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Lanosterol Analogs: Dual-Action Inhibitors of Cholesterol Biosynthesis

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CONTENTS

I. INTRODUCTION

A. CHOLESTEROL AND CORONARY HEART DISEASE

Coronary heart disease (CHD) constitutes a major medical problem. In the U.S., yearly deaths from heart disease outnumber those from any other single disease. The results of numerous clinical studies indicate that lowering serum cholesterol levels reduces the risk of CHD. For example, the Cholesterol-Lowering Atherosclerosis Study provides strong evidence that lowering LDL-cholesterol levels slows the progression and even fosters the regression of atherosclerotic lesions.[1] Obvious methods for the reduction of serum cholesterol levels include 1) reduction of the intake of dietary cholesterol, 2) increase in the rate of degradation of cholesterol, and 3) reduction in the rate of hepatic cholesterol biosynthesis. With many patients, restriction of dietary intake of cholesterol does not result in the reduction of serum cholesterol concentrations to recommended levels (American Heart Association recommends 200 mg/dl); therefore, other means for the control of their cholesterol levels must be available.[2] An effective method for reducing cholesterol levels is to increase the rate of cholesterol degradation by sequestering bile acids in the gut with cationic resins. This results in the induction of the enzymes associated with the conversion of cholesterol to bile acids.[2] This treatment is very effective in many patients but suffers from

serious patient compliance problems and common side effects.[2] A more recent approach to the reduction of serum cholesterol levels is the development of inhibitors of cholesterol biosynthesis.

B. REGULATION OF CHOLESTEROL BIOSYNTHESIS

1. 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase (HMGR)

The rate-limiting step in overall cholesterol biosynthesis is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase[3] (HMGR) (Figure 1); therefore, much of the effort towards the development of inhibitors of cholesterol biosynthesis has focused on this enzyme. This work culminated in the Food and Drug Administration (FDA) approval of lovastatin (Mevacor), a competitive inhibitor of HMGR, for the treatment of hypercholesterolemia.[2] However, HMGR catalyzes a step very early in the cholesterol biosynthetic pathway prior to the formation of dimethylallylpyrophosphate and isopentenylpyrophosphate, the building blocks of all of isoprene biosynthesis (Figure 1). Since these isoprene building blocks are required for the biosynthesis of physiologically important molecules other than sterols (e.g., the dolichols, ubiquinone, and prenylated proteins) (Figure 1), the inhibition of cholesterol biosynthesis at the level of HMGR may result in long-term problems. In addition, cultured human fibroblasts respond to treatment with lovastatin by accumulating increased amounts of HMGR.[4] This increase has been

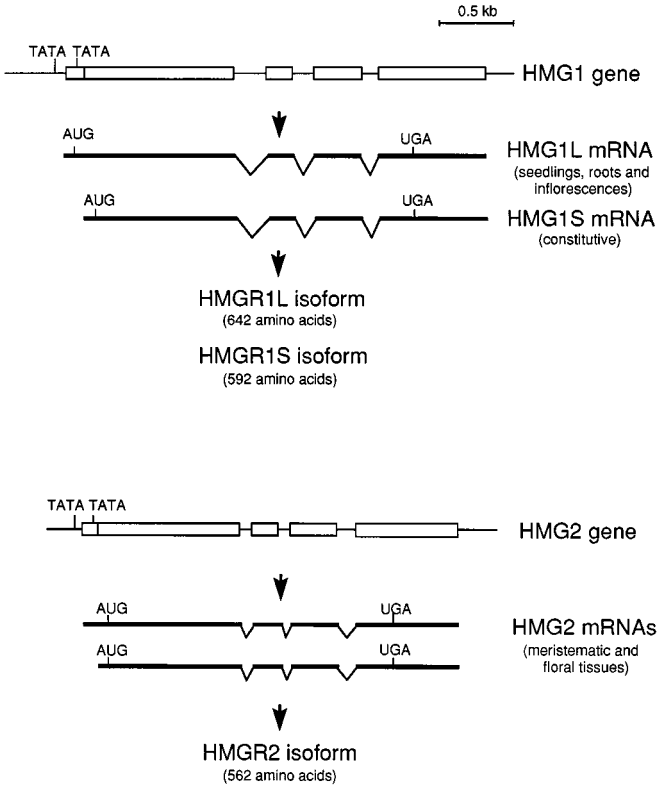
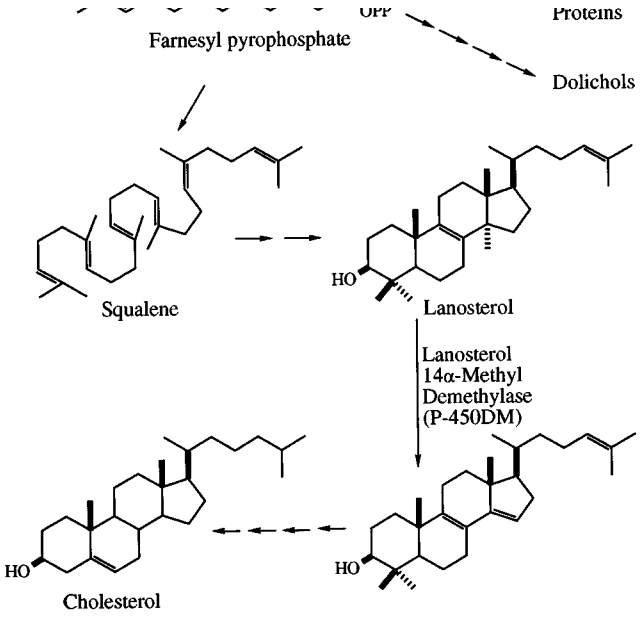


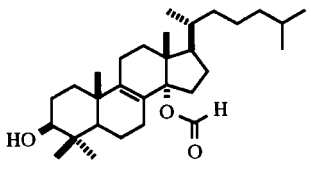
Figure 1 Cholesterol biosynthetic pathway.

attributed to an increase in the rate of transcription of the HMGR gene, an increase in the rate of translation of HMGR mRNA, and a decrease in the rate of HMGR degradation.[5]

2. Lanosterol 14α-Methyl Demethylase (P-450DM)

An enzyme which is involved in a step much later in the biosynthesis of cholesterol, past the formation of the isoprenes, is lanosterol 14α-methyl demethylase (P-450DM) (Figure 1).[6,7] This enzyme catalyzes the first step in the conversion of lanosterol **1** to cholesterol and is considered to be the rate limiting enzyme in this process. This cytochrome P-450 monooxygenase catalyzes the oxidative removal of the 14α-methyl group (C-32) of lanosterol **1** *via* three NADPH-O_2 dependent steps (eq 1). The 14α-methyl group is first oxidized to the hydroxymethyl moiety **2** followed by oxidation to the corresponding aldehyde **4** presumably *via* geminal diol **3**. The nature of the third oxidation, which results in the formation of 8,14-diene **5** with loss of the 15α-proton and formic acid, is still unclear, but appears to proceed *via* an enzyme bound peroxyhemiacetal intermediate.[8–10] Isolation of 14α-formyloxy-4,4-dimethylcholest-8-en-3β-ol **16** from the incubation of 3β-hydroxylanost-8-en-32-al **9** with rat liver microsomes suggests that removal of C-32 may proceed *via* a Baeyer-Villiger oxidation of aldehyde **9** followed by a *syn* elimination. Lanosterol **1**, 24,25-dihydrolanosterol **6**, and the Δ^7-isomer **11** appear to be substrates for P-450DM.[11–15] Lanost-8-en-3β,32-diol **7** and the corresponding Δ^7-isomer **12** have been shown to bind more tightly to mammalian P-450DM than 24,25-dihydrolanosterol **6** (K_M = 5.1 μ*M*, 5.7 μ*M*, and 32–35 μM for compounds **7**, **12**, and **6**, respectively).[14] In addition, aldehyde **9** has been shown to inhibit the loss of C-32 in rat liver microsomes.[16]





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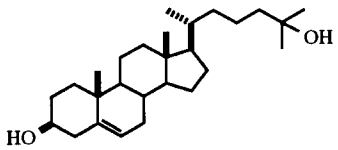
3. Endogenous Suppressors of HMGR

Interestingly, the oxygenated sterols (oxysterols) generated during the removal of the 14α-methyl group of dihydrolanosterol **6** by P-450DM (compounds **7** and **9**) and their Δ^7-analogs (compounds **12** and **14**) have been shown to be suppressors of HMGR activity.[17] As mentioned earlier, HMGR is considered to be the rate-limiting step in cholesterol biosynthesis.[3] Addition of highly purified cholesterol to cultured cells does not affect the activity of HMGR or the rate of sterol synthesis; however, numerous oxysterols, including compounds **7** and **9**, have been shown to be potent suppressors of HMGR activity.[18–20] These observations led Kandutsch and Chen to hypothesize that oxysterols, rather than cholesterol, may function as the natural regulators of HMGR activity and sterol synthesis.[21] More recently, it has been suggested that the activity of HMGR is regulated in both cultured mammalian cells[3,5] and in rat liver[22] through a multivalent feedback mechanism mediated by sterols along with an additional non-sterol metabolite(s) of mevalonate.



