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Dibenzodioxocinones—A new class of CETP inhibitors

David Brückner, Frank-Thorsten Hafner, Volkhart Li, Carsten Schmeck, Joachim Telser,* Alexandros Vakalopoulos and Gabriele Wirtz

Bayer Healthcare Pharma Research, Aprather Weg, 42096 Wuppertal, Germany

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Abstract—Derivatives of the natural product 11-hydroxy-3-[(*S*)-1-hydroxy-3-methylbutyl]-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[*b*,*g*][1,5]dioxocin-5-one **1** were studied as novel CETP inhibitors. Compound **2** was identified from HTS as a micromolar inhibitor. The compound suffered from very low stability in plasma. Optimisation by partial synthesis started from **1** and led to low-nanomolar inhibitors with good stability in rat plasma.

Coronary heart disease (CHD) is the leading cause of mortality and morbidity in the industrialised world.[1] Lowering of low-density lipoprotein cholesterol (LDL-C) is nowadays the primary focus of lipid-modifying therapy for the prevention of CHD. Nevertheless, a large portion of cardiovascular events cannot be prevented by LDL-C lowering.[2]

Several epidemiological studies clearly show that a low level of high-density lipoprotein cholesterol (HDL-C) is a strong and independent risk factor for the development of CHD.[3] Therefore, raising of HDL-C levels is expected to be fundamental for future treatment of the disease.[4]

Inhibition of cholesterol ester transfer protein (CETP) provides a promising mechanism of action for HDL enhancing drugs[5] and a number of inhibitors have entered preclinical[6] and clinical[7] studies in the last years (Fig. 1).

We set out for novel, orally bioavailable CETP inhibitors using a high-throughput screening approach identifying compound **2** as a lead. Compound **2** is a derivative of the natural product penicillide[8] (11-hydroxy-3-[(1*S*)-1-hydroxy-3-methylbutyl]-4-methoxy-9-methyl-5*H*,7*H*-dibenzo[*b*,*g*][1,5]dioxocin-5-one, compound **1**, Fig. 2). For some penicillide derivatives, ACAT inhibitory activity,[9] oxytocin antagonism,[10] and antihypertensive potential[11] had been described.

Compound **2** showed an IC_{50} of 1 μM in a CETP-fluorescence assay[12] but suffered from poor in vitro pharmacokinetic properties. Incubation in rat plasma[13] showed

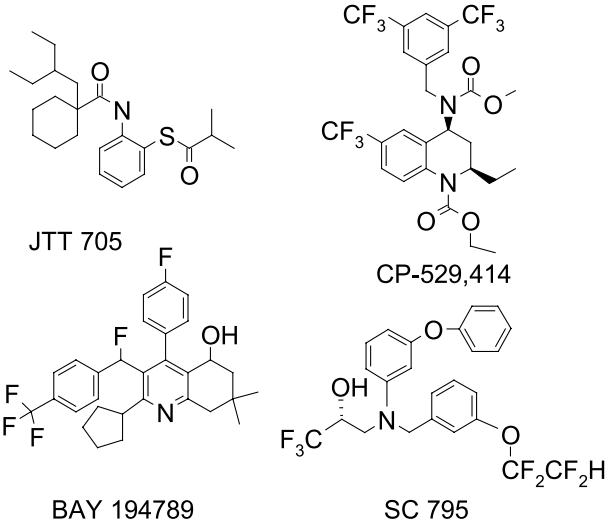

Figure 1. Experimental CETP inhibitors.

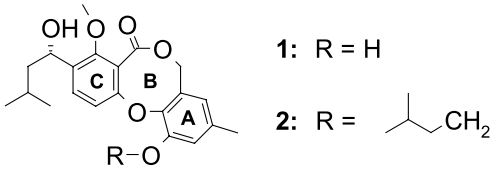

Figure 2. Dibenzodioxocinones.

