Bioorganic & Medicinal Chemistry Letters, Vol.2, No.3, pp. 223-228, 1992
Printed in Great Britain

0960-894X/92 $3.00 + .00

THE DESIGN AND BIOLOGICAL EVALUATION OF A SERIES OF 3-HYDROXY-3-METHYLGLUTARYL COENZYME A (HMG-CoA) REDUCTASE INHIBITORS RELATED TO DIHYDROMEVINOLIN.

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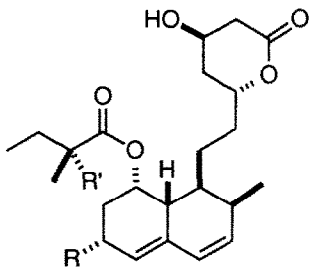
(*Received* 25 *November* 1991)

Abstract: HMG-CoA reductase inhibitors structurally related to dihydromevinolin have been designed and tested. It has been shown that for high inhibitory potency these compounds must possess a methyl group at the C-7 position, but several different alkenes can be tolerated at the C-3 position. These compounds show good activity both in vitro and in vivo.

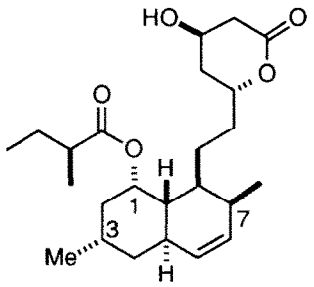
Coronary heart disease (CHD) and atherosclerosis are the major causes of mortality in the western world. Epidemiological studies have revealed that there is a strong correlation between the incidence of CHD and the level of cholesterol in the blood, particularly low density lipoprotein cholesterol. Over the past fifteen years compounds **1-3**, now known as mevinic acids, have been shown to be highly effective LDL-cholesterol lowering agents, through their ability to inhibit HMG-CoA reductase, the rate limiting enzyme in the biosynthesis of cholesterol.[1] Compactin[2] **1** and mevinolin[3] **2**, both fungal metabolites and the earliest mevinic acids to be discovered, have complex structures with several centres of asymmetry, and many groups have therefore designed and synthesised HMG-CoA reductase inhibitors in which the decalin portion of the natural products has been replaced by a simpler, substituted ring system, such as biphenyl[4] or indole.[5] In this letter we disclose some new structure-activity relationships of close analogues of the natural products themselves.

In all the known mevinic acids there is a methyl group at the C-7 position of the decalin ring.[6] In the first instance we were interested in examining compounds in which this substituent was altered and chose the C-7 isopropyl substituted and the unsubstituted compounds as initial



			Rel. Activity[a]
1	R = H ; R' = H	Compactin	100
2	R = Me ; R' = H	Mevinolin	254
3	R = Me ; R' = Me	Simvastatin	622

a) Relative activities taken from reference 7

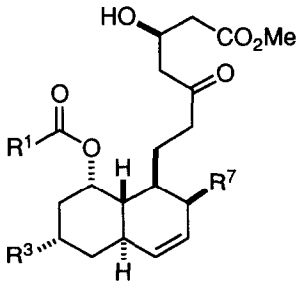


		Rel. Activity[b]
4	Dihydromevinolin	188

b) Calculated from IC_{50} values in reference 10

targets. Secondly, we noted the trend of increasing activity from compactin **1** through mevinolin **2** to simvastatin **3** (a semi-synthetic derivative of mevinolin[7]). It occurred to us that this may arise from the increasing 1,3 diaxial interaction as extra methyl groups were introduced first at the C-3 position (compactin to mevinolin) and then on the C-1 ester (mevinolin to simvastatin). This increasing interaction would in turn restrict the conformational space available to the carbonyl group, which is important for effective binding.[8] The Merck group has explored structural changes of the C-1 ester in the mevinolin/ simvastatin series and concluded that there is little correlation of activity with the log P or volume of the ester side chain, and that compact, branched aliphatic groups give maximum inhibitory potency.[7] This appeared to support our hypothesis that the 1,3 interaction is important. We therefore decided to make a series of changes at the C-3 position. However, since the increase in activity from compactin with a hydrogen atom at C-3 to mevinolin with a methyl group is only moderate, we selected the more rigid C-3 alkenes rather than the alkanes as our primary target molecules. Since our work was concluded, Clive has published the synthesis of the C-3 ethyl analogue of mevinolin that has indistinguishable biological activity from the parent compound.[9] Finally, we decided to prepare compounds in the 4,4a dihydro series. Although we expected that this would lead to a slight decrease in inhibitory potency, based on the comparison between mevinolin and dihydromevinolin[10] **4** (a minor co-metabolite produced with mevinolin), we felt that the dihydro-compounds would be less prone to oxidation in vivo and excretion.[11]