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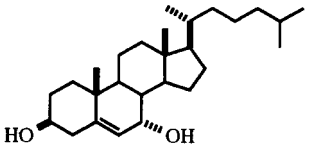
The commercially available oxysterol, 25-hydroxycholesterol **17**, has been shown to be a potent suppressor of HMGR activity in cultured cells[23] and has been utilized in most of the studies regarding the regulation of cholesterol biosynthesis by oxysterols. Although 25-hydroxycholesterol **17** has been isolated from cells, there is no direct evidence that this oxysterol functions as the natural regulator of cholesterol metabolism.[24] The suppression of HMGR activity by oxysterol **17** occurs *via* reduction in the concentration of HMGR protein, not by inhibition of the enzyme.[25] Suppression of transcription of the gene for HMGR accounts for most of the decrease in enzyme activity, although 25-hydroxycholesterol **17** also enhances the degradation of enzyme protein.[5] The transcription of other enzymes involved in the biosynthesis of cholesterol, as well as the LDL receptor (LDLR), also appears to be regulated by 25-hydroxycholesterol **17**. Oxysterol-mediated regulation of transcription of these genes occurs *via* an octanucleotide sequence in the promoter region. In the HMGR promoter, this sequence differs by one nucleotide from the core sterol regulatory element (SRE-1) found in the LDLR and HMG-CoA synthase promoters.[25]

A number of cellular oxysterols other than 25-hydroxycholesterol **17** are also potent suppressors of HMGR activity. Some of these are catabolites of cholesterol.[26] For example, 7α-hydroxycholesterol **18** and 26-hydroxycholesterol **19** are intermediates in the formation of bile acids from cholesterol and 20(*R*)- and 22(*S*)-hydroxycholesterol **20** and **21** are produced during the cleavage of the side chain of



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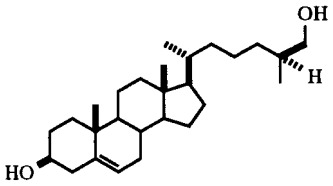

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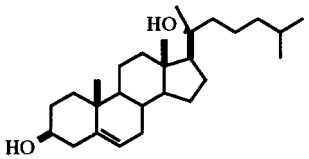

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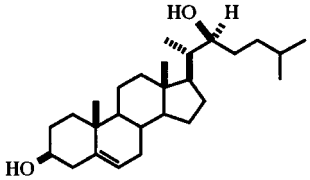

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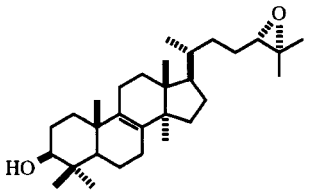
cholesterol in the formation of pregnanes. However, these oxycholesterols are not synthesized in most cells. In contrast, the oxysterols generated during the removal of the 14α-methyl group of lanosterol by P-450DM (i.e., lanost-8-ene-3β,32-diol **7** and 3β-hydroxylanost-8-en-32-al **9**) are obligatory precursors to cholesterol and are thus generated in most cells.[7] It has been proposed that oxysterols **7** and **9** may be endogenous regulators of cholesterol biosynthesis under normal physiological conditions.[27–29] However, other investigators have provided evidence that oxysterols generated *via* the cyclization of squalene diepoxide (i.e., compounds **22** and **23**) may also be involved in the regulation of cholesterol biosynthesis.[24]



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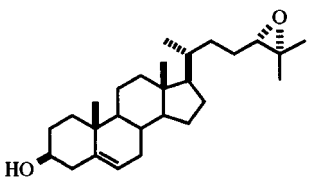

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4. Post-Transcriptional Regulation of HMGR

Initially, it was assumed that all oxysterols regulated HMGR through a combination of decreased rates of gene transcription and increased rates of enzyme degradation. However, recent reports have described a limited number of lanosterol analogs which regulate HMGR activity solely by post-transcriptional mechanisms.[30–33] Panini *et al.* have shown that 24(*S*),25-oxidolanosterol **22** can act as a post-transcriptional regulator of HMGR when P-450DM is inhibited by ketoconazole.[30] In the absence of ketoconazole, compound **22** is metabolized to 24(*S*),25-oxidocholesterol **23** which acts as a transcriptional regulator of HMGR. Treatment of cultured mammalian cells with the lanosterol analog, 15α-fluorolanost-7-en-3β-ol **24** (Table 1), a competitive inhibitor of P-450DM, also decreases HMGR activity. The suppression of enzyme activity by this compound is a result of translational rather than transcriptional regulation (*vide infra*).[31] The lanosterol demethylation intermediate **9** also suppresses HMGR activity *via* solely post-transcriptional mechanisms (decreased enzyme synthesis and enhanced enzyme degradation).[32] Therefore, a growing body of evidence suggests that oxylanosterols may regulate cholesterol biosynthesis in a manner much different from that of oxycholesterols.

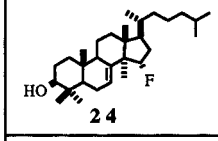
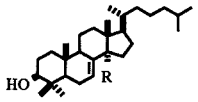
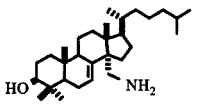
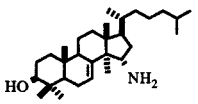
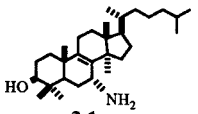
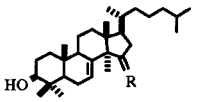
Recent evidence suggests that post-transcriptional regulation of HMGR is also important *in vivo*. In early studies cholesterol feeding markedly reduced hepatic HMGR activity and mRNA levels in rats on a diet containing lovastatin and cholestyramine, leading to the conclusion that dietary cholesterol was a transcriptional regulator of HMGR.[34] However, when normally fed rats were given cholesterol, the decreased abundance of HMGR mRNA could not account for the observed suppression of hepatic HMGR activity and protein levels.[22,35,36] This suggests that when competitive inhibitors of HMGR are not present, regulation of HMGR by dietary cholesterol occurs post-transcriptionally.

The mechanism of cholesterol regulation by oxycholesterols and oxylanosterols in cultured cells also differs with respect to their effects on LDL metabolism. Some oxylanosterol suppressors of HMGR activity have been shown to stimulate receptor-mediated binding, uptake, and metabolism of LDL,[37,38] while oxycholesterols (such as 25-hydroxycholesterol **17**) appear to suppress LDLR formation and thus decrease LDL uptake and metabolism.[3] These results are of obvious pharmacological relevance, since a drug which inhibited endogenous cholesterol biosynthesis while enhancing clearance of serum cholesterol *via* the LDLR would be of particular benefit. A hypothesis for the proposed regulation of HMGR and LDLR gene expression by oxycholesterols and oxylanosterols is summarized in Figure 2.

II. DUAL-ACTION INHIBITORS OF CHOLESTEROL BIOSYNTHESIS

Recent work in our laboratories has been directed toward the development of dual-action inhibitors of cholesterol biosynthesis.[7,33,39,40] These molecules were designed to act as competitive inhibitors of P-450DM and partial suppressors of HMGR activity. Utilizing this approach, we are pursuing the development of molecules that will effectively shut down cholesterol biosynthesis in hepatic tissue but allow for the buildup of the isoprenes needed for the biosynthesis of polyisoprenes other than sterols (Figure 1). Studies of either endogenous or side chain modified oxysterols are hampered by their rapid metabolism. Therefore, to circumvent this problem, we have designed compounds which should be relatively stable to demethylation by P-450DM.

Table 1 Summary of the Biochemical Evaluation of Selected Potential Dual-Action Inhibitors of Cholesterol Biosynthesis

	Inhibitor	IC_{50} (μM)				Reference
		P-450DM		HMGR		
		in vitro [a]	CHO	CHO	AR45	
TYPE 1	 **24**	>100	5.0	1.0	>20	31, 46
	 **9** R=CHO	NA[b]	1.9	3.0	2.0	16, 32
	25a R=CH(OH)CH_3 (less polar diastereomer)	0.33	0.3	3.0	>20	7, 39
	25b R=CH(OH)CH_3 (more polar diastereomer)	1.6	0.3	1.5	>20	7, 39
	26 R=$C(CH_3)O$	9.1	2.7	1.0	>20	7, 39
	27 R=NOH	8.9	4.5	1.0	2.0	40
	28 R=$NOCH_3$	100	>20	10	>20	40
TYPE 2	 **29**	4.0	>20	1.5	>20	
	 **30**	1.5	0.15	6.0	>20	
	 **31**	60	1.0	2.0	5.0	
TYPE 3	 **32** R=O	7.1	1.0	0.5	1.8	40
	33 R=NOH	3.0	2.0	0.6	1.5	40
	34 R=$NOCH_3$	77	>20	15	>20	40
	35 R=NOBn	100	>20	15	NA	40

[a] Experiments carried out using rat liver microsomal preparations.

[b] IC50 value is not available.

