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THE SQUALESTATINS: EFFECTS OF CHANGES AT THE ALLYLIC CENTRE IN THE C1 SIDECHAIN

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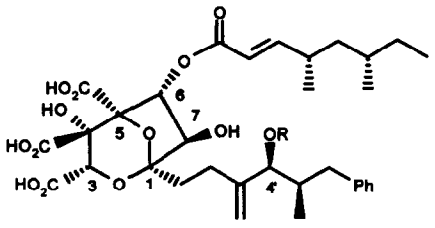
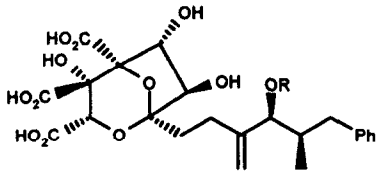
Abstract: The C4' acetoxy group in squalestatin **2a** has been replaced by alkoxy, acyloxy and acetamido groups. The ethers **5b**, **5c**, **5e** and **5g** retain SQS inhibitory activity equivalent to that of **2a** and are metabolically more stable. All other compounds tested are significantly less active.

Recently we have published the isolation[1] and structure elucidation[2] of the squalestatins, a novel group of fungal metabolites isolated from a previously unknown *Phoma* species. Squalestatin S1[3] (**1a**) incorporates the highly functionalised 2,8-dioxabicyclo[3.2.1]octane system having carboxyl groups at C3, C4 and C5, hydroxyl groups at C4 and C7 and two lipophilic sidechains at C1 and C6. **1a** is a potent and selective inhibitor of mammalian SQS (IC_{50} = 12 nM) with good lipid lowering properties in marmosets (ED_{50} = 10 mg/kg/d for 7 days *p.o.*).[4] In this species, **1a** has a profound and extended effect on lipids (50-60% decrease in serum cholesterol levels during 7 d after a single *i.v.* dose of 1 mg/kg).[5] Squalestatin H1 (**2a**), the 6-desacyl analogue, possesses SQS inhibitory activity closely similar to that of **1a** (IC_{50} = 26 nM) and retains reduced but significant lipid lowering ability (30%) following a single *i.v.* dose of 10 mg/kg.[5] Subsequent to our publications, the group at Merck reported the isolation of the zaragozic acids, zaragozic acid A[6,7] being structurally identical to squalestatin **1a**, and the group at Tokyo Noko University have reported the isolation of **1a** from a different source.[8]



1a R = Ac (IC_{50} = 12 nM)
1b R = H (IC_{50} = 5 nM)

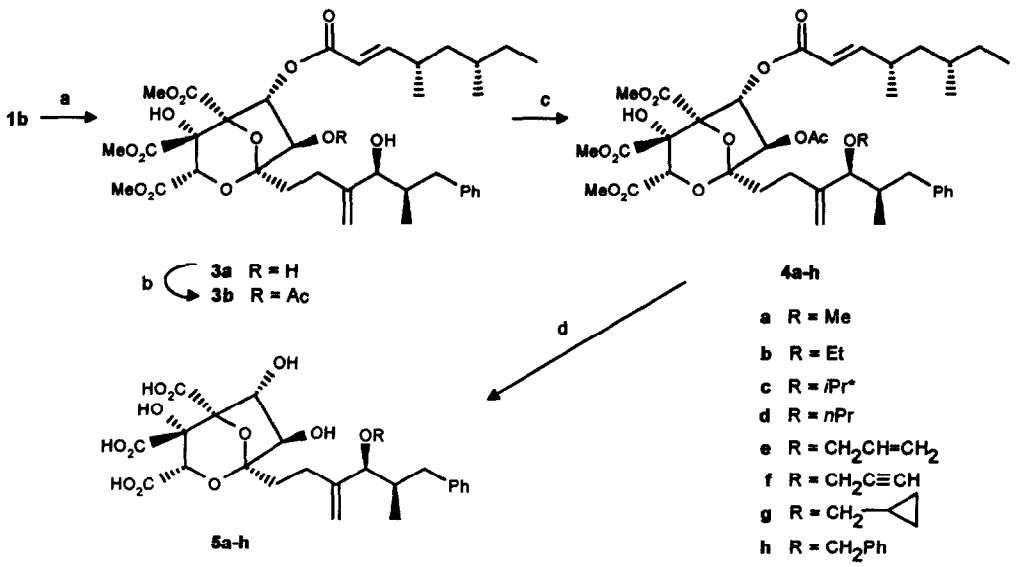


2a R = Ac (IC_{50} = 26 nM)
2b R = H (IC_{50} = 252 nM)

At Glaxo we have undertaken a chemical programme centred on identifying the key structural features essential for SQS inhibitory activity.[5,9-17] Differences in SAR have been observed between squalestatins possessing a 4,6-dimethyloctenoate chain at C6 and those with a hydroxyl at that position. In the former series greater structural changes are tolerated elsewhere in the molecule without significant loss of SQS inhibitory activity.[5,10,11,13,14,16,17] The deacetylated natural product **1b** retains potent enzyme inhibitory activity.[1] In contrast deacetylation of the natural product **2a** results in a tenfold reduction in activity in the product **2b.** As deacetylation has been found to be the primary metabolic fate of squalestatins,[18] this is a potential source of *in vivo* inactivation for compounds possessing a hydroxyl group at C6. Analogues in the 6-hydroxy series with alternative C4' substituents were prepared to identify the parameters affecting enzyme inhibition and to assess whether this centre offers the opportunity for incorporating a metabolically more stable group which might promote increased *in vivo* activity.

