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SEMI-SYNTHETIC SQUALESTATINS: SQUALENE SYNTHASE INHIBITION AND ANTIFUNGAL ACTIVITY. THE SAR OF C6 AND C7 MODIFICATIONS

Gerard M P Giblin*, Richard Bell, Ashley P Hancock, C David Hartley, Graham G A Inglis, Jeremy J Payne, Panayiotis A Procopiou, Anthony H Shingler, Colin Smith and Stephen J Spooner
Departments of Medicinal Chemistry, Glaxo Group Research, Greenford, Middx. UB6 0HE. UK

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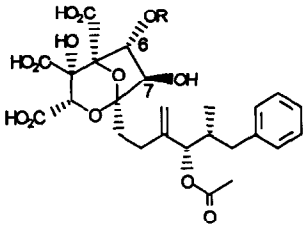
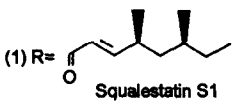
Abstract: We describe herein a protection/deprotection strategy that enables efficient transformation of natural Squalestatin S1 into C6 and C7 acyl analogues. We present the mammalian and fungal SQS enzyme activity and whole cell antifungal activity of the semi-synthetic Squalestatins.

The Squalestatins[1] are a series of natural products of a novel structural type.[2] They are potent inhibitors[3] of the enzyme squalene synthase (SQS), a key step in the sterol biosynthesis pathway[4] of eukaryotes and consequently are potential cholesterol lowering agents to rival HMGR inhibitors[5] in the treatment of hypercholesterolemia.

During a continuing biological investigation of these compounds we observed that Squalestatin S1 was a potent whole cell antifungal agent whereas Squalestatin H1, lacking the lipophilic C6 (Squalestatin numbering) substituent, was essentially devoid of any antifungal activity. We thus embarked on a medicinal chemistry programme to investigate the requirements for both *in-vitro* mammalian and fungal SQS inhibition and whole cell antifungal activity around the C6 and C7 positions.



