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The Squalestatins: Synthesis of C-4 Carboxamide Derivatives

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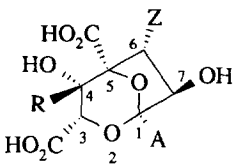
Abstract: Synthesis of squalestatin S1 C-4 carboxamide, **2**, as well as related C-4 amides and C-4 hydroxymethyl derivatives possessing a C-3 hydroxymethyl group (**15** and **19**) together with their SQS inhibitory activities are presented. Copyright © 1996 Elsevier Science Ltd

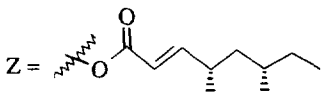
Squalestatins/zaragozic acids are a family of fungal metabolites which possess potent inhibitory activities against squalene synthase (SQS), an enzyme committed to cholesterol biosynthesis, and squalestatin **1**, S1, possesses a profound cholesterol lowering ability *in vivo*.[1] Previously we reported 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

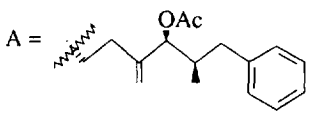


1, S1, R = CO_2H

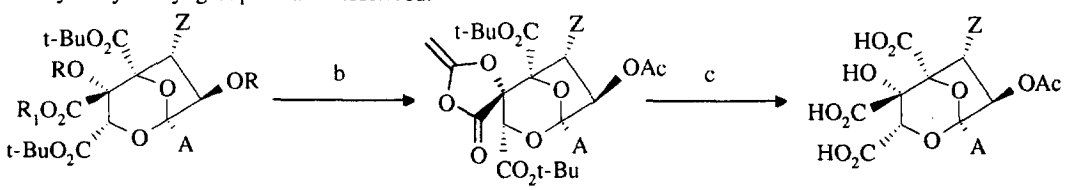
2, R = $CONH_2$

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.



