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THE SQUALESTATINS: NOVEL INHIBITORS OF SQUALENE SYNTHASE. THE OPTIMAL C1 CHAIN-LENGTH REQUIREMENTS.

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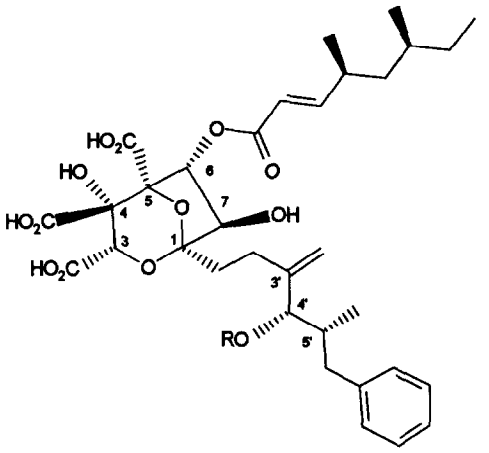
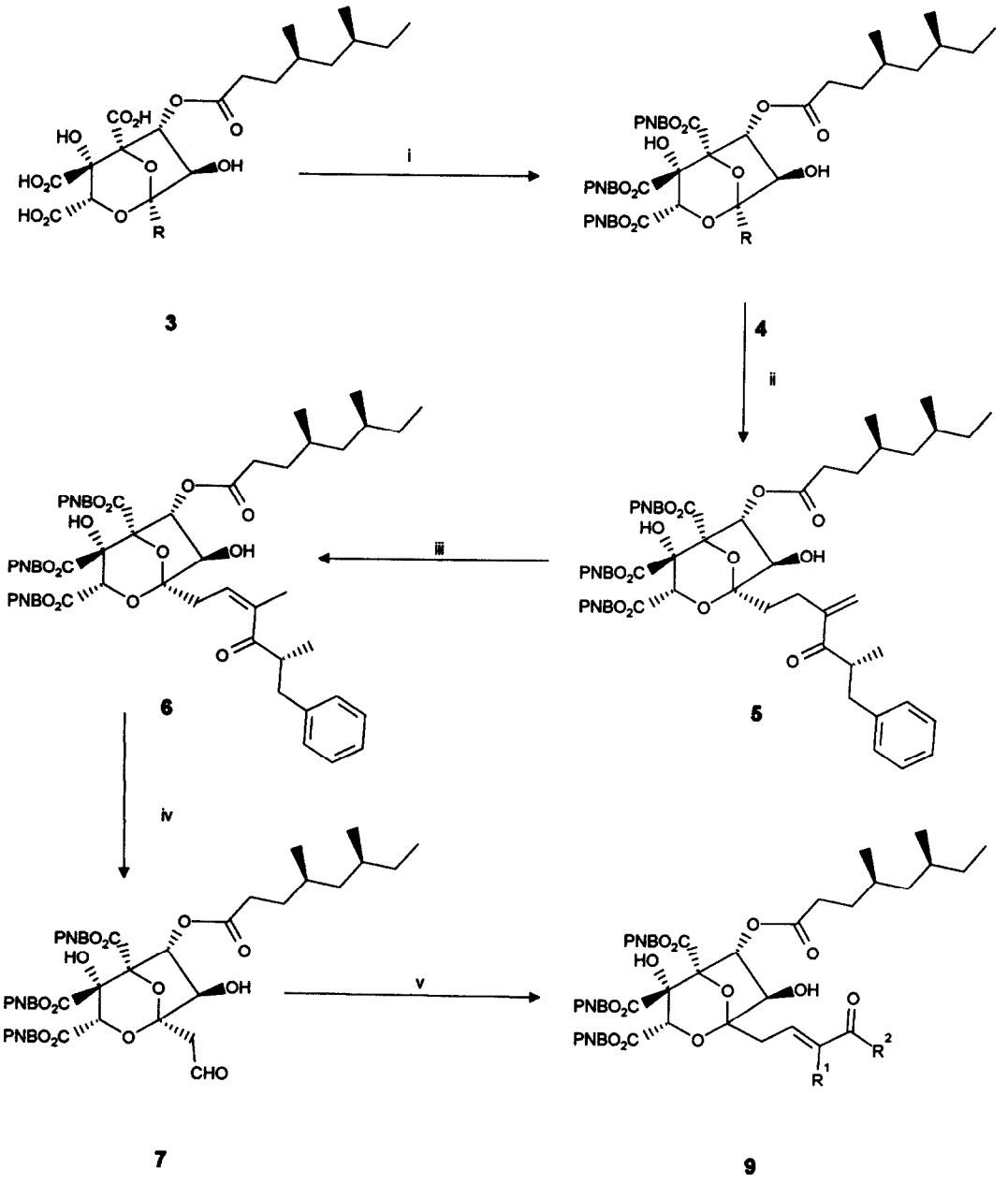
Abstract: Analogues of squalestatin **1** modified in the C1 side-chain were prepared and evaluated for their ability to inhibit squalene synthase *in vitro*. An appropriately substituted 6-phenylhexyl chain was found to be optimal for effective enzyme inhibition.

