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NOVEL OPTIMISED QUINUCLIDINE SQUALENE SYNTHASE INHIBITORS

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Abstract: Optimised quinuclidine squalene synthase (SQS) inhibitors are reported; 3-[2-(2-allyl-4-(2-ethoxy carbonylethyl)phenyl)ethynyl]quinuclidin-3-ol 1c, is a potent inhibitor of rat (KI = 6 nM) and human (KI = 43 nM) microsomal SQS; the oral ED₅₀ of 1c, for the inhibition of rat cholesterol biosynthesis was 1.3 ± 0.45 mg/kg and for the R-enantiomer 1m, 0.8 ± 0.2 mg/kg, with the corresponding R-carboxylic acid 6a, being 0.9 ± 0.25 mg/kg. © 1997 Elsevier Science Ltd. All rights reserved.

Recent clinical trials of the cholesterol lowering, HMGCoA reductase inhibitor drugs (HMGCoARIs) have shown significant reductions in patient mortality rates for both hypercholesterolaemic¹ patients and those with existing coronary heart disease². An even greater life-saving effect might be attainable if plasma cholesterol levels could be brought below those currently achieved by the HMGCoARI drugs; in this respect, the potential advantages of a drug which interrupts cholesterol biosynthesis at the squalene synthase (SQS) step have been pursued³, leading to the polyanionic natural product and phosphonate SQS inhibitors⁴. We have recently described^{5a, 5b} novel series of 3-substituted quinuclidines as inhibitors of rat microsomal SQS, and now report their optimisation.



In this context we have previously exemplified^{5a} the benzoic ester derivative **1a**, which we report as a very potent *in vitro* inhibitor of rat microsomal SQS (KI = 4 nM) and an orally active inhibitor of rat cholesterol biosynthesis from tritiated mevalonate ($ED_{50} = 6.0\pm 2.0 \text{ mg/kg}$, n = 5); these *in vitro* and *in vivo* biological tests have been reported^{5b, c} in detail. Our aim was to obtain a compound which could achieve a greater plasma Fax 01625 513910

cholesterol lowering effect than that attainable with an HMGCoARI, and despite the good inhibitory activity of 1a, we sought a compound with an oral $ED_{50} < 1 \text{ mg/kg}$ for the inhibition of rat cholesterol biosynthesis. The choice of synthetic targets was based on how quinuclidine SQS inhibitors might act at the enzyme site. SQS assembles two molecules of farnesyl pyrophosphate (FPP) into squalene in two distinct steps⁶; both steps have been envisaged⁷ to involve a cyclopropyl carbocationic intermediate, and protonated quinuclidine SQS inhibitors^{5b} may inhibit the enzyme by acting as carbocation mimics for either step of the FPP to squalene conversion. We postulated that the 3-phenylethynyl substituent in 1a, might interact with a lipophilic pocket on the enzyme in a similar manner to the isoprenyl subunits in a farnesyl chain, and synthetic target compounds were aimed at increasing this interaction with the enzyme, by placing lipophilic alkyl linking groups (TABLE 1) between the phenyl and carboxylic ester groups of 1a.

The compounds in TABLE 1 were prepared from known substituted phenols by alkylation with allyl bromide and subsequent Claisen rearrangement. Triflate derivatives of these phenols were reacted with 3-hydroxy-3-ethynylquinuclidine^{5a} in a palladium catalysed coupling reaction at 80 °C (Scheme 1). New



compounds analysed correctly ($\pm 0.4\%$) for C, H, and N, and gave ¹H-NMR spectral data consistent with the structures assigned⁸.

Placing a single CH₂ group (as in **1b**) between the phenyl and ester groups of **1a**, led to a significant decrease in SQS inhibitory potency (**1b**, KI = 100 nM), despite the lipophilicity of **1a**, CLOGP = 3.3 (higher than would be expected due to electron delocalisation around the phenyl ring from the carboxylic ester group) being similar to that of **1a**, (CLOGP = 3.0). For the 2 to 5 carbon methylene linked compounds **1c**-g, where the lipophilicity range was increased (CLOGP = 3.3-5.6), the SQS inhibitory potency was of the same order as for **1a**, (≤ 20 nM). Although these compounds did not exhibit an increase in SQS inhibitory activity, the oral ED₅₀ values for the inhibition of rat cholesterol biosynthesis were in the range 1.3-3.0 mg/kg, and in particular the ED₅₀ for the ester **1c**, was 1.3±0.45 mg/kg; i.e. the chain extended ester **1c**, was more effective *in vivo* than **1a**.

