Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 16, pp. 1931-1936, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00257-6

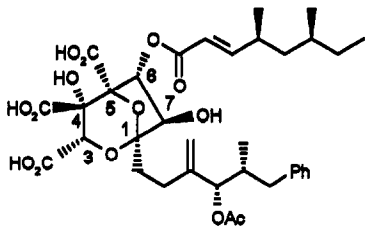
THE SQUALESTATINS: POTENT INHIBITORS OF SQUALENE SYNTHASE, 3-HYDROXYMETHYL DERIVATIVES.

Brian Cox *, Julie L. Hutson, Suzanne E. Keeling, Barrie E. Kirk, Anton R. P. Srikantha and Nigel S. Watson

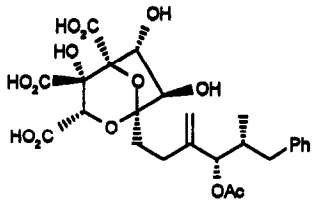
Glaxo Research and Development Ltd, Greenford, Middlesex, UB6 0HE, United Kingdom.

Abstract: A series of 3-hydroxymethyl derivatives of squalestatin 1 was prepared as inhibitors of squalene synthase. Potent *in vitro* inhibitory activity is retained in those analogues which possess C-6 and C-1 substituents analogous to those found in 1.

Squalestatin 1 and its 6-desacyl analogue 2, members of a family of novel fungal metabolites, are potent inhibitors of squalene synthase (SQS), with IC_{50} values of 12 and 6nM respectively against the rat enzyme.[1] Subsequently, a group at Merck reported[2] the isolation of the zaragozic acids, zaragozic acid A is identical to squalestatin 1. Extensive studies have been undertaken in order to identify the structural features necessary for SQS inhibitory activity. We have reported previously our studies on the role of the 3-carboxylic acid function and have shown the 3-methyl ester[3] and 3-decarboxylated[4] analogues of squalestatin 1 retain potent SQS inhibitory activity. In this communication we describe the synthesis and biological evaluation of a series of 3-hydroxymethyl derivatives of 1.[5]



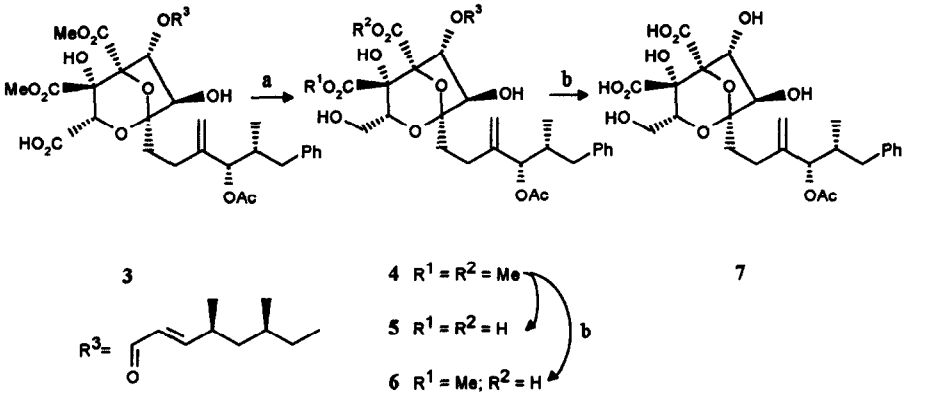
1



2

Selective reduction of the 3-carboxylate function of the 4,5-dimethyl ester 3[3] was achieved by activation as its 3-*N*-hydroxy succinimidyl ester followed by *in situ* treatment with sodium borohydride.[6] Treatment of the derived product 4 with lithium iodide in *sym*-collidine at 45°C under a stream of nitrogen,[7] resulted in selective cleavage of the methyl esters to yield the desired dicarboxylate 5; the 4-methyl ester 6 and the 6-hydroxy compound 7 were isolated as minor by-products (Scheme 1). Compound 5[8] possesses potent SQS inhibitory activity (IC_{50}=15nM) closely similar to that for the natural product 1. The 4-methyl ester 6 retains significant enzyme inhibitory activity (IC_{50}=79nM) similar to that observed for the 3,4-dimethyl ester of 1 (IC_{50}=134nM).[3] However, the 6-hydroxy analogue 7 possesses relatively weak enzyme inhibitory activity (IC_{50}=395nM) which is in contrast to that shown by the corresponding tricarboxylic acid 2. These data support previous findings[3,4] that the 3-carboxylate group is not essential for activity in derivatives of 1; the corresponding 3-methyl ester and the 3-decarboxylated analogues possess IC_{50} values of 7 and 23 nM

