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Identification and optimization of tetrahydro-2*H*-3-benzazepin-2-ones as squalene synthase inhibitors

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ABSTRACT

Novel squalene synthase inhibitors are disclosed. SAR and pharmacological profile of selected compounds are discussed.

Atherosclerosis is by far the most important pathological process in the development of ischemic cardiopathy and cerebral infarction, which are the most common causes of morbidity and mortality in industrialized countries.

Atherosclerosis is characterized by the accumulation of cholesterol, mainly LDL-cholesterol in macrophages, leading to lesion formation in large and medium-size arteries.[1] These lesions may develop into atheromatic plaques, which are prone to rupture and cause clinical events such as heart attack and stroke. Currently, treatment of atherosclerosis aims at reducing blood cholesterol and triglyceride content. A temporary interruption of cholesterol biosynthesis can lead to decreased plasma LDL-cholesterol levels by removing LDL-cholesterol from the circulation through up-regulation of hepatic LDL-receptors. Inhibitors of cholesterol biosynthesis via inhibition of HMGCo-A (3-hydroxy-3-methylglutaryl-CoA) reductase are presently the most effective means for blood LDL-cholesterol reduction.[2] However, inhibition of HMGCo-A reductase may block the formation of biologically necessary isoprenoids. Squalene synthase inhibition seems to offer a preferable alternative because squalene synthase catalyzed reductive dimerization of farnesyl pyrophosphate to squalene is the first specific step in cholesterol biosynthesis; thus, isoprenoid formation is not affected. Furthermore, squalene synthase inhibitors have been found to lower triglyceride levels in addition to the decrease of plasma cholesterol.[1c]

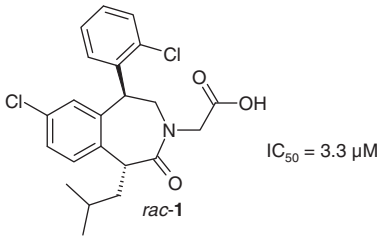

Figure 1. Initial lead structure **1**. IC_{50} was determined for the racemic compound. Relative stereochemistry was assigned based on the X-ray crystal structure analysis of compound **19**.

Therefore, squalene synthase inhibitors are a promising drug class for the treatment of hyperlipidemia and atherosclerosis.

Various squalene synthase inhibitors have been evaluated over the years as cholesterol lowering agents, with lapaquistat (TAK 475) advancing furthest in development. To date, the only squalene synthase inhibitor to have made it into phase III clinical trials is lapaquistat.[3] Our interest was aroused by the identification of **1** as a moderately potent squalene synthase inhibitor (Fig. 1). The IC_{50} values were determined by a biochemical assay measuring the conversion of farnesyl pyrophosphate to squalene by squalene synthase.[4]

The tetrahydro-2*H*-3-benzazepin-2-one scaffold was discovered during a structure based design approach derived from known inhibitors such as 4,1-benzoxazepine-3-acetic acid derivatives.[5]

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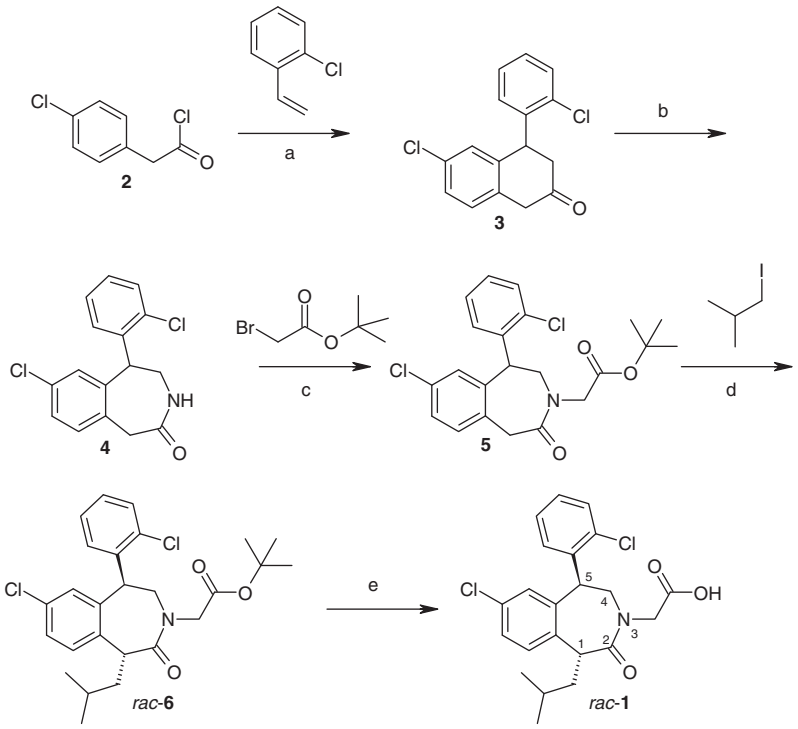


