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Chromanol derivatives—A novel class of CETP inhibitors

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ABSTRACT

Based on our former development candidate BAY 38-1315, optimization efforts led to the discovery of a novel chemical class of orally active cholesteryl ester transfer protein (CETP) inhibitors. The chromanol derivative **19b** is a highly potent CETP inhibitor with favorable pharmacokinetic properties suitable for clinical studies. Chemical process optimization furnished a robust synthesis for a kilogram-scale process.

Despite the wide use of HMG-CoA reductase inhibitors (statins) that lower low-density lipoprotein cholesterol (LDL-C), cardiovascular disease is still the most common cause of morbidity and mortality in developed countries.[1] Guidelines for the prevention of cardiovascular disease still focus on the management of LDL-C,[2,3] although a large portion of cardiovascular events cannot be prevented by LDL-C lowering.[4]

In addition, low levels of high-density lipoprotein cholesterol (HDL-C) are an independent and important risk factor for cardiovascular disease and HDL-C has been proposed to have potential atheroprotective effects.[5] Epidemiological studies suggest that the risk for coronary heart disease is 2–3% lower for each 0.1 mg/l increase in HDL-C.[6] Inhibition of cholesterol ester transfer protein (CETP) still represents a promising mechanism for HDL-C elevation, especially since currently available HDL-C-elevating drugs based on different mechanisms have limited efficacy and undesirable side effects.[7,8] CETP inhibitors have achieved remarkable elevations of HDL-C and marked decreases in LDL-C, thus clinical trials are ongoing to validate the therapeutic potential of CETP inhibition for the treatment of cardiovascular disease.[9]

More recently, phase III clinical studies of the CETP inhibitor torcetrapib (**1**) were terminated because of an increase in cardiovascular events and mortality.[10] Most likely, torcetrapib has some compound-specific and off-target effects, such as raising blood pressure and aldosterone, which could have led to an increase in cardiovascular events and mortality.[11] Nevertheless, clinical evaluation of other CETP inhibitors, such as anacetrapib, MK-0859 (**2**) and dalcetrapib, JTT-705 (**3**), which do not have the off-target effects of torcetrapib, for the treatment of cardiovascular disease is still ongoing[12,13] (see Fig. 1).

Our research program focused on identifying orally active CETP inhibitors suitable for clinical development. Tetrahydronaphtalene derivative BAY 38-1315 (**4**)[14] and tetrahydrochinoline compound BAY 60-5521 (**5**)[15] were found to be highly active CETP inhibitors and were selected as development candidates (see Fig. 2). Unfortunately, BAY 38-1315 later revealed unfavorable pharmacokinetic properties thus leading to termination of preclinical development while BAY 60-5521 entered clinical phase I.

In order to find a backup candidate for compound **4** we sought derivatives with lowered lipophilicity and reduced synthetic complexity. Replacing the tetrahydronaphtalene moiety by a chromanol system seemed to be a good starting point to fulfill both criteria.

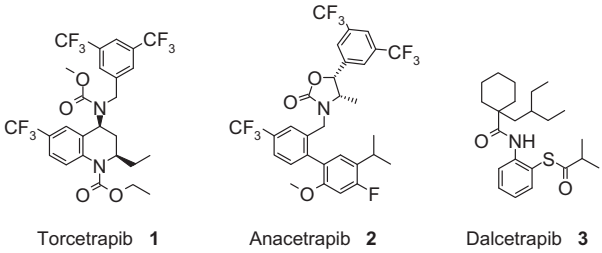

Figure 1. Former and current CETP inhibitors in clinical development.

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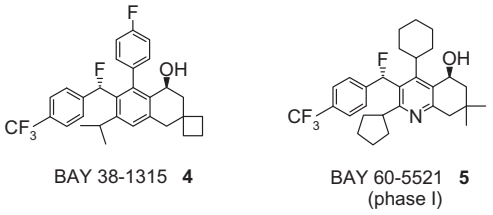
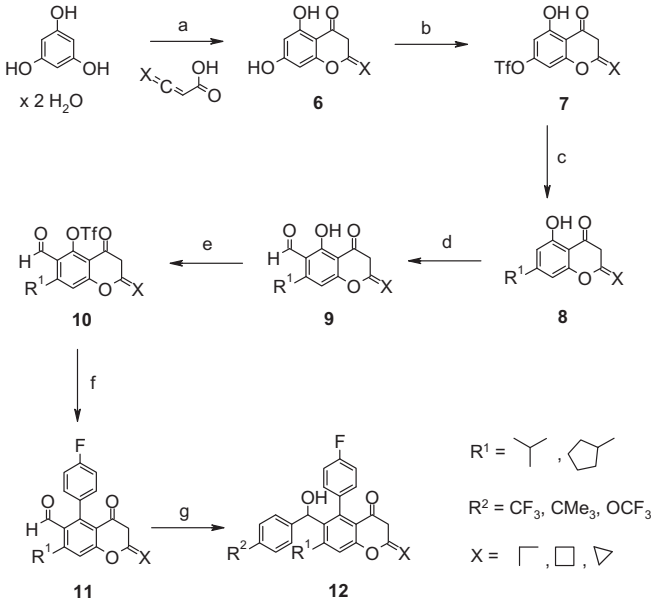