The syntheses of the novel compounds of formulae **5** and **6** will be described elsewhere. The compounds were hydrolysed to the sodium salts of the dihydroxy acids and their ability to inhibit microsomal rat liver HMG-CoA reductase evaluated.[12] The IC_{50} values are presented in



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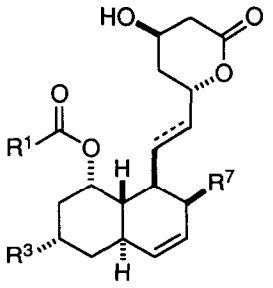

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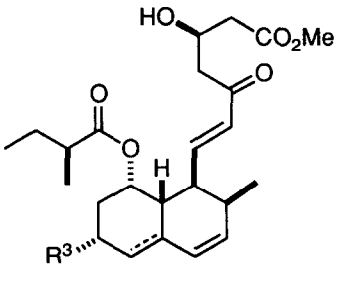

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a) R^3 = H ; 4,4a = double bond
b) R^3 = Me ; 4,4a = single bond

Table 1. In general the dimethylbutyrate esters are twice as active as those with the monomethylbutyrate group, and both are far more potent than the compound with an acetate ester at C-1 (**6e,f** vs **6d**). The compounds with a ketone group at C-5' have approximately the same activity as their reduced counterparts (**5a,b** vs **4,6b**), but the introduction of a double bond into the side chain at the 6',7' position reduces activity 30 fold (**6i** vs **6h**). These results are similar to those

TABLE 1

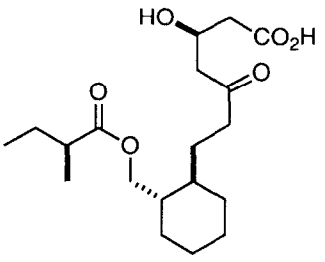
Compound	R^1	R^3	R^7	IC_{50}(nM)[a]	Selective[b]
Mevinolin[c]	(S)-$CH(CH_3)CH_2CH_3$	Me	Me	11	+
4	(S)-$CH(CH_3)CH_2CH_3$	Me	Me	30	
5a	(S)-$CH(CH_3)CH_2CH_3$	Me	Me	80	
6b	(S)-$CH(CH_3)CH_2CH_3$	Me	i-Pr	1000	
5b	(S)-$CH(CH_3)CH_2CH_3$	Me	i-Pr	6000	
6c	(S)-$CH(CH_3)CH_2CH_3$	Me	H	1500	
6d	CH_3	cis $CH{=}CHCH_3$	Me	1500	
6e	(S)-$CH(CH_3)CH_2CH_3$	cis $CH{=}CHCH_3$	Me	30	
6f	$C(CH_3)_2CH_2CH_3$	cis $CH{=}CHCH_3$	Me	15	
6g	(S)-$CH(CH_3)CH_2CH_3$	trans $CH{=}CHCH_3$	Me	4	-
6h	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CHCH_3$	Me	3	+
6i[d]	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CHCH_3$	Me	100	
6j	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CHCH_2CH_3$	Me	15	
6k	(S)-$CH(CH_3)CH_2CH_3$	trans $CH{=}CH(CH_2)_3CH_3$	Me	20	-
6l	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CH(CH_2)_3CH_3$	Me	8	-
6m	(S)-$CH(CH_3)CH_2CH_3$	trans $CH{=}CHCH_2Ph$	Me	7	-
6n	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CHCH_2Ph$	Me	20	
6o	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CHCH(CH_3)_2$	Me	1200	
6p[e]	$C(CH_3)_2CH_2CH_3$	trans $CH{=}CHCH_3$	Me	5	+

a) of sodium salt against microsomal rat liver HMG-CoA reductase, mean of at least 3 determinations;
b) In vivo rat assay + greater inhibition in liver than in spleen, testes, kidney,
- no significant diffence between organs;
c) has 4,4a double bond; d) has 6',7' double bond; e) also has equatorial methyl group at C-3

obtained by other workers.[7,13]