See Reference 16 for details of inhibition of P-450DM by compound **9**.

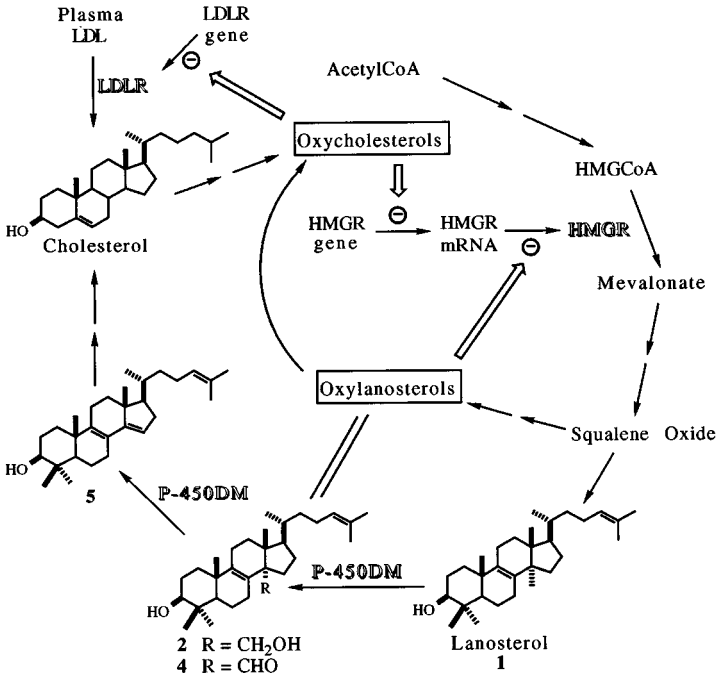
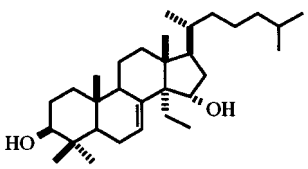


Figure 2 Proposed pathways for regulation of cholesterol metabolism by oxysterols.

When we began these studies, one compound, 14α-ethylcholest-7-ene-3β,15α-diol **36**, had been reported to act as both an inhibitor of P-450DM and as a suppressor of HMGR activity.[41–43] In studies utilizing this oxylanosterol, Schroepfer et al. found it to be more potent in the inhibition of overall cholesterol biosynthesis than could be explained by its ability to suppress HMGR activity.[41] This compound was later found to inhibit P-450DM.[43] These results supported our hypothesis that we could design molecules which would act as inhibitors of P-450DM and as suppressors of HMGR activity. Our results, along with recent reports on 3β-hydroxylanost-7-en-15-one **32**,[44,45] a novel carboxylic acid analog of lanosterol (compound **37**),[38] and a series of compounds prepared by DuPont-Merck[46] verify that it is indeed possible to design compounds which function as dual-action inhibitors of cholesterol biosynthesis.



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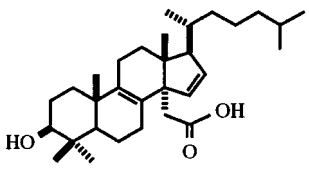

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Selected lanosterol analogs that have been identified as potential dual-action inhibitors of cholesterol biosynthesis are shown in Table 1. These compounds can be divided into three types: 1) analogs of the intermediates (sterols **7** and **9**) generated during the removal of the 14α-methyl group by P-450DM (type 1 inhibitors, compounds **24**-**28**); 2) aminolanosterols with the amine nitrogen placed in the vicinity of C-32 (type 2 inhibitors, compounds **29**-**31**); 3) lanosterol analogs with a ketone or oxime functionality at C-15 (type 3 inhibitors, compounds **32**-**35**).

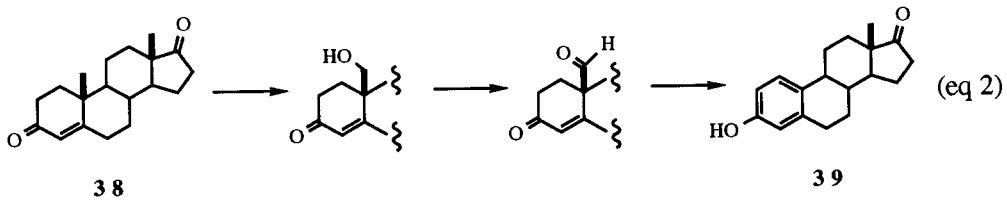
A. TYPE 1 INHIBITORS: ANALOGS OF THE INTERMEDIATES GENERATED DURING THE REMOVAL OF THE 14α-METHYL GROUP OF LANOSTEROL BY P-450DM

1. Design of 32-Methyllanosterol Analogs

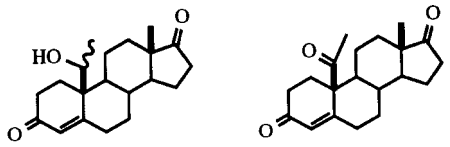
Compounds **25a**, **25b**, and **26** were expected to bind tightly to P-450DM since they are similar in structure to the natural intermediates **7** and **9**, which bind very tightly to P-450DM.[10–15] In addition, it was

anticipated that they would cause the suppression of HMGR activity either directly or by causing the buildup of the natural intermediates. In light of the fact that lanost-7-en-3β-ol **11** is a substrate for P-450DM[14] and that the Δ^{24}-double bond is not necessary for the demethylation,[11–15] the 24,25-dihydro-Δ^{7}-compounds were prepared since they were synthetically more easily accessible than the 8,24-dienes.[39]

We predicted that the C-14–C-32 bond of compounds **25a**, **25b**, and **26** would be stable to the lyase activity of P-450DM by analogy to the similar compounds studied as inhibitors of aromatase.[47] Aromatase is the cytochrome P-450 monooxygenase which oxidatively removes the 10β-methyl group (C-19) of androstenedione **38** to yield estrone **39** (eq 2). The methyl group is removed in a manner analogous to P-450DM, i.e., *via* hydroxylation, oxidation to the corresponding aldehyde, and removal of C-19 as formic acid. Androstenedione analogs **40a**, **40b**, and **41** have been shown by Beusen *et al.* to be competitive inhibitors of aromatase.[47] In addition, they demonstrated that the C-10–C-19 bond of these compounds was stable to the lyase activity of aromatase. Considering the similarity of aromatase to P-450DM, it was anticipated that the C-14–C-32 bond of lanosterol analogs **25a**, **25b**, and **26** would be stable to cleavage by P-450DM. The inability to cleave the C-14–C-32 bond should significantly enhance the metabolic stability of compounds **25a**, **25b**, and **26** relative to their naturally occurring analogs.



38 **39** (eq 2)



40a&b **41**

2. Biochemical Evaluation of 32-Methyllanosterol Analogs

Inhibition of P-450DM by lanosterol analogs was assessed using a modification of the assay developed by Trzaskos and coworkers[48] (rat liver microsomal preparations). The results of these studies are summarized in Table 1. As can be seen in the table, compounds **25a**, **25b**, and **26** appear to be inhibitors of P-450DM, since their IC_{50} values are lower than the K_M of 24,25-dihydrolanosterol **6** (K_M = 33 μ*M* under the assay conditions). Further evidence for the inhibition of P-450DM by these 32-methyl sterols was provided by [^{14}C]-acetate incorporation studies in CHO cells. All three of the 32-methyl analogs caused a concentration-dependent reduction of incorporation of radiolabel into C_{27} sterols with a concomitant increase in production of C_{30} sterols which is consistent with the inhibition of P-450DM. The concentration of inhibitor which results in equal accumulation of radiolabel into C_{27} and C_{30} sterols (IC_{50} value) for each of the three compounds is provided in Table 1.[39] For illustrative purposes, the experimental results for the more polar 32-methyl-32-alcohol **25b** are shown in Figure 3.

