Pergamon

Tetrahedron Letters, Vol. 37, No. 49, pp. 8925-8928, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)02049-7

The Squalestatins: Synthesis of C-4 Carboxamide Derivatives

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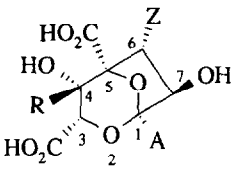
Abstract: Synthesis of squalestatin S1 C-4 carboxamide, **2**, as well as related C-4 amides and C-4 hydroxymethyl derivatives possessing a C-3 hydroxymethyl group (**15** and **19**) together with their SQS inhibitory activities are presented. Copyright © 1996 Elsevier Science Ltd

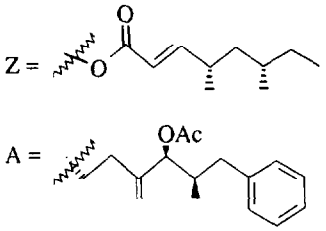
Squalestatins/zaragozic acids are a family of fungal metabolites which possess potent inhibitory activities against squalene synthase (SQS), an enzyme committed to cholesterol biosynthesis, and squalestatin **1**, S1, possesses a profound cholesterol lowering ability *in vivo*.[1] Previously we reported that the C-4 monomethyl ester[1] of S1 as well as C-4 decarboxy derivatives[2] retain potent SQS inhibitory activities. We now report on the synthesis of S1 C-4 carboxamide **2** and our efforts towards the C-4 hydroxymethylS1 **3** to assess



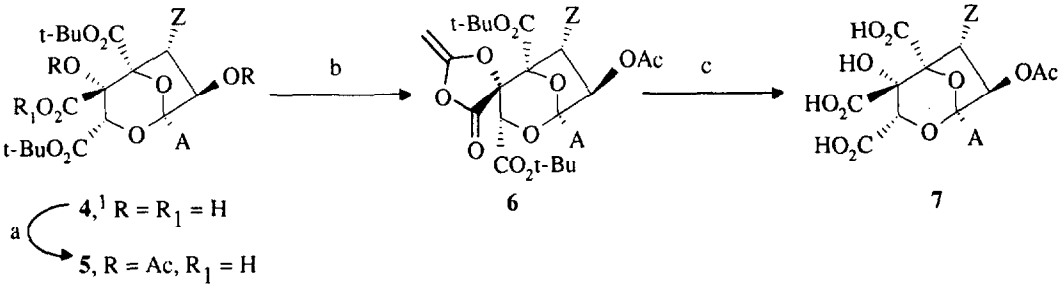
1, S1, R = CO_2H

2, R = $CONH_2$

3, R = CH_2OH



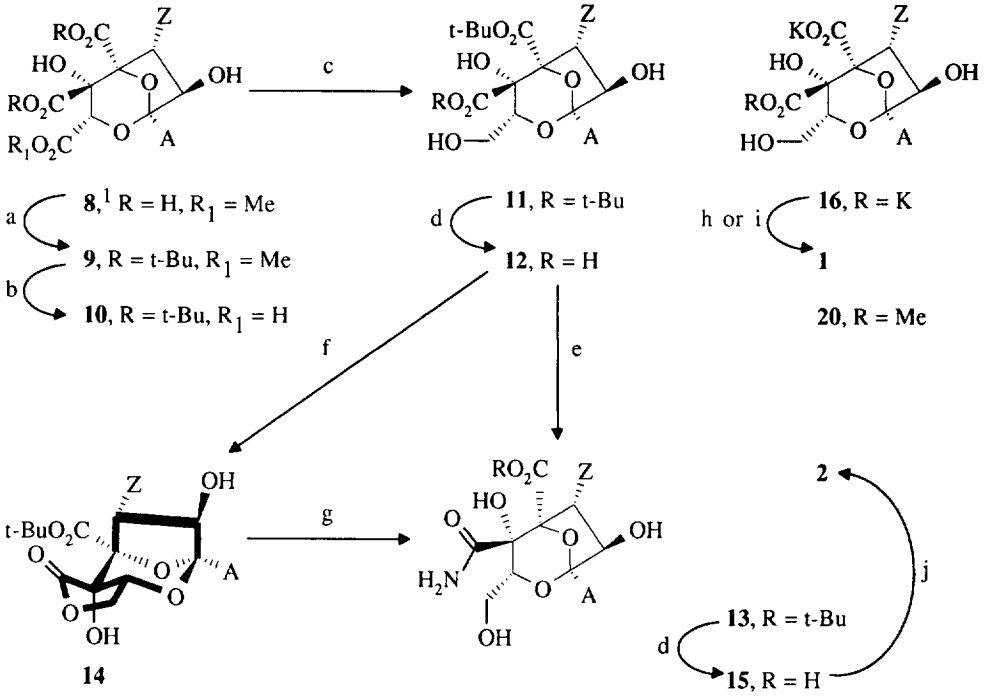
whether a hydrogen bond donating group is tolerated at C-4. Similarly we have reported that the good potency shown by C-3 hydroxymethylS1 is retained in its C-4 monomethyl ester,[1] related 4-modified analogues having a C-3 hydroxymethyl group are also described.



