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DESIGN AND BIOLOGICAL EVALUATION OF A SERIES OF THIOPHENE-BASED 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE INHIBITORS

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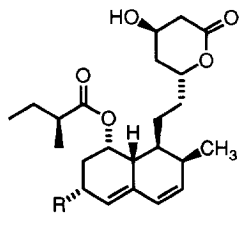
Abstract: A series of highly functionalized thiophene-based 3,5-dihydroxyheptenoic acid derivatives (**10**) were prepared from aldehydes **6** by homologation, aldol condensation with ethyl acetoacetate dianion and stereoselective β-hydroxyketone reduction. High levels of HMG-CoA reductase inhibitory activity have been found within the series. The most active analog in vitro was **10i** whereas in vivo, **10e**, **10i**, **10m**, **10n**, **10o**, and **10v** were approximately equipotent.

It has been clearly established that elevated levels of serum cholesterol increase the risk of coronary heart disease (CHD), which is one of the leading causes of death in Western civilization.[1-3] Consequently, in the past three decades pharmaceutical research has focused on discovering hypocholesterolemic agents that will reduce the circulating level of serum lipoprotein cholesterol.

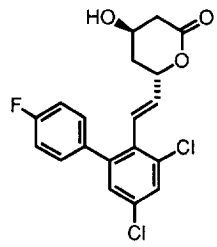
Targeting the major rate-controlling enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid in the cholesterol biosynthetic pathway, has proven to be an effective method for the reduction of low-density lipoprotein (LDL) cholesterol.[4]

The fungal metabolites compactin[5] (**1**) and mevinolin[6,7] (**2**) exhibit potent inhibitory activity on HMG-CoA reductase. Although these natural products possess many asymmetric centers, this level of complexity in the decalin segment of the molecules is not essential to retain biological activity. Interestingly, the replacement of the decalin moiety with a simple biphenyl nucleus results in a compound (**3**) that is equipotent to compactin.[8]

In this paper, we would like to describe our efforts toward the replacement of the decalin with a thiophene heterocycle and the investigation of the inhibitory properties of these compounds on HMG-CoA reductase.[9] Our target thiophenes **4** are in the 3,5-dihydroxyheptenoic acid form since it has been shown that analogous forms of compactin and mevinolin have microsomal inhibitory activity 6.5 and 14.8 times that of the respective lactonized natural products.[4]



1 R = H
2 R = CH_3



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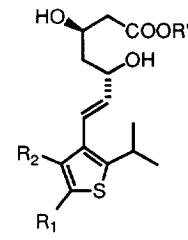

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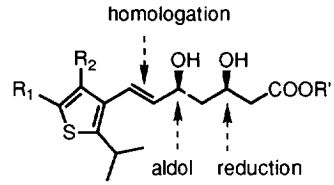
Chemistry

Retrosynthetic analysis of the target molecule **4** indicates that the heptenoic acid backbone can be assembled by two disconnections. Starting with a thiophene-3-carboxaldehyde, the aldehyde group can be homologated to an α,β-unsaturated aldehyde by known procedures. An aldol condensation with ethyl acetoacetate dianion would then introduce a δ-hydroxy-β-keto fragment where the keto functionality can be selectively reduced to the required *erythro*-β,δ-dihydroxy ester also by known procedures.