32-Methyllanosterol analogs (compounds **25a**, **25b**, and **26**) were also evaluated as suppressors of HMGR activity. As can be seen in Table 1, we found all three compounds to cause a concentration-dependent reduction of HMGR activity in CHO cells (Table 1).[39] Therefore, these compounds are indeed dual-action inhibitors of cholesterol biosynthesis. However, utilizing a P-450DM-deficient cell line (AR45 cells),[49] we have shown that the ability of compounds **25a**, **25b**, and **26** to suppress HMGR activity requires a functional P-450DM (Table 1, Figures 4 and 5). For example, the more polar 32-methyl-32-alcohol **25b**, exhibited a large difference in the suppression of HMGR activity in CHO *versus* AR45 cells (Figure 4). Similar results were seen for less polar diastereomer **25a** and 32-methyl ketone **26** (Figure 5), suggesting that compounds **25a**, **25b**, and **26** either require P-450DM for conversion to an active metabolite, which then suppresses HMGR activity, or they cause the accumulation of the natural intermediates **7** and **9**, resulting in the suppression of HMGR activity. The latter of these two

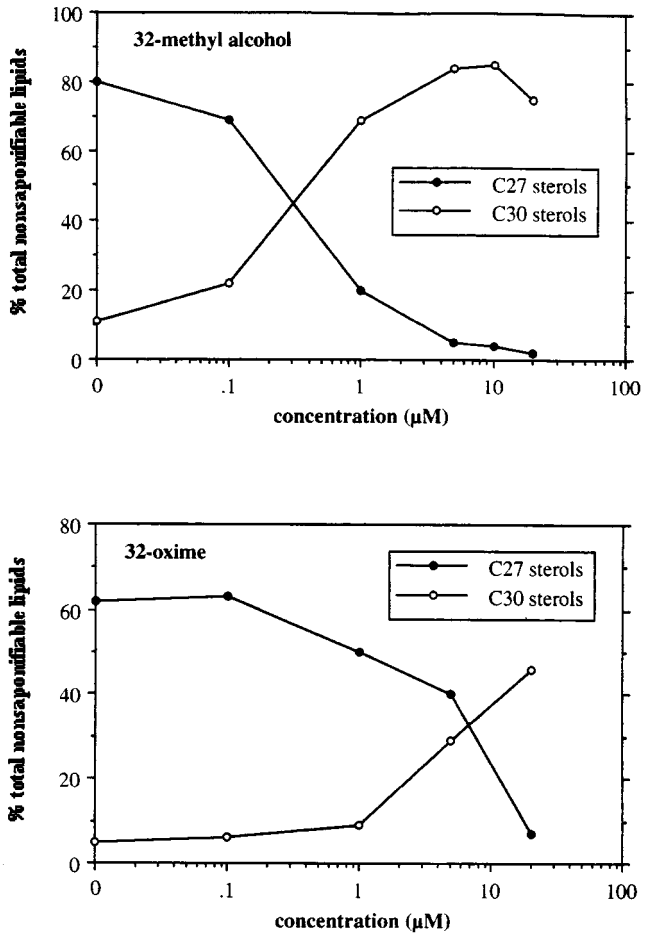
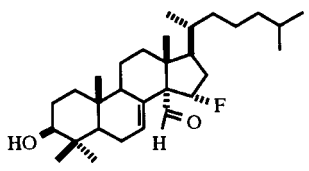


Figure 3 Changes in sterol labeling profiles in CHO cells treated with 32-methyl-32-alcohol (more polar diastereomer) **25b** or 32-oxime **27**. Cells were treated for 2 h with the indicated concentration of sterol and then labeled for an additional 2 h with [1-^{14}C]acetate. Nonsaponifiable lipids were extracted and analyzed by thin layer chromatography. Incorporation of radiolabel into C_{27} and C_{30} monohydroxysterols is expressed as a percentage of incorporation into total nonsaponifiable lipids. (Top panel reprinted with permission from Frye, L. L. and Leonard, D. A., in *Regulation of Isopentenoid Metabolism.*, ACS Symposium Series **497**, Nes, W. D. et al., Eds., American Chemical Society, Washington, D.C., 1992, 94. Copyright 1992 American Chemical Society.)

possibilities is the mechanism by which ketoconazole and miconazole cause the suppression of HMGR activity.[28,29] To address whether or not compounds **25a**, **25b**, and **26** are metabolized by P-450DM, we undertook studies using rat liver microsomal preparations. As expected, no evidence for the conversion of any of these compounds to diene **10** or diene **15** (UV-HPLC) was found, indicating that the C-14–C-32 bond is stable to the lyase activity of P-450DM. However, preliminary studies (TLC) do suggest that both alcohols (compounds **25a** and **25b**) are converted to ketone **26** by rat liver microsomes. In similar studies, 15α-fluorolanosterol analog **24** was also shown to exhibit HMGR suppressive activity in CHO cells but not in AR45 cells (Table 1 and Figure 5).[31] This sterol has no HMGR suppressive activity itself, but is metabolized by P-450DM to the 32-aldehyde analog **42** which has been shown to be the active metabolite.[31]



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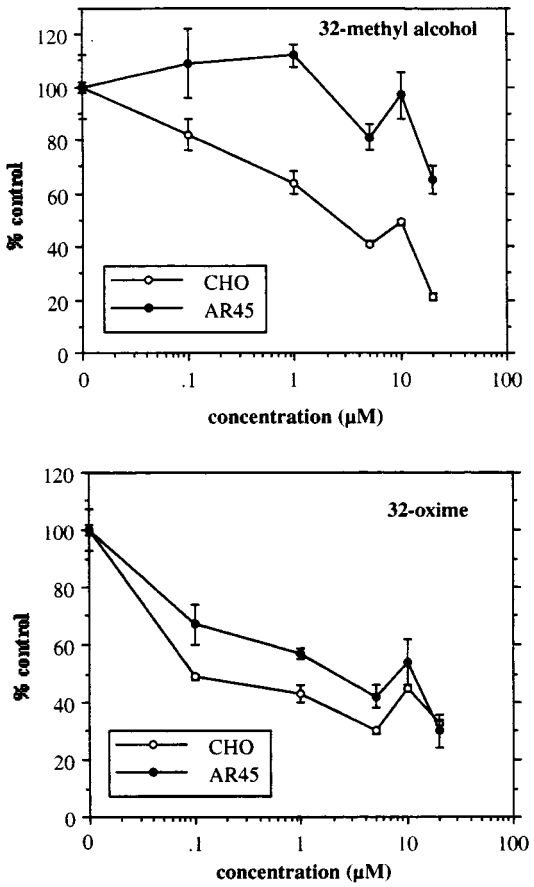


Figure 4 HMGR activity in CHO and in AR45 cells treated with 32-methyl-32-alcohol **25b** or 32-oxime **27**. Wild-type (CHO) cells and lanosterol 14α-methyl demethylase deficient (AR45) cells were treated for 6 h with increasing concentrations of sterol. HMGR activity was assayed in permeabilized cells by monitoring the conversion of [^{14}C]HMG-CoA to [^{14}C]mevalonate. Enzyme activity was calculated as pmol mevalonate per minute per 10^5 cells, expressed as a percentage of control values. Data represent the mean and standard error of four determinations. (Top panel reprinted with permission from Frye, L. L. and Leonard, D. A., in *Regulation of Isopentenoid Metabolism*, ACS Symposium Series **497**, Nes, W. D. et al., Eds., American Chemical Society, Washington, D.C., 1992, 94. Copyright 1992 American Chemical Society.)

3. Design and Evaluation of 32-Oximes

32-Oxime **27**, like compounds **25a**, **25b**, and **26**, is similar in structure to both the alcohol and the aldehyde intermediates generated during removal of the 14α-methyl group (i.e., compounds **7** and **9**). This compound was found to inhibit P-450DM in rat liver microsomal preparations and in CHO cells (Table 1).[40] A higher concentration of 32-oxime **27** as compared to 32-methylalcohol **25b** was required for equal accumulation of radiolabel into C_{27} and C_{30} sterols by cultured cells which is consistent with the higher IC_{50} value for this compound in the rat liver microsome assay (Figure 3). Surprisingly, 32-oxime **27** was found to suppress HMGR activity to a similar extent in CHO and in AR45 cells (Figure 4) indicating that, in contrast to the 32-methyllanosterols (compounds **25a**, **25b**, and **26**) and 15α-fluoro compound **24**, this dual-action inhibitor of cholesterol biosynthesis does not require activation by P-450DM for suppression of HMGR activity. This conclusion is supported by the observation that 32-oxime **27** is not metabolized during incubation with rat liver microsomes. The ability of 32-oxime **27** to suppress HMGR in demethylase-deficient AR45 cells also demonstrates that the suppression of HMGR by this compound is not a result of the buildup of the natural intermediates **7** and **9**. The observation that some lanosterol analogs require an active P-450DM for HMGR suppression while others do not (Figure 5) may provide important insights into the *in vivo* regulation of the cholesterol biosynthetic pathway.

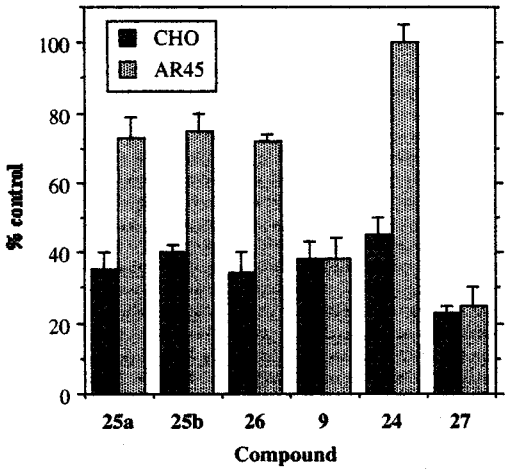


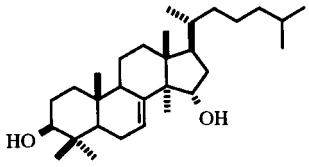
Figure 5 HMGR activity in CHO and in AR45 cells treated with type 1 inhibitors. Cells were treated with 5.0 μ*M* sterol for 6 h and assayed for HMGR activity as described in Figure 4. Values are expressed as a percentage of control and represent the mean and standard error of four determinations.

