Synthesis and Structure−Activity Studies of Novel Orally Active Non-Terpenoic 2,3-Oxidosqualene Cyclase Inhibitors

Henrietta Dehmlow, Johannes D. Aebi,* Synèse Jolidon, Yu-Hua Ji,† Elisabeth M. von der Mark, Jacques Himber, and Olivier H. Morand‡

Pharmaceuticals Division, Preclinical Research, F. Hoffmann-La Roche Ltd., Grenzacherstrasse 124, CH-4070 Basel, Switzerland

Received December 17, 2002

New orally active non-terpenoic inhibitors of human 2,3-oxidosqualene cyclase (hOSC) are reported. The starting point for the optimization process was a set of compounds derived from a fungicide project, which in addition to showing high affinity for OSC from *Candida albicans* showed also high affinity for human OSC. Common structural elements of these inhibitors are an amine residue and an electrophilic carbonyl C atom embedded in a benzophenone system, which are at a distance of about 10.7 Å. Considering that the keto moiety is in a potentially labile position, modifications of the substitution pattern at the benzophenone as well as annelated heteroaryl systems were explored. Our approach combined testing of the compounds first for increased binding affinity and for increased stability in vitro. Most promising compounds were then evaluated for their efficacy in lowering plasma total cholesterol (TC) and plasma low-density lipoprotein cholesterol (LDL-C) in hyperlipidemic hamsters. In this respect, the most promising compounds are the benzophenone derivative **1**·fumarate and the benzo[*d*]-isothiazol **24**·fumarate, which lowered TC by 40% and 33%, respectively.

Introduction

Causal risk factors that directly promote the development of coronary and peripheral atherosclerosis include elevated plasma low-density lipoprotein cholesterol (LDL-C), low plasma high-density lipoprotein cholesterol (HDL-C), hypertension, cigarette smoking, and diabetes mellitus. Other synergistic risk factors include elevated concentrations of plasma triglyceride (TG)-rich lipoproteins, small, dense, low-density lipoprotein particles, lipoprotein (a) and homocysteine. Predisposing risk factors such as obesity, physical inactivity, family history of premature cardiovascular disease (CVD), and male gender modify the causal or conditional risk factors and thus affect atherogenesis indirectly. The strong correlation between coronary heart disease (CHD) and high LDL-C levels in plasma, and the clinical benefit associated with the lowering of LDL-C levels are now well established.[1−3] Cholesterol-rich, sometimes unstable, atherosclerotic plaques lead to the occlusion of blood vessels, resulting in ischemia or infarct.

Primary prevention studies have shown that lowering of plasma LDL-C levels reduces the incidence of nonfatal CHD, while the overall morbidity remains unchanged.[4,5] The lowering of plasma LDL-C levels in patients with preestablished CHD (secondary prevention) reduces CHD mortality and morbidity; meta analysis of different studies shows that this decrease is proportional to the reduction of LDL-C.[6]

The clinical benefit of cholesterol lowering is greater for patients with preestablished CHD than for asymptomatic individuals with hypercholesterolemia. According to current guidelines, cholesterol lowering treatment is recommended for patients who had survived a myocardial infarct or patients suffering from angina pectoris or another atherosclerotic disease, with a target LDL-C level of 100 mg/dL.

Standard drug therapies include bile acid sequestrants, fibrates, nicotinic acid, probucol, and statins (HMG-Co-A reductase inhibitors). Statins, the most widely used lipid-modifying drugs, reduce plasma LDL-C by 30−45% at standard dosage and also plasma triglycerides, a synergistic risk factor, but less effectively. In contrast, fibrates reduce plasma triglycerides more effectively but not LDL-C. In humans, statins are well tolerated at standard dosage, but high doses may be associated with adverse effects such as elevated liver enzymes and rhabdomyolisis resulting in part from the reduction of isoprenoid intermediates of cholesterol synthesis and their metabolites, e.g., coenzyme Q10[7].

This has stimulated the search for compounds that inhibit cholesterol biosynthesis downstream of these important, non-sterol isoprenoid intermediates. 2,3-Oxidosqualene−lanosterol cyclase (OSC, EC 5.4.99.7), a microsomal enzyme, represents a unique target for a cholesterol-lowering drug.[8,9] OSC is located downstream of farnesyl pyrophosphate, beyond the synthesis of isoprenoids and coenzyme Q. In hamsters, pharmacologically active doses of an OSC inhibitor showed no adverse effects, in contrast to a statin that reduced food intake and body weight and increased plasma bilirubin, liver weight, and liver triglyceride content. In contrast to the statin, the OSC inhibitor did not lower liver and heart coenzyme Q10 tissue levels.[8]

In yeast, OSC inhibition or down-regulation stimulated the degradation of the HMG-CoA reductase pro-

* To whom correspondence should be addressed. Phone: ++41 61 688 7738. Fax: ++41 61 688 6459. E-mail: johannes.aebi@roche.com.
† Present address: Theravance Inc., 901 Gateway Boulevard, South San Francisco, CA 94065.
‡ Present address: Actelion Pharmaceuticals Ltd., Gewerbestrasse 16, CH-4123 Allschwil, Switzerland.

10.1021/jm021120f CCC: $25.00
Published on Web 06/18/2003

tein.[10] OSC inhibition does not trigger the overexpression of HMGR because of an indirect, negative feedback regulatory mechanism involving the production of 24(*S*),25-epoxycholesterol.[11,12] This negative feedback regulatory mechanism is essential to the concept of OSC inhibition because (i) it potentiates synergistically the primary inhibitory effect with an indirect down-regulation of HMGR and (ii) it minimizes the massive accumulation of the precursor monooxidosqualene in the liver. In addition, 24(*S*),25-epoxycholesterol is known to be a potent natural agonist of the nuclear receptor LXR.[13] Considering that 24(*S*),25-epoxycholesterol is a byproduct of inhibition of OSC, it is theoretically possible that OSC inhibitors could indirectly activate LXR-dependent pathways such as the ABC transporters.[14−17] This has been recently evidenced in cultured macrophages where incubation with OSC inhibitors resulted in upregulation of ABCA1 and ABCG1, which led to enhanced cholesterol efflux and reduced foam cell formation.[18]

Several mammalian OSCs have been purified, cloned, and expressed (for a summary, see Corey[19] and Abe[20]), but so far only one member of the related squalene cyclase family could be crystallized and structurally elucidated.[21,22] The squalene−hopene cyclase from the thermophilic bacterium *Alicyclobacillus acidocaldarius* is a dimer of 2 × 631 amino acid residues showing a peculiar monotopic association with the membrane. The catalytic center is located in a large cavity that is connected through a channel with the membrane interior from where the cyclase picks the substrate squalene or oxidosqualene and through which it releases the product hopene or lanosterol, respectively. The cyclization is thought to proceed through three discrete steps:[19] (i) enzymatic binding to induce the correct folding of 2,3-oxidosqualene; (ii) a conformational change, which correctly positions an electrophilic group for activation of the epoxide; (iii) cyclization starting the reaction by protonation of the epoxide to produce protosterol via cationic intermediates. Further skeletal rearrangements through 1,2-shifts of hydride and methyl substituents yield lanosterol.

The first compounds that represented a starting point for medicinal chemistry were found in the chemical collection of a fungicide project.[23,24] These compounds were further optimized to cholesterol-lowering agents such as Ro 48-8071 **1**, active on mammalian OSC.[8] A variety of OSC inhibitors have been published bearing amines,[9] quinuclidines,[25] isoquinolines,[26] and a lipophilic residue. The large lipophilic residues that fit into the cavity of the cyclase offer a wide range of possibilities for designing new OSC inhibitors. The amine that is protonated at physiological pH mimics the cation formed in the acid-catalyzed opening of the 2,3-oxirane ring of monooxidosqualene (MOS). A wide range of pK_a values are accepted,[27] and three major classes of OSC inhibitors with respect to the amines are known: tertiary amines mentioned before, Boehringer Ingelheim's oxazole derivatives such as BIBB-515 **2** (pK_a = 6.4),[28] and AstraZeneca's pyridine **3a** or pyrimidine **3b** (pK_a = 6.1−9.2)[27,29] (Figure 1).

In the absence of an X-ray structure of OSC, our approach for the design of new OSC inhibitors consisted of superimposing a prototype inhibitor on the high-

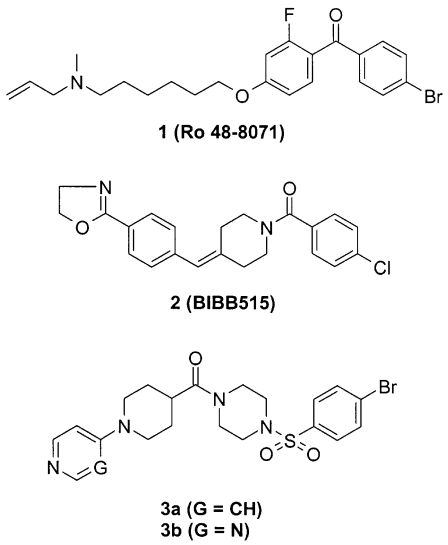
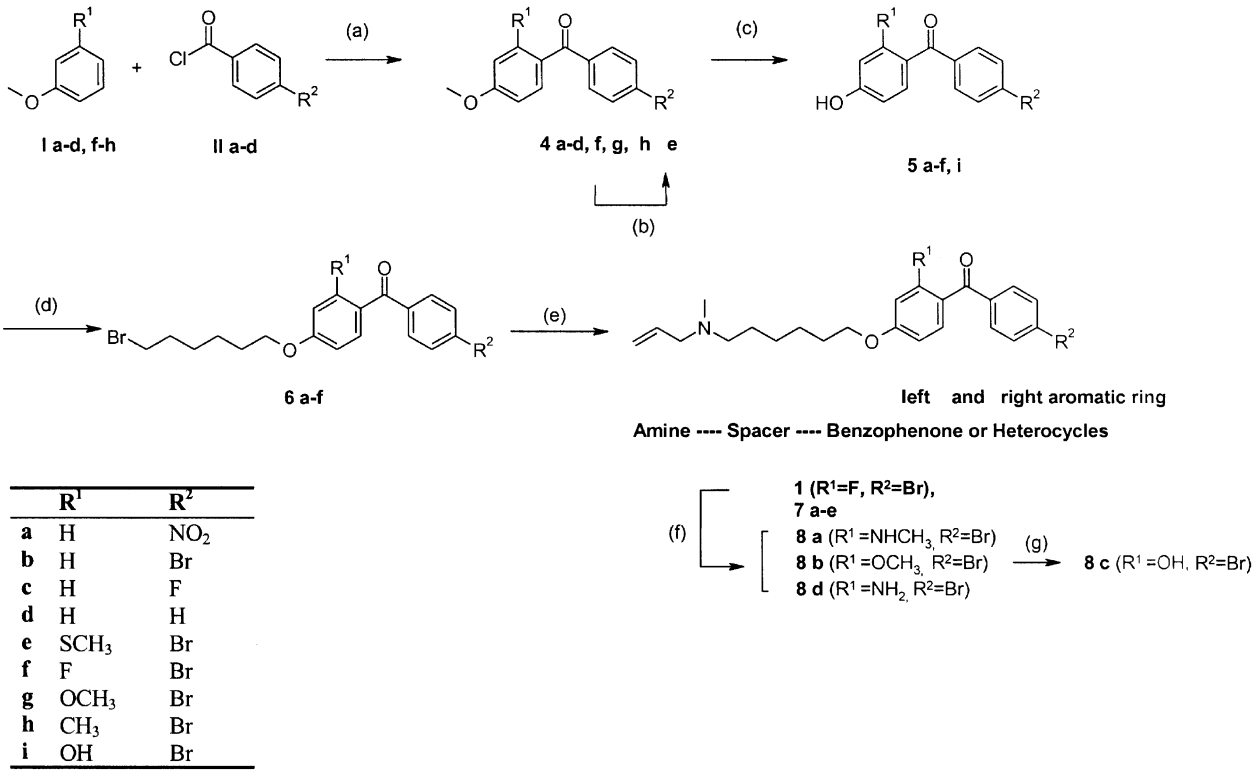


Figure 1.

energy intermediate of oxidosqualene[23,24] to optimize hydrophobic and ionic interactions with the transition state of the enzyme. The folded, high-energy intermediate of oxidosqualene cyclization was modeled as an opened protosterol, using the modeling program MOLOC.[30] The structure of human OSC was later modeled[31] from the X-ray structure of *Alicyclobacillus acidocaldarius* squalene−hopene cyclase (SHC).[21] The active site residues of SHC showed a similarity of 50% and an identity of 38% with respect to human OSC.[22,32] If residues forming the bottom (residues close to Trp196) and entrance tunnel are neglected and only the active site residues are aligned, the score is even higher (65% sequence similarity and 50% sequence identity).[31] The X-ray structure of Ro 48-8071 in SHC[33] revealed that the binding of **1** (Ro 48-8071) is largely identical to the expected binding site for squalene. Owing to the high similarity of residues in the active site between the SHC and OSC, observations from SHC crystals were extrapolated to the OSC model. The major part of the inhibitor is located in the hydrophobic tunnel with a nearly fixed amine position. In SHC, the positive charge of the amino group in **1** forms an ion pair with the DXDD motif and is stabilized by cation−π interactions with tryptophans and a phenylalanine. The carbonyl group of the benzophenone lacks a hydrogen-bonding partner. The carbonyl carbon is probably stabilized by the π system of Phe605. The bromophenyl group of the inhibitor occupies part of the entrance to the active site, which is surrounded by two phenylalanines and stabilized by edge-to-face interaction. To optimally fulfill the steric demands of the active site, inhibitors have to be flexible at the corner position. Ro 48-8071 features a benzophenone that is ideally bent. We investigated the influence of the substitution pattern of the benzophenone on the activity against human oxidosqualene cyclase (hOSC) and on the stability of the inhibitors in rat hepatocytes (Tables 1 and 2). Different substituted amines were synthesized to see the influence of polarity and pK_a on the inhibition of hOSC (Table 3). Finally, the benzophenone was replaced with new heterocyclic systems to give potent and stable hOSC inhibitors (Table 4). The SHC cocrystals of five heterocyclic inhibitors are discussed

Scheme 1. Synthesis of Benzophenone OSC Inhibitors[a]



	R^1	R^2
a	H	NO_2
b	H	Br
c	H	F
d	H	H
e	SCH_3	Br
f	F	Br
g	OCH_3	Br
h	CH_3	Br
i	OH	Br

[a] Reagents and conditions: (a) $AlCl_3$, nitrobenzene, 0 °C to room temp; (b) NaSMe, THF, room temp; (c) 62% aqueous HBr, AcOH, 120 °C or BBr_3, CH_2Cl_2, −78 °C to room temp; (d) 1,6-dibromohexane, 10% aqueous NaOH, *n*-Bu_4NBr, CH_2Cl_2, room temp or 1,6-dibromohexane, K_2CO_3, acetone, 60 °C; (e) NHMeAllyl, DMA, room temp; (f) for R^1 = NHMe, NH_2Me, EtOH/DMA, 120 °C; for $R^1 = NH_2$, (1) PMB-NH_2/K_2CO_3, toluene, 120 °C, (2) TFA, room temp; for R^1 = OMe, (1) NaOMe, MeOH, 70 °C; (g) for R^1 = OH, 62% aqueous HBr, AcOH, 90 °C.

in Lenhart et al.[34] The best compounds were then tested in hamsters for their cholesterol-lowering effects.

Chemistry

Preparation of Benzophenone Derivatives. For the preparation of the aminoalkylbenzophenones, a synthetic route was sought that would give the possibility of modifying the substitution pattern at either of the aromatic rings, the spacer moiety, and/or the amine residue in a rapid manner. This synthesis is outlined in Scheme 1 for the aminohexylbenzophenones. Starting from anisole derivatives **Ia**−**d**,**f**−**h**, Friedel−Crafts acylation with substituted benzoyl chlorides **IIa**−**d** in the presence of $AlCl_3$ in nitromethane gave the desired 4-methoxybenzophenones **4a**−**d** and **4f**−**h**. Reaction of the fluoro-substituted benzophenone **4f** with sodium methanethiolate yielded the methylsulfanyl derivative **4e**. Cleavage of the methyl ethers **4a**−**g** was achieved by treatment with hydrogen bromide in acetic acid or boron tribromide in dichloromethane to yield the 4-hydroxybenzophenones **5a**−**f**,**i**. These 4-hydroxybenzophenones were alkylated with an excess of 1,6-dibromohexane to give the intermediates **6a**−**f**, which were transformed to the final aminohexylbenzophenone inhibitor **1** and **7a**−**e** by amination with *N*-allylmethylamine.

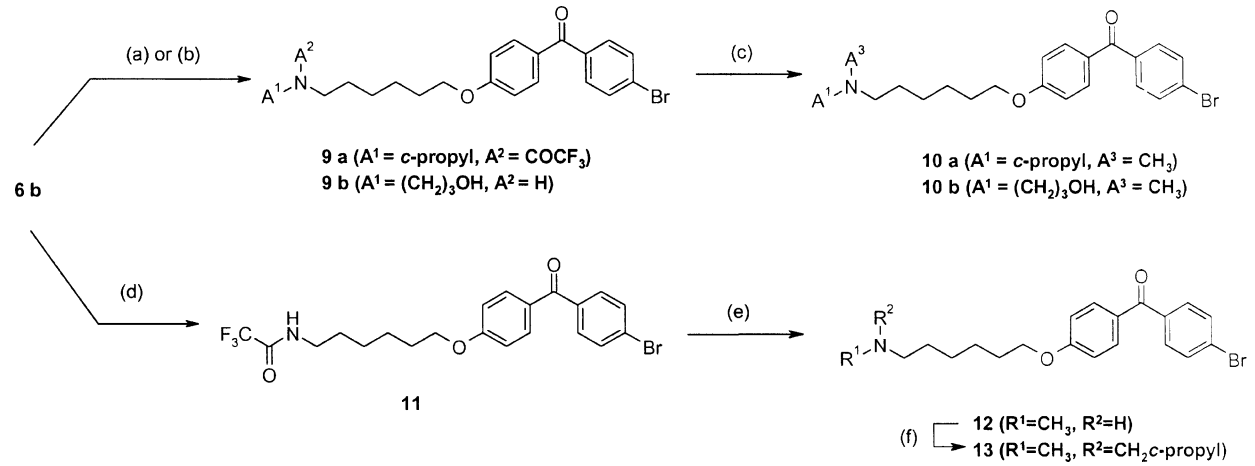
Further modification of the substitution pattern at the aromatic moiety was possible by nucleophilic substitution of the fluoro-substituted benzophenone **1**. For the introduction of amine or oxygene moieties, **1** was treated with the corresponding amine in DMA at 120 °C or with sodium methanolate in methanol at 70 °C to give the compounds **8a** or **8b**, respectively. The methyl ether of the derivative **8b** could be cleaved with 62% aqueous HBr in acetic acid at 90°C to give the compound **8c**. The aminobenzophenone **8d** was synthesized from **1** with *p*-methoxybenzylamine in the presence of potassium carbonate in toluene followed by debenzylation in trifluoroacetic acid.