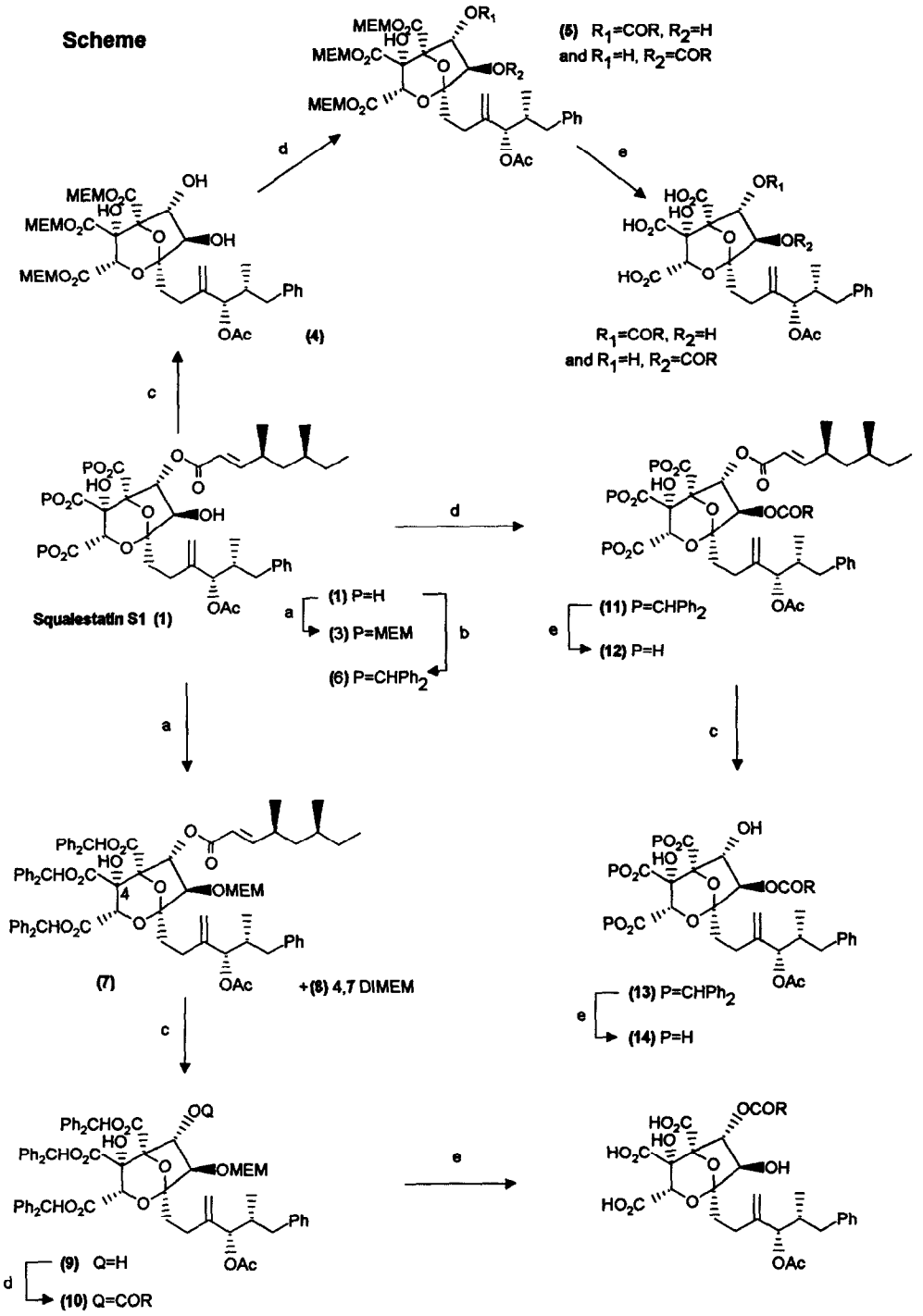


Squalestatin S1

(2) R= H, Squalestatin H1

In order to conveniently modify Squalestatin S1 (1) we sought suitable protection of the tri-carboxylic acid functionality of the molecule. Thus treatment of Squalestatin S1 with excess 2-methoxyethoxymethyl (MEM) chloride in refluxing dichloroethane in the presence of Hunig's base gave the tris-MEM ester (3) in quantitative yield. Removal of the C-6 side chain with N-methylhydroxylamine[6] in DMF gave the rather labile protected diol (4). Treatment of this diol with an acid chloride in the presence of DMAP furnished a mixture of C6 and C7 functionalised esters (5); these were separated by silica gel chromatography (SGC). Finally removal of the MEM protecting groups with aqueous formic acid followed by purification by reverse phase preparative HPLC gave the required C6 and C7 ester analogues of Squalestatin S1.

Scheme



Reagents: a, MEMCl, $^{i}Pr_2EtN$, DCE; b, Ph_2CN_2; c, MeNHOH.HCl, Et_3N, DMF; d, RCOCl, DMAP; e, HCO_2H, H_2O; then HPLC. For alkyl groups designated R, see table.

Although this procedure allowed rapid access to a number of analogues we were keen to develop a more selective synthetic route. Consequently treatment of Squalestatin S1 with 3 equivalents of diphenyldiazomethane gave the tris(diphenylmethyl) (DPM) ester (6) in quantitative yield. Protection of the free C7 hydroxyl with the MEM group gave the intermediate (7) in 75% yield together with its 4-MEM derivative (8) (7%) which were conveniently separated by SGC. Exposure of (7) to N-methylhydroxylamine gave the C6 alcohol (9) in 90% yield *with no trace of acetate cleavage in the C1 side chain.* Functionalisation of the free C6 hydroxyl was then accomplished with a suitable acid chloride or chloroformate to obtain fully protected C6 analogues (10) of Squalestatin S1 that were deprotected in a single step as before and purified by HPLC.

We modified this protection strategy to allow selective functionalisation of the C7 hydroxyl. Tris(DPM) ester (6) was derivatised at C7 using the standard acylation conditions to give protected C7 acyl derivatives (11) and simple DPM ester deprotection conditions gave C7 derivatives (12) of Squalestatin S1. Alternatively, cleavage of the C6 octenoate of (11) using N-methylhydroxylamine gave the C6 alcohol (13) which was deprotected to furnish C7 acyl derivatives (14) of Squalestatin H1. The semi-synthetic squalestatins synthesised using these methods are listed in the Table.

