Novel Inhibitors of Lanosterol 14α-Methyl Demethylase, a Critical Enzyme in Cholesterol Biosynthesis

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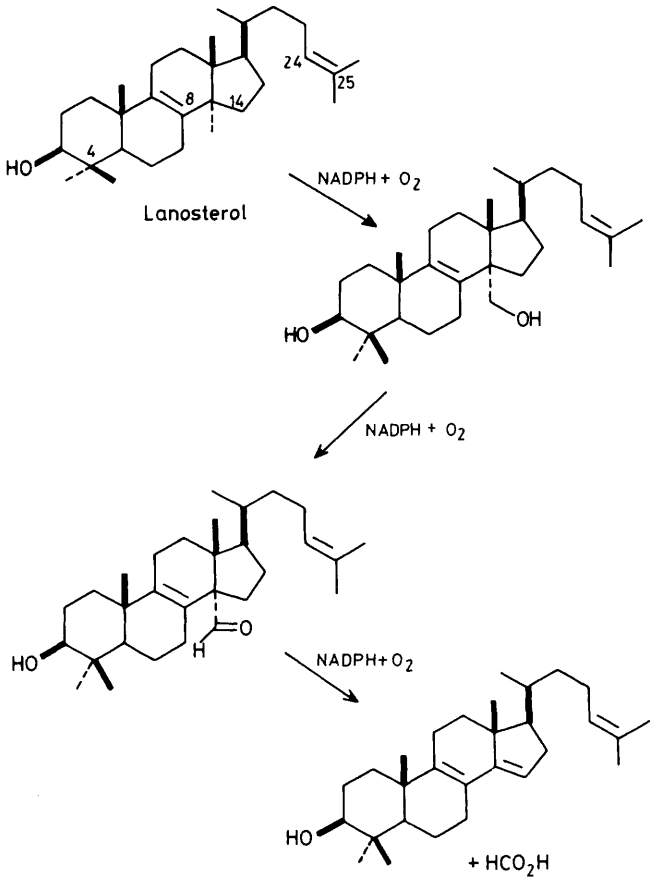
A series of novel 32-functionalised lanost-7-en-3-ols (**1**)—(**7**) has been synthesised; these compounds are all powerful inhibitors of lanosterol 14α-methyl demethylase, an important enzyme in cholesterol biosynthesis.

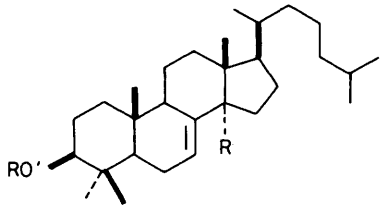
Lanosterol 14α-methyl demethylase ($P\text{-}450_{14DM}$) is the rate limiting enzyme in the degradation of lanosterol to cholesterol. $P\text{-}450_{14DM}$ is a cytochrome P-450 mono-oxygenase which oxidatively removes the 14α-methyl group of lanosterol *via* three O_2-NADPH dependent steps. The 14α-methyl group is first oxidised to a hydroxymethyl moiety followed by oxidation to the corresponding aldehyde; the nature of the third oxidation which results in the formation of the 8,14-diene and loss of formic acid, is still unclear (Scheme 1).[1] Mammalian and yeast $P\text{-}450_{14DM}$ have been purified by Gaylor[2] and Yoshida.[3] Inhibitors of this enzyme system are not only of potential use as cholesterol-lowering agents[4] but also as antimycotics since it has been shown[5] that clinically useful antifungal agents, such as ketoconazole and miconazole, cause the accumulation of 14α-methylsterols. We report here the synthesis of a series of novel steroidal inhibitors (**1**)—(**7**) of $P\text{-}450_{14DM}$.

The 32-functionalised lanost-7-en-3-ols (**1**)—(**7**)† were prepared from the hitherto undescribed protected aldehydes (**9**) and (**10**). The latter were obtained from the known diol (**8**)[6] by selective protection of the C-3 hydroxy group followed by

† All new compounds exhibited satisfactory spectral and analytical properties. Data for compounds (**1**)—(**7**) (all n.m.r. spectra in $CDCl_3$). (**1**): m.p. 106.5—107.0 °C; ^{1}H n.m.r. δ 5.95 (t, 1H), 5.37 (m, 1H), 3.25 (dd, 1H); ^{19}F n.m.r. δ −117.9 (dd), −123.6 (dd). (**2**): m.p. 111.5—112.5 °C; ^{1}H n.m.r. δ 5.63 (tdd, 1H), 5.33 (m, 1H), 3.26 (dd, 1H). ^{19}F n.m.r. δ −110.0 (ddd). (**3**): m.p. 142.0—142.5 °C, ^{1}H n.m.r. δ 5.28 (t, 1H), 3.26 (dd, 1H), 2.5 (dt, 1H), 2.16 (dd, 1H). (**4**): m.p. 161.0—162.0 °C, ^{1}H n.m.r. δ 5.45–5.25 (1m, 1H), 4.41 (brd, 1H), 3.25 (dd, 1H), 2.55 (d, 1H). (**5**): m.p. 135.0—136.0 °C, ^{1}H n.m.r. δ 5.6—5.4 (m, 1H), 4.6—4.5 (m, 1H), 3.4—3.1 (m, 1H), 2.4 (d, 1H). (**6**): m.p. 106.5—107.0 °C, ^{1}H n.m.r. δ 6.28 (dd, 1H), 5.3 (m, 1H), 5.01 (dd, 1H), 4.95 (dd, 1H), 3.24 (dd, 1H). (**7**): m.p. 168.0—168.5 °C, ^{1}H n.m.r. δ 5.42 (m, 1H) 3.28 (dd, 1H), 2.17 (s, 1H).