Scheme 1

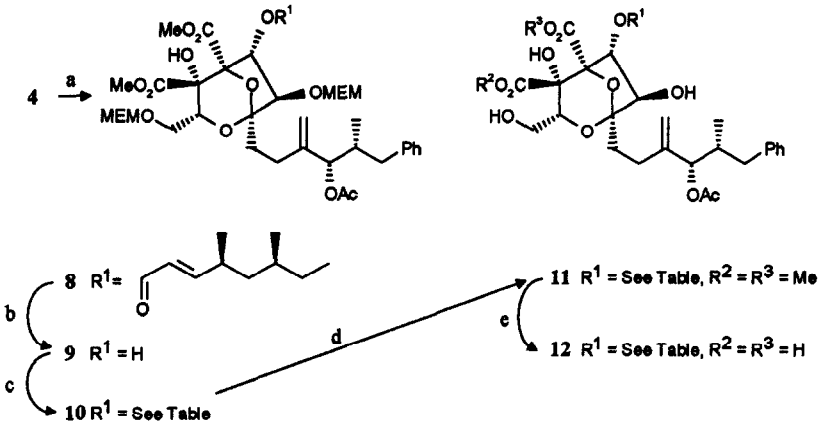


Conditions
a) (i) N-hydroxysuccinimide, 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide p-toluenesulfonate (CMC), THF, (ii) $NaBH_4$,THF; b) LiI (anhydrous), *sym*-collidine.

respectively. The 6-hydroxy derivatives of these two compounds showed much reduced inhibitory activity (IC_{50} values 220nM and 25μM respectively), paralleling the reduced activity noted for **7** in comparison with **5**.

In the light of the results for the 3-hydroxymethyl series, studies centred on establishing the minimal structural features required at C-6, for good *in vitro* activity and a series of C-6 esters was targeted. Protection of **4** as its 3,7-bis MEM derivative **8** followed by removal of the C-6 side-chain[9] provided the key intermediate **9**. Re-esterification at C-6 was achieved using the appropriate acid, acid chloride or anhydride; removal of the MEM groups from the derived products **10** by treatment with formic acid, then potassium carbonate[10] gave the dimethyl esters **11**, which after deprotection using the lithium iodide/collidine system (see Scheme 2),

Scheme 2

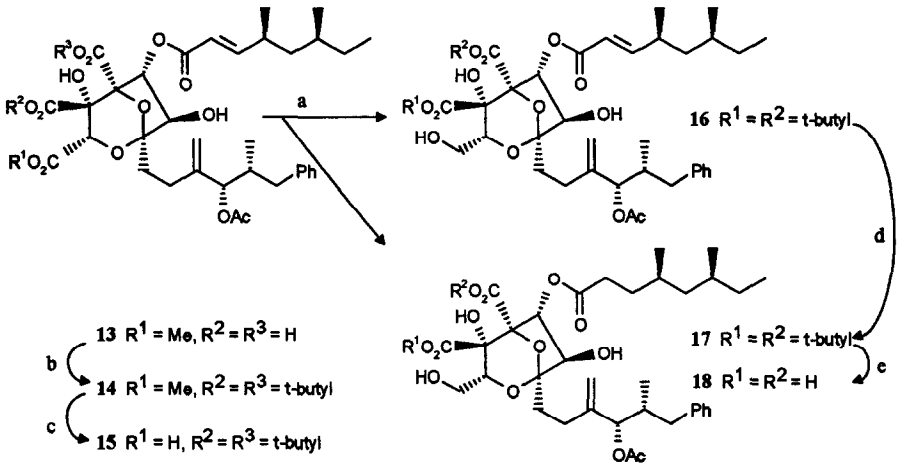


Conditions
a) i-Pr_2EtN, MEMCl, CH_2Cl_2; b) MeNHOH.HCl, Et_3N, DMF; c) RCOCl or $(RCO)_2O$ with CH_2Cl_2, DMAP or RCOOH, CMC, THF; d) (i) HCOOH, H_2O, (ii) $KHCO_3$, MeOH; e) LiI (anhydrous), *sym*-collidine.