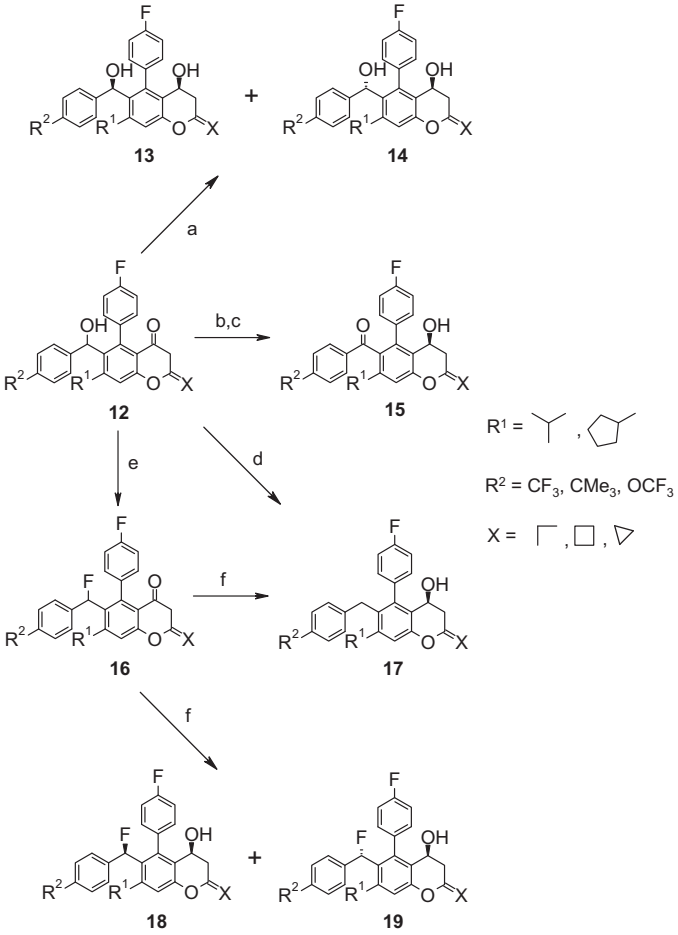
Figure 2. Tetrahydronaphtalene and tetrahydrochinoline derived CETP inhibitors.

For the synthesis of the complex pentasubstituted benzene ring we chose phloroglucinol dihydrate as starting material to establish a synthetic route to the highly substituted chromanols (Scheme 1). Chromanones **6** were formed by Michael addition/Friedel–Crafts acylation with the corresponding cycloalkylideneacetic acids in a single step. The *para*-phenolic group reacted selectively in the presence of the second *ortho*-phenolic group to form trifluoromethanesulfonates **7**, which turned out to be suitable compounds for palladium-catalyzed reactions using zinc reagents to introduce branched alkyl residues. Selective *ortho*-formylation of the remaining phenolic group was achieved by using dichloromethyl methyl ether in the presence of the Lewis acid $TiCl_4$, to give functionalized pentasubstituted chromanones **9** in only four steps from an inexpensive starting material. Activation of the phenols as triflates **10** followed by Suzuki cross-coupling reactions provided the aldehydes **11**, which were then subjected to Grignard reactions at low temperatures to obtain alcohols **12** as racemic mixtures.