The preparation of C4' ethers (Scheme 1) utilised the key intermediate C4' alcohol **3b**, prepared from the trimethyl ester **3a**[2] by selective[19] acetylation of the C7 hydroxyl group. Ethers **4a, 4b, 4d, 4e, 4f, 4g** and **4h** were formed selectively[20] from **3b** by a modification of the method of Wessel *et al.*[21] However this procedure failed for **4c** which was prepared, less selectively, with *i*-propyl iodide in the presence of silver oxide. Saponification of **4a-h** afforded the tricarboxylic acids **5a-h.**

Scheme 1

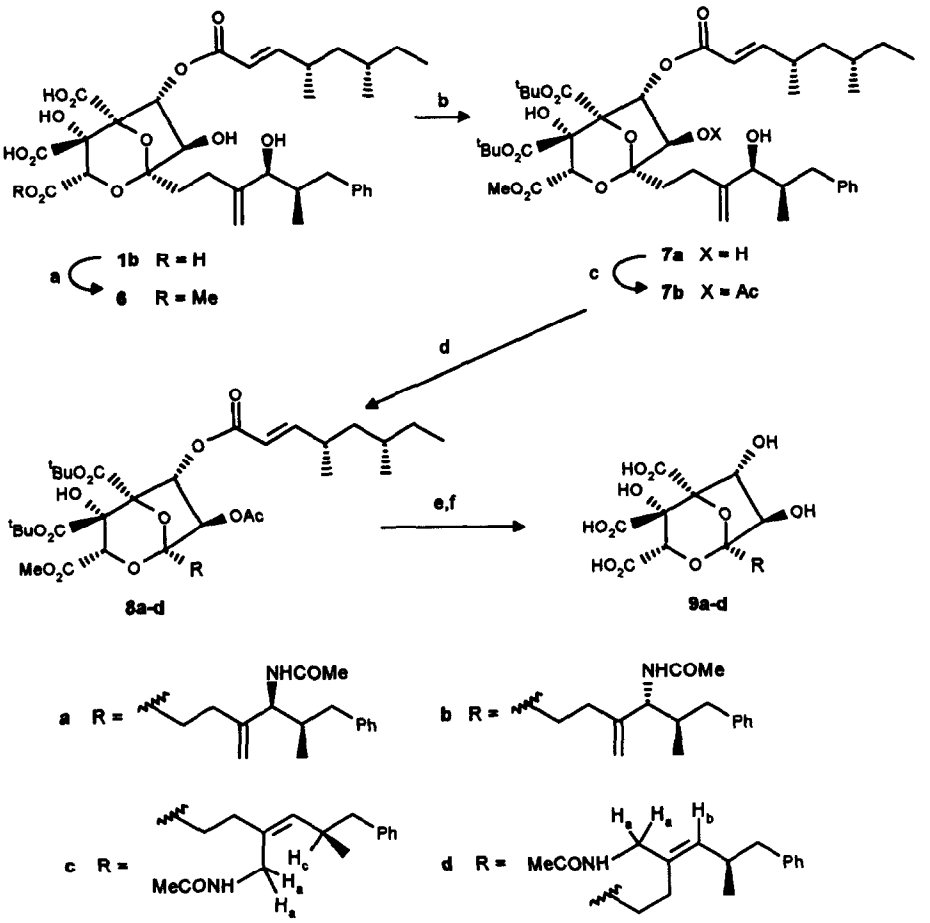


Reagents: a: MeI, $NaHCO_3$, DMF, 20°C; b: Ac_2O (13.7 equiv.), Et_3N (15 equiv.), CH_2Cl_2, 20°C; c: $CCl_3C(:NH)OR$ (3.5 equiv.), CF_3SO_3H (0.5 equiv.), CH_2Cl_2, cyclohexane, 20°C; d: NaOH (excess) H_2O, THF, reflux.

* 4c was prepared from **3b** by treatment with $(CH_3)_2CHI$, Ag_2O at 20°C.

Amides (Scheme 2) were prepared from a different intermediate (**7b**) which was obtained from **1b** by selective methyl esterification at C3 to afford **6**, di-*t*-butyl ester formation at C4 and C5 and selective acetylation at C7. Under modified Ritter conditions **7b** afforded the diastereoisomeric amides **8a** and **8b** together with the *Z* and *E* isomeric amides **8c** and **8d** derived from allylic rearrangement of the intermediate carbocation.[22] Acid-catalysed cleavage of the *t*-butyl esters in **8a-8d** followed by saponification of the ester functions at C3 and C7 afforded the corresponding tricarboxylic acids **9a-9d**.