provided the target compounds **12a-d** (see Table).[8]

The route to the corresponding 6-(4,6-dimethyloctanoate) compound is shown in Scheme 3; t-butyl esterification of the 4,5-dicarboxylate functions in the 3-methyl ester **13**[3] followed by saponification of the 3-methyl ester in the derived triester **14** gave the 4,5-di-t-butyl ester **15**. Activation followed by reduction of the 3-carboxylic acid using the procedure described above gave the 3-hydroxymethyl derivative **16** in good yield. Prolonged reaction time with sodium borohydride resulted in the formation of a proportion of the dihydro product **17**,[11] which could also be derived from **16** by the use of triphenyl phosphine copper hydride hexamer.[12] Treatment of this compound with dioxan/HCl gave the required compound **18**.[8]

Scheme 3

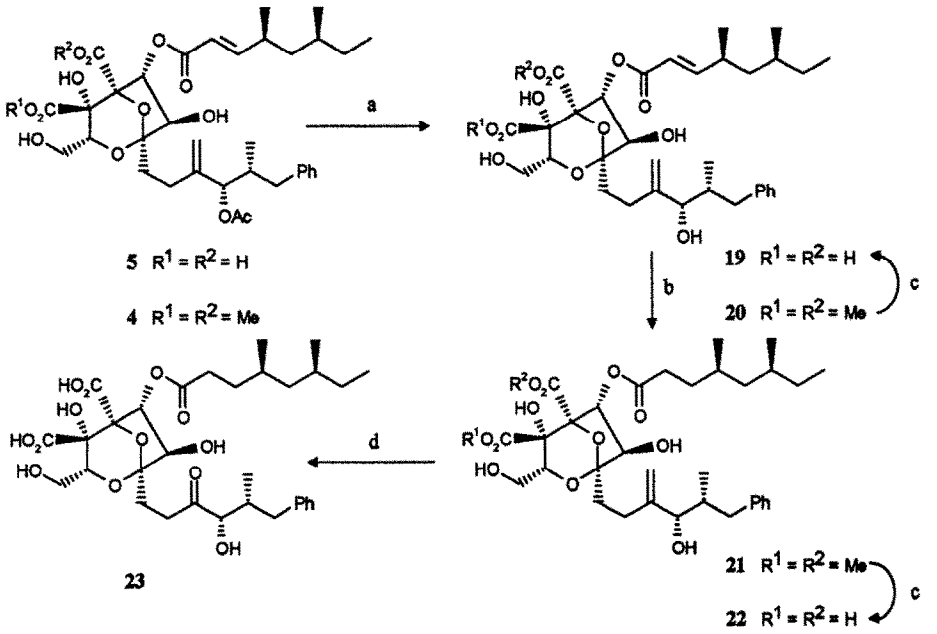


Conditions
a) (i) N-hydroxysuccinimide, 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide p-toluenesulfonate (CMC), THF, (ii) $NaBH_4$,THF; b) di-t-butyl, dimethylaminoacetal/toluene/Δ; c) $NaOH/H_20$,THF; d) $[Ph_3PCuH]_6$, wet toluene; e) 1,4-Dioxan, HCl.

