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A NOVEL APPROACH TO THE SITE SPECIFIC DELIVERY OF POTENTIAL HMG-CoA REDUCTASE INHIBITORS

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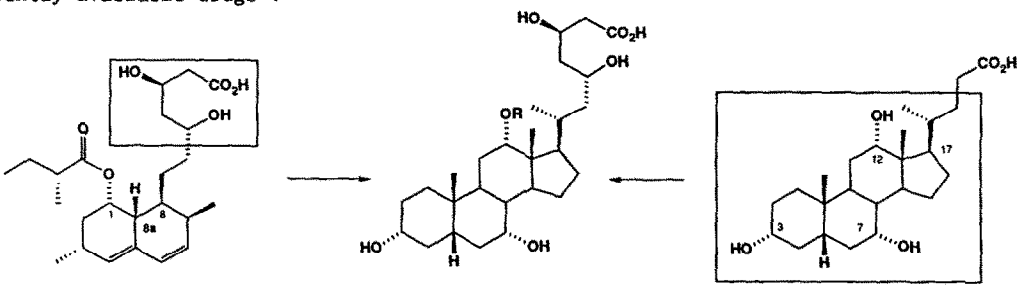
Abstract: A novel approach to the site specific delivery of potential HMG-CoA reductase inhibitors based on bile acid uptake is described. The synthesis of inhibitors **9** and **16** was achieved from cholic acid methyl ester. Both compounds **9** and **16** are weak inhibitors of HMG-CoA reductase with IC_{50} values of 39.2 and 12.3μM respectively. Compound **16** is transported non-specifically across an intestinal epithelial monolayer.

Elevated levels of serum cholesterol have been intimately linked to the occurrence of atherosclerosis and coronary heart disease[1,2]. Considerable attention has been directed towards the discovery and synthesis of hypolipidaemic agents which act as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme in cholesterol biosynthesis[3]. A major lead in this area was the discovery of the fungal metabolite mevinolin **1**[4], a highly potent inhibitor of HMG-CoA reductase which has as its major pharmacophore a 3,5-dihyroxyheptanoic acid side chain. Additionally, since the liver is the primary site for cholesterol homoeostasis[5] there is now seen to be great potential in enzyme inhibitors that do not affect extrahepatic HMG-CoA reductase; such compounds should elicit fewer side effects following chronic administration than currently available drugs[6].



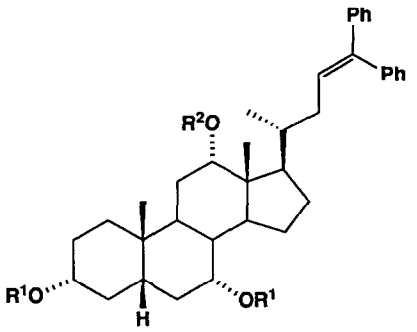
1 **2** **3**

SCHEME 1

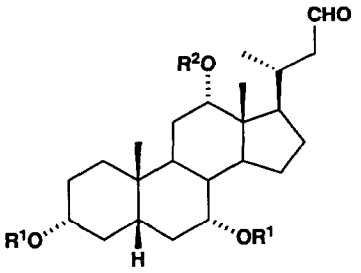
It is in the context of tissue selectivity that we have designed and synthesised a number of novel HMG-CoA reductase inhibitors **2** which utilise the remarkable affinity that bile acids, such as cholic acid **3** (Scheme 1), have for the enterohepatic circulation[7]. For example, in man bile acids are transported within the enterohepatic circulation; this system recycles between twelve and thirty six grams of bile acid per day with negligible loss through faecal excretion or through the circulation. The molecular requirements for bile acid transport are dependent on the general topology of the molecule, on the presence of a C-17 side chain carboxylic or sulphonic acid group which is extended from the ring junction with a chain length no greater than eight atoms, and on the C-3, C-7 and to a lesser extent C-12 β-hydroxyl groups[8]. Enzyme inhibition by mevinolin **1** and its analogues is critically dependent on the 3,5-dihydroxyheptanoic acid side chain although other secondary substrate-enzyme interactions contribute significantly to their potency[9].