Scheme 1. Synthetic route A. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2, 0 °C, 1.5 h (31%); (b) $TMSN_3$, H_2SO_4, CH_2Cl_2, rt, 1 h (29% pure isomer **4**); (c) Cs_2CO_3, DMF, rt, 18 h (51%); (d) *tert*-butyl-P_4, −78 °C, 2 h, (53%, ds >99:1); (e) TFA/CH_2Cl_2 (v/v = 1:2), rt, 1.5 h (99%). For details see Ref. 6.

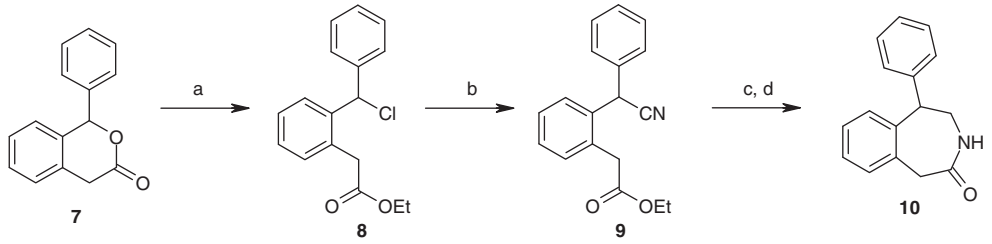
Compound **1** was built up in five linear steps (Scheme 1).[6] Initially, Friedel–Crafts reaction of (4-chlorophenyl)acetyl chloride and 1-chloro-2-vinylbenzene mediated by aluminum trichloride gave the tetralone **3**. Schmidt rearrangement[7] of tetralone **3** yielded the regioisomeric 1-benzazepin-2-one and 3-benzazepin-2-one **4** as a 1:1 mixture of isomers which were separated by chromatography.

N-Alkylation of tetrahydro-2*H*-3-benzazepin-2-one **4** with *tert*-butyl bromoacetate in the presence of cesium carbonate as base provided **5**, which was subsequently alkylated at the benzylic position with *iso*-butyl iodide mediated by the Schwesinger phosphazene base *tert*-butyl-P_4.[8,9] The *trans*-isomer was formed exclusively (ds >99:1).[10] Other bases such as sodium hydride and lithium bis(trimethylsilyl)amide were not effective. Finally, the *tert*-butyl ester **6** was cleaved with trifluoroacetic acid in dichloromethane (v/v = 1:2) to provide racemic **1**. However, the introduction of important electron-rich phenyl substituents at the 5-position[5a] of the 3-benzazepin-2-one core was not feasible via this synthetic route. In our hands, electron-rich styrenes, such as 1-methoxy-2-vinylbenzene or 1,2-dimethoxy-3-vinylbenzene, yielded side-products during Friedel–Crafts reaction with phenylacetyl chlorides. Thus, we envisaged to prepare the 3-benzazepin-2-one core via the intermediate 1-phenyl-1,4-dihydro-3*H*-isochromen-3-ones by a ring-opening and recyclization reaction sequence as first described by Busacca et al. in 1992 (Scheme 2).[11]