a. Ac_2O, Et_3N, DMAP, CH_2Cl_2. b. $(COCl)_2$, DMF, CH_2Cl_2, 0 °C with or without $NaBH_4$, DMF. c. HCl-dioxan

Synthesis of S1 C-4 carboxamide **2** and C-4 hydroxymethylS1 **3** *via* direct modifications of a suitably protected C-4 carboxyl group was attempted initially. Thus activation of the C-4 carboxy group in **5** (readily available in 92% yield from **4**) with the Vilsmeier salt followed by reduction with a DMF solution of $NaBH_4$ gave a product that was not inconsistent with a C-4 hydroxymethyl product by ^{1}H-NMR. However its

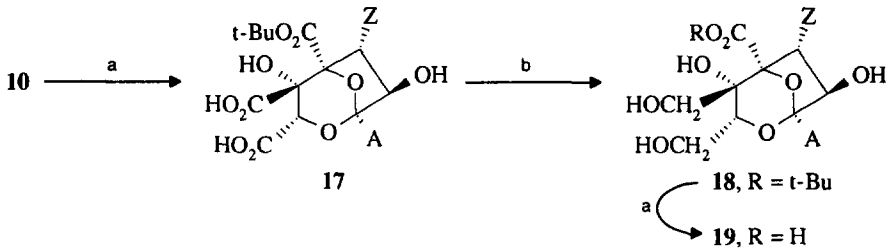
deprotection with HCl-dioxan gave S1 C-7 acetate **7**. Analysis of the "reduction" product by spectroscopic techniques revealed its identity as the spiroacetal **6**.[3] Indeed omitting $NaBH_4$ in the reaction of Vilsmeier salt with **5** also gave **6** (37%). A plausible explanation for the formation of **6** was the intramolecular cyclisation of the C-4 activated ester by the C-4 acetoxyl group. Similar treatment of the Vilsmeier-activated intermediate derived from **4**, or the related C-3 methyl ester, with gaseous ammonia also failed to give the corresponding C-4 amide and we believe steric crowding around the C-4 carbonyl group precluded nucleophilic attack by the external nucleophile. In order to reduce such steric congestion an indirect approach was investigated *via* **10**. Activation of the acid **10** with *N*-hydroxysuccinimide (NHS) and a water-soluble carbodimide (CMC) followed



a. $(t\text{-BuO})_2CHNMe_2$, toluene, Δ. b. 1 eq. aqueous NaOH, THF, r.t. c. N-hydroxysuccinimide (NHS), 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-p-toluenesulphonate (CMC), $NaBH_4$, THF. d. HCl-dioxan. e. DMF, $(COCl)_2$, CH_2Cl_2-MeCN, 0 °C then NH_3, -78 °C. f. CMC, NHS, THF, r.t., 23h. g. NH_3, THF, -78 °C. h. 4-5 atm. O_2, 10% Pt-C, H_2O, pH8, 90-100 °C, 13 d. i. 14 mol% $RuCl_3$, 2.5 eq. $K_2S_2O_8$, 14 eq. 2,4,6-collidine, H_2O, r.t. 5 d. j. As in i. except 16 mol% $RuCl_3$ and 6 d.

by reduction with $NaBH_4$ gave the C-3 hydroxymethyl derivative **11** (60%). Selective deprotection by controlled exposure of **11** to HCl-dioxan gave the C-4 acid **12** whose regiochemistry was confirmed by its conversion to the lactone **14** (*vide infra*). Vilsmeier activation of **12** followed by treatment with liquid ammonia in THF at -78 °C thereby gave the C-4 carboxamide **13** (57%).[4] It is of particular interest to note that treatment of **12** with NHS and CMC gave the *trans*-fused lactone **14**[5] (49%). We believe that amide **13** was formed *via* the intermediacy of **14** in which the reduced congestion about the lactone carbonyl group coupled with its altered orientation relative to the C-4 carboxyl in **4** made it more susceptible to attack by an external nucleophile. Indeed treatment of the *trans*-fused lactone **14** with ammonia in THF at -78 °C gave the C-4 carboxamide **13** in quantitative yield. **13** was deprotected to provide the acid **15**[3] (60%).