As can be seen from the *in vitro* results in the Table, enzyme inhibitory activity is critically dependant on the nature of the C-6 substituent; only those analogues incorporating the 4,6-dimethyloctenoate and its dihydro counterpart possess good SQS inhibitory activity. These data provide further evidence that in core modified series the binding of the natural C-6 side-chain or its reduced version plays a crucial role in the overall binding of the molecule. We have also reported structure-activity relationships for modification of the C-1 side chain in a tricarboxylic acid series,[13] therefore it was of interest to compare these findings with a related series of C-1 side-chain modified derivatives of **5**. The desacetyl compound **19** was prepared by treatment of **5** with methanol and hydrochloric acid.[14] Subsequently, the corresponding dimethyl ester **4** was similarly treated to give the allylic alcohol **20** from which further quantities of **19**[8] were prepared using established (LiI/collidine) conditions. Further manipulation of the C-1 side-chain was carried out using intermediates possessing the 4,6-dimethyloctanoate ester at C-6, ready access to which was achieved using an alternative procedure to that described above. Sodium borohydride/copper (II) sulphate has been reported[15] to reduce esters in the presence of carboxylic acid functions; attempts to use this reagent to prepare the 3-hydroxymethyl analogue **5** from the 3-methyl ester **13** resulted only in the 1,4-reduction of the C-6 unsaturated ester.[16] Application of this procedure to the diester **20** gave the dihydro diester **21**, deprotection of which (LiI/collidine) provided the

diacid **22**.[8] Ozonolysis of this material in methanol, followed by treatment with triphenylphosphine and acidic work-up yielded the keto-alcohol **23** (Scheme 4).[8]

Scheme 4



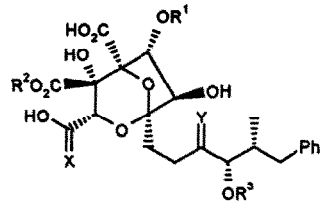
Conditions
a) MeOH, HCl; b) $NaBH_4$, aq. $CuSO_4$ (Catalytic), EtOH; c) LiI (anhydrous), *sym*-collidine; d) (i) O_3, MeOH, (ii) Ph_3P, acid work-up.

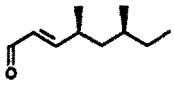
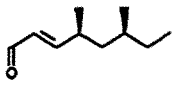
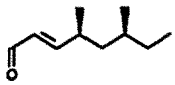
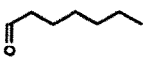
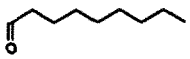
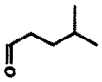
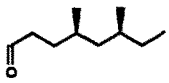
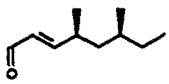
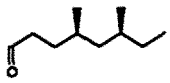
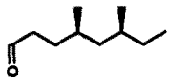
Although the allylic alcohols **19** and **22** possess potent SQS enzyme inhibitory activity, the keto-alcohol **23** was found to have substantially reduced activity (see Table). In contrast, these modifications when made in the tricarboxylate series, resulted in the retention of potent activity.[13]

The squalestatins possess three subunits, namely the substituents at C-6/C-7, the C-1 side-chain and the tricarboxylic acid moiety of the bicyclic core. We have reported previously that the nature of the grouping at C-6 can critically influence whether modifications made elsewhere in the molecule are well tolerated. Thus, in series retaining a 4,6-dimethyloctenoate or 4,6-dimethyloctanoate ester, modification to the substituent at C-7, to the C-1 side-chain or the carboxylic acid group at C-3 and C-4 can be well tolerated. In contrast, while potent activity is retained in the parent natural product possessing a hydroxyl group at C-6 while retaining the same substitution pattern throughout the rest of the molecule, modifications made at C-7, to the C-1 side-chain or the carboxylic acids at C-3 and C-4 lead to reduction in SQS inhibitory activity.[3,4,13,17] The data generated during this study reveal a similar dependence of activity in the 3-hydroxymethyl series on the nature of the substituents at C-6 and C-1; potent SQS inhibitory activity is retained only in those analogues which possess C-1 and C-6 substituents closely similar to those in the natural product **1**.

Acknowledgements: We are indebted to Miss Belinda J. Fitzgerald for expert technical assistance in conducting the enzyme inhibition assay.