The target structure **2** is a hybrid bile acid:HMG-CoA reductase inhibitor and has been designed to incorporate the necessary functionality for bile acid transport in the intestine and liver (both passive transport and active transport via the bile acid binding protein) together with the structural requirements necessary for HMG-CoA reductase inhibition. This has been achieved by elaboration of the C-17 side chain of cholic acid **3** to give a 3,5-dihydroxyheptanoic acid moiety suitable for enzyme inhibition which possesses the terminal carboxyl group and requisite chain length necessary for bile acid transport. Also noteworthy is a comparison of the three contiguous chiral centres at C-12, C-13 and C-17 in the bile acid **3** which have the equivalent stereochemistry to C-1, C-8a and C-8 in mevinolin **1**, further enhancing the structural relationship between bile acid and enzyme inhibitor. The synthesis and biological evaluation of the hybrid bile acid:HMG-CoA reductase inhibitors **9** and **16** are described.

The synthesis of two hybrid bile acid:HMG-CoA reductase inhibitors is outlined in Scheme 2. The diphenyl alkene **4** was readily available from cholic acid methyl ester after treatment with phenylmagnesium bromide (2 eq.) and dehydration in refluxing toluene-iodine. Exhaustive acylation of compound **4** in refluxing pyridine-acetic anhydride gave the 3,7,12-triacetyl alkene **5**. Ozonolysis of **5** (0°C, ethyl acetate) provided the seco-cholylaldehyde **6** after a reductive dimethyl sulphide work up[10]. Aldol condensation of the aldehyde **6** with methyl acetoacetate dianion (THF, -78°C 15 min., -30°C 30 min.)[11] gave the ketone **7** as a mixture of epimers about C-24 (1.27:1), with well resolved signals in the ^{1}H NMR for the methylene at C-27 for each epimer (δ3.48 and 3.50ppm) and readily separable by HPLC. Reduction of the ketone **7** with sodium borohydride (0°C, methanol) proceeded smoothly to give the alcohol **8** in 89% yield. Saponification with 1M sodium hydroxide (1eq., methanol), removal of the solvent, and trituration with ethanol-diethyl ether[12] gave the sodium salt **9** in 80% yield (mp. 159-161°C).



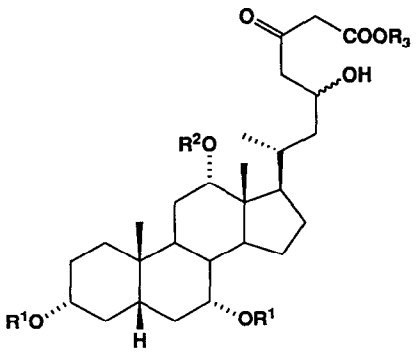
4 $R^1 = R^2 = H$
5 $R^1 = R^2 = Ac$



6 $R^1 = R^2 = Ac$

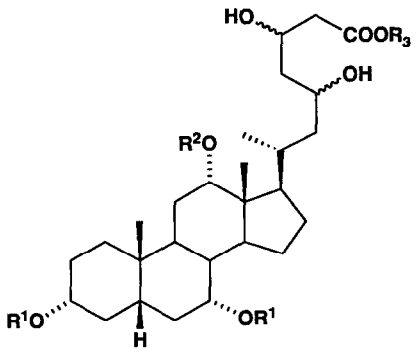
10 $R^1 = Ac$, $R^2 = H$
11 $R^1 = Ac$, $R^2 = COtBu$

12 $R^1 = Ac$, $R^2 = COtBu$



7 $R^1 = R^2 = Ac$, $R^3 = Me$

13 $R^1 = Ac$, $R^2 = COtBu$, $R^3 = Me$



8 $R^1 = R^2 = Ac$, $R^3 = Me$
9 $R^1 = R^2 = Ac$, $R^3 = Na^+$

14 $R^1 = Ac$, $R^2 = COtBu$, $R^3 = Me$
15 $R^1 = H$, $R^2 = COtBu$, $R^3 = H$
16 $R^1 = H$, $R^2 = COtBu$, $R^3 = Na^+$