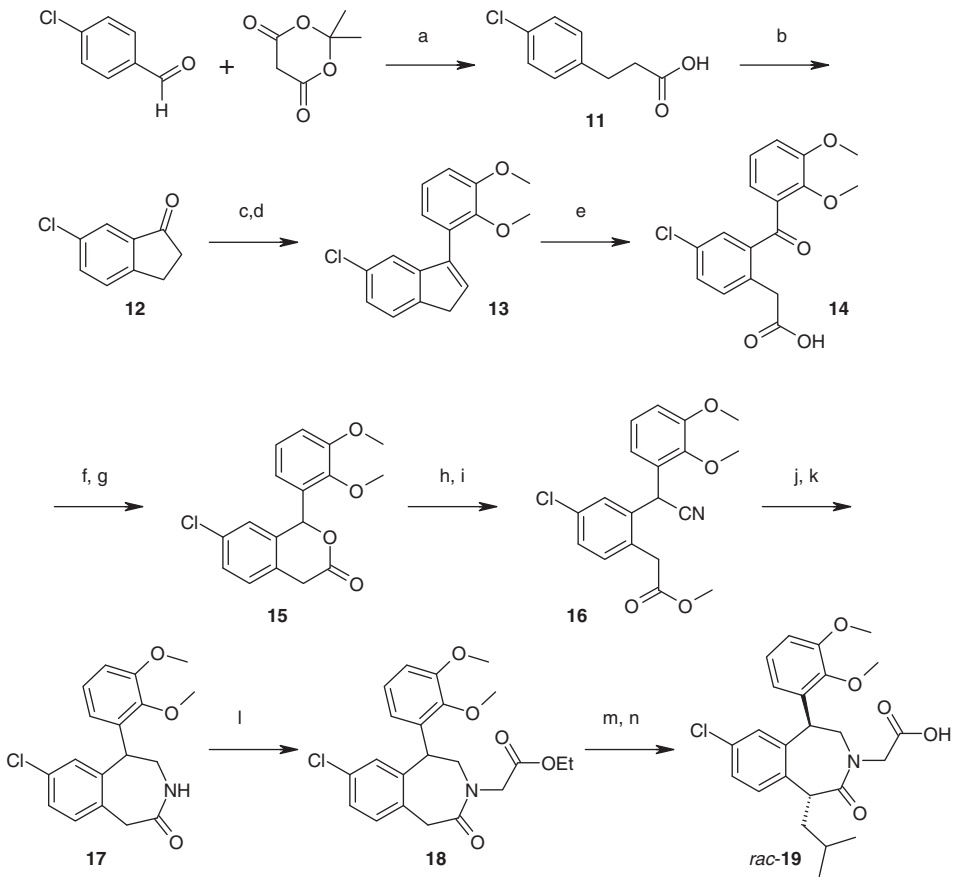
In detail, isochromen-3-one **7** was first reacted with ethanolic HCl to give **8** in high yield. Introduction of the cyano group under Lewis acid catalysis proceeded smoothly, providing cyanoester **9**. This species was then reduced to the crude amino ester with $NaBH_4/CF_3CO_2H$ and thermally cyclized to target lactam **10** in modest yield.

Our target intermediate isochromen-3-one **15** was built up in seven linear steps as outlined in Scheme 3. The synthesis started with a one pot reductive alkylation and decarboxylation to deliver 3-arylpropanoic acid **11**.[12] In detail, 4-chlorobenzaldehyde was reacted with Meldrum's acid in the presence triethylammonium formate at elevated temperature to yield **11**, which was thermally cyclized to indanone **12** upon treatment with polyphosphoric acid.[13]

Indanone **12** was reacted with (2,3-dimethoxyphenyl)lithium to form 6-chloro-1-(2,3-dimethoxyphenyl)indan-1-ol which was subsequently converted to indene **13** by acid catalyzed dehydration with *p*-toluenesulfonic acid. The 2-carboxymethylphenyl ketone **14** was obtained from indene **13** by oxidative cleavage with



Scheme 2. Reagents and conditions: (a) anhyd ethanolic HCl, 25 °C (99%); (b) TMSCN, cat. $SnCl_4$, CH_2Cl_2, 30 min (91%); (c) 10 equiv $NaBH_4/CF_3CO_2H$, 5 h, 25 °C; (d) $PhCH_3$, 8 h reflux (35% over two steps).