4

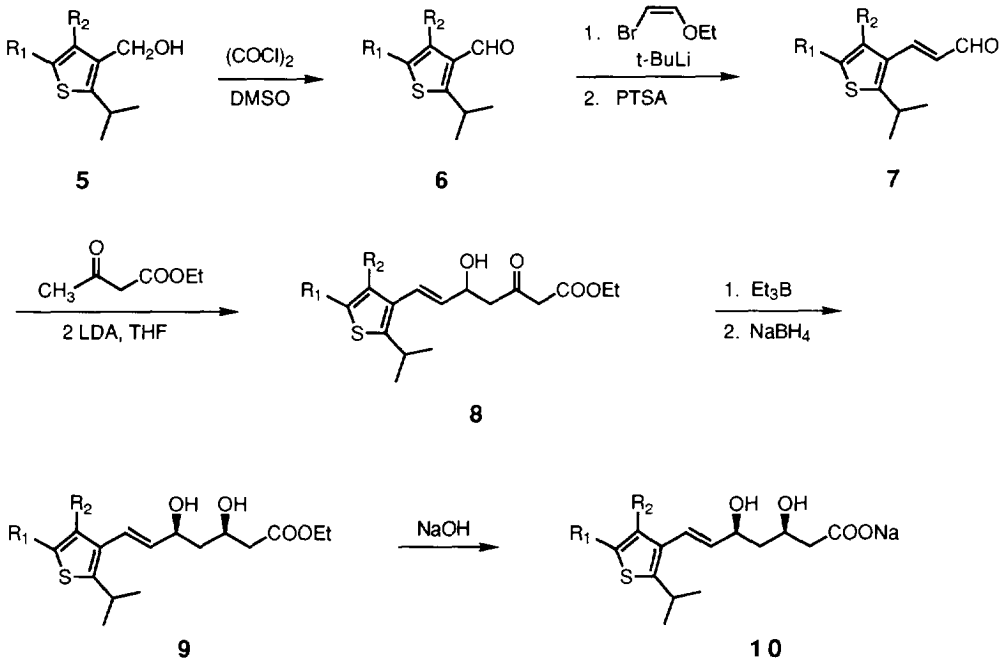
The highly functionalized thiophene alcohols **5** required as the starting point for our synthesis are readily prepared according to our previously published procedures.[10,11] Oxidation of the alcohol under Swern conditions (oxalyl chloride/DMSO, CH_2Cl_2, -78 °C, 1 h) produces nearly pure aldehyde **6** in high yield. Due to the propensity of these aldehydes to hydrate, they were carried on to the next step without chromatographic purification.

Homologation of **6** to α,β-unsaturated aldehyde **7** is accomplished with *cis*-2-ethoxyvinyllithium.[12] The reagent is generated from *cis*-2-ethoxyvinyl bromide by lithium-halogen exchange with *t*-butyllithium (THF, -78 °C, 2 h). Reaction of the lithiated species with **6** is complete within 10 min. Subsequent hydrolysis and dehydration of the intermediate adduct is effected with 0.25 equiv. of *p*-toluenesulfonic acid (90% THF/H_2O, rt, 1 h) to give the desired aldehyde **7** in >90% yield.

An aldol condensation of **7** with ethyl acetoacetate dianion (generated with 2 equiv. of LDA, THF, 0 °C, 1 h) at -20 °C for 1 h produces adduct **8** in >90% yield. A stereoselective chelation-controlled reduction[13] of the β-ketoester function to the desired *erythro*-diol **9** is accomplished by precomplexing the β-hydroxy ketone with triethylborane (THF/MeOH (4:1), rt, 1 h) followed by reduction with sodium borohydride at -78 °C for 4 h. Methanolysis of the intermediate borate complex (MeOH, rt, 24 h) furnishes **9** in approximately 40% yield with

>97:3 *syn*-selectivity. Hydrolysis of the ester with one equivalent of 1.0 N NaOH (EtOH, 65 °C, 4 h) followed by removal of the ethanol and lyophilization affords the sodium carboxylate **10** in >90% yield.

Synthetic Scheme



Biology

The compounds were tested for HMG-CoA reductase inhibitory activity in a microsomal preparation using a procedure described by Ackerman, et al.[14] Data are represented as IC_{50}s, which are the concentrations (in nM units) found to produce a 50% inhibition of the incorporation of labelled HMG-CoA into mevalonate. In vivo results are expressed as ED_{50}s, which are the doses (in mg/kg) necessary to produce a 50% inhibition of the incorporation of ^{14}C-acetate into cholesterol.

The analogs in Table 1 were designed to investigate the effect of substitution at the 4 and 5 positions of the thiophene ring on HMG-CoA reductase activity. In the first series of compounds (**10a-l**), R_1 was a phenyl group with variation in R_2. The in vitro activity was generally higher when R_2 was a phenyl substituent and lower with corresponding alkyl or alkenyl substitution. The most active compound was the 4-fluorophenyl analog **10i**. Comparison of **10e** with **10i** suggests that the 4-fluoro group is worth approximately a factor of 5 in potency.