SCHEME 2

By analogy with mevinolin **1** we reasoned that the regioselective introduction of a suitable side chain ester such as a trimethylacetyl ester at C-12 would lead to an analogue with enhanced potency over **9**[13]. The synthetic route to this C-12 monoacylated product requires the selective removal of protecting groups at C-3 and C-7. This would then furnish a bile acid derivative having the free hydroxyl groups at C-3 and C-7 which are necessary for transport within the enterohepatic circulation. To this end a dichloromethane solution of the diphenyl alkene **4** was treated with acetic anhydride (2 eq.) in the presence of 4-pyrrolidinopyridine (4 eq., 0°C-25°C) to afford selectively the 3,7-diacylated product **10** in 61% yield after flash chromatography. The 12-trimethylacetyl-3,7-diacetyl alkene **11** was obtained after treatment of the alkene **10** with trimethylacetyl chloride (10eq.) and N,N-dimethylaminopyridine (0.1 eq.) in refluxing pyridine, and was isolated as a white solid following recrystallisation from methanol-chloroform (mp. 188-189°C). Ozonolysis of **11** in methanol-chloroform gave substantial amounts of the dimethyl acetal. However, when the reaction was carried out in ethyl acetate the aldehyde **12** was readily formed after reduction of the ozonide with dimethyl sulphide. Aldol condensation of the aldehyde **12** with methyl acetoacetate dianion as before gave the the hydroxyketone **13** which underwent quantitative reduction with sodium borohydride to the alcohol **14**. Selective removal of the C-3 and C-7 acetyl and methyl ester protecting groups was achieved by the treatment of **14** with 10% methanolic potassium hydroxide at 80°C for 7 hours. The resulting 12-trimethylacetyl acid **15** was then converted directly to the corresponding sodium salt **16** according to the method described earlier for **9**. No evidence was found for the concomitant removal of the trimethylacetyl ester under these conditions and this method may prove useful for the selective protection of cholic acid derivatives.

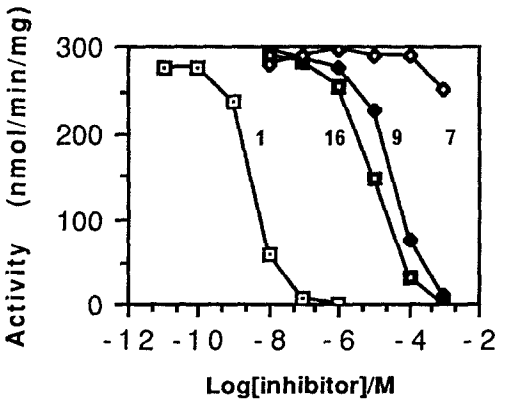


Figure 1. Comparative in vitro HMG-CoA reductase inhibition of compounds 1, 9 and 16. Compound 7 is included to show that no intrinsic activity resides with the bile acid nucleus.

The biological activity of compounds **9** and **16**, as unresolved diastereomeric pairs, was measured in a solubilised rat hepatic microsomal HMG-CoA reductase assay[14]. When compared to mevinolin **1** inhibitory activities are weak (**9**, IC_{50}=39.2μM and **16**, IC_{50}=12.3μM, cf. **1**, IC_{50}=3.69nM); however, they clearly show dose response profiles typical of this class of inhibitor (Figure 1). The ability of bile acid:HMG-CoA reductase inhibitors to be transported across the intestinal epithelia via the bile acid binding protein (specific transport) was examined in confluent, polarised layers of Caco-2 cells[15]. Compound **16**, labelled with tritium at C-25, is transported across this endothelial like cell layer non-specifically. This would indicate that the predominant mode of transport for inhibitor **16** does not involve the bile acid binding protein. Further biological and chemical profiling of these hybrid bile acid:HMG-CoA reductase inhibitors is under way.

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