We have recently published the isolation[1] and structure elucidation [2] of the squalestatins , a novel group of fungal metabolites isolated from a previously unknown *Phoma* species (Coelomycetes). Squalestatin **1** is a potent and selective inhibitor of both rat and *Candida* squalene synthase (SQS) enzymes; 50% inhibition of rat SQS activity is observed *in vitro* at a concentration of 12 nM. Furthermore when **1** is administered orally to marmosets for seven days 50% reduction in serum cholesterol levels is observed at a dose of 10 mg/kg/day. [3a] These findings could lead to the development of new therapies for elevated serum cholesterol in humans. Squalestatin **1** incorporates the highly substituted 2,8-dioxabicyclo[3.2.1]octane system possessing carboxylic acid groups at C3, C4 and C5, hydroxyl groups at C4 and C7, and two lipophilic side-chains at C1 and C6.



1 R=Ac

2 R=H



Reagents: i) p-nitrobenzyl bromide, Et_3N, DMF; ii) PCC, CH_2Cl_2 ; iii) $RhCl_3$, MeOH, H_2O, reflux
iv) a)O_3, CH_2Cl_2, -70° ; b) PPh_3 ; v) NaH, $(MeO)_2POCHR^1COR^2$ (8) , THF.

Currently we are engaged in a chemical programme aimed at the systematic modification of the squalestatins, and the identification of the pharmacophore. In this communication we report the optimal C1 chain-length requirements for effective SQS inhibitory activity.

The natural product **2**, available alternatively by selective acid catalysed hydrolysis of **1** (1:1 10% aqueous sulphuric acid-acetone), was a particularly attractive starting material as it possessed the allylic alcohol group which could be selectively exploited. We have shown [4] that potent SQS inhibitory activity is retained when the α,β-unsaturated ester of **1** is replaced with the 4,6-dimethyloctanoate group; the chemistry described in this paper required the presence of this group at C6. Partial hydrogenation of **2** over 5% Pd on $BaSO_4$ in ethanol for 1h followed by reverse-phase HPLC on a Spherisorb 5 ODS-2 column provided the allylic alcohol **3a** as the major product (80%); the regioisomeric allylic alcohol **3b** (10%), the hydrogenolysis product **3c** (isolated as a 1:4 mixture of *E*:*Z* olefins; 5%), and the diastereoisomeric ketones **3d** (5%) were isolated as minor byproducts. The diastereoisomeric ketones **3d** with shorter and longer retention times on HPLC were assigned as isomer 1 and 2, respectively. Compounds **3a**, **3b**, **3c**, and **3d** were evaluated for their inhibitory activity against rat liver SQS and the data are shown in the Table. The enzyme preparation and assay procedures used in this study were the same as those described in our earlier publications.[3a,3b] The allylic alcohols **3a** and **3b** were found to possess activities closely similar to that for the parent natural product **2** in line with our findings in the allylic acetate series.[4] Compound **3c** was found to possess a slightly reduced level of activity while there was a significant difference between the activities of diastereoisomeric dimethylketones **3d**. Although the configuration at C3' could not be established by circular dichroism or NMR studies it was evident from the NMR spectra that the C1 side-chains in the two diastereoisomers adopt different conformations from each other which could account for the observed difference in activity.

The importance of the C3' substituent was investigated by initial conversion of the crude hydrogenation mixture described above to the respective tris *p*-nitrobenzyl (PNB) esters **4** , followed by column chromatography to provide the allylic alcohol **4a**. Ozonolysis of **4a**, followed by removal of the PNB esters by catalytic hydrogenolysis gave the hydroxyketone **3e**, reduction of which provided diol **3f**. Both **3e** and **3f** were found to be equipotent with **2**, indicating that polar and hydrophilic groups are well tolerated at C3'.

In order to establish whether the C1 chain plays a critical role in the potency associated with the squalestatins, routes to analogues with truncated C1 side-chains were established. Selective oxidation of the allylic alcohol in **4a** was accomplished using pyridinium chlorochromate (PCC) to give the enone **5**, rhodium (III) chloride catalysed isomerisation [5] of which provided enone **6**. Ozonolysis of **6** gave the aldehyde **7**, which on reduction with $NaBH_4$, followed by removal of the PNB esters provided the hydroxyethyl analogue **3g**. Alcohol **3g** was devoid of any significant SQS inhibitory activity. Ozonolysis of **4c**, followed by removal of the PNB esters gave the truncated methyl ketone **3h** which possessed only modest inhibitory activity against SQS.