The intermediate chromanones turned out to be highly versatile compounds for further derivatisation and SAR exploration (Scheme 2). Thus, hydroxy ketones **12** undergo a CBS-type reduction, using (1*R*,2*S*)-1-aminoindan-2-ol as a source of chirality,[16] with high diastereoselectivity (>85% de) to provide the *syn*-diols **13** and *anti*-diols **14** (both *S*-configuration in the chromanol moiety). The hydroxy ketones with interchanged functionalities were prepared by oxidation with Dess–Martin periodinane to afford the diketones, which subsequently were reduced in the described usual manner with complete regioselectivity and moderate to high enantioselectivities (>70% ee), to yield hydroxy ketones **15**. The removal of the hydroxyl group in the bis-benzylic position in **12** was achieved in two steps by two different methods, which were employed depending on the electronic properties of the substituents R^2. For an electron-withdrawing group like trifluoromethyl group (R^2), lithium aluminum hydride was required for the reduction of the bis-benzylic chloride prepared from the hydroxyketone **12** with thionylchloride. On the other hand, electron-donating groups for R^2 (*t*-Bu, OCF_3) enabled a reduction of a bis-benzylic fluoride by a milder reagent, such as the 1-aminoindan-2-ol-borane complex. Both pathways led to the formation of alcohols **17** (>80% ee, *S*-configuration) which were assembled in two/three steps starting from the key intermediates **12**. However, in accordance with our previously observed SAR[15] the fluoro alcohols **18** and **19** were of further interest, which were easily prepared from the racemic ketones **16** by the described chiral reduction



Scheme 1. Synthesis of alcohols **12**. Reagents and conditions: (a) 1.2 equiv acid, 4 equiv BF_3–Et_2O, 70 °C, 3 h, 43–82%; (b) 1.05 equiv $PhNTf_2$, 1.1 equiv K_2CO_3, DMF, −20 °C, 2–5 h, 60–80%; (c) 2 equiv Zn(*i*-Pr)$_2$ or 4.5 equiv ZnBr(*c*-pentyl), 0.1–0.2 equiv $PdCl_2$(dppf)–CH_2Cl_2, 3–6 equiv LiCl, DMF, 0 °C to rt, 4 h, 78–96%; (d) 1.1 equiv dichloromethyl methyl ether, 2.5 equiv $TiCl_4$, CH_2Cl_2, −70 °C to 0 °C, 2.5–4 h, 56–96%; (e) 1.1 equiv $PhNTf_2$, 1.1 equiv K_2CO_3, DMF, −20 °C to rt, 2–4 h, 76–93%; (f) 1.3 equiv 4-fluorophenyl-boronic acid, 0.11 equiv $Pd(PPh_3)_4$, 1.7 equiv K_3PO_4, dioxane, 100 °C, 18 h, 62–86%; (g) 1.2–1.5 equiv R^2-Ph–MgBr, THF, −78 °C to 0 °C, 0.5–1.5 h, 42–67%.



Scheme 2. Synthesis of alcohols **13**–**15**; **17**–**19**. Reagents and conditions: (a) 0.10–0.15 equiv (1*R*,2*S*)-1-aminoindan-2-ol, 4 equiv *N*,*N*-diethylaniline–borane (1:1), THF, 0 °C or rt, 10–30 min, then **12**, rt, 6–18 h, 60–93% of **13** and **14**; (b) 1.5 equiv Dess–Martin periodinane, 1 equiv pyridine, −30 °C to 0 °C, 1–6 h, 78–99%; (c) 0.10–0.20 equiv (1*R*,2*S*)-1-aminoindan-2-ol, 4 equiv *N*,*N*-diethylaniline–borane (1:1), THF, 0 °C or rt, 10–30 min, then ketone, rt, 4–18 h, 54–83% of **15**; (d) for $R^2 = CF_3$: (i) 1.1 equiv $SOCl_2$, 1.6 equiv Et_3N, THF, rt, 1.5 h; (ii) 0.10 equiv (1*R*,2*S*)-1-aminoindan-2-ol, 4 equiv *N*,*N*-diethylaniline–borane (1:1), THF, rt, 30 min, then ketone, rt, 18 h; (iii) 15 equiv LAH, rt, 5 h, 16%; (e) 1.5 equiv DAST, toluene, −78 °C to −20 °C, 2 h, 61–83%; (f) 0.10–0.15 equiv (1*R*,2*S*)-1-aminoindan-2-ol, 4 equiv *N*,*N*-diethylaniline–borane (1:1), THF, 0 °C or rt, 10–30 min, then ketone, rt, 3–18 h, 22–38% of **17** (for $R^2 = OCF_3$, CMe_3) and 26–80% of **18** and **19** (for $R^2 = OCF_3$, CF_3).