Synthesis of Amine Analogues. For the synthesis of the cyclopropyl- and cyclopropylmethylamine analogues, two different synthetic routes were chosen (Scheme 2). The synthesis of **10a** and **10b** started with bromide **6b**. Reaction of *N*-cyclopropyl-2,2,2-trifluoroacetamide or 3-aminopropanol (to **9a** or **9b**) followed by basic deprotection of amide **9a** and reductive methylation with 37% aqueous formalin and 1 N NaH_2PO_3 in dioxane at 65 °C[35] gave the final compounds **10a** and **10b**.

Cyclopropylmethyl methylamine derivative **13** was synthesized from bromide **6b** with sodium azide in DMF, followed by Staudinger reduction and protection of the primary amine as a trifluoroacetamide to give intermediate **11**. N-methylation by treatment with NaH and MeI in DMF and deprotection of the tertiary amide under basic conditions gave derivative **12.** Alkylation of **12** with bromomethylcyclopropane in DMA in the presence of Hunig's base yielded the cyclopropylmethyl methylamine **13**.

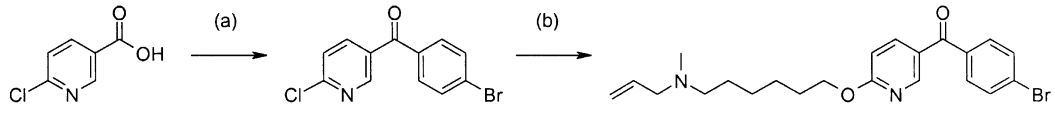
Synthesis of Pyridinyl Analogues. The synthesis of pyridinylphenyl methanone inhibitor **15** is depicted in Scheme 3. Starting from the chloropyridinecarboxylic acid **III**, the benzophenones **14** were prepared via treatment of the Weinreb amide with monolithiated 1,4-

Scheme 2. Synthesis of Different Aminobenzophenones[a]



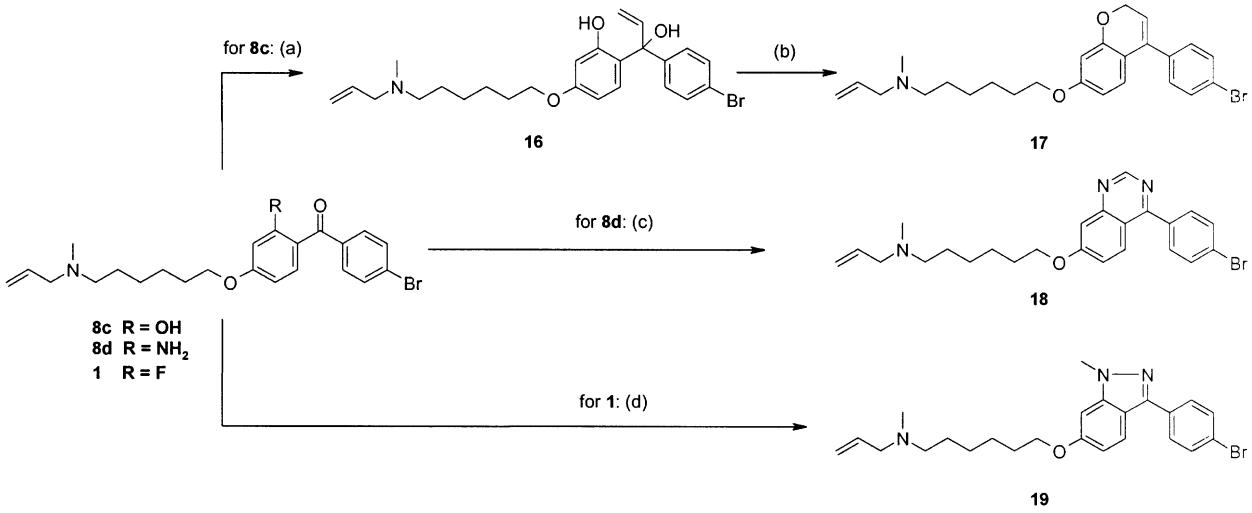
[a] Reagents and conditions: (a) *N*-cyclopropyl-2,2,2-trifluoroacetamide, K_2CO_3, benzyltriethylammonium bromide, MeCN, 80 °C; (b) 3-aminopropanol, DMA, room temp; (c) (1) 20% aqueous KOH, MeOH/THF, room temp (for **9a**), (2) 37% aqueous formaline, 1 N NaH_2PO_3, dioxane, 65 °C; (d) (1) NaN_3, DMF, 90 °C, (2) Ph_3P, THF/H_2O, room temp, (3) $(CF_3CO)_2O$, Et_2O, 0 °C to room temp; (e) (1) NaH, MeI, DMF, −20 °C to room temp, (2) 20% aqueous KOH, MeOH/THF, room temp; (f) bromomethylcyclopropane, *i*-Pr_2EtN, DMA, 50 °C.

Scheme 3. Synthesis of Pyridinyl Analogues[a]



[a] Reagents and conditions: (a) (1) $SOCl_2$, reflux, (2) MeNHOMe·HCl, Et_3N, CH_2Cl_2, 0 °C to room temp, (3) 1,4-dibromobenzene, *n*-BuLi, THF, −78 to 0 °C; (b) 6-(allylmethylamino)hexan-1-ol, KOH, K_2CO_3, dicyclohexano-18-crown-6, toluene, 80 °C.

Scheme 4. Synthesis of Chromene, Quinazoline, and Indazole OSC Inhibitors[a]



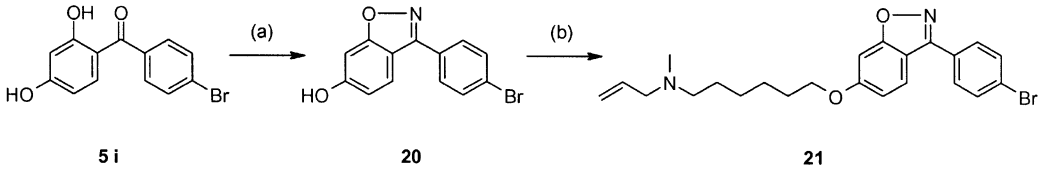
[a] Reagents and conditions: (a) vinylmagnesium chloride (1.7 M in THF), THF/Et_2O, 0 °C to room temp; (b) *o*-xylene (Dean−Stark), 170 °C; (c) HCOOH, $HCONH_2$, 165 °C; (d) $MeHNNH_2$, K_2CO_3, DMA, 120 °C.

dibromobenzene. Reaction of **14** with allylmethylaminohexanol, KOH, K_2CO_3, and dicyclohexano-18-crown-6 in toluene at 80 °C gave the final pyridinyl inhibitor **15**.

Synthesis of Heteroaryl Systems. For the replacement of the benzophenone moiety by heteroaryl systems, two alternative approaches were elaborated. The first approach used the fluoro-substituted benzophenone **1** as an intermediate, from which the new heteroaryl analogues were derived in one or two steps (Scheme 4). In the second one, the heteroaryl systems were assembled first and then modified according to the procedures described for compounds in Scheme 1 to yield the envisaged "amine−spacer−heteroaryl" systems. This approach would allow a rapid modification and optimization of both the spacer and the amine part of the products.

The first approach was used for the preparation of the heteroaryl compounds **17**−**19**. Hydroxy-substituted benzophenone **8c** (Scheme 4) was treated with vinylmagnesium chloride to yield the tertiary alcohol **16**. Cyclization and dehydration was achieved by heating the compound **16** in a Dean−Stark apparatus in *o*-xylene at 120 °C to give the 2*H*-chromene **17**.[36] Reaction of the *o*-aminobenzophenone **8d** in formamide and formic acid at 165 °C gave the quinazoline **18**.[37] Treat-

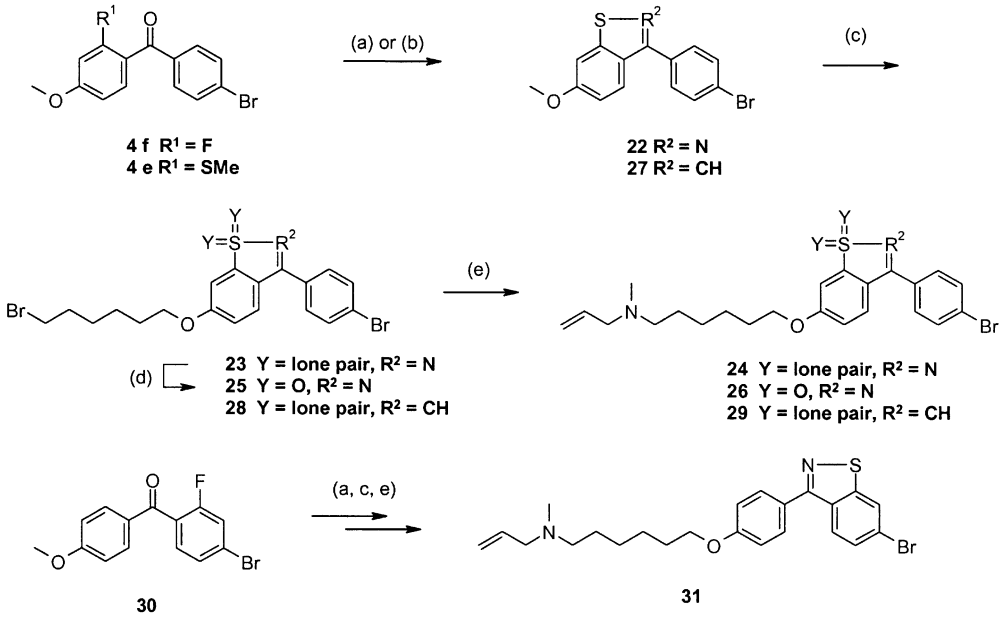
Scheme 5. Synthesis of Isoxazole OSC Inhibitors[a]



5 i **20** **21**

[a] Reagents and conditions: (a) (1) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, 85 °C, (2) *p*-TosOH, EtOH, 85 °C; (b) (1) 1,6-dibromohexane, K_2CO_3, acetone, 60 °C, (2) NHMe-allyl, K_2CO_3, acetone, room temp.

Scheme 6. Synthesis of Benzo[*d*]isothiazole, Dioxobenzo[*d*]isothiazole, and Benzo[*b*]thiophene OSC Inhibitors[a]



4 f R^1 = F
4 e R^1 = SMe

22 R^2 = N
27 R^2 = CH

23 Y = lone pair, R^2 = N
25 Y = O, R^2 = N
28 Y = lone pair, R^2 = CH

24 Y = lone pair, R^2 = N
26 Y = O, R^2 = N
29 Y = lone pair, R^2 = CH

30 **31**

[a] Reagents and conditions: (a) for R^1 = F, (1) KO*t*-Bu, BnSH, THF, room temp, (2) SO_2Cl_2, CH_2Cl_2, room temp, (3) NH_3, EtOH, room temp; (b) for R^1 = SMe, (1) KO*t*-Bu, THF, reflux, (2) TFA, toluene, 0 °C; (c) (1) BBr_3, CH_2Cl_2, −78 °C to room temp, (2) 1,6-dibromohexane, 10% aqueous NaOH, *n*-Bu_4NBr, CH_2Cl_2, room temp; (d) $KMnO_4$ on SiO_2, CH_2Cl_2, room temp; (e) NHMeAllyl, DMA, room temp.

ment of fluoro-substituted benzophenone **1** with methylhydrazine and potassium carbonate in DMA at 120 °C[38] gave indazole **19**.

For the preparation of benzo[*d*]isoxazole **21**, the second approach was chosen (Scheme 5). Dihydroxybenzophenone **5i** was treated with hydroxylamine·hydrochloride and sodium acetate in ethanol at reflux, followed by cyclization with *p*-toluenesulfonic acid to give benzo[*d*]isoxazole **20**. Transformation using the standard conditions described in Scheme 1 gave the final OSC inhibitor **21**.

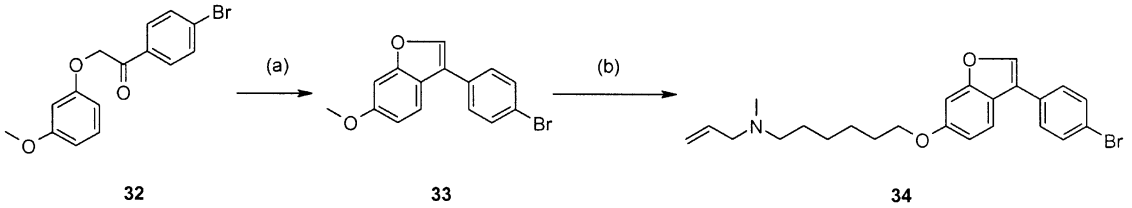
Benzo[*d*]isothiazole **24** was synthesized from the fluorobenzophenone **4f** by reaction with potassium benzylthiolate followed by S-chlorination and cleavage of the benzyl group with sulfuryl chloride to the sulfenyl chloride, which was quenched with ammonia to give benzo[*d*]isothiazole **22**[39] (Scheme 6). Methoxy deprotection and alkylation with an excess of 1,6-dibromohexane gave the intermediate **23** that was converted to the final amine **24** by amination with *N*-allylmethylamine. Furthermore, the intermediate **23** could be oxidized with potassium permanganate to give the bromohexyloxy dioxobenzo[*d*]isothiazole **25**, which was further converted to the *N*-allylmethylamine **26**.

Ortho thiomethylated derivative **4e** was cyclized by treatment with potassium *tert*-butylate in THF at reflux to the benzo[*b*]thiophene **27** and further transformed via bromide **28** to the final inhibitor **29** (Scheme 6). Analogously to compound **24**, the benzo[*d*]isothiazole **31** was derived from benzophenone **30**.

To investigate the importance of the sulfur in the heteroaryl system, the corresponding benzofuran **34** was synthesized (Scheme 7). Alkylation of 3-methoxyphenol with *p*-bromophenacylbromide gave compound **32**, which by heating in polyphosphoric acid at 80°C was converted to benzofuran **33**. Following the procedures already discussed for Scheme 1, compound **33** was transformed to benzofuran **34**. To explore the influence of the heteroaryl moiety further, dihydro- and isoquinolines were prepared (Scheme 8). Following the Bischler−Napieralski cyclization of the benzoylamide **35** gave the dihydroisoquinoline **36**,[40] which was then converted to the final OSC inhibitor **37**. Oxidation of the intermediate **36** with activated manganese(IV) oxide in the presence of anhydrous sodium sulfate gave isoquinoline **38**, which was transformed into the final inhibitor **39** (Scheme 8).

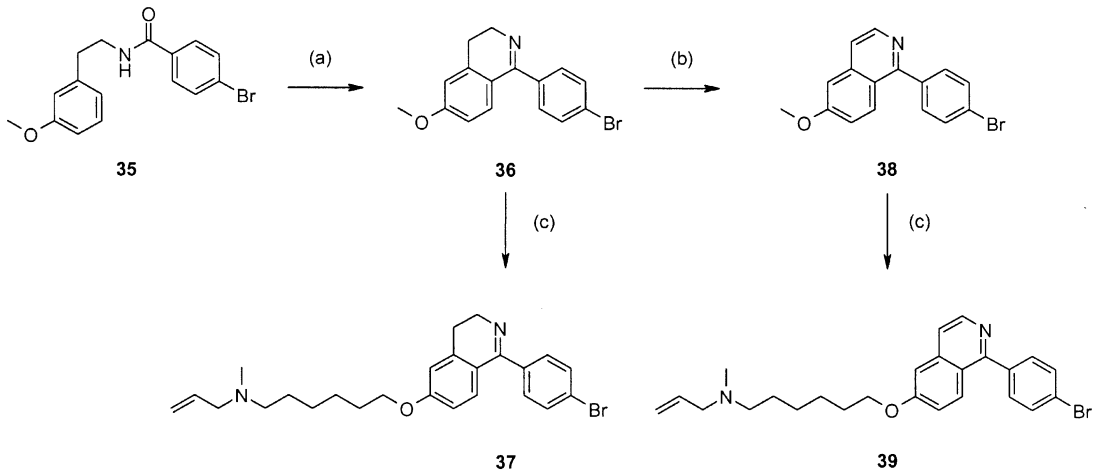
Other six-membered electron-poor heteroaryl compounds such as 1*H*-benzo[*d*][1,2]oxazines **43** were synthesized also (Scheme 9). The ortho-methylated benzophenone **4h** was selectively monobrominated with 1.2 equiv of *N*-bromosuccinimide in the presence of a catalytic amount of benzoyl peroxide and irradiation ($\lambda \geq 390$ nM) in tetrachloromethane to give **40**. Alkylation with *N*-hydroxycarbamide acid *tert*-butyl ester in the presence of sodium hydride in DMF gave **41**, which was

Scheme 7. Synthesis of Benzofuran OSC Inhibitors[a]



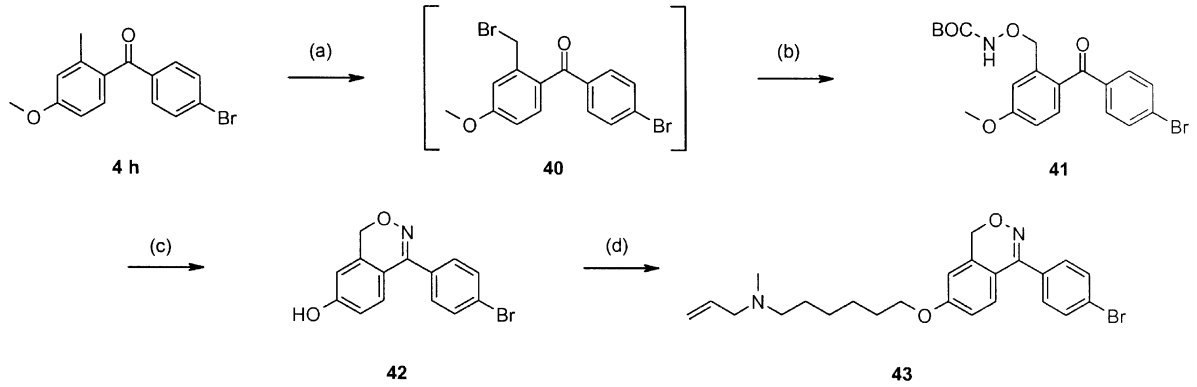
[a] Reagents and conditions: (a) polyphosphoric acid, 80 °C; (b) (1) BBr_3 (1 M), CH_2Cl_2, 0°C to room temp, (2) 1,6-dibromohexane, K_2CO_3, acetone, 60 °C, (3) NHMe-allyl, DMA, room temp.