Following these findings, a major objective was to establish the optimal C1 side-chain length for effective SQS inhibitory activity. Having established that a ketone group is well tolerated at C4' a general route was developed for the synthesis of analogues that utilised the reaction of aldehyde **7** with stabilised phosphonate carbanions **8** which provided *trans*-enones **9** in the key coupling step. Enones **9** were hydrogenated catalytically to provide the analogous ketones **3**. Studies were carried out on analogues without C3' or C5' substituents as preliminary investigations on ketones **3i**, **3j** and **3k** had shown these to possess closely similar SQS inhibitory activities; ketones **3i** and **3j** were isolated as diastereoisomeric mixtures at C3' and C5' respectively.

Table. ***In Vitro*** **SQS Inhibitory Activity**

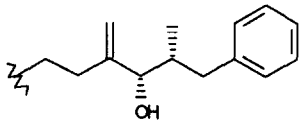
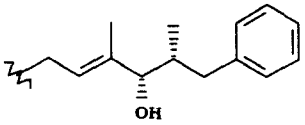
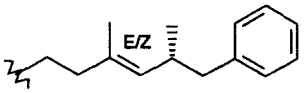
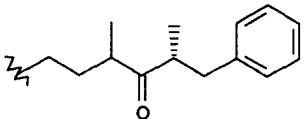
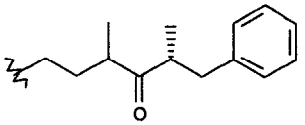
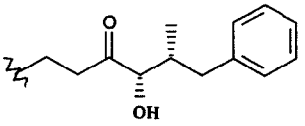
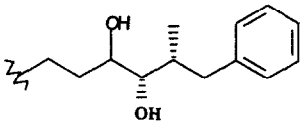
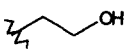
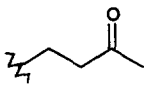
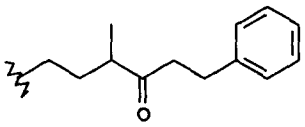
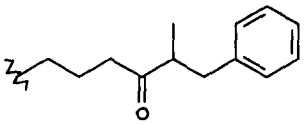
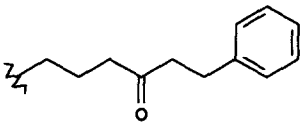
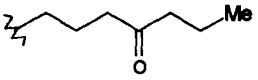
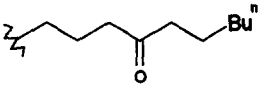
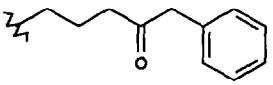
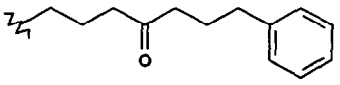
Compound	R	IC_{50} (nM)
3a		**2**
3b		**17**
3c		**63**
3d isomer 1		**16**
3d isomer 2		**130**
3e		**5**
3f		**4**
3g		**>500**

Table continued

Compound	R	IC_{50} (nM)
3h		**470**
3i		**48**
3j		**29**
3k		**56**
3l		**>500**
3m		**>500**
3n		**>500**
3o		**152**

SQS activity was measured using juvenile male rat liver microsomes as enzyme source. IC_{50} values were determined at least in duplicate at each concentration, and are expressed as mean values, using squalestatin 1 as a reference according to the assay procedure described in reference 3b. Squalestatins 1 and 2 possessed IC_{50} of 12 and 5 nM respectively.

Replacement of the phenyl ring present in the ketone **3k** with a methyl or butyl group **3l** and **3m** caused a dramatic loss of activity. This finding is in agreement with our observations[4] that replacement of the phenyl ring with a cyclohexyl ring causes a significant loss of activity. Replacement of the phenylhexanone chain present in **3k** by the phenylpentanone group provided **3n** which was without significant activity, while the homologous phenylheptanone **3o** is significantly less active than **3k**. Thus it is clear that in this homologous series an appropriately substituted phenylhexyl unit is optimal for securing potent SQS inhibitory activity.

We believe that squalestatins possessing the 4,6-dimethyloctenoate/dimethyloctanoate ester at C6 may be presqualene diphosphate (PSDP) mimetics, whereas those possessing only a hydroxyl group at C6 may be mimetics of farnesyl diphosphate (FPP). No investigations have appeared in the literature concerning the SAR of PSDP. However, SAR for FPP mimetics have indicated the critical dependence of inhibitory activity on both the presence of the double bonds and in the chain length of the farnesyl chain.[6] The tricarboxylic acid moiety of the squalestatins is thought to be mimicking the diphosphate moiety, whereas the two lipophilic chains of the squalestatins are thought to be mimicking the farnesyl derived side-chains of PSDP. The aromatic ring of the squalestatins might be providing additional binding to the enzyme analogous to that provided by the double bonds in the farnesyl chains of FPP. Thus removal of the phenyl group of the squalestatins resulted in the dramatic loss of activity described above. The loss of activity observed on shortening the C1 side-chain of the squalestatins is also consistent with the observation that truncated FPP analogues are poor inhibitors of SQS.[6]

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