In the second series of compounds (**10m-v**), R_2 was a 4-fluorophenyl group (except **10n** in which it was phenyl) while R_1 was varied. The best activity was obtained with R_1 as aryl, (**10t, u, v**) alkenyl (**10m**), or a small alkyl group (**10o**). Larger alkyl groups or polar (oxygenated) chains led to reduced levels of activity. None of these was more potent than **10i**.

In the in vivo assay, analogs **10e**, **10i**, **10m**, **10n**, **10o**, and **10v** (Table 2) are essentially equipotent and are somewhat more potent than **10u, d,** and **t**. No strong correlation exists between the in vitro and in vivo potency of this set of compounds, all of which demonstrated good levels of activity in both assays.

In summary, we have developed a series of thiophene-based 3,5-dihydroxyheptenoic acid derivatives which are potent HMG-CoA reductase inhibitors. Considerable flexibility exists in the substitution pattern around the thiophene ring, particularly the 5-position. Within the series the optimal analog in vitro is the 5-phenyl derivative **10i** (261 times as potent as compactin) whereas in vivo the 5-isopropyl analog **10o** is the most active (50 times as potent as compactin).

Table 1. In Vitro HMG-CoA Reductase Inhibitory Activity of Thiophenes **10**

10	R_1	R_2	IC_{50} (nM)[a]	Rel. Potency [b]
a	C_6H_5	n-C_8H_{17}	>10,000[c]	<0.1
b	C_6H_5	$(CH_3)_2C{=}CH$	1000 ± 150 (4)	1.2
c	C_6H_5	c-C_5H_9	417 ± 100 (5)	2.8
d	C_6H_5	c-C_6H_{11}	202 ± 46 (5)	5.7
e	C_6H_5	C_6H_5	26 ± 5 (5)	44
f	C_6H_5	3-CF_3-C_6H_4	2980 ± 520 (3)	0.4
g	C_6H_5	2-F-C_6H_4	233 ± 60 (5)	4.9
h	C_6H_5	3-F-C_6H_4	49 ± 16 (6)[c]	23
i	C_6H_5	4-F-C_6H_4	4.4 ± 0.5 (21)	261
j	C_6H_5	4-CH_3O-C_6H_4	2430 ± 344	0.5
k	C_6H_5	4-Ph-C_6H_4	>10,000	<0.1
l	C_6H_5	2-Thienyl	1420 ± 390 (3)	0.8
m	$(CH_3)_2C{=}CH$	4-F-C_6H_4	15 ± 5 (5)	77
n	CH_3	C_6H_5	94 ± 16	12
o	i-C_3H_7	4-F-C_6H_4	26 ± 4 (5)	44
p	n-C_8H_{17}	4-F-C_6H_4	>10,000	<0.1
q	$HOCH_2$	4-F-C_6H_4	325 ± 72 (5)	4
r	$CH_3O(CH_3)_2OCH_2$	4-F-C_6H_4	206 ± 78 (5)	5.6
s	c-C_5H_9	4-F-C_6H_4	570 ± 267 (5)	2
t	4-Cl-C_6H_4	4-F-C_6H_4	21 ± 7 (5)	55
u	4-CH_3-C_6H_4	4-F-C_6H_4	9.7 ± 3 (6)	119
v	4-CH_3O-C_6H_4	4-F-C_6H_4	6.3 ± 0.8 (11)	183
Compactin (lactone)			1150 ± 30 (232)[d]	1
Compactin (Na salt)			110 ± 26 (12)[d]	10.5
Mevinolin (lactone)			530 ± 70 (8)[d]	2.2
Mevinolin (Na salt)			160 ± 35 (7)[d]	7.2

[a]IC_{50} ± SE: Parameters were calculated using a logistic curve fit of dose response data with the indicated number (in parentheses) of dose points. The response at each dose is the mean response of duplicate determinations. [b]Relative to compactin = 1. [c]ester. [d]Mean and S.E. from the indicated number of separate dose response studies.

