgC1_2$, H_2O; (h) (i) iPrOCOCl, Py, 0 °C-rt, (ii) H_2, 10% Pd/C, 1 atm, EtOAc; (j) (i) CNBr, THF, iPr_2NEt, rt, (ii) NaN_3, Et_3N, toluene, 110 °C; (k) R-OH, PPh_3, or X-R, K_2CO_3, DMF, acetone, rt or X-R , Et_3N, acetonitrile, rt; (l) Diketene, THF, DMAP, rt; (m) X=N, NH_2NH_2, EtOH, P_2O_5, 90 °C or X=O, NH_2OH, NaOAc, MeOH.

derivatized to the N-benzyl amino derivatives **17** by reductive amination with bis(trifluoromethyl)benzaldehyde. Reaction of (2*R*,4*S*)-**17** with diketene and subsequent cyclization with hydrazine or hydroxylamine gave pyrazole **7** and isoxazole **8**, respectively. Treatment of **17** with cyanogen bromide in the presence of potassium *tert*-butoxide, followed by condensation with sodium azide in basic media produced the tetrazole **6a**. Deprotonation of **6a** with potassium carbonate followed by reaction with the corresponding alkyl halide, allowed the preparation of the alkyltetrazoles **6h–k** and **6o–q**. Alternatively, treatment of **6a** with the appropriate alcohol under Mitsunobu conditions gave rise to the tetrahydroquinolines **6b–g, 6l–m, 6r–t, 9, 10, 11** and **12**.

We next studied the replacement of the carbamate moiety at the exocyclic nitrogen of the tetrahydroquinoline core (Table 1). We first observed that the *iso*-propyl carbamate at N-1 improved the potency of the acetyl derivatives (**5a** vs **5b**). We decided then to continue the optimization with the *iso*-propyl carbamate at that position. Improvement of the CETP inhibition was achieved by replacing the acetyl (**5a–b**) or carbamate (**1**) moieties on the nitrogen at C-4 with 2-methyltetrazole (**6b**), while the unsubstituted tetrazole was detrimental for activity (**6a**). Other five membered heterocycles such as 3-methyl pyrazole (**7**) or 3-methylisoxazole (**8**) were also tolerated.

Since the 2-methyl tetrazole markedly increased the potency, we decided to explore the nature of the substitution at the tetrazole moiety (Table 2). We first found that longer and branched alkyl chains (**6c–h**) led to less potent compounds. Then, in order to reduce the lipophilicity and improve solubility of the scaffold, we introduced different polar groups onto the alkyl chain substitution on the tetrazole ring. We observed that cyano (**6i**, **6j**), hydroxy (**6l**, **6r**) or amino (**6m**, **6s**) groups were well tolerated, independent of the chain length. Introduction of a carboxylic acid (**6n**) onto the

Table 1
SAR study at exocyclic nitrogen

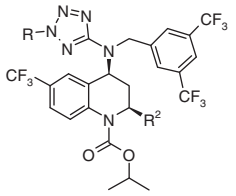


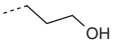
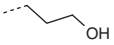
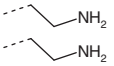
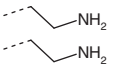
Compound	R	R_1	CETP IC_{50}[a] (nM)
1		Et	39
5a		Et	195[b]
5b		*i*Pr	57[b]
6a		*i*Pr	2310
6b		*i*Pr	13
7		*i*Pr	26
8		*i*Pr	24

[a] Human plasma CETP[13] IC_{50}.
[b] Racemic.

alkyl chain was detrimental for activity, however potency was recovered with the corresponding methyl ester (**6o**) and the primary amide (**6p**). Dimethyl substituted amide (**6q**) or amine (**6t**) reduced potency when compared with the unsubstituted analogues. We also attempted to determine the effect of substitution at position C-2 of the tetrahydroquinoline core. The results are summarized in Table 3, and showed that methyl (**9b**, **9r**, **9m**), propyl (**10b**), *iso*-propyl (**11b**, **11r**, **11m**) and cyclopropyl (**12b**, **12m**) substituents are well tolerated, with the appropriate substitution on the tetrazole ring.

Table 3
Influence of substitution at C-2 in combination with tetrazole substitution



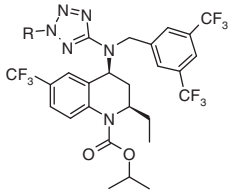
Compound[b]	R	R_2	CETP IC_{50}[a] (nM)
9b	Me	Me	48
10b	Me	Propyl	55
11b	Me	*iso*-Propyl	47
12b	Me		42
9r		Me	36
11r		*iso*-Propyl	37
9m		Me	47
11m		*iso*-Propyl	40
12m			54

[a] Human CETP plasma IC_{50}.
[b] All racemic.