Re-oxidation of the C-3 hydroxymethyl group was initially investigated with the readily available C-3 hydroxymethylS1 potassium salt **16**.[1] Prolonged treatment of **16** with oxygen[6] in the presence of 10% Pt-C afforded S1. A similar result was obtained with $RuCl_3$ in the presence of potassium persulphate buffered with 2,4,6-collidine[7] and this latter method was successfully applied to the oxidation of the potassium salt of **15** to provide S1 C-4 carboxamide **2**[3] (67%). These direct oxidation methodologies complement the two step procedures used by Carreira[8] and Nicolaou[9] in their total synthesis of squalestatins/zaragozic acids.



a. HCl-dioxan. b. (i) $(COCl)_2$, DMF, CH_2Cl_2 ; (ii) THF, MeCN, 0 to -30 °C, 1h; (iii) $NaBH_4$, DMF, -78 to -20 °C, 2h.

Synthesis of C-3,C-4 bis(hydroxymethyl)S1 **19** was achieved via controlled treatment of **10** with HCl-dioxan to give the diacid **17**. Reaction of the latter with excess Vilsmeier reagent followed by $NaBH_4$, under carefully controlled conditions,[10] gave the 3,4-bis(hydroxymethyl) product **18** (28%). Deprotection under standard conditions gave **19**[3] (37%). However attempts to oxidise **18** or its derivatives to **3** using the above conditions were unsuccessful.

Effects of the potassium salts of **2**, **15** and **19** on the conversion of [^{3}H]-farnesyl pyrophosphate to [^{3}H]-squalene by rat microsomal SQS[1] were evaluated. C-4 carboxamide **2** was 15 fold less active (IC_{50} 175 nM) than S1 **1** (IC_{50} 12 nM) and in contrast to the good activity shown by C-3 hydroxylmethylS1 **16** (IC_{50} 15 nM) and its C-4 methyl ester **20**[1] (IC_{50} 79 nM), the related C-4 carboxamide **15** and C-4 hydroxylmethyl analogue **19** were without significant activities (IC_{50} >1000 and 742 nM respectively). These data suggested that a hydrogen bond donating group is not well tolerated at C-4.

Acknowledgements The authors would like to thank Miss. Belinda J. Fitzgerald and Mrs. Surriya Faulkes for carrying out the rat microsomal SQS assays and Mr. Andy D. Roberts for carrying out NMR studies.

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3. Spectroscopic data for key compounds are shown below:
2: δ(d_6-DMSO) includes 0.75 - 0.85 (m, 9H, 3 Me), 0.98 (d, 3H, MeCHCH=CHCO$_2$, J = 7 Hz), 1.02 - 1.15 (m, 2H), 1.21 - 1.38 (m, 3H), 1.79 - 1.88 (m, 2H), 2.08 (s, 3H, MeCO$_2$), 2.62 (dd, 1H, proton of PhCH$_2$, J = 14 & 6 Hz), 3.82 (d, 1H, H-7, J = 2 Hz), 4.91 (s, 2H, C=CH$_2$), 4.98 (s, 1H, H-3), 5.0 (d, 1H, CHOAc, J = 5 Hz), 5.73 (d, 1H, CH=CHCO$_2$, J = 15 Hz), 5.90 (broad s, 1H, 7-OH), 6.37 (d, 1H, H-6, J = 2 Hz), 6.72 (dd, 1H,

CH=$CHCO_2$, J = 15 & 8 Hz), 6.93 and 7.06 (2 broad s, 2H, $CONH_2$), 7.10 - 7.2 & 7.25 - 7.36 (2m, 5H, Ph). MS: For $C_{35}H_{47}NO_{13}$, 661 (M - H).