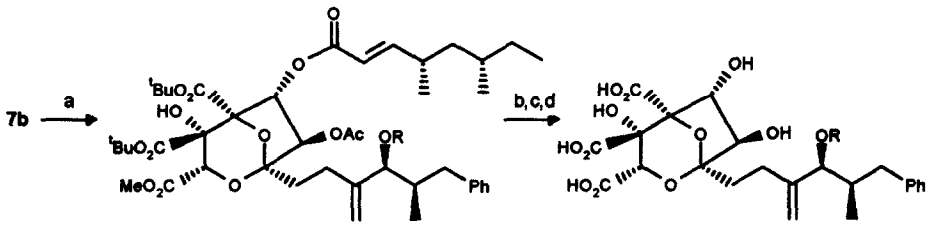
Scheme 2



Reagents: a: HCl, MeOH, 20°C. b: $Me_2NCH(O^tBu)_2$, toluene, reflux. c: Ac_2O (13 equiv.), Et_3N (15.5 equiv.), CH_2Cl_2, 20°C. d: CH_3CN, $(CF_3SO_2)_2O$ (3 equiv.), collidine (8.3 equiv.), 20°C. e: 6M-HCl, dioxane, 20°C. f: NaOH (excess) H_2O, 20°C.

The C4' pivaloate **10a** and benzoate **10b** esters (Scheme 3) were prepared by acylation of **7b**; selective removal of the C6 ester using N-methylhydroxylamine in DMF,[12] followed by unmasking of the C7 hydroxyl group and carboxylic acids as described above[23] generated the desired tricarboxylic acids **11a** and **11b**.

Scheme 3



10a R = CO^tBu
10b R = COPh

11a R = CO^tBu
11b R = COPh

Reagents: a: RCOCl, Et_3N, DMAP, CH_2Cl_2, 20°C. b: MeNHOH.HCl, Et_3N, DMF, 20°C. c: 6M-HCl, dioxane, 20°C. d: NaOH (excess), H_2O, 20°C.

The compounds listed in the table were evaluated for their inhibitory activity against SQS. The enzyme preparation and assay procedures used in this study were the same as those described in our earlier publications.[4,24] Activity comparable with **2a** was observed only with the ethyl, *i*-propyl, allyl and cyclopropylmethyl ethers **5b**, **5c**, **5e** and **5g** respectively, the remaining ethers, esters and amides tested having greatly diminished potency. The results indicate that a likely role of the C4' acetoxy group of providing increased binding *via* favourable hydrophobic interactions can be mimicked by certain small ethers.

Table. *In vitro* SQS Inhibitory Activity[25]

Structural type	Compound	IC_{50}(nM)	Structural type	Compound	IC_{50}(nM)
	5a	500	Amides	**9a**	2225
	5b	40		**9b**	>10000
	5c	49		**9c**	>10000
Ethers	**5d**	600		**9d**	>10000
	5e	27			
	5f	1200	Esters	**2a**	26
	5g	33		**11a**	2286
	5h	1418		**11b**	532

The limited scope for maximising favourable interactions at this centre is shown by the weak activity of the methyl, *n*-propyl and propynyl ethers (**5a**, **5d** and **5f**, respectively). Lack of tolerance of steric bulk is demonstrated by the poor activities of the pivaloate and benzoate esters **11a** and **11b** as well as that of the benzyl ether **5h**. The poor activity of amide **9a**, possessing the 'natural' stereochemistry at C4', suggests the need at C4' for a small lipophilic group devoid of hydrogen bond donor properties. The remaining amides, **9b**, **9c** and **9d**, were without demonstrable activity.

No evidence for metabolic degradation was found when ethers **5a**, **5c**, **5e** and **5h** were tested in rat liver microsomes.[18] However when evaluated in a single dose lipid lowering study in marmosets, **5e** showed no increased ability to lower lipids when tested in parallel with **2a**: both compounds reduced serum cholesterol levels by 30% after a single *i.v.* dose of 1 mg/kg.

In conclusion, we have observed that replacement of the C4' acetoxy group by certain small ethers [e.g. ethyl (**5b**), *i*-propyl (**5c**), allyl (**5e**) and cyclopropylmethyl (**5g**) ethers] results in retention of potent SQS inhibitory activity and that those ethers tested show increased metabolic stability when incubated with rat liver microsomes.[18] However the failure of **5e** to lower lipids more effectively than **2a** suggests that the promotion of increased *in vivo* potency in this series is unlikely to be achieved solely by incorporation of a metabolically more stable group at the allylic position and suggests that pharmacokinetic properties also influence *in vivo* potency in this series of compounds.

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