In order to assess the importance of the oxime hydrogen in the inhibitory properties of 32-oxime **27**, the corresponding 32-methyloxime, compound **28**, was prepared[40] and evaluated. This compound was significantly less active than 32-oxime **27** in its ability to suppress HMGR and P-450DM activities in cultured cells and was a poor inhibitor of microsomal P-450DM *in vitro* (Table 1).[40] These results suggest either that the oxime hydrogen is important for the inhibitory properties of compound **27** or that the larger methyl group is inhibiting the binding of the oxime to the active site of P-450DM as well as interfering with the ability of this compound to regulate HMGR gene expression.

B. TYPE 2 INHIBITORS: AMINOLANOSTEROLS WITH THE AMINE NITROGEN PLACED IN THE VICINITY OF C-32 OF LANOSTEROL

1. Design of Aminolanosterols

Compounds **29** through **31** were designed to be specific inhibitors of P-450DM. They are structurally similar to the natural substrate and have a heteroatom in the vicinity of C-32 for coordination to the heme iron of P-450DM which should facilitate binding to the active site of P-450DM.[50,51] Compounds **29** and **30** are nitrogen-containing derivatives of lanosterol that are similar in structure to oxysterols **7**[17] (eq 1) and **43**[44,45] which are known suppressors of HMGR activity. Hence, compounds **29** and **30** may also cause the suppression of HMGR activity.



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2. Biochemical Evaluation of Aminolanosterols

Evaluation of amino analogs **29**, **30**, and **31** as inhibitors of P-450DM in rat liver microsomal preparations gave IC_{50} values of 4.0 μ*M*, 1.5 μ*M*, and 60 μ*M*, respectively (Table 1, unpublished results). The lower IC_{50} values of 15α-amine **30** and 32-amine **29** as compared to the 7α-amine may indicate that the heme iron is closer to C-15 than to C-7. In contrast to its potency *in vitro*, 32-amine **29** caused only a modest accumulation of C_{30} sterols in CHO cells (Table 1), suggesting that it may be metabolized to a compound which is less capable of inhibiting P-450DM. This hypothesis is consistent with the suppression of HMGR activity by compound **29** in wild-type CHO cells and the lack of an effect on HMGR activity in the P-450-deficient cell line (Table 1). 15α-Amine **30** caused both the accumulation of C_{30} sterols and the suppression of HMGR activity in wild-type CHO cells (Table 1). Like 32-methylalcohols **25a**,

and **25b**, this compound had no effect on HMGR activity in P-450-deficient cells (Table 1). In contrast, 7α-amine **31** caused the suppression of HMGR activity in both cell lines indicating that the mechanism of HMGR suppression is independent of P-450DM activity (Table 1). The accumulation of C_{30} sterols in CHO cells treated with compound **31**, despite its relatively weak inhibition of P-450DM in rat liver microsomes, suggests that a cellular metabolite of this compound may be acting as an inhibitor.

C. TYPE 3 INHIBITORS: LANOSTEROL ANALOGS WITH KETONE OR OXIME FUNCTIONALITY AT C-15 OF LANOSTEROL

1. Design of 15-Oxime 33

15-Ketone **32** has been identified by Schroepfer *et al.* as an inhibitor of sterol biosynthesis and HMGR activity in mouse L cells.[44] This sterol was considerably more potent in the inhibition of sterol synthesis (IC_{50} = 0.09 μ*M*) than it was in the suppression of HMGR activity (IC_{50} = 0.8 μ*M*) indicating that this sterol must act at an additional site in the cholesterol biosynthetic pathway. The results of Sato *et al.* suggested that this other site of inhibition might be at the level of P-450DM.[45] They found ketone **32** to cause the inhibition of cholesterol synthesis from dihydrolanosterol **6** (87% inhibition at 40 μ*M*, [dihydrolanosterol] = 18 μ*M*). We have carried out mechanistic studies on the inhibition of cholesterol biosynthesis by 15-ketone **32**. In addition, we prepared 15-oxime **33** as an intermediate in the synthesis of aforementioned amine **30**. Due to its similarity to ketone **32**, 15-oxime **33** was evaluated as an inhibitor of P-450DM and as a suppressor of HMGR activity.

2. Biochemical Evaluation of 15-Ketone 32 and 15-Oxime 33

To verify the results of Schroepfer and Sato,[44,45] we first studied the inhibitory properties of 15-ketone **32**. As expected, 15-ketone **32** was found to inhibit rat liver P-450DM (IC_{50} = 7.1 μ*M*) and to cause the accumulation of radiolabeled C_{30} sterol from [^{14}C]acetate (IC_{50} = 1.0 μ*M*).[40] Also, in accordance with the results of Schroepfer et al.,[44] 15-ketone **32** was found to be a potent suppressor of HMGR activity in CHO cells (Figure 6). Its suppressive activity in AR45 cells was similar to that seen in the parental cell line, indicating that, like 32-oxime **27**, the ability of this compound to suppress HMGR activity is independent of P-450DM. Once we had verified that 15-ketone **32** was indeed a dual-action inhibitor of cholesterol biosynthesis, we turned our attention to the study of 15-oxime **33**.

15-Oxime **33** was found to be an effective inhibitor of P-450DM in rat liver microsomes (IC_{50} = 3.0 μ*M*) with a potency similar to that of ketone **32**. The accumulation of lanosterol in cells treated with 15-oxime **33** suggests that this lanosterol analog also inhibits P-450DM in intact cells (IC_{50} = 2.0 μ*M*). In addition, 15-oxime **33** was found to be a potent suppressor of HMGR activity in both wild-type and AR45 cells (Figure 6). Therefore, as with 15-ketone **32**, 15-oxime **33** does not require activation by P-450DM for its suppressive activity.

In order to assess the importance of the oxime hydrogen in the inhibitory properties of 15-oxime **33**, the corresponding15-methyl and 15-benzyloximes (i.e., compounds **34** and **35**) were studied.[40] As with the 32-methyloxime **28**, compounds **34** and **35** were significantly less active than 15-oxime **33** in their ability to inhibit P-450DM and HMGR activities.

III. MECHANISM OF REGULATION OF HMGR BY LANOSTEROL ANALOGS

A. POST-TRANSCRIPTIONAL REGULATION OF HMGR BY LANOSTEROL ANALOGS

In response to the interesting finding that 15-ketone **32** and 15-oxime **33** do not require activation by P-450DM for suppression of HMGR activity, we undertook studies to ascertain the mechanism by which these compounds affect the activity of this important enzyme. We found that the addition of either 15-ketone **32** or 15-oxime **33** to CHO cell sonicates at concentrations up to 5 μ*M* did not affect HMGR activity, demonstrating that the ability of these compounds to reduce HMGR activity is not due to classical inhibition. We then evaluated the effect of compounds **32** and **33** on HMGR protein concentrations. These compounds were found to cause parallel declines in HMGR activities and HMGR protein levels in both CHO and AR45 cells. These observations suggest that these compounds regulate HMGR activity at the level of gene expression.

Further studies were carried out to determine the level at which 15-ketone **32** and 15-oxime **33** regulate HMGR gene expression. Interestingly, we found no correlation between HMGR protein concentrations and HMGR mRNA levels in cells treated with either of these compounds (Figure 6), suggesting that they are regulating HMGR in a post-transcriptional manner. Clearly, our lanosterol

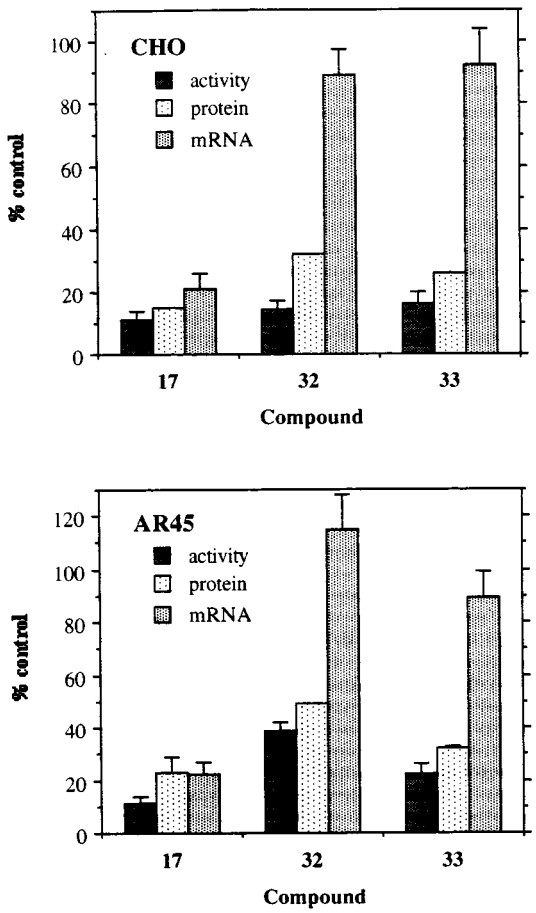


Figure 6 Sterol effects on HMGR activity, protein, and mRNA levels in CHO and in AR45 cells. Cells were treated with 2.3 μ*M* 25-hydroxycholesterol **17** or 5.0 μ*M* 15-ketone **32** or 15-oxime **33** for 6 h. Enzyme activity was determined as described in Figure 4. HMGR protein concentration was measured by immunoblotting, and mRNA levels were determined by Northern blot analysis. Values are the means and standard errors expressed as a percentage of control values. (Adapted from Anderson, J. A., Leonard, D. A., Cusack, K. P., and Frye, L. L., *Arch. Biochem. Biophys.*, 316, 190–196, 1995.)