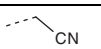
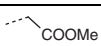
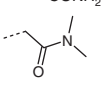
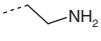
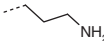
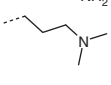
Finally we investigated alkylamino moieties as tetrazole ring substituents (Table 4). Constrained analogues such as piperidine **9w** were tolerated, but azetidine **9y** had reduced potency. Amine substitution also, gave a decrease in activity (**9u**, **9v**, **9x**).

Based on these in vitro SAR studies, we selected compounds **6j**, **6l**, **6m** and **6r** to test in our double transgenic mouse assay. Thus the animals were given a 30 mpk oral dose and blood was taken

Table 2
SAR study at tetrazole substitution

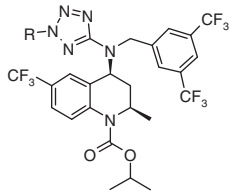


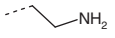
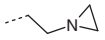
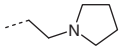
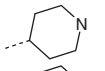
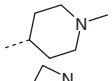
6a-t

Compound	R	CETP IC_{50}[a] (nM)	Compound	R	CETP IC_{50}[a] (nM)	Compound	R	CETP IC_{50}[a] (nM)
6b	Me	13	**6i**		23	**6o**		44
6c	Propyl	74	**6j**		15	**6p**		49
6d	Butyl	187	**6k**		58	**6q**		2350
6e	*iso*-Propyl	56	**6l**		16	**6r**		15
6f	*tert*-Butyl	>50000	**6m**[b]		29	**6s**[b]		56
6g	*iso*-Butyl	20000	**6n**[c]		3790	**6t**		162
6h		61						

[a] Human plasma CETP activity.
[b] Obtained from the corresponding isoindoline 1,3-dione by treatment with hydrazine.
[c] Obtained from the corresponding ester **6o** via saponification.

Table 4
SAR on the alkylamino substituent



Compound[b]	R	CETP IC_{50}[a] (nM)
9m		47
9u		151
9v		218
9w		78
9x		10100
9y		327

[a] Human CETP plasma IC_{50}.
[b] All racemic.

Table 5
Evaluation at 30 mg/Kg oral dose in hCETP/hAPO-A1 tg mice[a]

Compound	%CETP inhibition[b]			%HDL-c increase[c]	
	4 h	8 h	24 h	8 h	24 h
6j	102	105	80	226	97
6l	101	108	92	153	224
6m	100	107	80	194	109
6r	107	110	87	150	171

[a] Formulation: corn oil (79.5%), oleic acid (20%), labrafil (0.5%) Species: male CETP and ApoA1 heterozygote mice used for this experiment. Mice were orally dosed with reference CETP compound and blood was taken from the mice at different time point. Serum was aspirated off and tested for CETP activity.
[b] Ex vivo CETP inhibition.
[c] In vivo serum HDLc.

Table 6
Pharmacokinetic profile of **6m**

Compound	Species	CL (mL/min/Kg)	Vdss (L/Kg)	$t_{1/2}$ (h)	%F
6m	Rat	8	3	6.1	7
6m	Dog	2.5	2.5	6	15

Formulation: intravenous formulation: 20% solutol microemulsion/80% deionized H_2O.
Oral formulation: corn oil/oleic acid/labrafil 79.5:20:0.5.
Species: male sprague dawley cannulated rats.
Female beagle dogs dose: 1 mg/kg and 3 mg/kg.
Time course 0–72 h.

at different time points as shown in Table 5. All compounds showed demonstrated ex vivo CETP inhibition and a robust HDL-c elevation at 8 and 24 h.

Compound **6m** was selected for rat and dog pharmacokinetic studies (Table 6), due to its relatively higher in vitro kinetic solubility among the analogues tested in simulated intestinal fluid (data available in Supplementary data). **6m** exhibited an acceptable pharmacokinetic profile in dogs and rats, with a t1/2 of 6 h, relatively low clearance and Vdss and moderate bioavailability.

In summary we have identified a series of substituted tetrazoyl tetrahydroquinolines as potent CETP inhibitors that have demonstrated the ability to significantly raise HDL-c in the hCETP/hApoA1 dual heterozygous mouse model, with favorable pharmacokinetic profiles.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.042.

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