6: ^{1}H-NMR(400MHz): δ($CDCl_3$) includes 0.99 (d, 3H, McCHCH=$CHCO_2$, J = 7 Hz), 1.46 & 1.50 (2s, 18H, 2 t-Bu), 2.09 & 2.19 (2s, 6H, 2 $McCO_2$), 2.70 (dd, 1H, one proton of $PhCH_2$, J = 14 & 5 Hz), 3.85 (ABq, 2H, $(O)_2C=CH_2$, J = 5 Hz), 4.92 (s, 1H, H-3), 4.98 & 5.0 (2s, 2H, $C=CH_2$), 5.12 (d, 1H, CHOAc, J = 5.5 Hz), 5.29 (d, 1H, H-7, J = 2.5 Hz), 5.74 (d, 1H, CH=CHCO_2, J = 15.5 Hz), 6.05 (d, 1H, H-6, J = 2.5 Hz), 6.92 (dd, 1H, CH=$CHCO_2$, J = 15.5 & 8 Hz), 7.12 - 7.31 (m, 5H, Ph). ^{13}C-NMR(100MHz): δ($CDCl_3$) 11.0 ($McCH_2$), 13.8 ($McCHCH_2Ph$), 18.8 (McCHEt), 20.1 (CH=CHCHMc), 20.08 & 21.0 (2 McCO2), 25.1 ($CH_2C=CH_2$), 27.6 & 27.7 (2 Mc_3C), 29.7 (CH_2Mc), 31.7 (CHEt), 33.8 ($CH_2CH_2C=CH_2$), 34.4 ($CHC=CHCO_2$), 36.8 ($CHCH_2Ph$), 39.9 (CH_2Ph), 43.2 ($McCHCH_2CHEt$), 63.2 ($CH_2=C(O)_2$), 72.8 (C-3), 75.2 (C-6), 78.0 (C-4), 79.3 (CHOAc), 79.8 (C-7), 85.5 & 86.0 (2 Mc_3CO_2C), 87.5 (C-5), 105.3 (C-1), 112.1 ($C=CH_2$), 117.8 ($CH=CHCO_2$), 125.9 (para-C of Ph), 128.3 (2 ortho-C of Ph), 129.2 (2 meta-C of Ph), 140.4 (quaternary C of Ph), 145.2 ($C=CH_2$), 157.1 ($CH_2=C(O)_2$), 157.7 ($CH=CHCO_2$), 162.8 (C-3 CO_2tBu), 164.3 ($CH=CHCO_2$), 165.8 (C-4 CO_2), 169.0 & 170.0 (2 CH_3CO_2). ν_{max} (KBr) 1830, 1772, 1737, 1703 cm^{-1}. Accurate mass (+ve electrospray; MH^+ for $C_{47}H_{64}O_{15}$) found: 869.4358; calculated: 869.4323. Heteronuclear multiple bonds correlation (HMBC) studies showed a one bond C-H coupling of 165 Hz between the CH_2 protons at δ 3.85 and carbon at δ 63.2 consistent with a sp^2 exo-methylene group. Optimised at 6 Hz, these studies showed small correlations of the exo-methylene protons to the C-4 carbon (δ 78) and the C=O of the 1,3-dioxolan-4-one unit (δ 165.8). Together with correlations of the C-3 proton (δ 4.92) to the latter carbon and the C-3 ester C=O (δ 162.8), these data confirmed the identity of **6**. A similarly low δ values for the exo-methylene group of 5,5-dimethyl-2-methylene-1,3-dioxolan-4-one unit has been reported by Friary, R. *J. Heterocycl. Chem.* **1978**, *15*, 63-64.

15: δ (d_6-DMSO) includes 0.74-0.87 (m, 9H, 3 Me), 0.98 (d, 3H, McCHCH=$CHCO_2$, J = 6 Hz), 2.09 (s, 3H, $McCO_2$), 2.62 (dd, 1H, one proton of CH_2Ph, J = 13 & 6 Hz), 3.35-3.5 (m, 2H, CH_2OH), 3.84 (dd, 1H, H-7, J = 5 & 2 Hz), 4.46 (m, 1H, H-3), 4.71 (t, 1H, CH_2OH, J = 5 Hz), 4.89 (s, 2H, $C=CH_2$), 4.97 (d, 1H, CHOAc, J = 4 Hz), 5.76 (d, 1H, CH=CHCO_2, J = 15 Hz), 5.81 (d, H, 7-OH, J = 5 Hz), 6.32 (d, 1H, H-6, J = 2 Hz), 6.72 (dd, 1H, CH=$CHCO_2$, J = 15 & 8 Hz), 5.89 & 7.1 (2 broad s, 2H, $CONH_2$), 7.12 - 7.32 (m, 5H, Ph), 12.83 (broad s, 1H, CO_2H). ν_{max}($CHBr_3$) 3477 (OH), 1725 (ester & carboxylic acid C=O), 1702 (amide C=O), 1649 (amide II band) cm^{-1}. MS (DCI, NH3): For $C_{35}H_{49}NO_{12}$, 693 (MNH_4^+), 676 (MH^+).