analogs appear to regulate HMGR by a mechanism much different from that of the prototypical oxycholesterol, 25-hydroxycholesterol **17**, which, as mentioned earlier, regulates HMGR primarily by a transcriptional mechanism.[5] A limited number of other lanosterol analogs have also been reported to act as post-transcriptional regulators of HMGR. For example, 24(*S*),25-oxidolanosterol **22** was found to be a post-transcriptional suppressor of HMGR in CHO cells; however, this was only observed when P-450DM activity was inhibited by ketoconazole.[30] 15α-Fluorocompound **24** also appears to regulate HMGR in a post-transcriptional manner, but this compound requires P-450DM-mediated metabolism to 15α-fluoro-32-aldehyde **42** for its suppressive activity.[31] Thus, the study of the post-transcriptional regulation of HMGR by compounds **22** and **42** is complicated. Since 15-ketone **32** and 15-oxime **33** exhibit similar effects on HMGR activities, HMGR protein concentrations, and HMGR mRNA levels in CHO and in AR45 cells (Figure 6), they obviously do not require activation by P-450DM for their activity and they are presumably stable to metabolism by P-450DM. These characteristics should make our compounds particularly useful in the study of the cellular mechanisms involved in cholesterol metabolism; information which is essential for the development of successful therapies for the treatment of hypercholesterolemia.

The post-transcriptional regulation of HMGR by the lanosterol analogs described to date is due, at least in part, to translational regulation. In Figure 7, the effects of lanosterol analogs on HMGR mRNA levels are compared to their affects on K_s, the rate constant for the synthesis of HMGR. Using K_s as a

measure of relative rates of synthesis corrects for any effects on HMGR degradation.[30] While the transcriptional regulator 25-hydroxycholesterol **17** causes a coordinate decline in HMGR mRNA concentration and K_s, all of the lanosterol analogs decrease K_s without reducing HMGR mRNA levels. Thus, these compounds are all acting as translational regulators of HMGR. 25-Hydroxycholesterol **17**, in addition to inhibiting transcription, is also known to enhance HMGR degradation. We have seen similar effects on HMGR degradation in cells treated with 15-oxime **33** as well as endogenous 32-aldehyde **9** (Figure 8). 15α-Fluorocompound **24** is unique among the lanosterol analogs examined to date in that it inhibits HMGR synthesis despite an increase in levels of HMGR mRNA, but has no effect on HMGR degradation.[31]

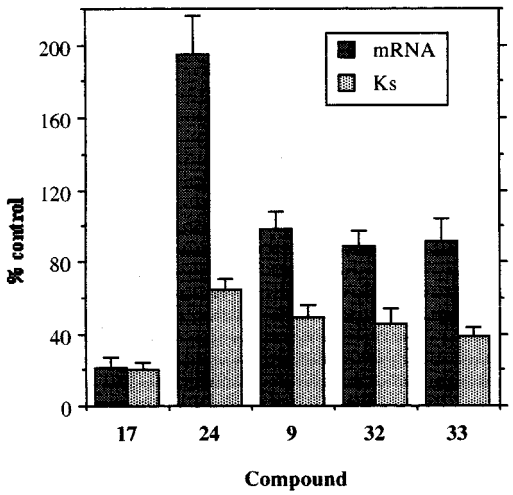
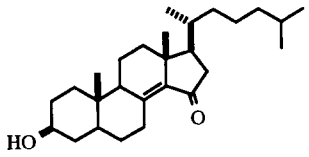


Figure 7 Lanosterol analogs have discordant effects on HMGR mRNA levels and synthetic rates. CHO cells were treated with 2.3 μ*M* 25-hydroxycholesterol **17** or 5.0 μ*M* 15α-fluorolanosterol **24**, lanosterol 32-aldehyde **9**, 15-oxime **33**, or 15-ketone **32**. HMGR mRNA levels were determined by Northern blot analysis. The relative rates of HMGR synthesis (K_s) were determined by immunoprecipitation of pulse-labeled HMGR. (Data adapted from references 31, 32, and 33.)

B. DISCORDANT EFFECTS ON HMGR AND LDL RECEPTOR (LDLR) GENE EXPRESSION

Coordinated transcriptional regulation of the LDLR and HMGR by sterols has been reported.[3] For example, the transcriptional regulator 25-hydroxycholesterol **17** causes comparable decreases in both LDLR and HMGR protein levels. This observation is of particular importance since the clearance of serum cholesterol is mediated by the LDLR and thus the optimal hypocholesterolemic drug should suppress HMGR activity without lowering LDLR protein levels. Interestingly, we have found 15-oxime **33** and 15-ketone **32** to show the desired effects on these two proteins, lowering HMGR levels without affecting LDLR protein concentration (Figure 9). 32-Carboxylic acid **37** and 24(*S*),25-oxidolanosterol **22** have also been reported to suppress HMGR activity without affecting cellular LDL metabolism.[30,38] The 15-ketosterol 3β-hydroxycholest-8(14)-en-15-one **44** has also been shown to suppress HMGR activity while stimulating LDL metabolism, but this effect was only observed at low or high inhibitor concentrations.[52] The ability of lanosterol analogs to suppress cholesterol synthesis without lowering LDLR activity suggests that this class of compound may prove to be useful as cholesterol lowering agents.



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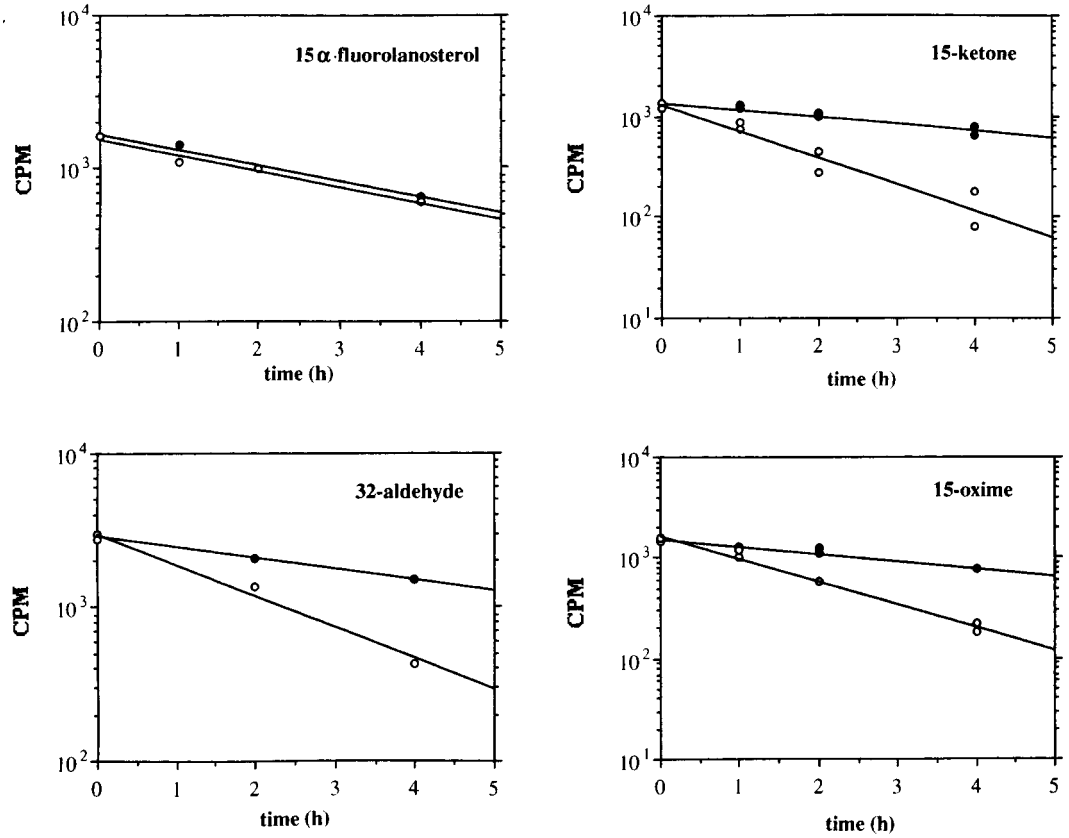

Figure 8 Effects of lanosterol analogs on HMGR degradation. CHO cells were pulse-labeled and chased for the indicated times in the presence or absence of 5.0 μ*M* 15α-fluorolanosterol **17**, lanosterol 32-aldehyde **9**, 15-ketone **32**, or 15-oxime **33**. Radiolabeled HMGR was isolated by immunoprecipitation and electrophoresis and quantitated with a liquid scintillation spectrophotometer. (Reprinted with permission from (1) Trzaskos, J. M. et al., *J. Biol. Chem.,* 268, 22591, 1993. Copyright 1993 American Society for Biochemistry and Molecular Biology; (2) Leonard, D. A., et al., *Arch. Biochem. Biophys.*, 310, 152, 1994. Copyright 1994 Academic Press; (3) Anderson, J. A. et al., *Arch. Biochem. Biophys.*, 316,190–196. Copyright 1995 Academic Press.)