Keywords: CETP; Dibenzodioxocinones.
* Corresponding author. Tel.: +49 202365518; fax: +49 2025461; e-mail: joachim.telser@bayerhealthcare.com

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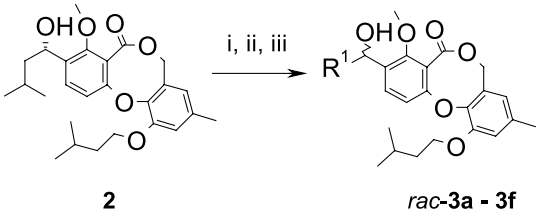
that **2** rapidly decomposed with a $t_{1/2} = 0.1$ h. Most probably enzymatic opening of the lactone ring occurred. Therefore, our optimisation programme focused on an increase in target activity as well as an increase in plasma stability.

The alkyl chain at the C-ring seemed to be a good starting point to establish a structure–activity relationship (SAR) by partial synthesis.

Elimination of the secondary alcohol of **2** followed by oxidative cleavage of the resulting double bond provided an aldehyde that was then reacted with Grignard reagents to obtain derivatives **3a**–**3e** as racemic mixtures (Scheme 1). Racemic **2**, synthesised by the same methodology as **3a**–**3e**, showed only half of the activity of enantiomerically pure **2**. After separation of the isomers and by comparison with natural product-derived **2**, it became evident that only the (*S*)-enantiomer is active.

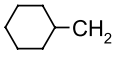
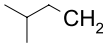
The SAR of the alkyl side chain was rather steep (Table 1). Chain shortening (compound **3d**) led to a 2-fold loss in activity and chain elongation by just one methylene group gave the almost inactive compound **3c**. A more dramatic loss was observed when the branched alkyl chain was replaced by cyclopropyl as in compound **3a**. Compound **3e** bearing the neopentyl group as R^1 was the only compound in this series that showed a slight improvement of activity.

The 11-hydroxy group at the A-ring of penicillide **1** turned out to be a very versatile site for derivatisation.[14]



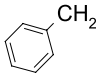
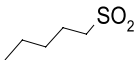
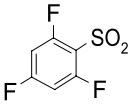
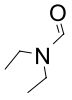
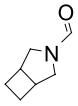
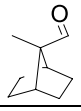
Scheme 1. Synthesis of alkyl side-chain substituted derivatives (**3a**–**3e** and *rac*-**2**). Reagents and conditions: (i) 0.1 equiv *p*-TsOH, toluene, reflux, 2 h, 65%; (ii) 0.1 equiv OsO_4, 5 equiv $NaIO_4$, 1,4-dioxane–water 1.6 + 1, 90 min, 85%; (iii) R^1–MgBr, THF, −78 °C—rt, 3 h, 16–40%.

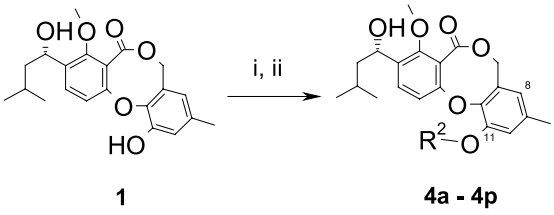
Table 1. SAR of the alkyl side chain

Compound	R^1=	IC_{50} (μM)
rac-**3a**		10
rac-**3b**		>20
rac-**3c**		>20
rac-**3d**	$(CH_3)_2CH$	4
rac-**2**		2
rac-**3e**		1.5

This phenolic hydroxy group reacted selectively in the presence of the unprotected secondary alcohol. Treatment of **1** with sodium hydride in THF followed by quenching of the phenolate with electrophiles such as alkyl halides, sulfonyl chlorides, carbamoyl chlorides or acyl chlorides gave sulfonic or carboxylic esters, carbamates or aliphatic ethers (compounds **4a**–**4p**, Table 2)