19: δ(d_4-MeOH) 0.8 - 0.95 (m, 9H, 3 Me), 1.06 (d, 3H, McCHCH=$CHCO_2$, J = Hz), 1.1 - 1.25 (m, 2H), 1.3 - 1.45 (m, 3H), 1.88 - 2.03 (m, 2H), 2.12 (s, 3H, $McCO_2$), 2.19 - 2.52 (m, 4H), 2.56 (dd, 1H, proton of $PhCH_2$, J = 14 & 6 Hz), 3.75 (dd, 1H, one proton of CH_2OH at 3, J = 12 & 5 Hz), 3.81 & 4.03 (2d, 2H, CH_2OH at 4, J = 12 Hz for both), 3.96 (dd, 1H, one proton of CH_2OH at 3, J = 12 & 2.5 Hz), 4.04 (s, 1H, H-7), 4.46 (m, 1H, H-3), 4.96 & 5.01 (2s, 2H, $C=CH_2$), 5.08 (d, 1H, CHOAc, J = 4 Hz), 5.82 (d, 1H, CH=CHCO_2, J = 16 Hz), 5.98 (d, 1H, H-6, J = 2 Hz), 6.87 (dd, 1H, CH=$CHCO_2$, J = 16 & 8 Hz), 7.16 - 7.20 & 7.22 - 7.30 (2m, 5H, Ph). MS (-ve FAB): For $C_{35}H_{50}O_{12}$, 661 (M - H).

4. The corresponding *N,N*-dimethylcarboxamide was also isolated as a by-product (16%) which was presumably formed by reaction with dimethylamine derived from DMF.
5. **14**: δ($CDCl_3$) includes 0.8-0.9 (m, 9H, 3 Me), 1.06 (d, 3H, McCHCH=$CHCO_2$, J = 7 Hz), 1.57 (s, 9H, t-Bu), 2.1 (s, 3H, $McCO_2$), 2.68 (dd, 1H, one proton of $PhCH_2$, J = 14 & 5.5 Hz), 3.38 (d, 1H, 7-OH, J = 3 Hz), 3.56 (s, 1H, 4-OH), 4.08 (t, 1H, H-7, J = 2 Hz), 4.89 (d, 1H, H-6, J = 2 Hz), 4.3 - 4.6 (m, 3H, $CHCH_2O$), 4.96 & 5.00 (2 s, 2H, $C=CH_2$), 5.08 (d, 1H, CHOAc, J = 5 Hz), 5.8 (d, 1H, CH=CHCO_2, J = 16.5 Hz), 6.95 (dd, 1H, CH=$CHCO_2$, J = 16.5 & 9.5 Hz), 7.1 - 7.3 (m, 5H, Ph). Inverse long range heteronuclear multiple bond correlation studies showed a correlation between 165.59 (lactone C=O at C-4) and 4.35-4.43 ($CHCH_2OCO$) confirming the lactone bond linkage to the C-3. Strong nOe from 4.89 (H-6) → 4.55 (H-3) confirmed the natural stereochemistries at these positions. ν_{max} ($CHBr_3$): 3540 (OH), 1808 (lactone C=O), 1731 (ester C=O) cm^{-1}. MS (DCI, NH_3): For $C_{39}H_{54}NO_{12}$, 732 (MNH_4^+), 676 (MH_4^+ - tBu), 674 (M - tBu). A similar *trans*-fused lactone was reported by the group at Merck: Kuo, C. H.; Plevyak, S. P.; Biftu, T.; Parsons, W. H.; Berger, G. D. *Tetrahedron Lett.* **1993**, *34*, 6863-6866.

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(*Received in UK* 23 *September* 1996; *accepted* 18 *October* 1996)