IV. SUMMARY

Drugs which suppress hepatic cholesterol biosynthesis are important therapeutic tools for lowering serum cholesterol, a major risk factor in coronary heart disease. With the goal of developing molecules that will effectively shut down cholesterol biosynthesis in hepatic tissue but allow for the buildup of the isoprenes needed for the biosynthesis of polyisoprenes other than sterols, we have designed and evaluated a series of lanosterol analogs to act as dual-action inhibitors of cholesterol biosynthesis. These sterols were predicted to act as competitive inhibitors of lanosterol 14α-methyl demethylase (P-450DM) and as partial suppressors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), the rate-limiting enzyme in the pathway.

Compounds which have been identified as dual-action inhibitors of cholesterol biosynthesis include analogs of the intermediates generated during the removal of the 14α-methyl group of lanosterol by P-450DM, aminolanosterols with the amine nitrogen placed in the vicinity of C-32, and lanosterol analogs with a ketone or oxime functionality at C-15. While some dual-action inhibitors require an active P-450DM for suppression of HMGR activity, others do not. The inability of some compounds to suppress HMGR activity in cells which lack P-450DM activity suggests either that these compounds require P-450DM for conversion to an active metabolite which then suppresses HMGR activity, or that they cause the accumulation of the natural demethylation intermediates resulting in the suppression of HMGR activity. Lanosterol analogs, in contrast to 25-hydroxycholesterol, do not inhibit transcription of the HMGR gene. Rather, they inhibit translation of the HMGR mRNA, and in most cases also accelerate

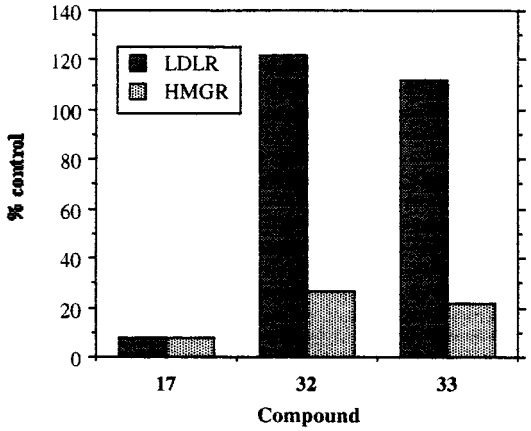

Figure 9 Lanosterol analogs suppress HMGR without altering LDLR protein concentration. Normal human fibroblasts were treated for 6 h with 2.3 μ*M* 25-hydroxycholesterol **17** or 5.0 μ*M* 15-oxime **33** or 15- ketone **32**. Quantitative immunoblot analysis was used to determine the relative concentrations of HMGR and LDLR protein. (Reprinted with permission from Anderson, J. A., Leonard, D. A., Cusack, K. P., and Frye, L. L., *Arch. Biochem. Biophys.*, 316, 190–196, 1995. Copyright 1995 Academic Press.)

the degradation of enzyme protein. The potential pharmacological utility of cholesterol biosynthesis inhibitors may be determined at least in part by their effects on LDL receptor (LDLR) activity. The transcriptional regulator 25-hydroxycholesterol suppresses both HMGR and LDLR activities, while the post-transcriptional regulatory lanosterol analogs exhibit a more desirable profile, lowering HMGR levels without suppressing LDLR expression, and in some cases actually enhancing cellular LDL metabolism. Lanosterol analogs which function as dual-action inhibitors of cholesterol biosynthesis promise to be useful not only as tools for dissecting the cellular regulation of cholesterol metabolism, but also as models for the development of safe, effective hypocholesterolemic agents.

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REFERENCES

1. **Blankenhorn, D. H., Nessim, S. A., Johnson, R. L., Sanmarco, M. E., Azen, S. P., and Cashin-Hempill, L.,** Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous by-pass grafts, *JAMA*, 257, 3233, 1987.
2. **Brown, M. S. and Goldstein, J. L.,** *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th ed., Pergamon Press, New York, 1990, 874.
3. **Goldstein, J. L. and Brown, M. S.,** Regulation of the mevalonate pathway, *Nature*, 343, 425, 1990.
4. **Brown, M. S., Faust, J. R., Goldstein, J. L., Kaneko, I., and Endo, A.,** Induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts incubated with compactin (ML-236B), a competitive inhibitor of the reductase, *J. Biol. Chem.*, 253 1121, 1978.
5. **Nakanishi, M., Goldstein, J. L., and Brown, M.S.,** Multivalent control of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Mevalonate-derived product inhibits translation of mRNA and accelerates degradation of enzyme, *J. Biol. Chem.*, 263, 8929, 1988.
6. **Gaylor, J. R.,** *Biosynthesis of Isoprenoid Compounds*, Vol. 1, John Wiley & Sons, New York, 1981, 481.
7. **Frye, L. L. and Leonard, D. A.,** Dual-action inhibitors of cholesterol biosynthesis: Lanosterol analogs that inhibit lanosterol 14α-methyl demethylase and suppress 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, in *Regulation of Isopentenoid Metabolism*, ACS Symposium Series 497, Nes, W. D., Parish, E. J., and Trzaskos, J. M. Eds., American Chemical Society, Washington, D.C., 1992, 94.