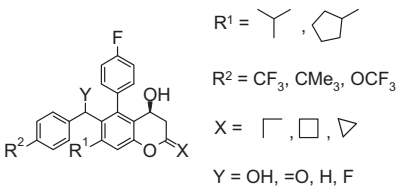
protocol to give both the *syn*- and *anti*-products **18** and **19** in moderate to high diastereoselectivities (>77% de).

SAR trends comparing the derivatives **13–15** and **17–19** are summarized in Table 1. CETP inhibition was determined using a CETP fluorescence assay.[17a] A rather steep SAR was observed for the *syn*-diols **13** and *anti*-diols **14**. While the *syn*-stereochemistry of the alcohol moieties is not favorable (compound **13a**), the chosen substituents at R^1 and R^2 seemed to be well tolerated in this assay. In particular, the combination of $R^1 = CF_3$, $R^2 = i$Pr and X = sprirocyclopropyl (compound **14d**) revealed a reduction of lipophilicity indicated by a decreased *c* log *P* value by three units comparing to compounds **4** and **5**. In addition, the replacement of the hydroxyl group in the bis-benzylic position by a ketone or a methylene group in the alcohols **15** and **17** showed similar activity. The spirocyclobutyl substitution adds additional potency compared to the *gem*-dimethyl pattern (IC_{50} = 19–59 nM). Finally, the bis-benzylic position could be substituted by a fluorine atom in **18** and **19** with a similar trend to the diols. The *syn*-stereochemistry of the fluorine and hydroxyl groups in **18a–c** resulted in compounds that were less potent when compared to the *anti*-stereochemistry of compounds **19a–c**, while the lipophilicity was slightly reduced on comparison with **4** and **5**.

Additional in vitro assays were performed to characterize all subseries (see Table 2). While CETP inhibition of the selected compounds in the scintillation proximity assay were comparable, the fluoro substituted compounds **19a–c** could be distinguished in the human plasma assay showing the best overall profile for compound **19b**.

The pharmacokinetic profile of **19b** was investigated in rats and dogs and exhibited good half-lives ($t_{1/2}$ = 3.0 and 6.6 h, respectively) and oral bioavailability (*F* = 79% in rats and 43% in dogs).

Table 1
IC_{50} data from CETP fluorescence assay for compounds **13–15**, **17–19**



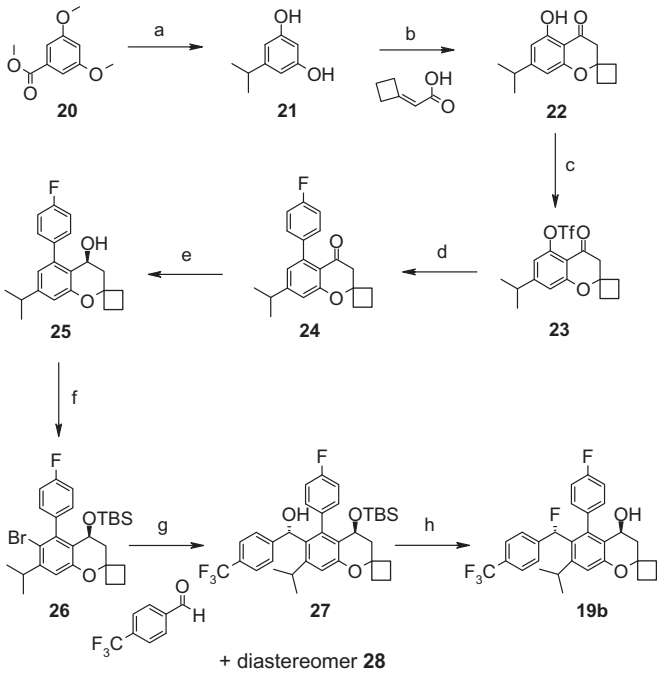
Compds	R^1=	R^2=	X=	Y=	IC_{50}[a] (nM)	*c* log *P*
1					18	7.55
4					24	8.63
5					25	8.77
13a	*t*Bu	*i*Pr	Spirocyclobutyl	OH, *syn*	800	7.11
14a	*t*Bu	*i*Pr	Spirocyclobutyl	OH, *anti*	14	7.11
14b	CF_3	*i*Pr	Spirocyclobutyl	OH, *anti*	77	6.17
14c	CF_3	cPent	Spirocyclobutyl	OH, *anti*	26	6.80
14d	CF_3	*i*Pr	Spirocyclopropyl	OH, *anti*	27	5.61
15a	*t*Bu	*i*Pr	Dimethyl	=O	176	8.64
15b	*t*Bu	*i*Pr	Spirocyclobutyl	=O	59	8.48
15c	OCF_3	cPent	Spirocyclobutyl	=O	42	8.40
15d	OCF_3	*i*Pr	Spirocyclobutyl	=O	36	7.77
15e	CF_3	*i*Pr	Spirocyclobutyl	=O	35	7.63
17a	*t*Bu	cPent	Spirocyclobutyl	H	30	9.50
17b	*t*Bu	*i*Pr	Spirocyclobutyl	H	28	8.87
17c	CF_3	*i*Pr	Dimethyl	H	90	8.09
17d	CF_3	*i*Pr	Spirocyclobutyl	H	19	7.93
18a	CF_3	*i*Pr	Dimethyl	F, *syn*	373	7.72
19a	CF_3	*i*Pr	Dimethyl	F, *anti*	32	7.72
18b	CF_3	*i*Pr	Spirocyclobutyl	F, *syn*	53	7.55
19b	CF_3	*i*Pr	Spirocyclobutyl	F, *anti*	18	7.55
18c	CF_3	*i*Pr	Spirocyclopropyl	F, *syn*	300	6.99
19c	CF_3	*i*Pr	Spirocyclopropyl	F, *anti*	14	6.99