Scheme 8. Synthesis of Dihydroisoquinoline and Isoquinoline OSC Inhibitors[a]



[a] Reagents and conditions: (a) $POCl_3$, MeCN, 80 °C; (b) MnO_2, Na_2SO_4, toluene, 110 °C; (c) (1) 62% aqueous HBr, AcOH, 120 °C, (2) 1,6-dibromohexane, K_2CO_3, acetone, 60 °C, (3) NHMe-allyl, DMA, room temp.

Scheme 9. Synthesis of 1*H*-Benzo[*d*][1,2]oxazine OSC Inhibitors[a]



[a] Reagents and conditions: (a) 1.2 equiv of NBS, catalytic $(PhCOO)_2$, $h\nu$, $\lambda \geq 390$ nM, CCl_4, room temp; (b) BocNHOH, DMF, 0 °C to room temp; (c) 62% aqueous HBr, AcOH, 120 °C; (d) (1) 1,6-dibromohexane, K_2CO_3, acetone, 60 °C, (2) NHMe-allyl, K_2CO_3, acetone, room temp.

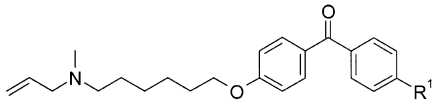
deprotected and cyclized with hydrogen bromide to give 1*H*-benzo[*d*][1,2]oxazine **42**. Standard reaction with 1,6-dibromohexane and *N*-allylmethylamine gave **43**.

Biological Results and Discussion

The strategy to optimize the hOSC inhibitors was as follows. First, we determined the percent inhibition of hOSC in the presence of 100 nM concentration of the compound; compounds exhibiting >50% inhibition at 100 nM were tested for IC_{50} and in a stability assay in rat hepatocytes. This stability assay was performed to determine the activity in the cells as well as the duration of action of the compound and its metabolites. The stability test was performed as a bioassay to select compounds retaining sufficient activity over time prior to testing efficacy in reducing cholesterol production while increasing MOS levels. The measurement of the substance metabolic stability was achieved by comparing the residual inhibitory activity after a preincubation of 8 or 24 h with rat hepatocytes with the activity following co-incubation of only 2 h; hence, reduction of cholesterol production was used there as a surrogate marker for the stability of the parent compound and/or the conversion to active metabolites. From each class of compounds, the most active and stable inhibitors were tested in hamsters for 10 days for plasma total cholesterol (TC) and LDL cholesterol lowering.

Compounds inhibiting OSC from *Candida albicans* were tested on human OSC. Although there was no correlation between the IC_{50} for human OSC and that

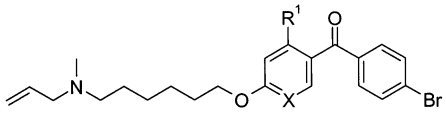
Table 1. Influence of the Modification of the Substituent R^1 on the Inhibitory Activity of the Compounds against hOSC and on the Stability of the Compounds in Rat Hepatocytes and Correlation between σ_p and IC_{50} of hOSC



compd	R^1	inhib hOSC[a] IC_{50} [nM]	residual inhibitory activity in rat hepatocytes[b] 2 h [%]	8 h [%]	24 h [%]	σ_p [c]
7a·HCl	NO_2	1.9	95	91	41	0.81
7b·fumarate	Br	5.4	92	93	78	0.26
7c·HCl	F	6.7	76	75	23	0.15
7d·HBr	H	22.5	87	54	3	0

[a] All potency measurements were performed in triplicate. The SEM ranged between 10% and 20%. [b] All stability assays were performed at 3 μM in duplicate. The SEM did not exceed 4% of the mean value when the mean value was >50% and did not exceed 15% when the mean value was <50%. [c] Hansch et al.[41]

Table 2. Influence of the Modification of the Substitution Pattern at the Left Aromatic Ring of the Benzophenone on the Inhibitory Activity of the Compounds against hOSC and on the Stability of the Compounds in Rat Hepatocytes



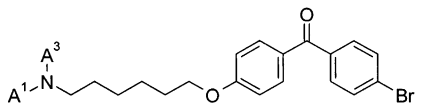
compd	R^1	X	inhib hOSC[a] IC_{50} [nM]	residual inhibitory activity in rat hepatocytes[b] 2 h [%]	8 h [%]	24 h [%]
8a·fumarate	$NHCH_3$	C	4.1	98	98	99
8b·fumarate	OCH_3	C	4.6	89	90	81
7b·fumarate	H	C	5.4	92	93	78
7e·fumarate	SCH_3	C	6.2	98	97	95
8c·fumarate	OH	C	6.3	95	95	91
1·fumarate	F	C	6.5	98	96	80
15·fumarate	H	N	8.7	95	90	38

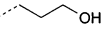
[a] All potency measurements were performed in triplicate. The SEM ranged between 10% and 20%. [b] All stability assays were performed at 3 μM in duplicate. The SEM did not exceed 4% of the mean value when the mean value was >50% and did not exceed 15% when the mean value was <50%.

for the fungal OSC, several of these compounds had motifs that were essential to the inhibition of both enzymes.[23,24] These are an amine function and an electrophilic carbonyl C atom embedded in a benzophenone system that are separated by about 10.7 Å. The best spacers between these two residues were either rigid benzyl or less rigid *trans*-butenyl or flexible alkyl chains. Hypothetically, the amine of the aminohexyloxybenzophenone inhibitors would interact with the catalytic aspartate of the enzyme in the epoxide-opening region. The carbonyl of the benzophenone system would then interact with the region of the OSC stabilizing the last protosterol cation. Because of chemical feasibility, we initially focused our synthetic program on alkyl spacers. In this report, only compounds with a hexyl spacer are discussed.

First, we investigated the influence of the substitution pattern at the right aromatic ring of the benzophenone (see description in Scheme 1) on the potency of the compounds against hOSC, (Table 1). Carbonyl-activating or deactivating groups were introduced in the

Table 3. Influence of the Modification of the Amino Groups on the Inhibitory Activity of the Compounds against hOSC and on the Stability of the Compounds in Rat Hepatocytes



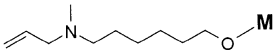
Compd	A^1	A^3	Inhib hOSC[a] IC_{50} [nM]	Residual inhibitory activity in rat hepatocytes[b] 2h [%]	8h [%]	24h [%]	pKa [c]
7b•Fumarate		CH_3	5.4	92	93	78	8.18
10b•Fumarate		CH_3	15.7	93	86	6	9.05
13•Fumarate		CH_3	31.8	98	95	69	9.37
10a•HCl		CH_3	35.3	93	91	51	7.48
12•HCl	H	CH_3	25%[d]	-	-	-	>10

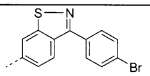
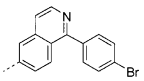
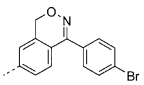
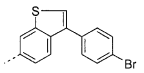
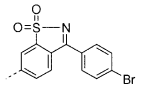
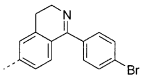
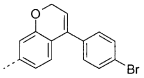
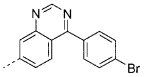
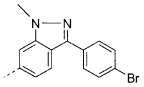
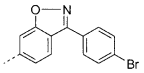
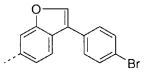
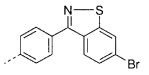
[a] All potency measurements were performed in triplicate. The SEM ranged between 10% and 20%. [b] All stability assays were performed at 3 μM in duplicate. The SEM did not exceed 4% of the mean value when the mean value was >50% and did not exceed 15% when the mean value was <50%. [c] Measured at 22 °C in 0.1 M KNO_3 containing 43 vol % methanol, following literature procedure.[53] [d] % inhibition of hOSC at 100 nM.

benzophenone system using various substituted benzoyl chlorides as starting material for the Friedel−Crafts acylation. With these modifications, it was possible to polarize the aromatic ring of the inhibitor concomitantly and thereby to change the properties of the interaction with the amino acid residues in this region of the enzyme. The changes observed can now be explained by the interactions seen in the crystal structure of **1** (Ro 48-8071) in SHC.[33] In SHC, the benzophenone extends into the putative substrate uptake channel and the bromophenyl is stabilized by two phenylalanines by edge-to-face interactions. A similar interaction probably occurs in hOSC, hence the higher degree of inhibition with compounds bearing electron-deficient aryl groups. It appeared that electron-withdrawing substituents such as nitro-**7a**·HCl (IC_{50} = 1.9 nM), bromo-**7b**·fumarate (IC_{50} = 5.4 nM), and fluoro groups of **7c**·HCl (IC_{50} = 6.7 nM) in the para position increased potency compared to the unsubstituted **7d**·HBr (IC_{50} = 22.5 nM). This could also be seen by comparing the corresponding σ values.[41] The modification of the substitution pattern at the aromatic ring also influenced the stability of these inhibitors in rat hepatocytes. So the residual inhibitory activity of the substance after a preincubation of 24 h with rat hepatocytes increased from 3% for **7d**·HBr to 78% for **7b**·fumarate. Subsequently, the most stable bromo-substituted **7b**·fumarate was taken for further optimization.

In a second step, the influence of the substitution pattern at the left aromatic ring of the benzophenone on the inhibitory activity of the compounds was investigated (Table 2). The electron density in this aryl part of the inhibitor was modified in order to improve the interaction with the phenylalanine-rich OSC channel. Introduction of a fluoro substituent in the ortho position of **7b** to give **1** did not increase the potency or the

Table 4. Influence of the Modification of Heteroaryl Systems on the Inhibitory Activity of the Compounds against hOSC and on the Stability of the Compounds in Rat Hepatocytes



Compd	M	Inhib hOSC[a]	Residual inhibitory activity in rat hepatocytes[b]		
		IC_{50} [nM]	2h [%]	8h [%]	24h [%]
24•Fumarate		2.9	99	97	98
39•HCl		3.5	97	98	96
43•Fumarate		4.1	84	90	94
29•Fumarate		5.6	94	96	95
26•Fumarate		7.8	94	97	93
37		7.9	97	96	93
17		11.4	87	90	85
18•Fumarate		12.3	98	98	92
19•Fumarate		19.6	87	92	83
21•Fumarate		21%[c]			
34•Fumarate		14%[c]			
31•Fumarate		5%[c]			

[a] All potency measurements were performed in triplicate. The SEM ranged between 10% and 20%. [b] All stability assays were performed at 3 μM in duplicate. The SEM did not exceed 4% of the mean value when the mean value was >50% and did not exceed 15% when the mean value was <50%. [c] % inhibition of hOSC at 100 nM.

stability. Compound **1** was an interesting synthetic intermediate for the preparation of derivatives bearing substituents such as amines, oxygens, and sulfurs. It was used later for the synthesis of new heteroaromatic systems. Surprisingly, the corresponding *N*-methylamine-**8a**·fumarate, the methansulfanyl-**7e**·fumarate, the methoxy-**8b**·fumarate, and the hydroxy derivatives **8c**·fumarate were potent inhibitors, all as active as the nonsubstituted **7b**·fumarate. This might be explained by an improved interaction of the polarized benzophenone system with the amino acids of the cavity and enough space in this region of the enzyme to accommodate the sterically demanding residues. The same was true for the electron-deficient pyridine compound **15**·fumarate (IC_{50} = 8.7 nM). All compounds with modified ortho substitution did not show a major improvement in terms of potency but led to, with the exception of fluoro substitution (**1**·fumarate) and methoxy substitution (**8b**·fumarate), more stable inhibitors as determined in the hepatocyte stability assay.

To evaluate the effect of basicity and polarity of the amine part on the activity of the inhibitors, different substituted amines were synthesized (Table 3). Tertiary amines were much more potent than the secondary amine **12**·HCl, which has a pK_a > 10. Good activities were obtained with hydroxypropylmethylamine **10b**·fumarate (pK_a = 9.05), though not as good as the *N*-allylmethylamine **7b**·fumarate (pK_a = 8.18). In addition, the hydroxyalkyl group of **10b**·fumarate increased the lability of the compounds in rat hepatocytes. Cyclopropylmethyl methylamine **13**·fumarate (pK_a = 9.37) with a slightly higher pK_a and cyclopropylmethylamine **10a**·HCl (pK_a = 7.48) with a lower pK_a were not as active as the original compound **7b**·fumarate. This indicates that for compounds from this benzophenone series an optimal pK_a value would be around 8.2. This is similar to the series described by Brown et al., in which inhibitors with a range of different pK_a values were tolerated by the enzyme, but for the optimal inhibition, only a small range of pK_a seemed acceptable.[27] The stability of the two cyclopropyl derivatives **10a**·HCl and **13**·fumarate was slightly lower compared to the one observed for **7b**·fumarate.

The wide variety of groups that were tolerated in the ortho position of the benzophenone led us to explore the potential of annelated heterocyclic systems as hOSC inhibitors that are devoid of the potentially metabolic labile carbonyl moiety (Table 4).[42−44] The best compound of this series was the benzo[*d*]isothiazole **24**·fumarate, which had an IC_{50} of 2.9 nM. It was more potent and also more stable in rat hepatocytes than the corresponding benzophenone derivatives **7b**·fumarate. The benzo[*b*]thiophene **29**·fumarate and the dioxobenzo[*d*]isothiazole **26**·fumarate were slightly less potent but as stable as the benzo[*d*]isothiazole **24**·fumarate. A completely different system, the arylisoquinoline **39**·HCl, was also a stable inhibitor with an IC_{50} of 3.5 nM. The same was true for dihydroisoquinoline **37** (IC_{50} = 7.9 nM). The diaza analogue, namely, the quinazoline **18**·fumarate, exhibited lower inhibitory activity with an IC_{50} of 12.3 nM. 2*H*-Chromene **17** with an IC_{50} of 11 nM had an intermediate activity. The potency of 1*H*-benzo[*d*][1,2]-oxazine **43**·fumarate (IC_{50} = 4.1 nM) was again comparable to the one of arylisoquinoline **39**·HCl. The N-methylated indazole **19**·fumarate (IC_{50} = 20 nM) was less potent but still better than the corresponding benzo[*d*]isoxazole **21**·fumarate (21% inhibition at 100 nM) and the benzofuran **34**·fumarate (21% inhibition at 100 nM).

The way of annelation was essential for optimal inhibition of hOSC. Thus, the compounds with the

Table 5. In Vitro Data and Total Plasma Cholesterol (TC) and LDL Cholesterol (LDL-C) Levels in Fat-Fed Hamsters ($n = 5$ per Group) Treated for 10 Days with OSC Inhibitors at 200 μmol/(kg·day)[a]

		residual inhibitory activity in rat hepatocytes[c]				
compd	inhib hOSC[b] IC_{50} [nM]	2 h [%]	8 h [%]	24 h [%]	LDL-C [%]	TC [%]
24·fumarate	2.9	99	97	98	-36 ± 14	-33 ± 4
1·fumarate	6.5	98	96	80	-41 ± 4	-27 ± 6
7b·fumarate	5.4	92	93	78	-27 ± 10	-25 ± 8
7c·HCl	6.7	76	75	23	-40 ± 4	-24 ± 2
39·HCl	3.5	97	98	96	ND[e]	-14 ± 7
7d·HBr	22.5	87	54	3	ND[e]	-12 ± 5
43·fumarate	4.1	84	90	94	ND[e]	-3 ± 4
simvastatin	*d*	*d*	*d*	*d*	-41 ± 9	-24 ± 4

[a] TC and LDL-C lowering is expressed as a percent ± SEM of day 0 (before compound administration) and corrected with respect to the variation of the untreated animals ($n = 10$). Simvastatin was used as a reference lipid-lowering agent at 20 μmol/(kg·day).[8] [b] All potency measurements were performed in triplicate. The SEM ranged between 10% and 20%. [c] All stability assays were performed at 3 μM in duplicate. The SEM did not exceed 4% of the mean value when the mean value was >50% and did not exceed 15% when the mean value was <50%. [d] Not applicable. [e] ND: not determined.

central heteroaryl moiety bearing a phenyl substituent were more potent than the compounds with a central phenyl group bearing a heteroaryl substituent. This is exemplified by the derivatives **24**·fumarate and **31**·fumarate. This can be explained by spatial restrictions of the cavity of hOSC and is in contrast to observations made for HSC, which are discussed in ref 34 In general, the six-membered rings tested were more potent than the five-membered ones with the exception of the thio-heteroaryl derivatives. This can be explained by the similar size of the corresponding thio-heteroaryl system and the six-membered rings, which is due to the larger van der Waals radius of sulfur compared to the radii of oxygen, nitrogen, and carbon. The heteroaryl compounds with two nitrogens were less potent, which might be attributed to a less optimal electron distribution in these systems.

There was a good correlation between IC_{50} for human and hamster OSC (not shown), allowing the testing of the efficacy on lipid lowering in vivo. Selected inhibitors were tested in hyperlipidemic hamsters[8] at a dose of 200 μmol/(kg·day), corresponding to about 70−90 mg/(kg·day) (Table 5). The compounds were given as food admixture for 10 days. Plasma TC and LDL-C levels were measured and compared to the control group. The inhibitors were well tolerated; no effect on food consumption was seen.[29] In the series of benzophenones, the plasma cholesterol lowering in vivo reflected the determined in vitro profile. The nonsubstituted benzophenone **7d**·HBr, with an IC_{50} of 22.5 nM and low stability in rat hepatocytes (54% and 3% residual inhibitory activity after 8 and 24 h), lowered TC by only 12%. Para fluoro substitution on the right aromatic ring (**7c**·HCl) increased the potency in vitro ($IC_{50} = 6.7$ nM) and the residual inhibitory activity in rat hepatocytes after 8 and 24 h (75% and 23%). This resulted in a more efficacious compound in vivo that decreased TC by 24% and LDL-C by 40%. The bromo-substituted **7b**·fumarate that had a similar potency but better stability (93% and 78% residual inhibitory activity after 8 and 24 h) in vitro had a similar efficacy profile in hamsters. The 2-fluoro analogue **1**·fumarate with a comparable activity and stability profile reduced TC by 27% and LDL-C by 41%. The heteroaryl compounds were very potent and also very stable in vitro. Three of these compounds, the benzo[*d*]isothiazole **24**·fumarate, the arylisoquinoline **39**·HCl, and the 1*H*-benzo[*d*][1,2]oxazine **43**·fumarate were tested in hyperlipidemic hamsters. The benzo[*d*]-isothiazole **24**·fumarate was quite efficacious in vivo, showing a lowering of TC of 33% and LDL-C of 36%. The modest efficacy of arylisoquinoline **39**·HCl (14% decrease of TC) and 1*H*-benzo[*d*][1,2]oxazine **43**·fumarate (3% decrease of TC) could not be explained, but might be due to a lower intestinal absorption.