Table 2. In Vivo Cholesterol Synthesis Inhibitory Activity of Thiophenes **10**

10	ED_{50} (mg/kg)[a]	Rel Potency [b]
d	0.57 ± 0.11 (5)	7.1
e	0.17 ± 0.02 (3)	24
i	0.11 ± .03 (21)	37
m	0.11 ± .03 (6)	37
n	0.19 ± 0.06 (9)	21
o	0.08 ± 0.03 (6)	50
t	0.64 ± 0.45 (3)	6.3
u	0.32 ± 0.11 (5)	13
v	0.15 ± 0.08 (3)	27
Compactin (lactone)	4.02 ± 1.13 (29)	1
Compactin (Na salt)	1.3 ± 0.4 (12)	3
Mevinolin (lactone)	0.38 ± 0.08 (50)	11
Mevinolin (Na salt)	0.33 (2)	12

[a]ED_{50} ± S.E.: Parameters were calculated using a logistic curve fit of dose response data with the indicated number (in parentheses) of dose points. The response at each dose was the mean response of a group of 6 animals. [b]Relative to compactin = 1

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15. Preparation of **6**. To a solution of oxalyl chloride (15 mmol) in 100 mL dichloromethane at -78 °C under Ar was added dropwise a solution of DMSO (28 mmol) in 40 mL dichloromethane. The mixture was stirred at -78°C for 10 min after which a solution of **5** (12 mmol) in 40 mL dichloromethane was added dropwise. The mixture was stirred at -78°C for an additional 1 h and triethylamine (6.0 g) was added. After warming to room temperature, the mixture was poured into water and extracted with dichloromethane. The organic phase was dried over sodium sulfate and concentrated under reduced pressure to give **6**. This material was used immediately in the next reaction.
16. Preparation of **7**. To a solution of *cis*-1-bromo-2-ethoxyethylene (15 mmol) in 75 mL THF at -78 °C under Ar was added *t*-butyllithium (30 mmol, 1.7M solution in pentane). The mixture was stirred at -78 °C for 2 h and a solution of **6** (10 mmol) on 30 mL THF was added dropwise. After 10 min, the mixture was quenched with ammonium chloride and extracted with dichloromethane. The solvent was removed under reduced pressure and the resulting oil was dissolved in THF-H_2O (9:1). *p*-Toluene sulfonic acid (700 mg) was added and the reaction was stirred at room temperature for 1 h. The solution was poured into aqueous sodium bicarbonate and extracted with dichloromethane. The organic phase was dried over sodium sulfate and the solvent removed under reduced pressure to give **7**.
17. Preparation of **8**. LDA (2.5 mmol) was prepared in 3 mL THF at 0 °C under Ar to which a solution of ethyl acetoacetate (1 mmol) in 0.5 mL THF was added dropwise. The solution was stirred at 0 °C for 1 h and was then cooled to -20 °C. A solution of **7** (1 mmol) in 2 mL THF was added dropwise and stirring was continued for 1 h. The mixture was quenched with saturated ammonium chloride and extracted with methylene chloride. The organic phase was dried over sodium sulfate and the solvent removed under reduced pressure to give **8**.
18. Preparation of **9**. To a solution of **8** (1 mmole) in 7 mL THF-MeOH (4:1) under Ar was added triethylborane (1 mmol, 1.0 M in THF). The mixture was stirred at room temperature for 3 h and was then cooled to -78 °C. To this solution was added sodium borohydride (1 mmol) and stirring was continued at -78 °C for 4 h. The mixture was quenched with saturated ammonium chloride and extracted with MTBE. The solvent was removed under reduced pressure and the residue stirred for 24 h in 25 mL MeOH. The solvent was removed under reduced pressure and the residue flash chromatographed to give pure **9**.
19. Preparation of **10**. A mixture of **9** (1 mmol) and 1.0 mL of 1.0M aqueous sodium hydroxide in 25 mL ethanol was stirred at 50 °C for 2 h. The ethanol was removed under reduced pressure and the remaining aqueous solution was lyophilized to give **10**.

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