Table . *In vitro* SQS Inhibitory Activity



	R1	R2	R3	X	Y	IC50 (nM)
1		H	Ac	O	CH_2	12
5		H	Ac	H_2	CH_2	15
6		Me	Ac	H_2	CH_2	79
7	H	H	Ac	H_2	CH_2	395
12a		H	Ac	H_2	CH_2	800
12b		H	Ac	H_2	CH_2	245
12c		H	Ac	H_2	CH_2	140
12d		H	Ac	H_2	CH_2	119
18		H	Ac	H_2	CH_2	44
19		H	H	H_2	CH_2	21
22		H	H	H_2	CH_2	22
23		H	H	H_2	O	740

IC_{50} values were determined at least in duplicate using Squalestatin 1 as a reference.

References

1 a) 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,G.; Buss, A.D.; Langley, D.; Hayes, M.V. *J. Antibiotics* **1992**, *45*, 639; b) Sidebottom, P. J.; Highcock, R.M.; Lane, S.J.; Procopiou, P.A.; Watson, N.S. *J. Antibiotics* **1992**, *45*, 648.

2 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 Watson, N.S.; Bell, R.; Chan, C.; Cox, B.; Hutson, J.L.; Keeling, S.E.; Kirk, B.E.; Procopiou, P.A.; Steeples, I.P.; Widdowson, J. *BioMed. Chem. Lett.* **1993**, *3*, 2541.

4 Chan, C.; Inglis, G.G.A.; Procopiou, P.A.; Ross, B.C.; Srikantha, A.R.P.; Watson, N.S. *Tetrahedron Lett.* **1993**, *34*, 6143.

5 During the preparation of this manuscript, a report appeared detailing the synthesis and biological activity of C-3 methyl analogues of zaragozic acid A together with an alternative synthesis of the C-3 hydroxymethyl analogue; Kuo, C.H.; Plevyak, S.P.; Biftu, T.; Parsons, W.H.; Berger G.D. *Tetrahedron Lett.* **1993**, *34*, 6863.

6 Nikawa, J.; Shiba, T. *Chem. Lett.* **1979**, 981.

7 A modification of the method of Elsinger, F.; Schreiber, J.; Eschenmoser, A. *Helv. Chim. Acta* **1960**, *43*, 113.

8 Converted to corresponding dipotasssium salt for biological evaluation; 1,4-dioxan, H_2O, $KHCO_3$-freeze-dried.

9 For selective procedure for the removal of the C-6 side-chain, see: Lester, M.G.; Giblin, G.M.P.; Inglis, G.G.A.; Procopiou, P.A.; Ross, B.C.; Watson, N.S. *Tetrahedron Lett.* **1993**, *34*, 4357.

10 The use of formic acid to remove the MEM groups results in the 3-formylation of a proportion of the desired products; deformylation is accomplished using potassium bicarbonate.

11 After 18 hours a 3:1 mixture of **16** and **17** was obtained, separated by reverse phase HPLC.

12 Mahoney, W.S.; Brestensky, D.M.; Stryker, J.M. *J. Am. Chem. Soc.* **1988**, *110*, 291.

13 Procopiou, P.A.; Bailey, E.J.; Hutson, J.L.; Kirk, B.E.; Sharratt, P.J.; Spooner, S.J.; Watson, N.S. *BioMed. Chem. Lett.* **1993**, *3*, 2527.

14 A procedure for the selective methanolysis of the C-1 side-chain acetate in the trimethyl ester of **1** has been reported[1b] and was observed during the preparation of **13** from **1**[3] with prolonged reaction times.

15 Yoo, S.E.; Lee, S.H. *Synlett.* **1990**, 419.

16 It has been suggested (Ravindranahay, M.; Kalyanam, N.; Sivaram, S. *J. Org. Chem.* **1982**, *47*, 4812) that this reagent is a copper (0) system. The conjugate reduction of α,β-unsaturated esters has been reported using Copper (II) chloride and sodium borohydride (Satoh, T.; Nanba, K.; Suzuki, S. *Chem. Pharm. Bull.* **1971**, *19*, 817).

17 Giblin, G.M.P.; Bell, R.; Hancock, A.P.; Hartley, C.D.; Inglis, G.G.A.; Payne, J.J.; Procopiou, P.A.; Shingler, A.H.; Smith, C.; Spooner, S. J. *BioMed. Chem. Lett.* **1993**, *3*, 2605.

(*Received in Belgium* 21 *April* 1994; *accepted* 14 *June* 1994)