Conclusion

Modification of the substitution pattern of the benzophenone at either of the aromatic rings led to the identification of the potent and stable hOSC inhibitors **7a**·HCl and **1**·fumarate. The most potent hOSC inhibitors in these series have tertiary amines, a pK_a of around 8.2, and small residues at the amine. On the basis of the X-ray structure of the inhibitors in SHC, the keto moiety of the benzophenone was not involved in hydrogen bonding. It is a potentially metabolic labile position, and in addition, a large variety of substituents in the ortho position of the benzophenones were tolerated for potent hOSC inhibition. These observations led us to explore annelated heteroaryl systems in more detail. The most active compounds in vitro were benzo-[*d*]isothiazole **24**·fumarate, arylisoquinoline **39**·HCl, 1*H*-benzo[*d*][1,2]oxazine **43**·fumarate, and benzo[*b*]-thiophene **29**·fumarate. For the benzophenones and the benzo[*d*]isothiazole **24**·fumarate, the cholesterol-lowering effect observed in hyperlipidemic hamsters reflects the in vitro potency against hOSC and the stability determined in rat hepatocytes. Thus, the nonsubstituted benzophenone **7d**·HBr lowered TC by only 12%. Better efficacy in TC lowering could be achieved with the appropriate substitution pattern at the benzophenone system, so **1**·fumarate showed a reduction of TC of 27% and LDL-C of 41%**.** Further improvement of stability of the compounds on rat hepatocytes led to the best heteroaryl derivative, the benzo[*d*]isothiazole **24**·fumarate, which lowered TC by 33% and LDL-C by 36%.

Experimental Section

General Methods. Reactions were carried out under an atmosphere of argon. Unless otherwise noted, NH_4Cl, $NaHCO_3$, and Na_2CO_3 solutions were saturated aqueous solutions. All compounds were characterized by 250 MHz ^{1}H NMR, IR, MS, and microanalyses. Melting points (uncorrected) were determined using a Büchi 510 apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AC250 spectrometer, and δ values are given in ppm relative to tetramethylsilane. In the ^{1}H NMR spectra, the acidic protons of the fumarate, HCl, and HBr ammonium salts were not seen unless listed. IR spectra of KBr pellets were recorded using a Nicolet 7199 FT-IR spectrometer. ATR (attenuated total reflection) spectra were recorded with an FT-IR spectrometer equipped with an IR microscope and a ZnSe ATR objective. The spectrometer is a Nicolet Magna 550/860 or equivalent (resolution of 2 cm^{-1}, 200 or 500 coadded scans, MCT detector). Mass spectra were obtained using the pneumatically assisted electrospray technique (Perkin-Elmer Sciex, type API-III). Results of elemental analyses were within 0.4%

of theoretical values. Unless indicated otherwise, commercially available chemicals and solvents were used without further purification. Silica gel 60 (0.04−0.063 mm; Merck) was used for flash chromatography.

General Procedure for the Formation of Fumarates. A solution of 1 equiv of the final amine·hydrobromide in methylene chloride was extracted with a $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated. The residue was dissolved in ethanol with 1.04 equiv of fumaric acid and crystallized or precipitated with ether to give the amine·fumarate (1:1).

General Procedure for the Formation of Hydrochlorides. A solution of 1 equiv of the final amine·hydrobromide in methylene chloride was extracted with a $NaHCO_3$ solution, dried (Na_2SO_4), and concentrated The residue was dissolved in methylene chloride, treated with 1 equiv of a 4.8 M HCl solution in ether, and crystallized to give the amine·hydrochloride (1:1).

General Procedure A. [4-[6-(Allylmethylamino)hexyloxy]phenyl](4-bromophenyl)methanone·Fumarate (1:1) (7b·Fumarate). Nitrobenzene (225 mL) was cooled in an ice bath and treated portionwise with aluminum chloride (72.5 g, 544.5 mmol) and 4-bromobenzoyl chloride (**IIb**) (109.5 g, 500 mmol) in nitrobenzene (100 mL) at a maximum of 8 °C. The mixture was stirred for 10 min, whereupon anisole (**Id**) (52.0 mL, 476.5 mmol) was added in such a manner that the temperature did not exceed 6 °C. The solution was left to warm to room temperature overnight, then poured onto ice−water and extracted with methylene chloride. The organic phase was washed with water and 10% NaCl solution, dried (Na_2SO_4), and concentrated. After crystallization from cyclohexane, (4-bromophenyl)(4-methoxyphenyl)methanone (**4b**) (128 g, 88%) was obtained: ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 6.96 and 7.79 (AA′-BB′-system, J_{AB} = 8.8 Hz, 4H), 7.62 (br s, 4H).

A solution of **4b** (128 g, 440 mmol) in acetic acid (870 mL) and 62% aqueous HBr solution (500 mL) was refluxed for 5 h, subsequently evaporated, reevaporated with toluene, taken up in ethyl acetate, washed with a $NaHCO_3$ solution and 10% NaCl solution, dried ($MgSO_4$), and evaporated. After crystallization from toluene, (4-bromophenyl)(4-hydroxyphenyl)-methanone (**5b**) (108.7 g, 89%) was obtained: ^{1}H NMR (DMSO-d_6) δ 6.90 and 7.66 (AA′-BB′-system, J_{AB} = 8.7 Hz, 4H), 7.61 and 7.75 (AA′-BB′-system, J_{AB} = 8.5 Hz, 4H), 10.52 (br s, 1H).

A 10% NaOH solution (80 mL) was added to 1,6-dibromohexane (13.7 mL, 90 mmol), **5b** (8.3 g, 30 mmol), and tetra-*n*-butylammonium bromide (1.8 g, 6 mmol) in methylene chloride (80 mL). The mixture was stirred at room temperature for 36 h, tetra-*n*-butylammonium bromide (1.8 g, 6 mmol) was added twice. Then the two phases were poured into water and extracted with ethyl acetate. The organic phase was washed with 10% NaCl solution, dried ($MgSO_4$), filtered, and evaporated. The residue was suspended in *n*-hexane, filtered, dissolved in methylene chloride/methanol, and treated with 40 g of Amberlite MB3 for 15 min. After filtration and evaporation of the solvents, [4-[6-bromohexyloxy]phenyl](4-bromophenyl)methanone (**6b**) (9.3 g, 71%) was obtained: ^{1}H NMR ($CDCl_3$) δ 1.46−1.62 (m, 4H), 1.79−1.97 (m, 4H), 3.44 (t, J = 6.7 Hz, 2H), 4.05 (t, J = 6.3 Hz, 2H), 6.95 and 7.78 (AA′-BB′-system, J_{AB} = 8.9 Hz, 4H), 7.62 (br s, 4H).

6b (6.2 g, 14.1 mmol) was taken up in DMA (47 mL) and stirred at room temperature for 3 days, adding every day *N*-allylmethylamine (2.7 mL, 28.2 mmol) and following the reaction by TLC (Silicagel, CH_2Cl_2/MeOH 98:2, R_f(starting material) = 0.73, R_f(product) = 0.27, UV). The solution was concentrated, and the residual oil was taken up in water and lyophilized. Crystallization from methylene chloride/ethyl acetate gave **7b**·HBr (1:1) (4.3 g, 60%) as colorless crystals: mp 117−119 °C; IR (KBr) 1640, 1602 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30−1.55 (m, 4H), 1.60−1.85 (m, 4H), 2.71 (s, 3H), 2.92−3.15 (m, 2H), 3.62−3.85 (m, 2H), 4.09 (t, J = 6.3 Hz, 2H), 5.45−5.62 (m, 2H), 5.82−6.05 (m, 1H), 7.09 and 7.74 (AA′-BB′-system, J_{AB} = 8.8 Hz, 4H), 7.62 and 7.77 (AA′-BB′-system, J_{AB} = 8.4 Hz, 4H); MS *m*/*z* 429 (M$^+$, 1Br). Anal. ($C_{23}H_{28}NO_2Br$·HBr) C, H, N, Br.

Following the general procedure for the formation of fumarates, **7b**·HBr (1:1) was converted to **7b**·fumarate (1:1) in 58% yield as colorless crystals: mp 85−86 °C; IR (ATR microscope) 1714, 1638, 1601, 1581 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.25−1.60 (m, 4H), 1.70−1.80 (m, 4H), 2.23 (s, 3H), 2.40−2.55 (m, 2H), 3.09 (d, J = 6.5, 2H), 4.09 (t, J = 6.3 Hz, 2H), 5.45−5.62 (m, 2H), 5.82−6.05 (m, 1H), 6.57 (s, 2H), 7.09 and 7.74 (AA′-BB′-system, J_{AB} = 8.8 Hz, 4H), 7.62 and 7.77 (AA′-BB′-system, J_{AB} = 8.4 Hz, 4H); HRMS calcd for $C_{23}H_{28}NO_2Br$ (M + H$^+$, 1Br) 430.1381, found 430.1385.

Analogously to the general procedure A described above, the following compounds were synthesized. **[4-[6-(Allylmethylamino)hexyloxy]phenyl](4-nitrophenyl)methanone·Hydrochloride (1:1) (7a·HCl). 7a**·HCl was obtained from 4-nitrobenzoyl chloride (**IIa**) and anisole (**Id**) in 33% yield: mp 79 °C; ^{1}H NMR (DMSO-d_6) δ 1.28−1.56 (m, 4H), 1.65−1.86 (m, 4H), 2.65 (s, 3H), 2.85−3.15 (m, 2H), 3.58−3.82 (m, 2H), 4.10 (t, J = 6.4 Hz, 2H), 5.42−5.60 (m, 2H), 5.92−6.15 (m, 1H), 7.11 and 7.77 (AA′BB′, J = 8.8 Hz, 4H), 7.91 and 7.37 (AA′BB′, J = 8.8 Hz, 4H), 10.98 (br s, 1H); MS *m*/*z* 396 (M$^+$). Anal. ($C_{23}H_{28}N_2O_4$·HCl) H, N, Cl. C: calcd, 63.81; found, 64.22.

[4-[6-(Allylmethylamino)hexyloxy]phenyl]-(4-fluorophenyl)methanone·HCl (1:1) (7c·HCl). 7c·HCl was obtained from (4-fluorophenyl)(4-hydroxyphenyl)methanone (**5c**) in 47% yield: mp 84 °C; ^{1}H NMR (DMSO-d_6) δ 1.26−1.56 (m, 4H), 1.65−1.86 (m, 4H), 2.65 (d, J = 4.6 Hz, 3H), 2.85−3.12 (m, 2H), 3.58−3.84 (m, 2H), 4.09 (t, J = 6.4 Hz, 2H), 5.42−5.60 (m, 2H), 5.92−6.15 (m, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.37 (d, J = 8.9 Hz, 2H), 7.41 (d, J = 8.9 Hz, 2H), 7.65−7.85 (m, 4H), 11.10 (br s, 1H); MS *m*/*z* 369 (M$^+$). Anal. ($C_{23}H_{28}NO_2F$·HCl) H, N, Cl, F. C: calcd, 68.05; found, 67.33.

[4-[6-(Allylmethylamino)hexyloxy]phenyl]phenylmethanone·Hydrobromide (1:1) (7d·HBr). 7d·HBr was obtained from (4-hydroxyphenyl)phenylmethanone (**5d**) in 29% yield: mp 89−91 °C; IR 1645, 1602 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.26−1.56 (m, 4H), 1.65−1.86 (m, 4H), 2.72 (s, 3H), 2.92−3.15 (m, 2H), 3.65−3.90 (m, 2H), 4.09 (t, J = 6.3 Hz, 2H), 5.48−5.62 (m, 2H), 5.86−6.15 (m, 1H), 7.09 (d, J = 8.9 Hz, 2H), 7.51−7.61 (m, 2H), 7.63−7.82 (m, 4H), 9.85 (br s, 1H); MS *m*/*z* 351 (M$^+$). Anal.($C_{23}H_{29}NO_2$·HBr) C, H, N, Br.

General Procedure B. [4-[6-(Allylmethylamino)hexyloxy]-2-methylsulfanylphenyl](4-bromophenyl)-methanone·Fumarate (1:1) (7e·Fumarate). NaSMe (95%, 1.45 g, 19.6 mmol) was suspended in THF (80 mL) and treated over a period of 1.5 h with (4-bromophenyl)(2-fluoro-4-methoxyphenyl)methanone (**4f**) (5.51 g, 17.82 mmol)[8] in THF (100 mL). The solution was stirred at room temperature, again treated with NaSMe (264 mg, 3.56 mmol), stirred, and treated after an interval of 10 min with a NH_4Cl solution (50 mL) and a $NaHCO_3$ solution (100 mL). The aqueous phase was extracted with methylene chloride, and the organic phases were washed with a $NaHCO_3$ solution as well as brine and dried (Na_2SO_4). After evaporation, the crude product was purified by column chromatography (silica gel, ethyl acetate/*n*-hexane 1:2). (4-Bromophenyl)(4-methoxy-2-methylsulfanylphenyl)-methanone (**4e**) (5.88 g, 89%) was obtained as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H), 3.89 (s, 3H), 6.64 (dd, 1H), 6.89 (d, 1H), 7.43 (dd, 1H), 7.59 (m, 4H).

Compound **4e** was deprotected in a manner similar to **4b** to give 96% (4-bromophenyl)(4-hydroxy-2-methylsulfanylphenyl)methanone (**5e**) as brown crystals: ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 6.55−6.66 (m, 1H), 6.83 (d, 1H), 7.33 (d, J = 8.5 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 10.42 (s, 1H).

5e (450 mg, 1.39 mmol) was taken up in acetone (7 mL) and treated with K_2CO_3 (1.24 g, 8.98 mmol) and 1,6-dibromohexane (0.53 mL, 3.48 mmol). The suspension was heated under reflux overnight, cooled, filtered, and concentrated. After removal of the excess 1,6-dibromohexane under reduced pressure, [4-[6-(bromo)hexyloxy]-2-methylsulfanylphenyl](4-bromophenyl)methanone **(6e)** (710 mg, quantitative) was obtained as a brown oil: ^{1}H NMR ($CDCl_3$) δ 1.42−1.60 (m, 6H), 1.74−1.96 (m, 2H), 2.43 (s, 3H), 3.43 (t, 2H), 4.04 (t, 3H), 6.62 (dd, 1H), 6.86 (d, 1H), 7.40 (d, 1H), 7.58 (m, 4H).

Following the general procedure A described for **6b**, **6e** and *N*-allylmethylamine gave **7e**·HBr, which was converted with fumaric acid to **7e**·fumarate and isolated after precipitation from EtOH with Et_2O as a viscous oil in a total yield of 84%: IR (KBr) 2484, 1703, 1587, 1391, 1276, 845 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.22−1.56 (m, 6H), 1.76 (m, 2H), 2.24 (s, 3H), 2.42 (s, 3H), 3.11 (d, J = 6.5 Hz, 2H), 4.09 (t, J = 7 Hz, 2H), 5.19 (d, J = 9.9 Hz), 5.24 (d, J = 15.4 Hz), 5.85 (m, 1H), 6.58 (s, 1.6H), 6.81 (dd, J = 8.6 Hz, 1H), 6.92 (d, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.55 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.5 Hz, 1H), 13.0 (s, br, 2H); MS m/z 476 ($M + H^+$, 1Br). Anal. free base ($C_{24}H_{30}NO_2BrS$·$0.8C_4H_4O_4$) C, H, N.

[4-[6-(Allylmethylamino)hexyloxy]-2-methylaminophenyl](4-bromophenyl)methanone·Fumarate (1:1) (8a·Fumarate). A solution of **1** (2.6 g, 5.8 mmol)[8] in DMA (115 mL) was boiled at 120 °C for 3 h with methylamine (7.2 mL, 58 mmol, 8.03 M in ethanol). The solution was concentrated and the residue was chromatographed over silica gel with methylene chloride/methanol (2.5−10%) to give **8a** (2.21 g, 83%). The resulting oil (0.5 g, 1.09 mmol) was dissolved in methylene chloride and stirred with fumaric acid (0.12 g, 1.03 mmol) overnight to give **8a**·fumarate (0.33 g, 53%): mp 72 °C, dec; IR (KBr) 1680, 1617, 1580, 1566 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25−1.55 (m, 6H), 1.65−1.85 (m, 2H), 2.23 (s, 3H), 2.36−2.60 (m, 2H), 2.90 (d, J = 6 Hz, 3H), 2.90 (d, J = 6 Hz, 3H), 4.06 (t, J = 6.6, 2H), 5.15−5.32 (m, 2H), 5.74−5.94 (m, 1H), 6.10−6.25 (m, 2H), 6.57 (s, 2H), 7.25 (d, J = 8.4 Hz), 7.42 and 7.69 (AA′-BB′-system, J_{AB} = 8.4, 4H); MS m/z 429 (M^+, 1Br). Anal. ($C_{24}H_{31}N_2O_2Br$·$C_4H_4O_4$) C, H, N, Br.

[4-[6-(Allylmethylamino)hexyloxy]-2-methoxyphenyl]-(4-bromophenyl)methanone·Fumarate (8b·Fumarate). Analogously to **8a**, **1** (8.97 g, 20 mmol) in THF (450 mL) was stirred with 5.4 M sodium methanolate (37 mL, 200 mmol) in methanol at room temperature for 14 h and under reflux for 1 h. The solution was evaporated, and the residue was taken up in methylene chloride/10% NaCl solution. The organic phase was dried (Na_2SO_4), evaporated, dissolved in ether, and stirred overnight with fumaric acid (2.08 g, 18 mmol). **8b**·fumarate (8.17 g, 71%) was obtained: mp 108−113 °C; IR (KBr) 1649, 1602 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28−1.55 (m, 4H), 1.70−1.85 (m, 4H), 2.21 (s, 3H), 2.35−2.50 (m, 2H), 3.08 (d, J = 6.0 Hz, 2H), 3.64 (s, 3H), 4.06 (t, J = 6.0 Hz, 2H), 5.14−5.28 (m, 2H), 5.75−5.94 (m, 1H), 6.57 (s, 2H), 6.61−6.71 (m, 2H), 7.32 (d, J = 8.4 Hz), 7.57 and 7.70 (AA′-BB′-system, J_{AB} = 8.6, 4H); MS m/z 460 ($M + H^+$, 1Br). Anal. ($C_{24}H_{30}NO_3Br$·$0.95C_4H_4O_4$) H, N, Cl, Br.