Scheme 3. Synthetic route B. Reagents and conditions: (a) NEt_3, HCO_2H, 95 °C, 2 h, (88%); (b) polyphosphoric acid, 100 °C, 1 h (60%); (c) veratrol, *n*-BuLi, −78 °C to rt, 3 h; (d) *p*-toluenesulfonic acid, CH_2Cl_2, rt, 16 h (22% over two steps); (e) $RuCl_3{\cdot}H_2O$, $NaIO_4$, hexane/ACN/H_2O (2:2:3), rt, 16 h, (49%); (f) $NaBH_4$, ethanol, reflux, 2 h; (g) 10% aq HCl, 40 °C, 1 h, (86% over two steps); (h) TMSCN, cat. I_2, CH_2Cl_2, rt, 18 h; (i) TMSCl, MeOH, rt, 16 h, (54% over two steps); (j) $NaBH_4$, $CoCl_2{\cdot}6H_2O$, MeOH, rt, 1 h; (k) toluene, reflux, 16 h, (41% over two steps); (l) ethyl 2-bromoacetate, Cs_2CO_3, DMF, rt, 16 h (30%); (m) *iso*-butyliodide, *tert*-butyl-P_4, −78 °C, 2 h, ds >99:1, (50%); (n) NaOH aq, THF, MeOH, rt, 16 h (99%).

sodium periodate and ruthenium(III) chloride.[14] Reduction of the ketone **14** with sodium borohydride followed by acid-promoted lactonization of the thereby formed alcohol provided the target isochromen-3-one **15**.

In analogy to the reaction protocol of Busacca et al. we tried ring-opening reaction of isochromen-3-one **15** with ethanolic HCl. However, the starting material underwent decomposition under these reaction conditions. Therefore, we investigated the direct introduction of the cyano group. Inspired by the efficiency of the iodine catalyzed cyanation of carbonyl compounds with trimethylsilyl cyanide,[15] we considered to adopt this reaction protocol for the ring-opening of **15**. Indeed, iodine promoted cyanation of **15** with trimethylsilyl cyanide proceeded smoothly to give the ring-opened species, which was treated with trimethylsilyl chloride and methanol to yield the cyanoester **16**.

This intermediate was then converted to the crude aminoester with a cobalt(II) chloride-promoted sodium borohydride reduction[16] and thermally cyclized to lactam **17** in moderate yield. Lactam **17** was elaborated further to the N- and C-alkylated 3-benzazepin-2-one **19** as described for compound **4**.

X-ray crystal structure analysis of racemic compound **19** revealed *trans*-orientation of the *iso*-butyl and the 2,3-dimethoxyphenyl substituent (Fig. 2).

Using the route outlined in Scheme 1 (synthetic route A) we briefly explored variation at the C-7 position of the lead **1** (Table 1). SAR at the C-7 position of the 3-benzazepin-2-one revealed that a chlorine substituent is clearly favored over other substituents such as methyl, fluorine and hydrogen (see **21**, **22**, **23**). Migration of the 7-chloro atom in the fused benzene ring to the 6- and 8-positions yielded compounds which had poor potencies (data not shown). Modification at the C-1 position of the seven-membered ring had a great influence on potency. The 1-benzyl compound **26** was