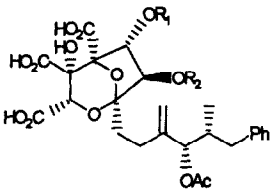
The semi-synthetic analogues were tested for inhibition of rat liver SQS and *Candida albicans* SQS. In addition compounds were evaluated in an antifungal whole cell assay to obtain minimum inhibitory concentrations (MIC) against representative phenotypes of *Candida, Aspergillus* and *Cryptococcus* gena. The results are collated in the Table.

Squalestatin S1 inhibits rat liver SQS[7] with an IC_{50} of 12nM. The closest analogues of Squalestatin S1, the C6 esters (15)-(23) retain potency at the nanomolar level. Various degrees of substitution along the chain are well tolerated; however the least active of this group (20) bears a secondary carbon centre adjacent to the ester. The most active C6 ester is the acetate (15) although chain length appears relatively unimportant. In the related carbonate series (32)-(34) the C6 methyl carbonate (32) is the most active.

C7 substitution is less well tolerated; small substituents such as the acetate (24) and the methyl carbonate (35) retain nanomolar activity only in the presence of the lipophilic C6 side chain of Squalestatin S1. C7 acetate (25) and methyl carbonate (36) in the Squalestatin H1 series are much less active. Derivatives containing larger C7 substituents (for example (26)) are essentially inactive irrespective of the substituent at C6 (compare for example (26), (27) and (30)). Shorter chain diesters (29) and (31) are 10-fold less active than Squalestatin S1 (1). Activity against the fungal enzyme[8] closely mirrors the mammalian system which suggests significant homology between the two enzymes.[9]

We observe that Squalestatin S1 is a potent antifungal agent whereas Squalestatin H1 is essentially devoid of activity, despite equipotent enzyme activity. A possible explanation for this effect is that lipophilicity is an important factor in fungal cell wall penetration of these agents. The semi-synthetic Echinocandins show antifungal activity that varies with the chain length of a lipophilic side chain.[10] In our series we find that short chain C6 esters (15) and (16) show no antifungal activity whereas the unsubstituted C6 octanoate (17) is significantly less active than Squalestatin S1 against Candida. Increasing chain length by two carbons (C6 decanoate (18)) increases activity, suggesting further evidence for the lipophilicity contributing to antifungal activity. The nonyl

Table. The SQS enzyme activity and whole cell antifungal activity of C6 and C7 modified Squalestatins.



				IC_{50} nM		MIC[2] μgml^{-1}		
No	R_1	R_2	SYNTH METH[1]	RAT SQS	FUNGAL SQS	CAND.	ASPER.	CRYPT.
1	S*	OH	A	12[3]	5	8	16	0.5
2	OH	OH	A	6	NT	>125	>125	>125
15	OAc	OH	B	2	NT	>125	>125	>125
16	$OCO(CH_2)_3Me$	OH	C	6	26	>125	>125	>125
17	$OCO(CH_2)_6Me$	OH	B	3	NT	31	8	1
18	$OCO(CH_2)_8Me$	OH	B	8	NT	16	2	2
19	$OCO(CH_2)_6Ph$	OH	C	9	7	125	62	2
20	$OCO(C_6H_{11})$	OH	C	17	17	>62	>62	31
21	OCO(CH=CH)Ph	OH	C	4	10	>125	>125	31
22	$OCO(CH_2)(C_6H_{11})$	OH	C	8	4	62	62	2
23	OCOPh	OH	C	8	8	>125	>125	>125
24	S*	OAc	C	10	NT	>125	8	1
25	OH	OAc	B	NT	>200	>125	>125	NT
26	S*	$OCO(CH_2)_6Me$	C	>500	>200	>32	>32	>32
27	OH	$OCO(CH_2)_6Me$	C	>500	>200	>125	>125	>125
28	OH	$OCO(CH_2)_8Me$	B	303	NT	>125	>125	>125
29	$OCO(CH_2)_2Me$	$OCO(CH_2)_2Me$	B	59	>147	>125	>125	>125
30	$OCO(CH_2)_6Me$	$OCO(CH_2)_6Me$	B	280	>200	>125	>125	>125
31	OAc	OAc	B	56	>200	>125	>125	ND
32	OCO_2Me	OH	C	3	NT	>125	>125	>125
33	$OCO_2(CH_2)_3Me$	OH	C	4	8	>125	>125	>125
34	$OCO_2(CH_2)_8Me$	OH	C	7	2	1	0.25	0.06
35	S*	OCO_2Me	C	15	15	125	>125	>125
36	OH	OCO_2Me	C	380	>200	>125	>125	>125