[4-[6-(Allylmethylamino)hexyloxy]-2-hydroxyphenyl]-(4-bromophenyl)methanone·Fumarate (8c·Fumarate). **8b**·fumarate was taken up in methylene chloride/$NaHCO_3$ solution, and the organic phase was dried and concentrated. The thus-obtained **8b** (3.09 g, 6.72 mmol) was boiled in acetic acid (13 mL)/62% HBr solution (7.7 mL) at 90 °C for 2 h. The reaction mixture was concentrated, and the residue was converted into the free base with methylene chloride/$NaHCO_3$ solution. The residue (3.14 g, quantitative) was dissolved in ethanol with fumaric acid (0.74 g, 6.4 mmol) and precipitated with ether. **8c**·fumarate (2.05 g, 49%) was obtained as a yellowish oil: IR (KBr) 1705, 1624, 1586 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25−1.55 (m, 4H), 1.65−1.82 (m, 4H), 2.24 (s, 3H), 2.35−2.50 (m, 2H), 3.12 (d, J = 6.0 Hz, 2H), 4.05 (t, J = 6.0 Hz, 2H), 5.14−5.32 (m, 2H), 5.78−5.94 (m, 1H), 6.57 (s, 2H), 6.49−6.65 (m, 2H), 7.40 (d, J = 8.4 Hz), 7.59 and 7.75 (AA′-BB′-system, J_{AB} = 8.5, 4H); MS m/z 446 ($M + H^+$, 1Br). Anal. ($C_{23}H_{28}NO_3Br$·$1.1C_4H_4O_4$) C, H, N, Br.

[2-Amino-4-[6-(Allylmethylamino)hexyloxy]phenyl](4-bromophenyl)methanone·Fumarate (1:1) (8d·Fumarate). Analogous to **8a**, **1** (17.55 g, 39.03 mmol) was boiled under reflux for 23 h with 4-methoxybenzylamine (50.7 mL, 307.55 mmol) and K_2CO_3 (6.5 g, 46.83 mmol) in toluene (600 mL). After filtration, evaporation, and purification over silica gel with methylene chloride/methanol (2.5−10%), [4-[6-(allylmethylamino)hexyloxy]-2-(4-methoxybenzylamino)phenyl]-(4-bromophenyl)methanone (17.43 g, 79%) was obtained. A solution of this material in trifluoroacetic acid (200 mL) was stirred at room temperature for 45 h and evaporated, and the residue was converted into the free base with methylene chloride/$NaHCO_3$ solution. After purification over silica gel with methylene chloride/methanol (9:1), **8d** (13.23 g, 96%) was obtained.

8d (9.1 g, 20.43 mmol) was dissolved in methylene chloride/ether and stirred with fumaric acid (2.25 g, 19.38 mmol) overnight to give after filtration **8d**·fumarate (7.28 g, 64%): mp 78 °C (with decomposition); IR (KBr) 1698, 1616, 1585 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25−1.55 (m, 4H), 1.65−1.82 (m, 4H), 2.24 (s, 3H), 2.35−2.50 (m, 2H), 3.12 (d, J = 6.0 Hz, 2H), 4.05 (t, J = 6.0 Hz, 2H), 5.14−5.32 (m, 2H), 5.78−5.94 (m, 1H), 6.57 (s, 2H), 6.49−6.65 (m, 2H), 7.40 (d, J = 8.4 Hz), 7.59 and 7.75 (AA′-BB′-system, J_{AB} = 8.5, 4H); MS m/z 445 ($M + H^+$, 1Br). Anal. ($C_{23}H_{28}NO_3Br$·$1.1C_4H_4O_4$) C, H, N, Br.

[6-[6-(Cyclopropylmethylamino)hexyloxy]phenyl](4-bromophenyl)methanone·HCl (10a·HCl). A suspension of **6b** (1 g, 2.27 mmol), *N*-cyclopropyl-2,2,2-trifluoroacetamide (0.7 g, 4.54 mmol)[45] in acetonitrile (50 mL) was heated at reflux for 19 h. Additional *N*-cyclopropyl-2,2,2-trifluoroacetamide (0.7 g, 4.54 mmol), K_2CO_3 (0.63 g, 4.54 mmol), and benzyltriethylammonium bromide (52 mg, 0.23 mmol) were added, and the mixture was heated for a further 23 h. After cooling to room temperature, the suspension was filtered. The filtrate was concentrated, and the residue was dissolved in a mixture of methylene chloride (45 mL) and methanol (5 mL) and the mixture was stirred with Amberlite MB3 (4.5 g) for 15 min, subsequently filtered and washed with methylene chloride. The organic phase was washed with water, dried (Na_2SO_4), concentrated, and purified by chromatography on silica gel (*n*-hexane/ethyl acetate 8:2) to afford *N*-{6-[4-(4-bromobenzoyl)phenoxy]hexyl}-*N*-cyclopropyl-2,2,2-trifluoroacetamide (**9a**) (0.56 g, 48%) as a yellowish solid: MS m/z 512 ($M + H^+$, 1Br).

A solution of **9a** (0.78 g, 1.52 mmol) in methanol (30 mL) and THF (10 mL) was treated with 20% aqueous potassium hydroxide solution (2.13 mL) while cooling with ice. The mixture was brought to room temperature and stirred for 2 days and concentrated under reduced pressure. The residue was treated with water/methylene chloride (3×). The organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to give (4-bromophenyl)-[4-(6-cyclopropylaminohexyloxy)-phenyl]methanone (0.32 g, 50%): ^1H NMR ($CDCl_3$) δ 0.29−0.37 (m, 2H), 0.39−0.50 (m, 2H), 1.33−1.61 (m, 7H), 1.76−1.91 (m, 2H), 2.08−2.17 (m, 1H), 2.70 (t, J = 7.2 Hz, 2H), 4.04 (t, J = 6.5 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 7.62 (s, br, 4H), 7.78 (d, J = 8.8 Hz, 2H); MS m/z 415 (M^+, 1Br).

This product (0.25 g, 0.6 mmol) was taken up in dioxane (5 mL), treated with 1 N NaH_2PO_3 (5 mL) solution and a 37% aqueous solution of formaldehyde (5 mL). The mixture was heated to 65 °C for 5 h. The mixture was adjusted to pH >11 with 10% aqueous NaOH and extracted with ether. Evaporation and chromatography of the residue on silica gel with ethyl acetate/*n*-hexane/triethylamine (40:60:1) gave **10a** (0.22 g, 85%), which was converted following the general procedure for the formation of hydrochlorides into the hydrochloride **10a**·HCl: ^1H NMR (DMSO-d_6) δ 0.82 (m, 2H), 1.01, 1.08 (2m, 2H), 1.43 (m, 4H), 1.77 (m, 4H), 2.79 (m, 4H), 3.13 (m, 2H), 4.10 (d, J = 6.3 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.77 (d, J = 8.4 Hz, 2H), 10.22 (br s, HCl); MS m/z 430 ($M + H^+$, 1Br). Anal. ($C_{23}H_{28}BrNO_2$·HCl) C, H, N, Br, Cl.

(4-Bromophenyl)[4-[6-[(3-hydroxypropyl)methylamino]-hexyloxy]phenyl]methanone·Fumarate (10b·Fumarate). **10b**·fumarate was synthesized from (4-bromophenyl)-[4-[6-[(3-hydroxypropyl)amino]hexyloxy]phenyl]methanone **9b** (which was obtained by treatment of **6b** with 3-aminopropanol in analogy to **7b** (general procedure A) in 99% yield) followed by N-methylation (analogous to **10a**) in a yield of 68%. Following the general procedure for the formation of fumarates, **10b**·fumarate was obtained: ^1H NMR ($CDCl_3$) δ 1.36−1.56 (m, 4H), 1.67−1.98 (2m, 6H), 2.65 (s, 3H), 2.90 (t, J = 6 Hz, 2H), 3.07 (t, J = 4.8 Hz, 2H), 3.75 (d, J = 5 Hz, 2H), 4.24 (d, J = 6.3 Hz, 2H), 5.3 (s, br, 3H), 6.77 (s, 2H), 6.94 (d, J = 8.8 Hz, 2H), 7.61

(s, 4H), 7.77 (d, J = 8.8 Hz, 2H); MS m/z 448 ($M + H^+$, 1Br). Anal. ($C_{23}H_{30}BrNO_3 \cdot C_4H_4O_4$) C, H, N.

(4-Bromophenyl)[4-(6-methylaminohexyloxy)phenyl]-methanone·HCl (1:1) (12·HCl). A solution of **6b** (10 g, 22.6 mmol) in DMF (200 mL) was treated with sodium azide (14.7 g, 22.6 mmol) and stirred at 90 °C for 24 h. After filtration and concentration, the residue was taken up in ethyl acetate (200 mL) and washed with a 10% aqueous $NaHCO_3$ solution. The organic phase was dried (Na_2SO_4) and concentrated. [4-(6-Azidohexyloxy)phenyl](4-bromophenyl)methanone (9.12 g, 99%) was obtained: ^{1}H NMR ($CDCl_3$) δ 1.51 (m, 4H), 1.66 (m, 2H), 1.85 (m, 2H), 3.30 (t, J = 6.8 Hz, 2H), 4.05 (t, J = 6.4 Hz, 2H), 6.90 (d, J = 8.9 Hz, 2H), 7.62 (s, 4H), 7.80 (d, J = 8.9 Hz, 2H).

Triphenylphosphine (7.1 g, 27 mmol) was added to a solution of the derived azide (8.7 g, 21.6 mmol) in THF/water (4:1) (100 mL). The mixture was stirred at room temperature for 5 h and subsequently evaporated. The residue was taken up in methylene chloride and treated with hydrochloric acid in ether. The hydrochloride was filtered off and washed with ether. [4-(6-Aminohexyloxy)phenyl](4-bromophenyl)methanone·hydrochloride (8.0 g, 90%) was obtained: ^{1}H NMR (DMSO-d_6) δ 1.44 (m, 4H), 1.60 (m, 2H), 1.77 (m, 2H), 2.78 (m, 2H), 4.09 (t, J = 6.4 Hz, 2H), 7.09 (d, J = 8.8 Hz, 2H), 7.63 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.77(d, J = 8.5 Hz, 2H), 8.03 (s, br, 3H).

The free amine (1.1 g, 3 mmol) (obtained from the hydrochloride described above by extraction with a $NaHCO_3$ solution/methylene chloride) was suspended in ether (30 mL), and trifluoroacetic anhydride (1.25 mL) was slowly added dropwise at 4 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature for 24 h. The solution was poured into water (100 mL) and neutralized with a $NaHCO_3$ solution. The aqueous phase was separated and extracted with ethyl acetate. The organic phases were washed with brine, dried, and evaporated. The residue was taken up in methylene chloride and filtered off. The filtrate was concentrated, dissolved in methylene chloride, and treated with n-hexane. After filtration of the precipitated crystals, N-[6-[4-(4-bromobenzoyl)-phenoxy)]hexyl]-2,2,2-trifluoroacetamide (**11**) (0.8 g, 56%) was obtained: ^{1}H NMR ($CDCl_3$) δ 1.38−1.60 (m, 4H), 1.64 (m, 2H), 1.83 (m, 2H), 3.39 (q, J = 6.8 Hz, 2H), 4.04 (t, J = 6.4 Hz, 2H), 6.42 (s, br, 1H), 6.94 (d, J = 8.9 Hz, 2H), 7.62 (s, 4H), 7.79 (d, J = 8.9 Hz, 2H).

At −20 °C, **11** (0.65 g, 1.4 mmol) was added to a suspension of sodium hydride (55% in oil, 0.08 g, 1.7 mmol) in DMF (20 mL). The mixture was stirred for 30 min at that temperature. Subsequently, methyl iodide (0.24 g, 1.7 mmol) was added at room temperature and the mixture was stirred for 1 h. The reaction mixture was treated with an NH_4Cl solution, adjusted to pH ∼4 with 1 M hydrochloric acid solution, and extracted with methylene chloride. The organic extracts were dried and concentrated. After chromatography of the residue on silica gel with ethyl acetate/n-hexane (2:8), N-[6-[4-(4-bromobenzoyl)-phenoxy)]hexyl]-N-methyl-2,2,2-trifluoroacetamide (0.45 g, 65%) was obtained: ^{1}H NMR ($CDCl_3$) δ 1.40, 1.54, 1.64, 1.83 (4m, 8H), 3.02, 3.12 (2s, 3H), 3.45 (m, 2H), 4.04 (t, J = 6.4 Hz, 2H), 6.94 (d, J = 8.9 Hz, 2H), 7.62 (s, 4H), 7.78 (d, J = 8.9 Hz, 2H).

Analogous to the hydrolysis of **9a**, (4-bromophenyl)-[4-(6-methylaminohexyloxy)phenyl]methanone (**12**) was obtained from N-[6-[4-(4-bromobenzoyl)phenoxy)]hexyl]-N-methyl-2,2,2-trifluoroacetamide in a yield of 89%. For the in vitro test, it was converted to the **12**·HCl following the general procedure for the formation of hydrochlorides: ^{1}H NMR of free amine ($CDCl_3$) δ 1.50 (m, 6H), 1.83 (m, 2H), 2.04 (s, br, 1H), 2.46 (s, 3H), 2.62 (d, J = 7.1 Hz, 2H), 4.04 (t, J = 6.4 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 7.62 (s, 4H), 7.78 (d, J = 8.8 Hz, 2H), MS m/z 389 ($M + H^+$). Anal. ($C_{20}H_{24}BrNO_3$) C, H, N, Br.

(4-Bromophenyl)[4-[6-(cyclopropylmethylmethylamino)hexyloxy]phenyl]methanon·Fumarate (1:1) (13·Fumarate). A mixture of **12** (0.1 g, 0.26 mmol), diisopropylethylamine (0.13 mL, 0.78 mmol), and bromomethylcyclopropane (0.06 mL, 0.63 mmol) in DMA (20 mL) was stirred at 50 °C for 24 h and concentrated, and the residue was treated with a $NaHCO_3$ solution and extracted with methylene chloride. The organic phases were washed with brine, dried, and evaporated. The residue was purified over silica gel with methylene chloride/MeOH/NH_4OH (94:5.4:0.6 to 85:12.5:2.5) to obtain **13** (0.77 g, 76%), which was converted into **13**·fumarate, following the general procedure for the formation of fumarates: ^{1}H NMR of the free amine ($CDCl_3$) δ 0.12, 0.53 (2m, 4H), 0.88 (m, 1H), 1.40, 1.52, 1.82 (3m, 8H), 2.25 (d, J = 6.5 Hz, 2H), 2.32 (s, 3H), 2.42 (d, J = 7.1 Hz, 2H), 4.04 (t, J = 6.4 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 7.62 (s, 4H), 7.78 (d, J = 8.8 Hz, 2H); MS m/z 443 (M^+, 1Br). Anal. ($C_{24}H_{30}BrFNO_2 \cdot C_4H_4O_4$) C, H, N.

6-[6-(Allylmethylamino)hexyloxy]pyridin-3-yl](4-bromophenyl)methanone·Fumarate (1:1) (15·Fumarate). 1-Bromo-6-hexanol (1 mL, 7.6 mmol) was taken up in DMA (26 mL), treated with N-allylmethylamine (1.47 mL, 15.3 mmol) and stirred at room temperature for 16 h. The reaction mixture was concentrated, and the residue was lyophilized overnight. 6-(Allylmethylamino)hexan-1-ol·hydrobromide (1.9 g, 100%) was obtained as the crude product: ^{1}H NMR of the HBr salt (DMSO-d_6) δ 1.23−1.50 (m, 4H), 1.58−1.75 (m, 2H), 2.70 (s, 3H), 2.95−3.10 (m, 2H), 3.30−3.46 (m, 2H), 3.70−3.82 (m, 2H), 4.32−4.45 (m, 1H), 5.46−5.62 (m, 2H), 5.88−6.08 (m, 1H), 9.70 (m, 1H), MS m/z 171 (M^+).

6-Chloronicotinic acid (**III**) (10 g, 63.4 mmol) was dissolved in thionyl chloride (50 mL), and the mixture was refluxed for 3.5 h. The solution was cooled, and the excess thionyl chloride was removed under reduced pressure. The crude product was taken up in methylene chloride (60 mL) and treated with N,O-dimethylhydroxylamine·hydrochloride (7.22 g, 73.96 mmol). Triethylamine (25 mL) in methylene chloride (30 mL) was added while cooling with ice, and the solution was stirred at room temperature for 1.5 h. The suspension was filtered, and the filtrate was washed with a diluted NaOH solution and brine and dried (Na_2SO_4). After concentration of the solution, crude 6-chloro-N-methoxy-N-methylnicotinamide (13.12 g, quantitative) was obtained: ^{1}H NMR ($CDCl_3$) δ 3.39 (s, 3H), 3.56 (s, 3H), 7.39 (d, 1H), 8.02 (dd, 1H), 8.78 (d, 1 H).

This amide (6.7 g, 33.3 mmol) in THF (40 mL) was added dropwise at −78 °C to a solution of n-BuLi (1.6M in n-hexane, 40 mL, 64 mmol) and 1,4-dibromobenzene (15 g, 63.58 mmol) in THF (140 mL), which was previously stirred at −78 °C (20 min). The solution was stirred at −78 °C for 2 h and at 0 °C for 1 h and then treated with 2 M HCl (60 mL). The phases were separated, the aqueous phase was extracted with ether (50 mL), and the organic phases were washed with a $NaHCO_3$ solution and brine and dried. The crude product was purified over silica gel with ethyl acetate/n-hexane (6:1) and recrystallized from ethyl acetate/n-hexane. (4-Bromophenyl)(6-chloropyridin-3-yl)methanone (**14**) (6.75 g, 68%) was obtained: mp 127.4 °C; IR (KBr) 1652, 1582, 1458, 851 cm^{-1}; ^{1}H NMR (DMSO) δ 7.68−7.88 (m, 5H), 8.17 (dd, 1H), 8.72 (d, 1 H); MS m/z 295 (M^+, 1Br).