[a] Chiral chromatography was employed to purify all compounds prior to biological testing (>95% ee or de).

Table 2
IC_{50} data from CETP SPA assay and from CETP fluorescence assay in human plasma for selected compounds

Compds	SPA assay[17c] IC_{50} (nM)	Human plasma assay[17b] IC_{50} (nM)
1	3	20
5	7	50
14c	22	100
14d	4	449
15b	16	1000
17d	4	437
19a	20	216
19b	12	41
19c	6	74

In order to provide a reliable large scale synthesis for the preparation of **19b**, avoiding the use of a sensitive zinc reagent, we switched to an alternative synthetic route starting from methyl ester **20**, which was transformed to an isopropyl group in a two step procedure (Scheme 3). Reaction with methylmagnesium bromide, hydrogenation of the tertiary alcohol under acidic conditions and cleavage of the methoxy groups gave the diphenol **21** in excellent yields. The chromanone **22** was formed by the aforementioned Michael addition/Friedel–Crafts acylation with cyclobutylidenacetic acid; however it became clear that the sequence to introduce the side chain had to be modified in order to ensure high reaction yields. The 4-fluorophenyl head group was installed and the subsequent reduction using (1*R*,2*S*)-1-aminoindan-2-ol gave excellent yields of **25** with a very high enantioselectivity (95% ee). Alcohol



Scheme 3. Synthesis of alcohol **19b**. Reagents and conditions: (a) (i) 2.4 equiv MeMgBr, THF, rt, 3 h, 93%; (ii) H_2, 1 bar, Pd/C (10%), 1.35 eqiuv H_3PO_4, rt, 3 d, 92%; (iii) HBr (50%), AcOH, rt, 2 h, 87%; (b) 1.2 equiv cyclobutylideneacetic acid, 0.1 equiv CF_3SO_3H, *o*-xylene, 140 °C, $-H_2O$, 2 h, 64%; (c) 1.1 equiv $(Tf)_2O$, 1.25 equiv *i*-Pr_2EtN, CH_2Cl_2, 0 °C, 3.5 h, 86%; (d) 1.8 equiv (4-fluorophenyl)boronic acid, 0.04 equiv $Pd(PPh_3)_4$, 1.7 equiv K_3PO_4, dioxane, 100 °C, 18 h, 78%; (e) 0.2 equiv (1*R*,2*S*)-1-aminoindan-2-ol, 2 equiv *N*,*N*-diethylaniline–borane (1:1), THF, rt, 15 min, then ketone, rt, 2.5 h, 97%; (f) (i) 1.05 equiv *N*-bromosuccinimide, CH_2Cl_2, 10 °C to rt, 1.5 h, 94%; (ii) 3.5 equiv TBSCl, 1.75 equiv DMAP, 3.5 equiv Et_3N, DMF, 110 °C, 24 h, 87%; (g) 1.25 equiv *n*-BuLi, THF, −78 °C, 1 h, then 1.25 equiv 4-(trifluoromethyl)-benzaldehyde, THF, −78 °C to rt, 18 h, 36% of **27** and 35% of diastereomer **28**; (h) (i) 2 equiv DAST, toluene, −78 °C to 10 °C, 5 h, 88%; (ii) 4 equiv TBAF, THF, rt, 18 h, 85%.