8. **Fischer, R. T., Trzaskos, J. M., Magolda, R. L., Ko, S. S., Brosz, C. S., and Larsen, B.,** Lanosterol 14α-methyl demethylase. Isolation and characterization of the third metabolically generated oxidative demethylation intermediate, *J. Biol. Chem.*, 266, 6124, 1991.
9. **Vaz, A. D. N., Roberts, E. S., and Coon, M. J.,** Olefin formation in the oxidative deformylation of aldehydes by cytochrome P-450. Mechanistic implications for catalysis by oxygen-derived peroxide, *J. Am. Chem. Soc.*, 113, 5886, 1991.
10. **Cole, P. A. and Robinson, C. H.,** A peroxide model reaction for placental aromatase, *J. Am. Chem. Soc.*, 110, 1284, 1988.
11. **Trzaskos, J. M., Fischer, R. T., and Favata, M. F.,** Mechanistic studies of lanosterol C-32 demethylation. Conditions which promote oxysterol intermediate accumulation during the demethylation process, *J. Biol. Chem.*, 261, 16937, 1986.
12. **Akhtar, M., Freeman, C. W., Wilton, D. C., Boar, R. B., and Copsey, D. B.,** The pathway for the removal of the 15α-methyl group of lanosterol. The role of lanost-8-ene-3β,32-diol in cholesterol biosynthesis, *Bioorg. Chem.*, 6, 473, 1977.
13. **Akhtar, M., Alexander, K., Boar, R. B., McGhie, J. F., and Barton, D. H. R.,** Chemical and enzymatic studies on the characterization of intermediates during the removal of the 14α-methyl group in cholesterol biosynthesis, *Biochem. J.*, 169, 449, 1978.
14. **Bossard, M. J., Tomaszek, T. A., Jr., Metcalf, B. W., and Adams, J. L.,** A novel, convenient assay of lanosterol 14α-methyl demethylase, *Bioorganic Chem.*, 17, 385, 1989.
15. **Sekigawa, Y., Sonoda, Y., and Sato, Y.,** Metabolism of 32-hydroxylated 24,25-dihydrolanosterols by partially purified cytochrome P-450_{14DM} from rat liver microsomes, *Chem. Pharm. Bull.*, 36, 3049, 1988.
16. **Gibbons, G. F., Pullinger, C. R., and Mitropoulos, K. A.,** Studies on the mechanism of lanosterol 14α-demethylation, *Biochem. J.*, 183, 309, 1979.
17. **Gibbons, G. F., Pullinger, C. R., Chen, H. W., Cavenee, W. K., and Kandutsch, A. A.,** Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols, *J. Biol. Chem.*, 255, 395, 1980.
18. **Kandutsch, A. A. and Taylor, F. R.,** Control of de novo cholesterol biosynthesis, in *Lipoproteins and Cholesterol Metabolism in Steroidogenic Tissues*, Straus, J. F., III, Menon, K. M. J., Eds., George F. Stickley Co., Philadelphia, PA, 1985, 1.
19. **Gibbons, G. F.,** Molecular control of 3-hydroxy-3-methylglutaryl coenzyme A reductase: The role of oxygenated sterols, in *3-Hydroxy-3-methylglutaryl Coenzyme A Reductase*, Sabine, J. R., Ed., CRC Press, Boca Raton, FL, 1983, 153.
20. **Kandutsch, A. A., Chen, H. W., and Heiniger, H.-J.,** Biological activity of some oxygenated sterols, *Science*, 201, 498, 1978.
21. **Kandutsch, A. A. and Chen, H. W.,** Inhibition of sterol synthesis in cultured mouse cells by 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol, *J. Biol. Chem.*, 248, 8408, 1973.
22. **Ness, G. C., Eales, S., Lopez, D., and Zhao, Z.,** Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression by sterol and nonsterols in rat liver, *Arch. Biochem. Biophys.*, 308, 420, 1994.
23. **Kandutsch, A. A. and Chen, H. W.,** Inhibition of sterol synthesis in cultured mouse cells by cholesterol derivatives oxygenated in the side chain, *J. Biol. Chem.*, 249, 6057, 1974.
24. **Saucier, S. E., Kandutsch, A. A., Taylor, F. R., Spencer, T. A., Phirwa, S., and Gayen, A. K.,** Identification of regulatory oxysterols, 24(*S*),25-epoxycholesterol and 25-hydroxycholesterol in cultured fibroblasts, *J. Biol. Chem.*, 260, 14571, 1985.
25. **Osborne, T. S., Goldstein, G. L., and Brown, M. S.,** 5′-End of HMG-CoA reductase gene contains sequence responsible for cholesterol mediated inhibition of transcription, *Cell*, 42, 203, 1985.
26. **Taylor, F. R., Saucier, S. E., Shown, E. P., Parish, E. J., and Kandutsch, A. A.,** Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase, *J. Biol. Chem.*, 259, 12383, 1984.
27. **Gibbons, G. F., Pullinger, C. R., Chen, H. W., Cavenee, W. K., and Kandutsch, A. A.,** Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols, *J. Biol. Chem.*, 255, 395, 1980.
28. **Trzaskos, J. M., Favata, M. F., Fischer, R. T., and Stam, S. H.,** In situ accumulation of 3β-hydroxylanost-8-en-32-al in hepatocyte cultures, *J. Biol. Chem.*, 262, 12261, 1987.
29. **Favata, M. F., Trzaskos, J. M., Chen, H. W., Fischer, R, T., and Greenberg, R. S.,** Modulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by azole antimycotics requires lanosterol demethylation, but not 24,25-epoxylanosterol formation, *J. Biol. Chem.*, 262, 12254, 1987.
30. **Panini, S. R., Delate, T. A., and Sinensky, M.,** Post-transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by 24(*S*),25-oxidolanosterol, *J. Biol. Chem.*, 267, 12647, 1992.
31. **Trzaskos, J. M., Magolda, R. L., Favata, M. F., Fischer, R. T., Johnson, P. R., Chen, H. W., Ko, S S., Leonard, D. A., and Gaylor, J. L.,** Modulation of 3-hydroxy-3-methyl-CoA reductase by 15α-fluorolanost-7-en-3β-ol. A mechanism-based inhibitor of cholesterol biosynthesis, *J. Biol. Chem.*, 268, 22591, 1993.
32. **Leonard, D. A., Kotarski, M. A., Tessiatore, J. E., Favata, M. F., and Trzaskos, J. M.,** Post-transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by 3β-hydroxylanost-8-en-32-al, an intermediate in the conversion of lanosterol to cholesterol, *Arch. Biochem. Biophys.*, 310, 152, 1994.

33. **Anderson, J. A., Leonard, D. A., Cusack, K. P., and Frye, L. L.,** 15-Substituted lanosterols: Post-transcriptional suppressors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, *Arch. Biochem. Biophys.*, 316, 190, 1995.
34. **Liscum, L., Luskey, K. L., Chin, D. J., Ho, Y. K., Goldstein, J. L., and Brown, M. S.,** Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and its mRNA in rat liver as studied with a monoclonal antibody and cDNA probe, *J. Biol. Chem.*, 258, 8450, 1983.
35. **Ness, G. C., Keller, R. K., and Pendleton, L. C.,** Feedback regulation of hepatic 3-hydroxy-3-methylglutaryl-CoA reductase activity by dietary cholesterol is not due to altered mRNA levels, *J. Biol. Chem.*, 266, 14854, 1991.
36. **Spady, D. K. and Cuthbert, J. A.,** Regulation of hepatic sterol metabolism in the rat, *J. Biol. Chem.*, 267, 5584, 1992.
37. **Ko, S. S., Brosz, C. S., Chen, H. W., Favata, M. F., Gaylor, J. L., Johnson, P. R., Magolda, R. L., Stam, S. H., and Trzaskos, J. M.,** Regulation of 3-hydroxy-3-methylglutaryl-CoA reductase gene expression — a new approach to cholesterol biosynthesis inhibitors. Presented at the FASEB/ACS Satellite Symposium, Biosynthesis and Utilization of Isoprenoids (Reductase V), April 1991, Atlanta, GA.
38. **Mayer, R. J., Adams, J. L., Bossard, M. J., and Berkhout, T. A.,** Effects of a novel lanosterol 14α-methyl demethylase inhibitor on the regulation of 3-hydroxy-3-methylglutaryl-CoA reductase in HepG2 cells, *J. Biol. Chem.*, 266, 20070, 1991.
39. **Frye, L. L., Cusack, K. P., and Leonard, D. A.,** 32-Methyl-32-oxylanosterols: Dual-action inhibitors of cholesterol biosynthesis, *J. Med. Chem.*, 36, 410, 1993.
40. **Frye, L. L., Cusack, K. P., Leonard, D. A., and Anderson, J. A.,** Oxolanosterol oximes: Dual-action inhibitors of cholesterol biosynthesis, *J. Lipid Res.*, 35, 1333, 1994.
41. **Schroepfer, G. J., Jr., Parish, E. J., Tsuda, M., Raulston, D. L., and Kandutsch, A. A.,** Inhibition of sterol biosynthesis in animal cells by 14α-alkyl-substituted 15-oxygenated sterols, *J. Lipid Res.*, 20, 994, 1979.
42. **Pinkerton, F. D., Izumi, A., Anderson, C. M., Miller, L. E., Kisic, A., and Schroepfer, G. J., Jr.,** 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol, a potent inhibitor of sterol biosynthesis, has two sites of action in cultured mammalian cells, *J. Biol. Chem.*, 257, 1929, 1982.
43. **Raulston, D. L., Pajewski, T. N., Miller, L. R., Phillip, B. W., Shapiro, D. J., and Schroepfer, G. J., Jr.,** Inhibition of cholesterol biosynthesis in cell-free preparations of rat liver by 14α-ethyl-5α-cholest-7-ene-3β,15α-diol, *Biochem. Int.*, 1, 113, 1980.
44. **Schroepfer, G. J., Jr., Parish, E. J., Tsuda, M., and Kandutsch, A. A.,** Inhibitors of sterol synthesis. Chemical syntheses, properties and effects of 4,4-dimethyl-15-oxygenated sterols on sterol synthesis and on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured mammalian cells, *Chem. Phys. Lipids*, 47, 187, 1988.
45. **Morisaki, M., Sonoda, Y., Makino, T., Ogihara, N., Ikekawa, N., and Sato, Y.,** Inhibitory effect of 15-oxygenated sterols on cholesterol synthesis from 24,25-dihydrolanosterol, *J. Biochem.*, 99, 597, 1986.
46. **Gaylor, J. L., Johnson, P. R., Ko, S. S., Magolda, R. L., Stam, S. H., and Trzaskos, J. M.,** *Steroid derivatives useful as hypocholesterolemics*, U.S. Patent 5 041 432, 1991.
47. **Beusen, D. D., Carrell, J. L., and Covey, D. F.,** Metabolism of 19-methyl-substituted steroids by human placental aromatase. *Biochemistry*, 26, 7833, 1987.
48. **Trzaskos, J. M., Bowen, W. D., Shafiee, A., Fischer, R. T., and Gaylor, J. L.,** Cytochrome P-450-dependent oxidation of lanosterol in cholesterol biosynthesis, *J. Biol. Chem.*, 259, 13402, 1984.
49. **Chen, H. W., Leonard, D. A., Fischer R. T., and Trzaskos, J. M.,** A mammalian mutant cell lacking detectable lanosterol 14α-methyl demethylase activity, *J. Biol. Chem.*, 263, 1248, 1988.
50. **Sheets, J. J. and Vickery, L. E.,** Active site-directed inhibitors of cytochrome P-450scc. Structural and mechanistic implications of a side chain-substituted series of amino-steroids, *J. Biol. Chem.*, 258, 11446, 1983.
51. **Wright, J. N., Calder, J. R., and Akhtar, M.,** Steroidal C-19 sulphur and nitrogen derivatives designed as aromatase inhibitors, *J. Chem. Soc. Chem. Commun.*, 1733, 1985.
52. **Pinkerton, F. D., Spilman, C. H., Via, D. P., and Schroepfer, G. J., Jr.**, Differing effects of three oxysterols on low density lipoproteins metabolism in HepG2 cells, *Arch. Biochem. Biophys.*, 193, 1091, 1993.