Table 2. Derivatives with different 11-*O* substituents

Compound	R^2=	IC_{50} (μM)	$t_{1/2}$ (rat plasma) (h)
1	H	>20	
4a		13.0	
4b		1.5	
4c	CF_3SO_2	10.0	
4d		4.0	
4e		6.0	
4f		0.7	
4g		3.0	
4h		0.8	2.9
4i		0.7	>5
4j		0.4	
4k		0.2	3.9
4l		0.8	<0.1
4m		0.6	
4n		0.2	<0.1
4o		0.2	0.64
4p		0.2	4.2



Scheme 2. Synthesis of alkyl side-chain substituted derivatives (**4a**–**4p**). Reagents and conditions: (i) 1.05 equiv NaH, THF, 0 °C, 10 min, then R^2–X; **4a**, **4b**: 2–4 equiv R^2–Br, 60 °C, 2 h, 17–80%; **4c**–**4p**: 1.05–1.5 equiv of R^2–Cl, rt, 2 h, 26–86%.

depending on the electrophile (Scheme 2). Some very close analogues of **2** featured different alkyl ethers in the 11-*O* position. Soon it became clear that benzylic ethers such as **4a** gave poor IC_{50} values, whereas better activity was observed when branched alkyl ethers as in compound **4b** were introduced.

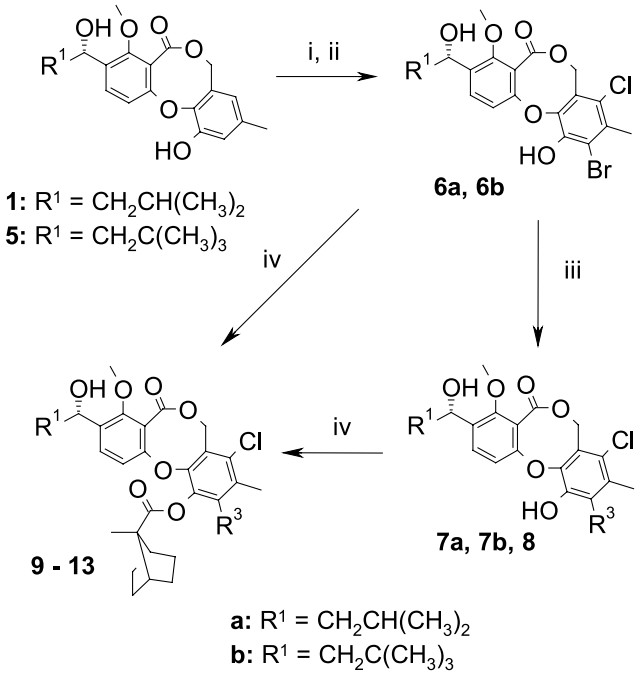
Generally, compounds in the ether series did not give a substantial gain in activity over **2**. Therefore, we tried sulfonic acid esters. The SAR obtained indicates that chain length is optimal at about three to five carbon atoms with branched substituents yielding the most potent compounds in this series (compounds **4c**, **4d** and **4f**). The aryl moiety in **4e** did not lead to satisfying activity.

The carbamate series (compounds **4g**–**4k**) compared favourably with the ether and sulfonic ester series. Within the carbamate series, aliphatic rings were clearly superior to linear alkyl residues. For example, the difference in activity between **4g** and **4h** which both possess four carbon atoms adjacent to the nitrogen is more than 3-fold. The somewhat more bulky substituent of **4i** gave a comparable increase in potency. The apparent superiority of rigid-branched residue structures led us to the conclusion that bicyclic structures might give even better results. Indeed, with compounds **4j** and **4k**, IC_{50} values well below 1 μM were obtained. Steric bulk in this part of the molecule showed an additional benefit, namely a significant increase in plasma stability. With these data in hand, a couple of aliphatic carboxylic acid esters with sterically demanding hydrocarbon moieties were synthesised. Their SAR paralleled that of the carbamates: compounds **4m** and **4h** featuring the same five-membered ring are almost equipotent, the bicyclic derivative **4n** is equipotent to **4k**. On the other hand, plasma half-lives of the ester series were inferior to those observed with the carbamates. Even highly bulky or bicyclic compounds such as **4l** or **4n** underwent almost instantaneous enzymatic cleavage in rat plasma. A first step towards longer half-life could be taken by using an α-branched bicyclic substituent (compound **4o**). Finally, the asymmetric bicyclic acid could be substituted by a symmetric one leading to **4p** with an IC_{50} of 0.2 μM and a plasma half-life of 4.2 h.