4,[1] R = R_1 = H

5, R = Ac, R_1 = H

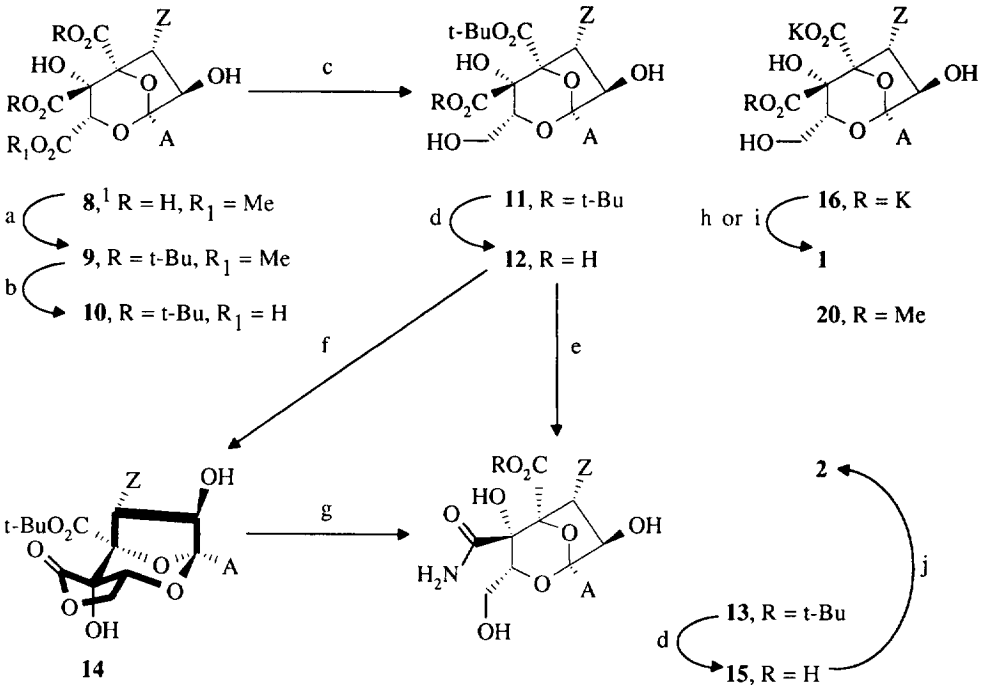
6

7

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

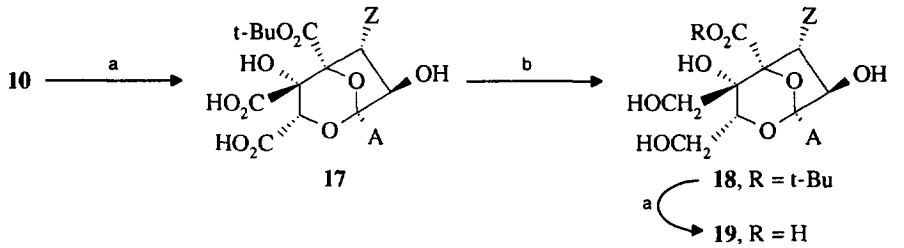
deprotection with HCl-dioxan gave S1 C-7 acetate **7**. Analysis of the "reduction" product by spectroscopic techniques revealed its identity as the spiroacetal **6**.[3] Indeed omitting $NaBH_4$ in the reaction of Vilsmeier salt with **5** also gave **6** (37%). A plausible explanation for the formation 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.

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References and Notes

1. Watson, N. S.; Procopiou, P. A. *Prog. Med. Chem.* **1996**, *33*, 331-378.
2. Chan, C.; Andreotti, D.; Cox, B.; Dymock, B. W.; Hutson, J. L.; Keeling, S. E.; McCarthy, A. D.; Procopiou, P. A.; Ross, B. C.; Sareen, M.; Scicinski, J. J.; Sharratt, P. J.; Snowden, M. A.; Watson, N. S. *J. Med. Chem.* **1996**, *39*, 207-216.
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, McCHCH=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, McCO$_2$), 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, MeCHCH=CHCO$_2$, J = 7 Hz), 1.46 & 1.50 (2s, 18H, 2 t-Bu), 2.09 & 2.19 (2s, 6H, 2 MeCO$_2$), 2.70 (dd, 1H, one proton of PhCH$_2$, J = 14 & 5 Hz), 3.85 (ABq, 2H, (O)$_2$C=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 (MeCH$_2$), 13.8 (MeCHCH$_2$Ph), 18.8 (MeCHEt), 20.1 (CH=CHCHMe), 20.08 & 21.0 (2 MeCO2), 25.1 (CH$_2$C=CH$_2$), 27.6 & 27.7 (2 Me$_3$C), 29.7 (CH$_2$Me), 31.7 (CHEt), 33.8 (CH$_2$CH$_2$C=CH$_2$), 34.4 (CHC=CHCO$_2$), 36.8 (CHCH$_2$Ph), 39.9 (CH$_2$Ph), 43.2 (MeCHCH$_2$CHEt), 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 Me$_3$CO$_2$C), 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$_2$tBu), 164.3 (CH=CHCO$_2$), 165.8 (C-4 CO$_2$), 169.0 & 170.0 (2 CH$_3$CO$_2$). 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, MeCHCH=CHCO$_2$, J = 6 Hz), 2.09 (s, 3H, MeCO$_2$), 2.62 (dd, 1H, one proton of CH$_2$Ph, J = 13 & 6 Hz), 3.35-3.5 (m, 2H, CH$_2$OH), 3.84 (dd, 1H, H-7, J = 5 & 2 Hz), 4.46 (m, 1H, H-3), 4.71 (t, 1H, CH$_2$OH, 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$_2$H). 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, MeCHCH=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, MeCO$_2$), 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$_2$OH at 3, J = 12 & 5 Hz), 3.81 & 4.03 (2d, 2H, CH$_2$OH at 4, J = 12 Hz for both), 3.96 (dd, 1H, one proton of CH$_2$OH 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, MeCHCH=CHCO$_2$, J = 7 Hz), 1.57 (s, 9H, t-Bu), 2.1 (s, 3H, MeCO$_2$), 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$_2$O), 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$_2$OCO) 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.

6. Heyns, K.; Alpers, E.; Weyer, J. *Chem. Ber.* **1968**, *101*, 4199-4208.
7. Green, G.; Griffith, W. P.; Hollinshead, D. M.; Ley, S. V.; Schröder, M. *J. Chem. Soc. Perkin Trans. 1* **1984**, 681-686.
8. Carreira, E. M.; Du Bois, J. *J. Am. Chem. Soc.* **1994**, *116*, 10825-10826.
9. Nicolaou, K.C.; Nadin, A.; Leresche, J. E.; La Greca, S.; Tsuri, T.; Yue, E. W.; Yang Z. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2187-2190.
10. Fujisawa, T.; Mori, T.; Sato, T. *Chem Lett.* **1983**, 835-839.

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