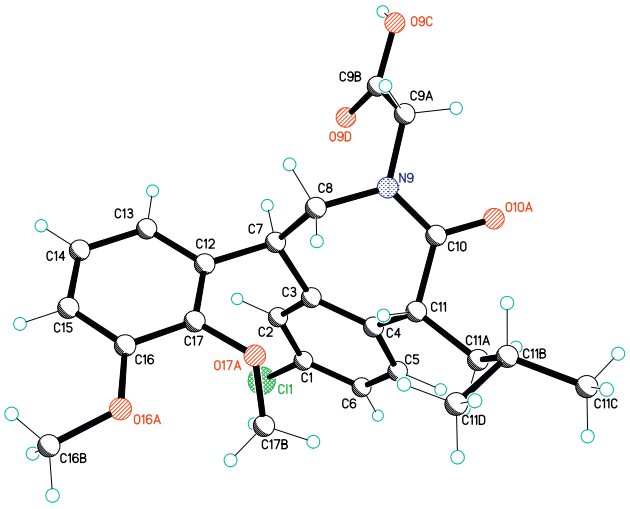
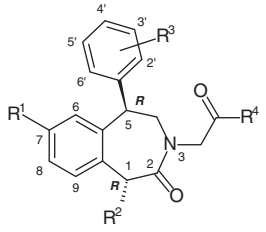
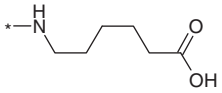
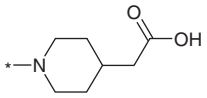
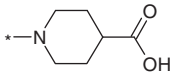
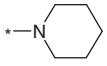
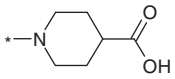
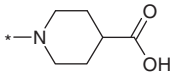
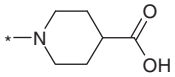
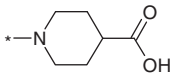


Figure 2. The molecular structure of the racemic compound **19** obtained by X-ray diffraction analysis.

Table 1
SAR of the tetrahydro-2*H*-3-benzazepin-2-ones



Compd	Route	R^1	R^2	R^3	R^4	IC_{50}[a] (μM)	HLM[b] (%)	Sterol biosynthesis[c] (%)
rac-**1**	A	Cl	*iso*-Butyl	2′-Cl	OH	3.3	60	nd
rac-**20**	A	Cl	*iso*-Butyl	2′-Cl	OEt	>20	nd	nd
rac-**21**	A	Me	*iso*-Butyl	2′-Cl	OH	>20	nd	nd
rac-**22**	A	F	*iso*-Butyl	2′-Cl	OH	>20	nd	nd
rac-**23**	A	H	*iso*-Butyl	2′-Cl	OH	>20	nd	nd
rac-**24**	A	Cl	2-Methylprop-2-en-1-yl	2′-Cl	OH	5.5	nd	nd
rac-**25**	A	Cl	2-Hydroxy-2-methylpropyl	2′-Cl	OH	>20	nd	nd
rac-**26**	A	Cl	Benzyl	2′-Cl	OH	>20	nd	nd
rac-**27**	A	Cl	*iso*-Butyl	H	OH	15.3	nd	nd
rac-**28**	A	Cl	*iso*-Butyl	2′-Br	OH	2.2	nd	nd
rac-**29**	B	Cl	*iso*-Butyl	2′-OMe	OH	1.9	nd	nd
rac-**19**	B	Cl	*iso*-Butyl	2′,3′-diOMe	OH	1.2	nd	nd
rac-**30**	A	Cl	*iso*-Butyl	2′-Cl		2.4	nd	nd
rac-**31**	A	Cl	*iso*-Butyl	2′-Cl		1.4	70	25
rac-**32**	A	Cl	*iso*-Butyl	2′-Cl		0.93	53	25
rac-**33**	B	Cl	*iso*-Butyl	2′-OMe		0.37	2	nd
rac-**34**	B	Cl	*iso*-Butyl	2′-OMe		0.50	72	39
rac-**35**	B	Cl	*iso*-Butyl	2′,3′-diOMe		0.45	78	nd
(1*S*,5*S*)-**36**	B	Cl	*iso*-Butyl	2′,3′-diOMe		>20	nd	nd
(1*R*,5*R*)-**37**	B	Cl	*iso*-Butyl	2′,3′-diOMe		0.22	89	47

[a] Values are means of three experiments. For a detailed description of the biochemical assay see Ref. 4.
[b] Human liver microsomal stability, % compound remaining after 60 min.
[c] Inhibition of sterol biosynthesis in %. For details see Refs. 6,18.

completely devoid of any activity. In the series of C-alkyl derivatives (**1**, **24**, **25** and **26**), *iso*-butyl compound **1** was found to be the most potent inhibitor of squalene synthase. Biological data for compounds having various substituents on the 5-phenyl ring are also shown in Table 1. Compound **27** with no substituent at the 2′-position was significantly less potent than the 2′-chloro analogue **1**.