Additional substituents at the A-ring were then explored (Table 3). The synthesis of compounds bearing chlorine in the 8-position and an additional substituent—bromine or alkyl—in the 10-position is outlined in Scheme 3. Compounds **1** and **5**[15] could be regioselectively halo-

Table 3. A-ring substituted derivatives

Compound	R^1	R^3	IC_{50} (μM)	$t_{1/2}$ (rat plasma) (h)
9	CH_2	Br	0.060	≫3
10	CH_2	CH_3	0.030	≫3
11	CH_2	Br	0.032	
12	CH_2	CH_3	0.030	
13	CH_2	CH_2CH_3	0.015	



Scheme 3. Synthesis of A-ring optimised derivatives (**9**–**13**). Reagents and conditions: (i) 1 equiv NCS, 0.97 equiv $FeCl_3$, ethanol–water 1 + 1, 84%; (ii) 1.1–2 equiv NBS, ethanol, 67–98%; (iii) for **7a**, **7b**: 5.6 equiv Me_4Sn, 0.05 equiv $Pd(PPh_3)_4$, DMF, microwave, 120 °C, 1 h, 88%; for **8** (R3 = CH_2CH_3): 3 equiv Et_2Zn, 0.3 equiv CuI, 0.1 equiv $Pd(dppf)_2Cl_2$, 1,4-dioxane, 90 °C, 16 h, 42%; (iv) 1.2 equiv NaH, THF, 0 °C, then 1.2 equiv 7-methylbicyclo[2.2.1]heptane-7-carbonyl chloride, rt, 1–4 h, 72–97%.

genated in the 8-position with *N*-chloro succinimide and iron (III) chloride in aqueous ethanol.[11b] Subsequent bromination gave **6a** and **6b**, respectively. These could be acylated giving compounds **9** and **11**. Alternatively, palladium-catalysed cross-coupling with tetramethyl stannane led to **7a** and **7b** (R^3 = methyl). Another palladium-catalysed method using diethyl zinc gave rise to compound **8** (R^3 = ethyl).[14]

These modifications of the A-ring substitution pattern caused a substantial increase of the in vitro activity. Furthermore, plasma-induced cleavage was below the detection limit as could be shown for **9** and **10**. Highest activity was achieved when the optimal R^1 was combined with ethyl as R^3 (see Table 3).

With compound **13** in hand, we synthesised a highly potent CETP inhibitor that shows sufficient stability in plasma to be a promising candidate for in vivo studies.

Acknowledgments

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12. To assess CETP activity, a microemulsion-based assay according to Bisgaier et al., *J. Lipid Res.* **1993**, *34*, 1625–1634, was used with the following modifications: (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 and 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 min 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 (1 volume donor, 1 volume buffer and 2 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. As a reference, the IC_{50} of compound CP-529,414 (see Fig. 1) was 18.5 nM in this assay.
13. Method for the determination of $t_{1/2}$ in rat plasma: samples were incubated in rat plasma at 37 °C in a water bath at a starting concentration of 10,000 ng/mL. Aliquots of 100 μL are taken, diluted with a 10-fold excess of acetonitrile and analysed by RP-HPLC with UV detection at 230 nm.
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15. Compound (*S*)-**5** was synthesised using the methodology outlined in Scheme 1 starting from the free phenol **1** followed by chiral HPLC: Daicel Chiralpak AD, isopropanol–methanol 8 + 2 with 0.2% diethylamine, detection at 220 nm; ee = 99.3%.