The potencies of the compounds with changes at the C-7 position are revealing. Both the unsubstituted (**6c**) and the isopropyl substituted (**6b**) compounds are very much less potent than the methyl substituted analogue, dihydromevinolin **4**. Molecular modelling[14] indicates that the C-7 substituent does not significantly affect the conformational space available to the dihydroxy acid side chain, nor the low energy conformation of the decalin ring. These results are corroborated by the NMR spectra in which the chemical shifts and coupling constants for the ring protons are similar for the three compounds. We therefore conclude that the enzyme active site has a pocket that binds tightly to the methyl group, since the loss of binding energy when the methyl group is removed (approximately 9.6 kJ/mol) is reasonable and well within the limits discussed by Fersht.[15] The isopropyl group of **6b** is simply too large to fit into the enzyme pocket and results in poor activity. Compound **6i** and the previously reported[13] enones **7a** and **7b**, in which the dihydroxy acid side chain is more rigid because of the double bond, may be less potent since it is not possible for the C-7 methyl group to fit properly into its pocket.

This hypothesis also allows us to explain the difference in activity of some recently reported mono-cyclic derivatives of mevinic acids. Heathcock[16] has showed that the disubstituted cyclohexane **8** is some 10,000 fold less potent than compactin **1**, a result confirmed by the data obtained by Bristol-Myers-Squibb with **9**.[17] In contrast, the methyl substituted cyclohexane **10** has good activity and is 658 times more potent than **9**.[17] This increased potency is similar in magnitude to the difference between **4** and **6c** and therefore is probably due to the presence of the crucial methyl group at the "C-7" position in **10**, which is missing in both **9** and the Heathcock compound.



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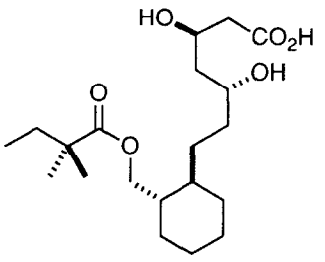

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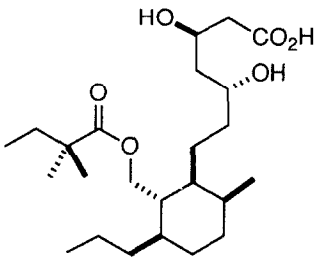

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Interpretation of the results from the inhibitors with C-3 substituents is more difficult. Except for the isopropyl substituted trans alkene **6o**, all the compounds have a similar potency. Although trans alkene substituted compounds are somewhat more potent than the cis isomers

(**6g,h** vs **6e,f**), the changes in activity do not correlate with log P or size parameters. This probably indicates that there is a large, irregularly shaped hydrophobic region on the enzyme surface, possibly the binding pocket for a portion of the coenzyme A molecule, which gives some interaction with our inhibitors. Heathcock[16] has postulated a similar binding region for the C-2, C-3, C-4 carbons of the decalin ring. That the surface available for hydrophobic binding is restricted is shown by the severely reduced activity of **6o**, and by the inverted order of activity of the benzylic substituted alkenes in which, unusually, the dimethylbutyryl ester **6n** is less potent that the monomethyl ester **6m**.

Selected compounds were tested for their ability to inhibit cholesterol synthesis in the rat.[18] In these experiments the animals were orally pre-treated with the test compound at 1 mg/kg and injected one hour later with sodium [1-^{14}C] acetate i.p. The animals were subsequently killed and the liver, kidneys, spleen and testes removed. These organs were homogenised and the sterols saponified, extracted, precipitated with digitonin and quantified by liquid scintillation counting. In this way the *organ* selectivity of the test compounds could be established (Table 1). Since the major site of cholesterol biosynthesis is the liver, a desirable property of any inhibitor would be liver selectivity. Although it has been shown that *tissue* selectivity of a wide range of HMG-CoA reductase inhibitors in an assay using tissue cubes is related to the calculated log P,[19] in our in vivo experiments no such trend is apparent.

In conclusion, we have shown that the active site of HMG-CoA reductase has two important features in addition to the dihydroxy acid side chain and C-1 ester binding clefts. These are a pocket that binds tightly to the methyl group found at C-7 of the natural mevinic acids, and a large hydrophobic surface near to C-3. Compounds that take advantage of both of these features are highly potent cholesterol biosynthesis inhibitors.

Acknowledgement

We would like to thank Y. Hase (Japan Tobacco Inc.) and P. Huxley for confirming the molecular modelling results and for useful discussion.

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