25 could be regioselectively brominated in the *para*-position of the ether function with *N*-bromosuccinimide and treatment with *t*-butyldimethylsilyl-chloride in a basic mixture of DMAP and triethylamine at high temperature furnished key intermediate **26**. Metal-halogen exchange at low temperature followed by quenching with 4-(trifluoromethyl)benzaldehyde gave a mixture of the corresponding alcohols which were easily separated by chromatography to provide the desired protected *anti*-diol **27** in 36% isolated yield. Diastereoselective fluorination with diethylaminosulfur trifluoride (DAST) in toluene at low temperature proceeded with complete retention of the configuration at this sterically encumbered site. In contrast to the usual S_N2 pathway, the observed S_Ni mechanism has also been reported in the literature.[18] Final deprotection with TBAF provided the desired target compound **19b** in high overall yield.

In summary, the structures of our previous development compounds **4** and **5** were successfully modified to afford chromanol derivatives with reduced lipophilicity. Compound **19b** displays good overall in vitro properties, a favorable pharmacokinetic profile and a remarkable in vivo profile in human CETP-transgenic mice by increasing HDL-cholesterol dose dependently,[19] and was therefore selected as a candidate for further development. The optimized synthesis of **19b** comprises 12 robust chemical steps applicable for a kilogram-scale process.

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17. (a) To assess CETP activity a microemulsion based assay according to Bisgaier et al., (*J. Lipid R.*, **1993**, *34*, 1625) was used with the following modification: (1) Donor liposomes were prepared applying 1 mg cholesteryl 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-dodecanoate (cholesteryl BODIPY® FL C_{12}, from Molecular Probes), 5.35 mg triolein (Sigma–Aldrich), and 6.67 mg POPC (Sigma–Aldrich), respectively, dissolved in a total volume of 600 μl dioxane and were slowly injected into 63 ml buffer (50 mM Tris/HCl pH 7.3, 150 mM NaCl, 2 mM EDTA) in a water bath sonicator. This suspension is then sonicated with 50 W (Branson Sonifier 450 with a cup-horn resonator) for 30 minutes under a nitrogen atmosphere at room temperature. (2) Acceptor liposomes were prepared in the same manner as donor liposomes using 86 mg cholesteryl-oleate, 20 mg triolein, and 100 mg POPC dissolved in 1.2 ml dioxane and injected into 114 ml buffer. (3) In a total test volume of 100 μl test compounds dissolved in DMSO (2 μl) were incubated at 37 °C for 4 h with 50 μl of a CETP containing sample (1–3 μg CETP, enriched from human plasma) and 48 μl of a liposome emulsion (one volume donor, one volume buffer, and two volumes acceptor, respectively). The increase of the fluorescence intensity (excitation 485 nm, emission 535 nm) is proportional to the cholesterol ester transfer. The inhibition of the transfer is followed in comparison with a DMSO control. (b) To assess CETP activity in the presence of human plasma 6 μl (12% v/v) of donor liposomes and 1 μl (2% v/v) of a solution of the substance to be tested in DMSO are added to 42 μl (86% v/v) of human plasma (Sigma P 9523). The mixture is incubated at 37 °C for 24 h. The change in the fluorescence at 510/520 nm is a measure of the cholesterol ester transfer. The inhibition of the transfer is followed in comparison with a DMSO control. (c) To assess CETP activity a scintillation proximity assay the transfer of ^{3}H-cholesterol ester from human HD lipoproteins to biotinylated LD lipoproteins is measured. In the test batch, 10 μl of HDL-^{3}H-cholesterol ester (~50,000 cpm) are incubated at 37 °C for 18 h with 10 μl of biotin-LDL (Amersham) in 50 nM Hepes/0.15 M NaCl/0.1% bovine serum albumin/0.05% NaN_3 pH 7.4 containing 10 μl of CETP (1 mg/ml) and 3 μl of a solution of the substance to be tested (dissolved in 10% DMSO/1% BSA). Two hundred microliters of the SPA-streptavidin bead solution (TRKQ 7005) are then added, incubated further with shaking for 1 h and then measured in a scintillation counter. Corresponding incubations with 10 μl of buffer, 10 μl of CETP serve as controls.
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