Notes:* S = Squalestatin S1 C6 side chain. [1] Method of synthesis: A= Natural Squalestatin; B= MEM protection; C= DPM protection. See scheme and text. [2] Minimum inhibitory concentrations in μgml^{-1} against *Candida albicans* C316, *Aspergillus niger* 48238 and *Cryptococcus neoformans* 2867E. [3] Squalestatin S1 has an IC50 of 12 +/- 5 nM. Enzyme activities were determined at least in duplicate using Squalestatin S1 as a control. NT = not tested.

carbonate (34) has increased antifungal activity over Squalestatin S1 although it seems unlikely that this is simply a lipophilic effect.[11] Nonyl carbonate (34) is the most potent antifungal Squalestatin in this series.

In conclusion we have developed a selective protection strategy that allows efficient transformation of Squalestatin S1 to C6 and C7 analogues.[12] All of the C6 analogues retain nanomolar SQS enzyme activity in both the mammalian and fungal systems; only sterically small C7 acyl derivatives that retain the Squalestatin S1 C6 side chain are active at the enzyme. The Squalestatin analogues are potent growth inhibitors of fungi provided the C6 substituent is a sufficiently lipophilic chain.

Acknowledgements

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References and Notes

1. Dawson, M.J.; Baxter, A.; Tait, R.M.; Watson, N.S.; Noble D.; Shuttleworth, A.; Wildman, H.G.; Hayes, M.V. *International Patent Publication* No. WO92/12156, 23rd July **1992**.

2. Dawson, M.J.; Farthing, J.E.; Marshall, P.S.; Middleton, R.F.; O'Neill, M.J.; Shuttleworth, A.; Stylli, C.; Tait, R.M.; Taylor, P.M.; Wildman, H.G.; Buss, A.D.; Langley, D.; Hayes, M.V. *J. Antibiotics*, **1992**, *45*, 639; Sidebottom, P.J.; Highcock, R.M.; Lane, S.J.; Procopiou, P.A.; Watson, N.S. *J Antibiotics*, **1992**, *45*, 648. The Merck group have recently described a series of similar natural products: Bergstrom, J.D.; Kurtz, M.M.; Rew, D.J.; Amend, A.M.; Karkas, J.D.; Bostedor, R.G.; Bansal, V.S.; Dufresne, C.; VanMiddlesworth, F.L.;Hensens, O.D.; Liesch, J.M.; Zink, D.L.; Wilson, K.E.; Onishi, J.; Milligan, J.A.; Bills, G.; Kaplan, L.; Nallin Omstead, M.; Jenkins, R.G.; Huang, L.; Meinz, M.S.; Quinn, L.; Burg, R.W.; Kong, Y.L.; Mochales, S.; Mojena, M.; Martin, I.; Palaez, F.; Diez, M.T.; Alberts, A.W.; *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 80; Hensens, O.D.; Dufresne, C.; Liesch, J.M.; Zink, D.L.; Reamer, R.A.; VanMiddlesworth, F. *Tetrahedron Lett.*,**1993**, *34*, 399.

3. Baxter, A.; Fitzgerald, B.J.; Hutson, J.L.; McCarthy, A.D.; Motteram, J.M.; Ross, B.C.; Sapra, M.; Snowden, M.A.; Watson, N.S.; Williams, R.J.; Wright, C.; *J. Biol. Chem.*, **1992**, *267*, 11705.

4. Brown, M.S.; Goldstein, J.L. *J. Lipid Res.*, **1980**, *21*, 505.

5. Mayer, V.M.G.; Thompson, G.R. *Q. J. Med.*, **1990**, *74*, 165.

6. For a related 2-step process see Baldwin, J.E.; Harwood, L.W.; Lombard, H.J. *Tetrahedron*, **1984**, *25*, 4363.

7. Tait, R.M. *Anal. Biochem.*, **1992**, *203*, 310.

8. Candida albicans C316 SQS prepared according to ref. 7.

9. Robinson, G.W.; Tsay, Y.H.; Kienzle, B.K.; Smithmonroy, C.A.; Bishop, R.W. *Molecular and Cellular Biology*, **1993**, *13*, 2607; Biller, S.A.; Sofia, M.J.; Abt, J.W.; DeLange, B.; Dickson, J.K.; Forster, C.; Gordon, E.M.; Harrity, T.; Magnin, D.R.; Marretta, J.; Rich, L.C.; Ciosek, C.P. *ACS Symp. Ser.*, **1992**, *497*, 65.