Replacement of the chloro atom at the 2′-position with a bromo and a methoxy group led to even more active compounds **28** and **29**. Best potency was obtained by a 2′,3′-dimethoxy substitution, prepared via synthetic route B (see compound **19**). Other substitution patterns were found to be less promising (data not shown).

Finally SAR was investigated varying mainly the carboxylic acid (R^4) as outlined in Table 1. Amide formation was accomplished under standard coupling conditions (EDC, HOBt, DIEA).

Esters such as **20** were found to be inactive, whereas the piperidine derivative **33** showed improved potency but suffered from inferior stability in human liver microsomes. The piperidine-4-carboxylic acid derivatives **32**, **34** and **35**, finally, combined good potencies with markedly improved microsomal stabilities. Best properties regarding potency and stability were displayed by compound **35**. It was therefore separated into its enantiomers **36** and **37** by chiral HPLC; only the (1*R*,5*R*)-isomer[17] **37** was found to be potent against squalene synthase.

Compound **37** was progressed further to in vivo animal studies. Inhibition of hepatic cholesterol biosynthesis was investigated in NMRI-mice. After po administration of 3 mg/kg, **37** showed a 47% reduction in sterol biosynthesis (Table 1).[18]

Inhibitor **37** exhibited nearly the same in vitro potency compared with lapaquistat (**37**: IC_{50} = 0.22 μM; lapaquistat:

IC_{50} = 0.21 μM).[19] Furthermore **37** has a higher stability in human liver microsomes than the pharmacologically active metabolite of lapaquistat T-91485l (**37**: 89% remaining after 60 min, T-91485: 58% remaining after 60 min). However lapaquistat showed a 1.5-fold higher reduction in sterol biosynthesis than **37** in NMRI-mice.[20]

In conclusion, we have identified a novel series of substituted tetrahydro-2*H*-3-benzazepin-2-ones as potent squalene synthase inhibitors. Two complementary synthetic routes have been established, which allowed for the flexible decoration of the scaffold. Lead optimization of **1** resulted in the identification of **37** with favorable potency and microsomal stability suitable for in vivo studies. Lead **37** showed the expected profile of a squalene synthase inhibitor regarding sterol biosynthesis after oral administration. Further pharmacological studies of **37** will be reported in due course.

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4. Squalene synthase catalyses the reductive condensation of farnesyl pyrophosphate to squalene during cholesterol biosynthesis. Turnover of *trans*,*trans*-[1-^{3}H]-farnesyl pyrophosphate to [^{3}H]-squalene by squalene synthase was determined under the following conditions: 0.03 μg recombinant SQS, 1 mM NADPH, 5 mM DTT, 10% PBS, 10 mM sodium fluoride, 0.5 mM $MgCl_2$, pH 7.5. Test compounds were dissolved in DMSO and added in defined concentrations. The assay was started by adding farnesyl pyrophosphate (final concn 5 μM) and 0.2 nM *trans*,*trans*-[1-^{3}H]-farnesyl pyrophosphate and incubated for 10 min at 37 °C. Subsequently, 100 μl solution was extracted with 200 μl chloroform, 200 μl methanol and 60 μl 5 N sodium hydroxide and adjusted to 2 mM squalene. An aliquot of the organic phase was mixed with scintillation fluid and measured for beta-emission.
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17. Absolute stereochemistry at the C-5 position of the 3-benzazepin-2-one **37** was assigned by analogy to the stereoselective inhibitory potencies of 4,1-benzoxazepine derivatives described in Ref. [5b].
18. The compounds were administered orally to NMRI-mice (n = 8) one hour before monitoring of the cholesterol synthesis started. Biosynthesis of labeled cholesterol and its precursors was monitored after ip application of [^{14}C]-mevalonolactone. One hour after mevalonolactone application, livers were removed and the amount of hepatic cholesterol synthesis was determined by sterol extraction.
19. In-house data from the biochemical assay described in Ref. [4].
20. After po administration of 3 mg/kg (**37**: 47% reduction, lapaquistat: 73% reduction).