14 (1.11 g, 3.74 mmol), 6-(allylmethylamino)hexan-1-ol (696 mg, 3.96 mmol) (the hydrobromide described above was converted into the free base with methylene chloride/$NaHCO_3$ solution), KOH (880 mg, 15.6 mmol), K_2CO_3 (552 mg, 3.96 mmol), and dicyclohexano-18-crown-6 (200 mg, 0.51 mmol) were dissolved in toluene (50 mL), and the mixture was heated to 80 °C overnight. The suspension was treated with additional 6-(allylmethylamino)hexan-1-ol (323 mg, 1.87 mmol) and heated for a further 5 h. The reaction mixture was treated with water and extracted with methylene chloride. The organic phases were washed with a $NaHCO_3$ solution and brine and dried. The crude product obtained was chromatographed in methylene chloride/MeOH (95:5). **15** (1.02 g) was obtained as a yellow-brown oil, which was dissolved in ethanol (15 mL) and treated with fumaric acid (261 mg, 2.25 mmol) in ethanol (5 mL). The solution was stirred at room temperature for 1 h and concentrated, and the oil was evaporated several times and lyophilized. **15**·fumarate (1.2 g, 59%) was obtained as a yellow oil: IR (KBr) 2509, 1707, 1652, 1593, 1394, 1284, 982, 845 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.25−1.55 (m, 6H), 1.74 (m,

2H), 2.26 (s, 3H), 2.44−2.54 (m, 2H), 3.14 (d, J = 5 Hz, 2H), 4.36 (t, J = 5 Hz, 2H), 5.21 (d, J = 9 Hz), 5.27 (d, J = 17.5 Hz), 5.83 (m, 1H), 6.58 (s, 2H), 6.96 (d, 1H), 7.63−7.85 (m, 4H), 8.07 (dd, 1H), 8.53 (d, 1H); MS m/z 430 (M^+, 1Br). Anal. ($C_{22}H_{27}N_2O_2Br \cdot C_4H_4O_4$) C, H, N, Br.

Allyl[6-[4-(4-bromophenyl)-2*H*-chromen-7-yloxy]hexyl]methylamine (17). 8c (1.0 g, 2.34 mmol) in THF/ether (1:1) (9 mL) was added dropwise over a period of 45 min to vinylmagnesium chloride solution (1.7 M in THF, 4.8 mL, 8.19 mmol) at 0 °C. The solution was left to warm to room temperature overnight, treated with acetic acid/water (1:1) (3 mL), and worked up with $NaHCO_3$ solution/methylene chloride. After the mixture was dried (Na_2SO_4), the organic phase was concentrated and the residue was purified over silica gel with methylene chloride/methanol (95:5). (*RS*)-5-[6-(Allylmethylamino)hexyloxy]-2-[1-(4-bromophenyl)-1-hydroxyallyl]-phenol (**16**) (0.82 g, 73%) was isolated: mp 78 °C (with decomposition); IR (KBr) 1621, 1584, 1508 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.21−1.51 (m, 6H), 1.60−1.76 (m, 2H), 2.14 (s, 3H), 2.32 (t, J = 6.6 Hz, 2H), 2.98 (d, J = 6.0 Hz, 2H), 3.86 (t, J = 6.0 Hz, 2H), 5.09−5.25 (m, 4H), 5.71−5.91 (m, 1H), 6.29 (d, J = 3 Hz, 1H), 6.35 (dd, J = 8.4 Hz, J = 3 Hz, 1H), 6.55 (dd, J = 16.8 Hz, J = 11.4 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.18 and 7.48 (AA′-BB′-system, J_{AB} = 8.5 Hz, 4H); MS m/z 474 ($M + H^+$, 1Br). Anal. ($C_{25}H_{32}NO_3Br$) C, H, N.

A solution of **16** (0.26 g, 0.5 mmol) in *o*-xylene (50 mL) was boiled at 170 °C in a Dean−Stark apparatus for 2 h and evaporated, and the residue was purified over silica gel with methylene chloride/methanol 95:5 to give **17** (0.18 g, 72%) as a yellow oil: IR 1612, 1566 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.23−1.50 (m, 6H), 1.60−1.75 (m, 2H), 2.12 (s, 3H), 2.31 (t, J = 10.8 Hz, 2H), 2.95 (d, J = 6 Hz, 2H), 3.92 (t, J = 6 Hz, 2H), 4.75 (d, J = 3.6 Hz, 2H), 5.08−5.25 (m, 2H), 5.70−5.81 (m, 2H), 6.44−6.52 (m, 2H), 6.81 (d, J = 9 Hz, 1H), 7.28 and 7.65 (AA′BB′, J = 9 Hz, 4H); MS m/z 456 ($M + H^+$, 1Br). Anal. ($C_{24}H_{30}N_3OBr$) C, H, N.

Allyl[6-[4-(4-bromophenyl)quinazolin-7-yloxy]hexyl]-methylamine·Fumarate (1:1) (18·Fumarate). In analogy to Uff et al.,[37] a solution of **8d** (222 mg, 0.5 mmol) in formic acid (0.5 mL) and formamide (2 mL) was boiled at 165 °C for 25 min and then concentrated and converted into the free amine (title compound) with methylene chloride/$NaHCO_3$ solution. After purification (silica gel, methylene chloride/methanol 2.5−10%), **18** (103 mg, 0.23 mmol) was dissolved in methylene chloride/ether and treated with fumaric acid (23.7 mg, 0.20 mmol). After the mixture was stirred overnight and filtered, **18**·fumarate (30 mg, 11%) was obtained: mp 90−95 °C; IR 1706, 1614, 1590, 1569 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.25−1.62 (m, 6H), 1.72−1.92 (m, 2H), 2.31 (s, 3H), 2.50−2.55 (m, 2H), 3.21 (d, J = 6 Hz, 2H), 4.24 (t, J = 6 Hz, 2H), 5.20−5.34 (m, 2H), 5.74−5.94 (m, 1H), 6.60 (s, 2H), 7.32 (dd, J = 9 Hz, J = 2.4 Hz, 1H), 7.45 (d, J = 2.4 Hz, 1H), 7.72 and 7.85 (AA′BB′, J = 8.4 Hz, 4H), 7.94 (d, J = 9 Hz, 1H), 9.22 (s, 1H); MS m/z 454 ($M + H^+$, 1Br). Anal. ($C_{24}H_{28}N_3OBr \cdot C_4H_4O_4$) C, H, N.

Allyl[6-[3-(4-bromophenyl)-1-methyl-1*H*-indazol-6-yloxy]-hexyl]methylamine·Fumarate (1:1.6) (19·Fumarate). A mixture of methylhydrazine (4.61 mL, 87.5 mmol) and K_2CO_3 (1.45 g, 10.5 mmol) was stirred in DMA (40 mL) at room temperature. **1** (3.92 g, 8.75 mmol) in DMA (30 mL) was added, and the mixture was heated for 1.5 h at 120 °C. After filtration and concentration at 80 °C/0.3 Torr, the residue was taken up in methylene chloride, filtered over Na_2SO_4, and concentrated. The residue was dissolved in ethanol, treated with fumaric acid (1.02 g, 8.75 mmol), and stirred. After the mixture was filtered and dried (Na_2SO_4), **19**·fumarate (1:1.6) (3.1 g, 78%) was obtained: mp 135−137 °C, dec; IR 1705, 1623 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28−1.60 (m, 6H), 1.65−1.80 (m, 2H), 2.25 (s, 3H), 2.35−2.60 (m, 2H), 3.13 (d, J = 5.5 Hz, 2H), 4.04 (s, 3H), 4.08 (d, J = 5.5 Hz, 1H), 5.20−5.30 (m, 2H), 5.76−5.95 (m, 1H), 6.58 (s, 2H), 6.87 (dd, J = 9 Hz, J = 1 Hz, 1H), 7.20 (d, J = 1, 1H), 7.67 and 7.90 (AA′BB′, J = 8 Hz, 4H), 7.91 (d, J = 9 Hz, 1H); MS m/z 456 ($M + H^+$, 1Br). Anal. ($C_{24}H_{30}N_3OBr \cdot 1.6C_4H_4O_4$) C, H, N, Br.

Allyl[6-[3-(4-bromophenyl)benzo[*d*]isoxazol-6-yloxy]-hexyl]methylamine (21). A mixture of (4-bromophenyl)(2,4-dihydroxyphenyl)methanone (**5i**) (4.25 g, 14.5 mmol) (synthesized from 1,3-dimethoxybenzene and 4-bromobenzoyl chloride followed by deprotection with HBr in AcOH; see general procedure A, **5b**), hydroxylamine·hydrochloride (3.22 g, 46.4 mmol), and sodium acetate (2.85 g, 34.8 mmol) in ethanol (100 mL) was refluxed. The reaction mixture was concentrated, treated with a $NaHCO_3$ solution (100 mL), and extracted with ethyl acetate. The organic extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was dissolved in ethanol (200 mL), treated with *p*-toluenesulfonic acid (1.1 g, 5.8 mmol), and heated to 85 °C for 24 h. The reaction mixture was concentrated, and the residue was redissolved in ethyl acetate (250 mL) and washed with a $NaHCO_3$ solution and with brine. The organic phase was dried (Na_2SO_4) and evaporated. 3-(4-Bromophenyl)benzo[*d*]isoxazol-6-ol (**20**) (3.7 g, 98%) was obtained: ^1H NMR (DMSO-d_6) δ 6.87 (dd, J_1 = 8.6 Hz, J_2 = 2.3 Hz, 1H), 7.10 (d, J = 2.3 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.79 (d, J = 8.58 Hz, 2H), 8.05 (d, J = 8.58 Hz, 2H), 9.95 (s, br, ArOH, 1H); MS m/z 289 ($M + H^+$, 1Br).

A mixture of **20** (1.82 g, 6.27 mmol), 1,6-dibromohexane (2.96 mL, 18.82 mmol), and K_2CO_3 (2.6 g, 18.82 mmol) in acetone (80 mL) was stirred at 65 °C for 18 h. The reaction mixture was filtered, concentrated, and purified by chromatography (flash column on silica gel, toluene). 6-(6-Bromohexyloxy)-3-(4-bromophenyl)benzo[*d*]isoxazole (3.27 g, quantitative) was obtained: ^1H NMR ($CDCl_3$) δ 1.47−1.62 (m, 4H), 1.79−1.98 (m, 4H), 3.44 (t, J = 6.4 Hz, 2H), 4.03 (t, J = 6 Hz, 2H), 6.96 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.3 Hz), 7.10 (1H, d, J = 2.3 Hz), 7.61 (1H, d, J = 8.8 Hz), 7.65 (d, J = 8.7 Hz, 2H), 8.08 (d, J = 8.7 Hz, 2H); MS m/z 451 (M^+, 2Br).

This compound (2.0 g, 4.41 mmol) was dissolved in acetone (40 mL) and treated at room temperature with *N*-allylmethylamine (1.27 mL, 13.24 mmol) and K_2CO_3 (1.83 g, 13.24 mmol). After 6 h, additional *N*-allylmethylamine (4.22 mL, 44.13 mmol) was added. The mixture was stirred at room temperature for 29 h, filtered, concentrated, and chromatographed over silica gel with toluene/acetone (8:2) and 1% of triethylamine as eluent. **21** (0.99 g, 51%) was obtained, which was converted to the fumarate **21**·fumarate, following the general procedure for the formation of fumarates: ^1H NMR (DMSO-d_6) δ 1.30−1.52 (6H, m), 1.78 (2H, m), 2.20 (3H, s), 2.43 (2H, m), 3.08 (2H, m), 4.08 (2H, m), 5.15−5.26 (2H, m), 5.82 (1H, m), 6.57 (2H, s), 7.02 (1H, dd, J_1 = 8.8 Hz, J_2 = 2.3 Hz), 7.42 (1H, d, J = 2.3 Hz), 7.71 (1H, d, J = 8.8 Hz), 7.83 (2H, d, J = 8.6 Hz), 8.08 (2H, d, J = 8.6 Hz); MS m/z 443 ($M + H^+$, 1Br). Anal. ($C_{23}H_{27}N_2O_2Br \cdot 0.9C_4H_4O_4$) C, H, N.

General Procedure C. Allyl[6-[3-(4-bromophenyl)benzo[*d*]isothiazol-6-yloxy]hexyl]methylamine·Fumarate (1:1) (24·Fumarate). Potassium *tert*-butylate (4.3 g, 38.3 mmol) was dissolved in THF (160 mL) and treated slowly with benzyl mercaptan (4.5 mL, 38.3 mmol).[39] The suspension was stirred at room temperature for 30 min and then treated with **4f** (10 g, 32.35 mmol) in THF (200 mL). The solution was stirred at room temperature (1.5 h) and treated with NH_4Cl solution (100 mL) as well as a $NaHCO_3$ solution (200 mL). The phases were separated, the aqueous phase was extracted with ethyl acetate, and the organic phases were washed with a $NaHCO_3$ solution and brine and dried (Na_2SO_4). (2-Benzylsulfanyl-4-methoxyphenyl)(4-bromophenyl)methanone (15.8 g, quantitative) was obtained, which was taken up in methylene chloride (160 mL), treated with sulfuryl chloride (3.4 mL, 41.9 mmol), and stirred at room temperature (1 h). After distillation of the sulfuryl chloride, the residue was taken up in THF (160 mL), treated with a saturated ammonia solution in ethanol (120 mL), and stirred at room temperature overnight. A 10% $NaHCO_3$ (100 mL) solution was added. The solvent was removed, and the residue was again taken up in $NaHCO_3$ solution and ethyl acetate. The phases were separated, and the aqueous phase was extracted with ethyl acetate and ether. The organic phases were washed with brine and dried (Na_2SO_4). Recrystallization from ethyl acetate/ethanol yielded 3-(4-bro-

mophenyl)-6-methoxy[*d*]benzisothiazole (**22**) (7.52 g, 73%): IR (KBr) 2840, 1600, 1499, 1236, 1124, 1017, 829 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.94 (s, 3H), 7.07 (dd, J = 8.8 Hz, 1H), 7.36 (d, 1H), 7.62−7.78 (m, 4H), 7.97 (d, J = 9 Hz, 1H); MS m/z 318 (M^+, 1Br).

22 (6.32 g, 19.7 mmol) was dissolved in methylene chloride (395 mL), and the mixture was cooled to −78 °C and treated with a boron tribromide solution (1 M in methylene chloride, 49.3 mL, 49.3 mmol). The solution was thawed overnight and then stirred at room temperature. The solution was added to a $NaHCO_3$ solution, the phases were separated, and the aqueous phase was extracted with methylene chloride (150 mL). The organic phases were washed with brine, dried (Na_2SO_4), and evaporated. (4-Bromophenyl)benzo[*d*]isothiazol-6-ol (1.5 g, 25%) was crystallized from the crude product using methylene chloride as solvent. The mother liquor was concentrated, dissolved in methylene chloride (320 mL), and treated at −78 °C with boron tribromide solution (1 M in methylene chloride, 40 mL, 40 mmol). After workup and crystallization, further 3-(4-bromophenyl)benzo[*d*]isothiazol-6-ol (2.24 g, 37%) was obtained: ^1H NMR (DMSO-d_6) δ 7.04 (dd, J = 11 Hz, 1H), 7.51 (d, 1H), 7.79 (m, 4H), 8.0 (d, J = 8.9 Hz, 1H), 10.38 (s, 1H).

This compound (3.0 g, 9.8 mmol) was treated as discribed for **6e** in general procedure B to give 6-(6-bromohexyloxy)-3-(4-bromophenyl)benzo[*d*]isothiazole (**23**) (4.18 g, 91%) as a pale-brown substance: ^1H NMR ($CDCl_3$) δ 1.56 (m, 4H), 2.9 (m, 4H), 3.44 (t, 3H), 4.08 (t, 3H), 7.07 (dd, 1H), 7.47 (d, 1H), 7.69 (m, 4H), 7.96 (d, J = 9 Hz, 1H), 10.38 (s, 1H).

23 (4.15 g, 8.84 mmol) was treated as described for **7b** in general procedure A to give colorless crystals of **24**·fumarate (3.43 g, 67%): mp 122−123.5 °C; IR (KBr) 2591, 2504, 1671, 1589, 1391, 1237, 960 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31−1.58 (m, 4H), 1.66 (m, 2H), 1.81 (m, 2H), 2.69 (s, 3H), 3.05 (t, 2H), 3.70 (d, 2H), 4.12 (t, 2H), 5.50 (d, 1H), 5.54 (d, 1H), 5.90 (m, 1H), 6.62 (s, 2H), 7.15 (dd, J = 8.9 Hz, 1H), 7.80 (m, 5H), 8.06 (d, J = 9 Hz, 1H); MS m/z 459 ($M + H^+$, 1Br). Anal. ($C_{23}H_{27}N_2OBr{\cdot}C_4H_4O_4$) C, H, N, Br, S.

Allyl[6-[4-(6-bromobenzo[*d*]isothiazol-3-yl)phenoxy]hexyl]methylamine·Fumarate (1:1) (31·Fumarate). **31**·fumarate was synthesized from 4-bromo-2-fluorobenzoyl chloride and anisole (**Id**) via (4-bromo-2-fluorophenyl)(4-methoxyphenyl)methanone (**30**) following general procedure A (63% yield) and general procedure C (38% yield): mp 134−135 °C; IR (KBr) 2643, 1696, 1608, 1581, 1516, 1462, 1252, 840 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28−1.58 (m, 6H), 1.76 (m, 2H), 2.24 (s, 3H), 2.38−2.58 (m, 2H), 3.10 (d, J = 5 Hz, 2H), 4.06 (t, J = 8 Hz, 2H), 5.18 (d, J = 9 Hz, 1H), 5.25 (d, J = 15.4 Hz, 1H), 5.82 (m, 1H), 6.57 (s, 2H), 7.13 (d, J = 8.7 Hz, 2H), 7.70 (dd, J = 8.7 Hz, 1H), 7.81 (d, J = 8.7 Hz, 2H), 8.12 (d, J = 8.7 Hz, 1H), 8.61 (dd, 1H), 13.0 (s, br, 2H); MS m/z 458 (M^+, 1Br). Anal. ($C_{23}H_{27}N_2OBrS{\cdot}2C_4H_4O_4$) C, H, N, Br, S.

Allyl[6-[4-(6-bromo-1,1-dioxobenzo[*d*]isothiazol-3-yl)-phenoxy]hexyl]methylamine·Fumarate (1:1) (26·Fumarate). **23** (2.34 g, 4.99 mmol) was taken up in methylene chloride (40 mL) and treated with potassium permanganate (8.5 g, 5.38 mmol) adsorbed on silica gel.[46] The suspension was stirred at room temperature and then treated with Na_2SO_4 and filtered through silica gel. After concentration, the crude product was crystallized from ethyl acetate and *n*-hexane to give 6-(6-bromohexyloxy)-3-(4-bromophenyl)benzo[*d*]isothiazole 1,1-dioxide (**25**) (650 mg, 28%) and starting material **23** (1.23 g, 52%). **25**: ^1H NMR ($CDCl_3$) δ 1.54 (m, 4H), 1.9 (m, 4H), 3.44 (t, 2H), 4.13 (t, 2H), 7.14 (dd, 1H), 7.47 (d, 1H), 7.67−7.77 (m, 3H), 7.84 (d, 2H); MS m/z 499 (M^+, 1Br).