10. Debono, M.; Abbott, B.J.; Turner, J.R.; Howard, L.C.; Gordee, R.S.; Hunt, R.S.; Barnhart, M.; Molloy, R.M.; Willard, K.E.; Fukuda, D.; Butler, T.F.; Zeckner, D.J. *Annals of the New York Academy of Sciences*, **1988**, *544*, 152.

11. Hansch has found that esters and carbonates of the same carbon chain length have identical logD, Hansch, C.; Anderson, S.M. *J. Org. Chem.*, **1967**,*32*,2583.

12. Key experimental data for intermediates is as follows:

(3) δ (CD_3OD) includes 0.80-0.90 (m,3H), 1.03 (d J=7Hz,3H), 4.05 (d J=2Hz,1H), 4.22 (s,1H), 4.98 (d,2H), 5.09 (d J=5Hz,1H), 5.30 (s,1H), 5.38 (d J=11Hz,2H), 5.43 (s,2H), 5.54 (s,2H), 5.77 (d J=16Hz,1H), 5.88 (d J=2Hz,1H), 6.88 (dd J=9,16Hz,1H), 7.10-7.30 (m,5H).

(4) δ (CD_3OD) includes 0.83 (d J=7Hz,3H), 2.08 (s,3H), 3.35 (s,3H), 3.37 (s,3H), 3.40 (s,3H), 4.17 (d J=2Hz,1H), 4.98 (s,1H), 5.03 (s,1H), 5.15 (d J=2Hz,1H), 5.18 (s,1H), 5.28 (d J=5Hz,1H), 5.37 (s,2H), 5.46 (d J=5Hz,1H), 5.53 (d J=5Hz,1H), 5.62 (d J=5Hz,1H), 7.10-7.34 (m,5H).

(6) δ ($CDCl_3$) includes 0.80-1.05 (m,12Hz), 2.06 (s,3H), 3.25 (d J=2Hz,1H), 3.92 (s,1H), 3.95 (t J=2Hz,1H), 4.96 (d J=12Hz,1H), 4.99 (m,2H), 5.1 (d J=4Hz,1H), 5.34 (s,3H), 5.81 (d J=2Hz,1H), 6.68 (dd, J=8Hz,12Hz,1H), 6.81 (s,1H), 6.82 (s,1H),6.87 (s,1H), 7.05-7.35 (m,35H). Found: C,74.4%;H,6.4%. $C_{74}H_{76}O_{16}$ requires C,74.7%;H,6.4%.

(7) δ ($CDCl_3$) includes 0.79-0.97(m,12H), 2.08 (s,3H), 3.30 (s,3H), 3.43 (m,2H), 3.67(m,2H), 3.89 (s,1H), 4.06 (m,7H), 4.77 (d J=6Hz,2H), 4.84 (d J=12Hz,1H), 4.96 (d J=6Hz,2H), 5.00 (brs,2H), 5.15 (d J=4Hz,1H), 5.34 (s,3H), 6.33 (brs,6H), 6.65 (dd J=7Hz 12Hz, 1H), 6.65 (s,1H), 6.83 (s,1H), 6.85 (s,1H), 7.05-7.32 (m,35H). Found: C,72.4%;H,6.6%.$C_{78}H_{84}O_{16}$.0.25CH_2Cl_2 requires C,72.4%;H,6.6%.

(9) δ ($CDCl_3$) includes 0.81 (d J=7Hz,3H), 2.05 (s,3H), 3.15-3.55 (m,2H), 3.40-3.55 (m,2H), 3.24 (s,3H), 3.86 (s,1H), 3.88 (brs,7H), 4.05 (d J=3Hz,1H), 4.68 (d J=8Hz,2H), 4.74 (d J=8Hz,2H), 5.00 (brs,2H), 5.10-5.18 (m,2H), 5.13 (s,3H), 6.58 (s,1H), 6.80 (s,1H), 6.87 (s,1H), 7.0-7.3 (m,35H). Found: C,72.7%;H, 6.1%. $C_{68}H_{68}O_{15}$ requires C,72.6%;H,6.1%.