Scheme 1



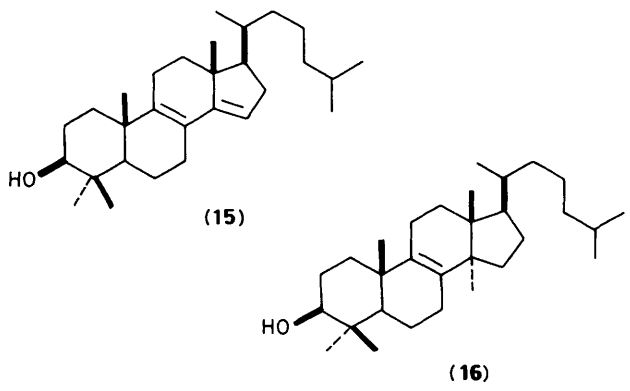
(1) R = CHF_2, R′ = H
(2) R = CH_2CHF_2, R′ = H
(3) R = $CH_2C{\equiv}CH$, R′ = H
(4) and (5) R = $CHOHC{\equiv}CH$, R′ = H
(6) R = $CH{=}CH_2$, R′ = H
(7) R = $C{\equiv}CH$, R′ = H
(8) R = CH_2OH, R′ = H
(9) R = CHO, R′ = THP
(10) R = CHO, R′ = Ac
(11) R = CH_2CHO, R′ = THP
(12) R = CH_2CHO, R′ = Ac
(13) R = CH=CHOMe, R′ = H
(14) R = CH_2CHO, R′ = H

Figure 1

oxidation at C-32 with Fetizon's reagent.[7] The difluoromethyl analogue (**1**) was prepared from aldehyde (**10**) by reaction with neat diethylaminosulphur trifluoride (DAST)[8] at 80 °C followed by hydrolysis (KOH/EtOH). The easily separable diastereoisomeric propynyl alcohols (**4**) and (**5**) were synthesised by treatment of the THP-aldehyde (**9**) (THP = tetrahydropyran-2-yl) with the Grignard reagent of acetylene[9] followed by removal of the THP-protecting group with pyridinium toluene-*p*-sulphonate (PPTS)/EtOH.[10] The vinyl compound (**6**) was prepared using Oshima's reagent[11] ($CH_2Br_2/TiCl_4/Zn$) followed by treatment with PPTS/EtOH.

Table 1. The concentration of inhibitor which results in 50% reduction of the rate of 8,14-diene formation (IC_{50}) was determined using a substrate concentration of 25 μM.

Compound	R	IC_{50}
(1)	CHF_2	7.3 μM
(2)	CH_2CHF_2	7.6 μM
(3)	$CH_2C{\equiv}CH$	1.2 μM
(4)	$CHOHC{\equiv}CH$	5.0 nM
(5)	$CHOHC{\equiv}CH$	570 nM
(6)	$CH{=}CH_2$	2.3 μM
(7)	$C{\equiv}CH$	1.2 μM



The acetylenic analogue (**7**) was obtained by reaction of aldehyde (**9**) with the ylide of chloromethyltriphenylphosphonium chloride followed by treatment with n-butyl-lithium and removal of the protecting group at C-3 with PPTS/EtOH.[12]

The synthesis of the difluoroethyl and propynyl compounds (**2**) and (**3**) necessitated the preparation of the homologated aldehydes (**11**) and (**12**). The THP-protected aldehyde (**9**) was treated with the ylide of methoxymethyltriphenylphosphonium chloride in tetrahydrofuran (THF) giving methyl enol ether (**13**).[13] Cleavage of this enol ether ($HClO_4$/Et_2O, 0 °C) gave the deprotected homologated aldehyde (**14**) which was reprotected as the 3β-THP-ether (**11**) (DHP/PPTS/CH_2Cl_2)[10] or the 3β-acetate (**12**) (Ac_2O/pyridine). Treatment of homologated aldehyde (**12**) with DAST[8] followed by hydrolysis (KOH/EtOH) gave the difluoroethyl analogue (**2**). The propynyl compound (**3**) was prepared from the homologated aldehyde (**11**) in a manner analogous to the synthesis of the acetylenic compound (**7**).[12]

The 32-functionalised lanost-7-en-3-ols (**1**)—(**7**) were tested as inhibitors of lanosterol 14α-methyl demethylase in rat liver microsomes using a modification of an assay developed by Gaylor.[1a] This assay involves the inhibition of Δ^{14}-reductase by AY-9944[14] and 4-methylsterol oxidase by NaCN resulting in the build-up of the 8,14-diene (**15**). The latter is readily quantified by u.v.–h.p.l.c. The K_M of dihydrolanosterol (**16**), a substrate for P-450_{14DM}, was determined to be 23 μM using this assay.[15] The IC_{50} values for the lanosterols (**1**)—(**7**) are given in Table 1. Each of the lanosterol derivatives (**1**)—(**7**) proved to be a very powerful inhibitor of P-450_{14DM}. Although P-450_{14DM} is similar to aromatase in many ways,[16] recent results with yeast P-450_{14DM} indicate that the hydroxylated intermediate, lanost-8-ene-3,32-diol, is more tightly bound than lanosterol;[17] the opposite trend is observed[18] for aromatase. A comparison of the IC_{50} values for the propynyl

compound (**3**) and the propynyl alcohols (**4**) and (**5**) (Table 1) suggests that mammalian P-450$_{14DM}$ may follow the same trend as the corresponding yeast system.

These are the first reported steroidal inhibitors of lanosterol 14α-methyl demethylase. Compounds (**1**)—(**7**) were designed as potential irreversible inactivators of P-450$_{14DM}$; therefore, more detailed studies into the mechanism of inhibition for each of these compounds are underway.

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