Analogous to general procedure A for compound **7b**, **26**·fumarate was isolated as an oil in a yield of 55%: IR (KBr) 2640, 1714, 1604, 1169, 1011, 963, 844 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33−1.59 (m, 4H), 1.68 (m, 2H), 1.85 (m, 2H), 2.50 (s, 3H), 2.74 (m, 2H), 3.40 (d, J = 8 Hz, 2H), 4.10 (t, J = 6 Hz, 2H), 5.32 (d, J = 6 Hz, 1H), 5.36 (d, J = 10 Hz, 1H), 5.93 (m, 1H), 6.79 (s, 2H), 7.16 (dd, 1H), 7.46 (d, 1H), 7.69−7.77 (m, 3H), 7.79−7.86 (m, 2H)); MS m/z 491 ($M + H^+$, 1Br). Anal. ($C_{23}H_{27}N_2O_3BrS{\cdot}C_4H_4O_4$) C, H, N, Br, S.

Allyl[6-[3-(4-bromophenyl)benzo[*b*]thiophen-6-yloxy]-hexyl]methylamine·Fumarate (1:1) (29·Fumarate). Potassium *tert*-butylate (2.55 g, 22.7 mmol) was placed in THF (15 mL), and the solution was treated with **4e** (2.50 g, 7.4 mmol) in THF (12 mL). The mixture was heated under reflux and then treated with water and NH_4Cl solution. The water phases were extracted with ethyl acetate. The organic phases were washed with a $NaHCO_3$ solution and brine and dried (Na_2SO_4). The crude product obtained after concentration (2.08 g) was dissolved in toluene (14 mL) and treated with trifluoroacetic acid (6 mL) at 0 °C (2 h). The mixture was neutralized with a $NaHCO_3$ solution. The aqueous phase was extracted with ethyl acetate. The organic phases were washed with a $NaHCO_3$ solution and brine and dried (Na_2SO_4). The crude product obtained was purified by column chromatography (silica gel, ethyl acetate/*n*-hexane 1:6) and crystallized from ethyl acetate/*n*-hexane. 3-(4-Bromophenyl)-6-methoxybenzo-[*b*]thiophene (**27**) (476 mg, 20%) was obtained as white crystals: mp 102.5−104.5 °C; IR (KBr) 2830, 1601, 1460, 1267, 1049, 825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.89 (s, 3H), 7.02 (dd, J = 8.9, 2.4 Hz, 1H), 7.21 (s, 1H), 7.37 (d, J = 2.4 Hz, 1H), 7.43 (m, 2H), 7.60 (m, 2H), 7.72 (d, J = 8.9 Hz, 1H); MS m/z 318 (M^+, Br).

Analogous to general procedure C, **29**·fumarate was obtained from **27** in 35% yield: IR (KBr) 2496, 1704, 1270, 1233, 1068, 984, 824 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.20−1.58 (m, 6H), 1.76 (m, 2 H), 2.25 (s, 3H), 2.38−2.56 (m, 2H), 3.12 (d, J = 6.4 Hz, 2H), 4.05 (t, J = 5 Hz, 2 H), 5.19 (d, J = 10 Hz, 1H), 5.25 (d, J = 17.5 Hz, 1H), 5.82 (m, 1H), 6.58 (s, 2H), 7.05 (dd, J = 8.9 Hz, 1H), 7.5−7.77 (m, 2H), 13.0 (s, br, 2H); MS m/z 457 ($M + H^+$, Br). Anal. ($C_{24}H_{28}NOBrS{\cdot}C_4H_4O_4$) C, H, N, S.

Allyl[6-[3-(4-bromophenyl)benzofuran-6-yloxy]hexyl]-methylamine·Fumarate (34·Fumarate). 4-Bromophenacyl bromide (11.5 g, 41.5 mmol) was dissolved in acetone (250 mL), and after the addition of K_2CO_3 (5.75 g, 41.5 mmol) and 3-methoxyphenol (4.5 mL, 41.5 mmol), the reaction mixture was stirred at room temperature for 18 h and the mixture was subsequently filtered. The filtrate was concentrated and chromatographed over silica gel with ethyl acetate/*n*-hexane (5:95 to 10:90). 1-(4-Bromophenyl)-2-(3-methoxyphenoxy)ethanone (**32**) (7.3 g, 55%) was isolated as an off-white solid: ^1H NMR ($CDCl_3$) δ 3.78 (s, 3H), 5.19 (s, 2H), 6.50−6.57 (m, 3H), 7.19 (t, J = 8.8 Hz, 1H), 7.65 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.6 Hz, 2H); MS m/z 320 (M^+, 1Br).

32 (5.0 g, 15.6 mmol) was dissolved in polyphosphoric acid (53 g), and the mixture was heated for 1.5 h at 80 °C. The mixture was then adjusted to pH ∼8 with a Na_2CO_3 solution, cooled, and subsequently extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4) and concentrated. 3-(4-Bromophenyl)-6-methoxybenzofuran (**33**) (4.6 g, 98%) was obtained: ^1H NMR ($CDCl_3$) δ 3.88 (s, 3H), 6.95 (dd, J_1 = 8.6 Hz, J_2 = 2.1 Hz, 1H), 7.07 (d, J = 2.1 Hz, 1H), 7.49 (d, J = 8.4 Hz, 2H), 7.58 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.6 Hz, 1H), 7.70 (s, 1H); MS m/z 302 (M^+, 1Br).

33 (3.0 g, 9.9 mmol) in methylene chloride (100 mL) was treated at 0 °C with a boron tribromide solution (1 M in methylene chloride, 100 mL, 100 mmol). The ice bath was removed, and the reaction mixture was stirred for 4 h, subsequently poured into a Na_2CO_3 solution, and extracted with methylene chloride. The organic phases were washed with brine, dried (Na_2SO_4), and evaporated to give 3-(4-bromophenyl)benzofuran-6-ol (2.83 g, 99%): ^1H NMR ($CDCl_3$) δ 6.86 (dd, J_1 = 8.6 Hz, J_2 = 2.1 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.6 Hz, 1H), 7.69 (s, 1H); MS m/z 288 (M^+, 1Br).

Analogous to general procedure B, **34** was obtained in a total yield of 37% and the compound was converted into **34**·fumarate, following the general procedure for the formation of fumarates: ^1H NMR (DMSO-d_6) δ 1.37 (m, 2H), 1.50 (m, 4H), 1.75 (m, 2H), 2.28 (s, 3H), 2.50 (m, 2H), 3.17 (d, J = 6.6 Hz, 2H), 4.03 (t, J = 6.5 Hz, 2H), 5.20−5.30 (m, 2H), 5.77−5.92 (m, 1H), 6.57 (s, 2H), 6.97 (dd, J_1 = 8.6 Hz, J_2 = 2.1 Hz, 1H), 7.27 (d, J = 2.1 Hz, 1H), 7.68 (s, 4H), 7.77 (d, J = 8.6 Hz,

1H), 8.31 (s, 1H); MS *m*/*z* 442 (M + H$^+$, 1Br). Anal. ($C_{24}H_{28}BrNO_2 \cdot C_4H_4O_4$) C, H, N, Br.

Allyl[6-[1-(4-bromophenyl)-3,4-dihydroisoquinolin-6-yloxy]hexyl]methylamine·Fumarate (37·Fumarate). To a solution of 4-bromobenzoyl chloride (43.9 g, 200 mmol) in methylene chloride (500 mL) was added dropwise 2-(3-methoxyphenyl)ethylamine (29.2 mL, 200 mmol) at 0 °C followed by the addition of diisopropylethylamine (34.2 mL, 200 mmol). The reaction mixture was left to warm to room temperature and stirred for 26 h, subsequently diluted with methylene chloride and treated with a $NaHCO_3$ solution. The organic phase was washed with a $NaHCO_3$ solution and brine, dried (Na_2SO_4), and concentrated. 4-Bromo-*N*-[2-(3-methoxyphenyl)-ethyl]benzamide (**35**) (66.1 g, 99%) was obtained: ^{1}H NMR ($CDCl_3$) δ 2.91 (t, J = 6.8 Hz, 2H), 3.71 (q, J = 6.8 Hz, 2H), 3.79 (s, 3H), 6.11 (s, br, 1H), 6.78−6.84 (m, 3H), 7.25 (t, J = 7.8 Hz, 1H), 7.55 (s, 4H); MS *m*/*z* 333 (M$^+$, 1Br).

35 (50 g, 150 mmol) was dissolved in acetonitrile (750 mL) and treated under argon with phosphorus oxychloride (55 mL, 598 mmol). After the mixture was heated at 80 °C for 4 h, the solvent was removed, adjusted to pH >12 with concentrated ammonia solution and extracted with ethyl acetate. The organic extracts were washed with brine, dried, and concentrated. 1-(4-Bromophenyl)-6-methoxy-3,4-dihydroisoquinoline (**36**) (46.4 g, 98%) was obtained: ^{1}H NMR ($CDCl_3$) δ 2.77 (m, 2H), 3.81 (m, 2H), 3.86 (s, 3H), 6.75 (dd, J_1 = 8.5 Hz, J_2 = 2.4 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 7.16 (d, J = 8.5 Hz, 1H), 7.47 (d, J = 8.7 Hz, 2H), 7.56 (d, J = 8.7 Hz, 2H); MS *m*/*z* 314 (M$^+$, 1Br).

Treatment of **36** (15 g, 47.4 mmol) in acetic acid (95 mL) with 62% aqueous HBr solution (65 mL) at 100 °C (46 h) (analogous to general procedure A, compound **5b**) gave 1-(4-bromophenyl)-3,4-dihydroisoquinolin-6-ol (13.7 g, 96%): ^{1}H NMR ($CDCl_3$) δ 2.72 (m, 2H), 3.75 (m, 2H), 4.83 (s, br, 1H), 6.56 (m, 2H), 7.03 (d, J = 8.5 Hz, 1H), 7.41 (d, J = 8.5 Hz, 2H), 7.54 (d, J = 8.5 Hz, 2H); MS *m*/*z* 301 (M$^+$, 1Br).

Analogous to general procedure B for compound **7e**, **37** was obtained in a yield of 50% and converted into the **37**·fumarate, following the general procedure for the formation of fumarates: ^{1}H NMR of free base ($CDCl_3$) δ 1.35−1.59 (m, 6H), 1.82 (m, 2H), 2.21 (s, 3H), 2.34 (m, 2H), 2.76 (m, 2H), 2.99 (d, J = 6.5 Hz, 2H), 3.80 (m, 2H), 4.00 (t, J = 6.5 Hz, 2H), 5.11−5.20 (m, 2H), 5.87 (m, 1H), 6.73 (dd, J_1 = 8.5 Hz, J_2 = 2.1 Hz, 1H), 6.78 (d, J = 2.1 Hz, 1H), 7.14 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H); MS *m*/*z* 455 (M + H$^+$, 1Br). Anal. ($C_{25}H_{31}BrN_2O$) C, H, N, Br.

Allyl[6-[1-(4-bromophenyl)isoquinolin-6-yloxy]hexyl]-methylamine·2HCl (39·2HCl). 36 (10 g, 31.6 mmol) was dissolved in toluene (500 mL), treated with anhydrous Na_2SO_4 (60 g) and γ-manganese dioxide (15 g, 172.5 mmol),[47,48] and boiled under reflux for 24 h. After cooling, the reaction mixture was filtered and concentrated to give 1-(4-bromophenyl)-6-methoxyisoquinoline (**38**) (9.2 g, 92%) as an off-white solid: ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3H), 7.13 (d, J = 2.1 Hz, 1H), 7.17 (dd, J_1 = 9 Hz, J_2 = 2.2 Hz, 1H), 7.56 (m, 3H), 7.66 (d, J = 8.5 Hz, 2H), 7.94 (d, J = 9 Hz, 1H), 8.52 (d, J = 5.7 Hz, 1H); MS *m*/*z* 313 (M$^+$, 1Br).

38 (8.1 g, 25.8 mmol) was deprotected to give 1-(4-bromophenyl)isoquinolin-6-ol (6.7 g, 86%) following procedure A (compound **5b**): ^{1}H NMR (DMSO-d_6) δ 7.48 (dd, J_1 = 9.2 Hz, J_2 = 2 Hz, 1H), 7.52 (d, J = 2.0 Hz, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.95 (d, J = 8.5 Hz, 2H), 8.15 (d, J = 9.2 Hz, 1H), 8.23 (d, J = 6.6 Hz, 1H), 8.48 (d, J = 6.6 Hz, 1H), 11.75 (s, br, 1H); MS *m*/*z* 298 [(M − H)$^-$, 1Br].

Analogous to general procedure B for compound **7e**, 1-(4-bromophenyl)isoquinolin-6-ol gave **39** in 72% yield as a yellow oil, which was converted into the **39**·2HCl, following the general procedure for the formation of hydrochlorides: ^{1}H NMR as dihydrochloride (DMSO-d_6) δ 1.47 (m, 4H), 1.74 (m, 2H), 1.85 (m, 2H), 2.66 (d, J = 4.9 Hz, 3H), 3.02 (m, 2H), 3.71 (m, 2H), 4.28 (t, J = 6.5 Hz, 2H), 5.47−5.56 (m, 2H), 6.02 (m, 1H), 7.51 (dd, J_1 = 9.3 Hz, J_2 = 2.1 Hz, 1H), 7.75 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 2.1 Hz, 1H), 7.92 (d, J = 8.5 Hz, 2H), 8.01 (d, J = 9.3 Hz, 1H), 8.22 (d, J = 6.4 Hz, 1H), 8.55 (d, J = 6.4 Hz, 1H), 10.89 (s, br, 2H); MS *m*/*z* 453 (M + H$^+$, 1Br). For **39**: Anal. ($C_{25}H_{29}BrN_2O$) C, H, N, Br.

Allyl-6-[4-(4-bromophenyl)-1*H*-benzo[*d*][1,2]oxazin-7-yloxy]hexylmethylamine·Fumarate (43·Fumarate). A solution of (4-bromophenyl)(2-methyl-4-methoxyphenyl)methanone (**4h**) (3 g, 9.8 mmol) (prepared from 3-methylanisole (**Ih**) and 4-bromobenzoyl chloride (**IIb**) by Friedel−Crafts reaction, analogous to general procedure A, compound **4b**) in tetrachloromethane (100 mL) was treated with *N*-bromosuccinimide (2.1 g, 11.8 mmol) and a spatula tip of dibenzoyl peroxide. The reaction mixture was stirred at room temperature for 6 h under irradiation with a Hg lamp TQ 150 (150 W, Heraeus, Hanau, FRG) with light $\lambda \geq$ 390 nM, subsequently diluted with methylene chloride (100 mL), and washed in succession with a $NaHCO_3$ solution and brine. The organic phase was dried (Na_2SO_4), filtered, and immediately reacted. The solution of **40** was added dropwise to a mixture of *tert*-butyl *N*-hydroxycarbamate (2.13 g, 16 mmol) and sodium hydride (55% in oil, 0.38 g, 8.7 mmol) in DMF (30 mL), previously stirred at 0 °C (20 min). The reaction mixture was warmed to room temperature and stirred overnight. The mixture was treated with an NH_4Cl solution (60 mL) and extracted with methylene chloride. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. After chromatography of the residue (silica gel, ethyl acetate/*n*-hexane, 10:90 to 25:75), *tert*-butyl [2-(4-bromobenzoyl)-5-methoxybenzyloxy]carbamate (**41**) (2.65 g, 61%) was obtained: ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 3.89 (3H, s), 5.08 (2H, s), 6.85 (1H, dd, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 7.13 (1H, s, br), 7.21 (1H, d, J = 2.5 Hz), 7.36 (1H, d, J = 8.5 Hz), 7.61 (4H, m). MS *m*/*z* 436 (M + H$^+$, 1Br).

A solution of **41** (1.9 g, 4.36 mmol) was treated with 62% aqueous HBr solution and acetic acid as described in procedure A (compound **5b**) for 3.5 days to give 4-(4-bromophenyl)-1*H*-benzo[*d*][1,2]oxazin-7-ol (**42**) (1.2 g, 88%): ^{1}H NMR ($CDCl_3$) δ 4.97 (s, 2H), 5.52 (s, br, 1H), 6.72 (d, J = 2.5 Hz, 1H), 6.80 (dd, J_1 = 8.4 Hz, J_2 = 2.5 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 2H); MS *m*/*z* 302 [(M − H)$^-$, 1Br].

Analogously to compound **21**, **43** was obtained from **42** in 56% yield. For the in vitro test, it was converted to the **43**·fumarate, following the general procedure for the formation of fumarates: ^{1}H NMR of the free amine ($CDCl_3$) δ 1.35−1.56 (m, 6H), 1.82 (m, 2H), 2.21 (s, 3H), 2.35 (m, 2H), 3.00 (m, 2H), 4.01 (t, J = 6.2 Hz, 2H), 4.98 (s, 2H), 5.11−5.21 (m, 2H), 5.86 (m, 1H), 6.75 (d, J = 2.5 Hz, 1H), 6.84 (dd, J_1 = 8.4 Hz, J_2 = 2.5 Hz, 1H), 7.12 (d, J = 8.4 Hz, 1H), 7.50 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 2H); MS *m*/*z* 457 (M + H$^+$, 1Br). Anal. for **43** ($C_{24}H_{29}BrN_2O_2$) C, H, N, Br.

Biological Assays. Inhibition of Human Liver Microsomal 2,3-Oxidosqualene−Lanosterol Cyclase (OSC). A section of liver from an 18-year-old healthy donor was obtained at the hepatic transplantation unit of Hôpital Bicêtre, Paris, and frozen at −80 °C.[8] Liver microsomes were prepared in sodium phosphate buffer (pH 7.4). The OSC activity was measured in the same buffer, which also contained 1 mM EDTA and 1 mM dithiothreitol. The microsomes were diluted to 0.8 mg/mL protein in cold phosphate buffer. [^{14}C]*R*,*S*-monooxidosqualene (MOS, 12.8 mCi/mmol) was diluted to 20 nCi/μL with ethanol and mixed into phosphate buffer/1% BSA (bovine serum albumin). A stock solution of 1 mM test substance in DMSO was diluted to the desired concentration with phosphate buffer/1% BSA. An amount of 40 μL of microsomes was mixed with 20 μL of a solution of the test substance, and the reaction was subsequently started with 20 μL of the [^{14}C]*R*,*S*-MOS solution. The final conditions were the following: 0.4 mg/mL of microsomal proteins and 30 μL of [^{14}C]*R*,*S*-MOS in phosphate buffer, pH 7.4, containing 0.5% albumin, <0.1% DMSO, and <2% ethanol for a total volume of 80 μL.

After 1 h at 37 °C, the reaction was stopped by the addition of 0.6 mL of 10% KOH/methanol, 0.7 mL of water, and 0.1 mL of *n*-hexane/ether (1:1), which contained 25 μg of nonradioactive MOS and 25 μg of lanosterol as carriers. After shaking, 1 mL of *n*-hexane/ether (1:1) was added to each test

tube, and these were again shaken and then centrifuged. The upper phase was transferred into a glass test tube, and the lower phase was again extracted with *n*-hexane/ether and combined with the first extract. The entire extract was evaporated to dryness with nitrogen, the residue was suspended in 50 μL of *n*-hexane/ether (1:1) and applied to a silica gel plate for chromatographic separation using *n*-hexane/ether (1:1) as the eluent. The R_f values for the MOS substrate and the lanosterol product were 0.91 and 0.54, respectively. After the sample was dried, radioactive MOS and lanosterol were detected on the silica gel plate using a phosphor imager (Molecular Dynamics). The ratio of MOS to lanosterol was determined from the radioactive bands in order to determine the yield of the reaction and OSC inhibition.

Inhibition of Cholesterol Synthesis in Primary Rat Hepatocytes. This assay was performed to determine the cellular activity and the metabolic stability of the compounds prior to testing in hyperlipidemic hamsters. The stability test was performed as a bioassay and to assess the efficacy and potency of each test substance in reducing cholesterol production while increasing monooxidosqualene (MOS) levels. Also, it allowed the measurement of substance stability by comparing the residual inhibitory activity after preincubation for 8 or 24 h or after co-incubation for 2 h. Hepatocytes were isolated from collagenase perfused rat liver and seeded in collagen-coated cell-culture plates in medium containing 10% FCS. At 4 h after seeding, the medium was replaced with medium containing 5% lipoprotein deficient serum (LPDS), and the cells incubated overnight. In one set of wells, hepatocytes were incubated for 2 h with 3 μM test substance in the presence of ^{14}C-acetate to trace cholesterol biosynthesis. In another set of wells, test compound was applied for 8 and 24 h and tracing of cholesterol biosynthesis was performed by adding ^{14}C-acetate to these wells for the last 2 h. At the end of the 2-h labeling period, cellular lipids were extracted twice from each well using 2-propanol followed by *n*-hexane/2-propanol (3:2). After evaporation of solvent under nitrogen, lipids were saponified in KOH/methanol/water, and nonsaponifiable lipids were extracted with *n*-hexane/ether (1:1). After drying under nitrogen, nonsaponifiable lipids were separated by thin-layer chromatography (TLC) using *n*-hexane/ether/acetic acid (60:40:1). Radioactive lipids were identified using radioactive standards running in separate lanes, and radioactivity was quantified using a phosphor imager (Molecular Dynamics). The results are given as the percentage of inhibition of cholesterol synthesis after 2, 8, or 24 h of incubation in the presence of test compound.

Cholesterol Lowering in Fat-Fed Hamsters. Hamsters were treated as described previously.[49] Briefly, male golden hamsters (Fume SPF from BRL, Füllinsdorf, Switzerland) weighing 120−130 g were housed individually with 12 h of alternating periods of light and darkness. Animals had free access to standard rodent chow (12 cal % fat) and to water. During the 7 days preceding drug treatment, hamsters received every morning 9 g of a 40 cal % fat diet as 18 wt % coconut kernel instead of cereals. The daily serving of 9 g of powered fat diet was mixed with 9 mL of water to produce a paste. Then animals were assigned to treatment groups such that the groups had approximately identical average body weights. Each group ($n = 5$) received 9 g of fat diet containing the test compound. The control group ($n = 10$) received only the 9 g of fat diet. First, each test substance was homogenized in water and subsequently mixed with the powered fat diet. Food consumption and body weight were monitored throughout the treatment period. The animals were treated for 10 days with a test substance (200 μmol/(kg·day), corresponding to about 70−90 mg/(kg·day). Blood samples were collected on EDTA via the jugular vein under light isoflurane anesthesia on the last day of the pretreatment (day 0) and 1 day after the last administration of test substance. The total plasma cholesterol concentration was determined using a colorimetric enzymatic method.[50] To calculate the amount of cholesterol in the LDL and HDL fractions, the plasma lipoproteins from 20 μL of plasma aliquots were separated and identified by size-exclusion Superose-6 gel chromatography[51] and total cholesterol was quantified in each 50 μL elution fraction using a fluorometric enzymatic assay.[52] The effect of each compound on plasma total cholesterol and LDL cholesterol is expressed as a percent of day 0 (prior to compound administration) and corrected with respect to the variation of the untreated animals (Table 5).

Acknowledgment. We sincerely thank Daniel Egger, Gisela Flach, Corinne Handschin, Marie-Paule Imhoff, Isabelle Kaufmann, Hans-Jakob Krebs, Anke Kurt, Bettina Roser, and Heidi Stoll for their excellent and dedicated assistance, and Wolf Arnold, André Bubendorf, Walter Meister, Roger Rueher, and Björn Wagner for spectroscopic determination and analysis.

References

(1) Gotto, A. M., Jr. Management of dyslipidemia. *Am. J. Med.* **2002**, *112*, 10S−18S.
(2) Talbert, R. L. New therapeutic options in the National Cholesterol Education Program Adult Treatment Panel III. *Am. J. Manage. Care* **2002**, *8*, S301−S307.
(3) Wilson, P. W. Established risk factors and coronary artery disease: the Framingham Study. *Am. J. Hypertens.* **1994**, *7*, 7S−12S.
(4) Clearfield, M. B. Statins: balancing benefits, efficacy and safety. *Expert Opin. Pharmacother.* **2002**, *3*, 469−477.
(5) Pedersen, T. R.; Olsson, A. G.; Faergeman, O.; Kjekshus, J.; Wedel, H.; Berg, K.; Wilhelmsen, L.; Haghfelt, T.; Thorgeirsson, G.; Pyorala, K.; Miettinen, T.; Christophersen, B.; Tobert, J. A.; Musliner, T. A.; Cook, T. J. Lipoprotein changes and reduction in the incidence of major coronary heart disease events in the Scandinavian simvastatin survival study (4S). *Circulation* **1998**, *97*, 1453−1460.
(6) Tonkin, A. M. Clinical relevance of statins: their role in secondary prevention. *Atheroscler. Suppl.* **2001**, *2*, 21−25.
(7) Pedersen, T. R.; Tobert, J. A. Benefits and risks of HMG-CoA reductase inhibitors in the prevention of coronary heart disease: A reappraisal. *Drug Saf.* **1996**, *14*, 11−24.
(8) Morand, O. H.; Aebi, J. D.; Dehmlow, H.; Ji, Y. H.; Gains, N.; et al. Ro 48-8071, a new 2,3-oxidosqualene:lanosterol cyclase inhibitor lowering plasma cholesterol in hamsters, squirrel monkeys, and minipigs: comparison to simvastatin. *J. Lipid Res.* **1997**, *38*, 373−390.
(9) Mark, M.; Muller, P.; Maier, R.; Eisele, B. Effects of a novel 2,3-oxidosqualene cyclase inhibitor on the regulation of cholesterol biosynthesis in HepG2 cells. *J. Lipid Res.* **1996**, *37*, 148−158.
(10) Gardner, R. G.; Shan, H.; Matsuda, S. P. T.; Hampton, R. Y. An oxysterol-derived positive signal for 3-hydroxy-3-methylglutaryl-CoA reductase degradation in yeast. *J. Biol. Chem.* **2001**, *276*, 8681−8694.
(11) Peffley, D. M.; Gayen, A. K.; Morand, O. H. Down-regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels and synthesis in syrian hamster C100 cells by the oxidosqualene cyclase inhibitor [4′-(6-allyl-ethyl-amino-hexyloxy)-2′-fluorophenyl]-(4-bromophenyl)-methanone (Ro 48-8071): comparison to simvastatin. *Biochem. Pharmacol.* **1998**, *56*, 439−449.
(12) Panini, S. R.; Sexton, R. C.; Gupta, A. K.; Parish, E. J.; Chitrakorn, S.; Rudney, H. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol biosynthesis by oxylanosterols. *J. Lipid Res.* **1986**, *27*, 1190−1204.
(13) Janowski, B. A.; Grogan, M. J.; Jones, S. A.; Wisely, G. B.; Kliewer, S. A.; Corey, E. J.; Mangelsdorf, D. J. Structural requirements of ligands for the oxysterol liver X receptors LXR.alpha. and LXR.beta. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 266−271.
(14) Venkateswaran, A.; Repa, J. J.; Lobaccaro, J.-M. A.; Bronson, A.; Mangelsdorf, D. J.; Edwards, P. A. Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols. *J. Biol. Chem.* **2000**, *275*, 14700−14707.
(15) Costet, P.; Luo, Y.; Wang, N.; Tall, A. R. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **2000**, *275*, 28240−28245.
(16) Ordovas, J. M. ABC1: the gene for Tangier disease and beyond. *Nutr. Rev.* **2000**, *58*, 76−79.
(17) Schmitz, G.; Kaminski, W. E. ABC transporters and cholesterol metabolism. *Front. Biosci.* **2001**, *6*, D505−D514.
(18) Rowe, A. H.; Argmann, C. A.; Edwards, J. Y.; Sawyez, C. G.; Morand, O. H.; Hegele, R. A.; Huff, M. W. Enhanced endogenous synthesis of the oxysterol. 24(*S*)-25-Epoxycholesterol in macrophages is a novel strategy for inhibiting foam cell formation. *Circ. Res.*, submitted.

(19) Corey, E. J.; Virgil, S. C.; Cheng, H.; Baker, C. H.; Matsuda, S. P. T.; Singh, V.; Sarshar, S. New Insights Regarding the Cyclization Pathway for Sterol Biosynthesis from (*S*)-2,3-Oxidosqualene. *J. Am. Chem. Soc.* **1995**, *117*, 11819−11820.
(20) Abe, I.; Liu, W.; Oehlschlager, A. C.; Prestwich, G. D. Mechanism-Based Active Site Modification of Oxidosqualene Cyclase by Tritium-Labeled 18-Thia-2,3-Oxidosqualene. *J. Am. Chem. Soc.* **1996**, *118*, 9180−9181.
(21) Wendt, K. U.; Poralla, K.; Schulz, G. E. Structure and function of a squalene cyclase. *Science* **1997**, *277*, 1811−1815.
(22) Wendt, K. U.; Lenhart, A.; Schulz, G. E. The Structure of the Membrane Protein Squalene−Hopene Cyclase at 2.0 .ANG. Resolution. *J. Mol. Biol.* **1999**, *286*, 175−187.
(23) Jolidon, S.; Polak-Wyss, A.; Hartman, P. G.; Guerry, P. Inhibitors of 2,3-Oxidosqualene−lanosterol Cyclase as Antifungal Agents. *Molecular Aspects of Chemotherapy*; Polish Scientific Publishers PWN: Warszawa, Poland, 1991; pp 143−152.
(24) Jolidon, S.; Polak-Wyss, A.; Hartman, P. G.; Guerry, P. 2,3-Oxidosqualene−lanosterol cyclase: an attractive target for antifungal drug design. *Recent Advances in the Chemistry of Anti-infective Agents*; Royal Society of Chemistry: Cambridge, 1993; pp 223−233.
(25) Brown, G. R.; Hollinshead, D. M.; Stokes, E. S. E.; Clarke, D. S.; Eakin, M. A. Foubister, A. J.; Glossop, S. C.; Griffiths, D.; Johnson, M. C.; McTaggart, F.; Mirrlees, D. J.; Smith, G. J.; Wood, R. Quinuclidine inhibitors of 2,3-oxidosqualene cyclase−lanosterol synthase: optimization from lipid profiles. *J. Med. Chem.* **1999**, *42*, 1306−1311.
(26) Binet, J.; Thomas, D.; Benmbarek, A.; de Fornel, D.; Renaut, P. Structure activity relationships of new inhibitors of mammalian 2,3-oxidosqualene cyclase designed from isoquinoline derivatives. *Chem. Pharm. Bull.* **2002**, *50*, 316−329.
(27) Brown, G. R.; Hollinshead, D. M.; Stokes, E. S. E.; Waterson, D.; Clarke, D. S. Foubister, A. J.; Glossop, S. C.; McTaggart, F.; Mirrlees, D. J.; Smith, G. J.; Wood, R. A Novel Series of 4-Piperidinopyridine and 4-Piperidinopyrimidine Inhibitors of 2,3-Oxidosqualene Cyclase−Lanosterol Synthase. *J. Med. Chem.* **2000**, *43*, 4964−4972.
(28) Eisele, B.; Budzinski, R.; Muller, P.; Maier, R.; Mark, M. Effects of a novel 2,3-oxidosqualene cyclase inhibitor on cholesterol biosynthesis and lipid metabolism in vivo. *J. Lipid Res.* **1997**, *38*, 564−575.
(29) Brown, G. R.; Foubister, A. J.; Johnson, M. C.; Newcombe, N. J.; Waterson, D.; Wells, S. L. Novel 4-piperidinopyridine inhibitors of oxidosqualene cyclase−lanosterol synthase derived by consideration of inhibitor pK_a. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2213−2216.
(30) Gerber, P. R.; Mueller, K. MAB, a generally applicable molecular force field for structure modeling in medicinal chemistry. *J. Comput.-Aided Mol. Des.* **1995**, *9*, 251−268.
(31) Schulz-Gasch, T.; Stahl, M. Mechanistic Insights into Oxidosqualene Cyclisation through Homology Modelling. *J. Comput. Chem.* **2003**, *24*, 741−753.
(32) Wendt, K. U.; Schulz, G. E.; Corey, E. J.; Liu, D. R. Enzyme mechanisms for polycyclic triterpene formation. *Angew. Chem., Int. Ed.* **2000**, *39*, 2812−2833.
(33) Lenhart, A.; Weihofen, W. A.; Pleschke, A. E. W.; Schulz, G. E. Crystal Structure of a Squalene Cyclase in Complex with the Potential Anticholesteremic Drug Ro48-8071. *Chem. Biol.* **2002**, *9*, 639−645.
(34) Lenhart, A.; Reinert, D. J.; Aebi, J. D.; Dehmlow, H.; Morand, O. H.; Schulz, G. E. Binding Structures and Potencies of Oxidosqualene Cyclase Inhibitors with the Homologous Squalene−Hopene Cyclase. *J. Med. Chem.***2003**, *46*, 2083−2092.
(35) Loibner, H.; Pruckner, A.; Stuetz, A. Reductive methylation of primary and secondary amines with formaldehyde and phosphorous acid salts. *Tetrahedron Lett.* **1984**, *25*, 2535−2536.
(36) Hug, R.; Hansen, H. J.; Schmid, H. Thermal cyclodehydration of salicyl alcohols simple synthesis of 4-substituted 2*H*-chromenes. *Helv. Chim. Acta* **1972**, *55*, 1675−1691.
(37) Uff, B. C.; Joshi, B. L.; Popp, F. D. Reissert compound studies. Part LV. Studies with Reissert compounds. Part 17. Mono-Reissert compound formation at the 1,2-position of the quinazoline system. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2295−2303.
(38) Shutske, G. M.; Allen, R. C.; Forsch, M. F.; Setescak, L. L.; Wilker, J. C. Heterocyclic oxyacetic acid diuretics: indazole, benzisothiazole, and benzisothiazole 1,1-dioxide analogues of [[7-chloro-3-(2-fluorophenyl)-1,2-benzisoxazol-6-yl]oxy]acetic acid. *J. Med. Chem.* **1983**, *26*, 1307−1311.
(39) Fink, D. M.; Strupczewski, J. T. Preparation of 6-fluorobenzisothiazoles via a regioselective nucleophilic aromatic substitution reaction. *Tetrahedron Lett.* **1993**, *34*, 6525−6528.
(40) von Nussbaum, F.; Miller, B.; Wild, S.; Hilger, C. S.; Schumann, S. Zorbas, H.; Beck, W.; Steglich, W. Synthesis of 1-(2-Aminophenyl)isoquinolines and the Biological Activity of Their cis-Dichloro Platinum(II) Complexes. *J. Med. Chem.* **1999**, *42*, 3478−3485.
(41) Hansch, C.; Leo, A.; Hoekman, D. *Exploring QSAR*; ACS Professional Reference Book; American Chemical Society: Washington, DC, 1995.
(42) Landsiedel, R.; Kuhlow, A.; Glatt, H. R. Possible Bioactivation of Benzophenone Sunscreens by Enzymatic Reduction and Sulfonation. *Toxicol. Lett.* **2000**, *116*, 57−58.
(43) Weil, A.; Caldwell, J.; Strolin-Benedetti, M. The metabolism and disposition of 14C-fenofibrate in human volunteers. *Drug Metab. Dispos.* **1990**, *18*, 115−120.
(44) Robinson, D.; Williams, R. T. Metabolism of benzophenone. *Biochem. J.* **1957**, *66*, 46P−47P.
(45) Westland, R. D.; Holmes, J. L.; Green, B.; Dice, J. R. N-substituted derivatives of 2-aminoethanethiol and 2-hydrazinoethanethiol. *J. Med. Chem.* **1968**, *11*, 824−829.
(46) Tercio, J.; Ferreira, B.; Cruz, W. O.; Vieira, P. C.; Yonashiro, M. Carbon−carbon double bond cleavage using solid-supported potassium permanaganate on silica gel. *J. Org. Chem.* **1987**, *52*, 3698−3699.
(47) Fatiadi, A. J. Active manganese dioxide oxidation in organic chemistry. Part I. *Synthesis* **1976**, 65−104.
(48) Fatiadi, A. J. Active manganese dioxide oxidation in organic chemistry. Part II. *Synthesis* **1976**, 133−167.
(49) Himber, J.; Missano, B.; Rudling, M.; Hennes, U.; Kempen, H. J. Effects of stigmastanyl-phosphocholine (Ro 16-6532) and lovastatin on lipid and lipoprotein levels and lipoprotein metabolism in the hamster on different diets. *J. Lipid Res.* **1995**, *36*, 1567−1585.
(50) Weingand, K. W.; Daggy, B. P. Quantification of high-density-lipoprotein cholesterol in plasma from hamsters by differential precipitation. *Clin Chem.* **1990**, *36*, 575.
(51) Hennes, U.; Gross, W.; Edelmann, A. Changes of lipoprotein patterns in hamsters under different metabolic states analysed following separation by micropreparative chromatography on Smart from multiple microplasma samplings. *Sci. Tools* **1992**, *36*, 10−12.
(52) Gamble, W.; Vaughan, M.; Kruth, H. S.; Avigan, J. Procedure for determination of free and total cholesterol in micro- or nanogram amounts suitable for studies with cultured cells. *J. Lipid Res.* **1978**, *19*, 1068−1070.
(53) Allen, R. I.; Box, K. J.; Comer, J. E. A.; Peake, C.; Tam, K. Y. Multiwavelength spectrophotometric determination of acid dissociation constants of ionizable drugs. *J. Pharm. Biomed. Anal.* **1998**, *17*, 699−712.

JM021120F