The ester 1c, was selected for further study and structural variation as an example of the new compounds 1c-g, because of its relative ease of synthesis; yields from the palladium coupling reaction fell as the methylene

Compound	R	mp ⁰C	KI nM, (n = 2)
1b	CH ₂ CO ₂ Et	84-86	100
1c	(CH ₂) ₂ CO ₂ Et	55-56	6
1d	$(CH_2)_2CO_2Me$	63-65	20
1e	$(CH_2)_3CO_2Me$	34-35	2
1f	(CH ₂) ₄ CO ₂ Me	35-37	8
1g	(CH ₂) ₅ CO ₂ Me	oil	20
8 1h	(CH ₂) ₂ CONH ₂	137-137.5	>250
1i	(CH ₂) ₂ CONHMe	115-115.5	>250
1j	(CH ₂) ₂ CONEt ₂	oil	>250
1k	(CH ₂) ₂ CN	123-124	>250
2	Squibb 32377	oil	<u>30 μM</u>

TABLE 1: Inhibition of rat microsomal SQS

(Squibb 32377 was used as the standard SQS inhibitor, lit.⁹ IC₅₀ = 9 μ M.)

chain length was increased (e.g. 1c, 64% and 1g, 18% yield). The enantiomers of 1c, were prepared as in Scheme 1 (after kinetic enzymatic hydrolysis^{5a} of the O-butyrate ester of 3-ethynylquinuclidin-3-ol with pig liver esterase) in >99.5% ce (HPLC, n-hexanc/EtOH/Et₃N, 99:5:0.2; Chiralcel OD column) to give as oils 1l, (S-enantiomer, $|\alpha|^{25}_{D} = -21.6^{\circ}$; c = 0.314, EtOH) and 1m, (R-enantiomer, $|\alpha|^{25}_{D} = +21.8^{\circ}$; c = 0.316, EtOH). The R-enantiomer 1m, gave an 84% inhibition of rat microsomal SQS at 25 nM, with a 6% inhibition being found for the S-enantiomer 1l, indicating a steric requirement for enzyme inhibition. The oral ED₅₀ of 1m, for the inhibition of rat cholesterol biosynthesis was 0.8±0.2 mg/kg. Inhibitory potency fell considerably for the amide and nitrile analogues 1h-k, of the ester 1c, with KI values >250 nM. Compounds 1h-1k, were obtained by treating 1c, with excess of amines in MeOH for 40 h., to give the primary, secondary and tertiary amides, and



by replacing the ester group by nitrile in Scheme 1. Inclusion of an oxadiazole ring e.g.3, (prepared as in Scheme 1 via 4, and 5) as a 'methyl ester isostere' also afforded lower SQS inhibition (KI > 250 nM). A variety of ester analogues of the ethyl ester 1c, gave similar oral ED_{50} values for the inhibition of rat cholesterol

biosynthesis (e.g. ethyl 1c, = 1.3 mg/kg; n-hexyl 1n, = 1.5 mg/kg (mp 39-41 $^{\circ}$ C); and benzyl 1o, = 1.6 mg/kg (mp 47-49 $^{\circ}$ C)), and prompted the examination of the rate of ester hydrolysis in rat blood plasma. The plasma t_{1/2} hydrolysis values for 1c, 1n, 1o, were all \leq 5 min, indicating that the active species *in vivo* might be the corresponding carboxylic acid. This acid 6, (mp 41-44 $^{\circ}$ C, HCl salt) was derived from the ester 1c, by hydrolysis with KOH/EtOH, and shown to have an oral ED₅₀ for the inhibition of rat cholesterol biosynthesis of 1.8±0.6 mg/kg; the corresponding R-enantiomer 6a, (mp 161-163 $^{\circ}$ C, HCl salt) gave an ED₅₀ of 0.9±0.25 mg/kg compared to 0.8±0.2 mg/kg for the R-ester 1m.

In summary, optimised 3-substituted quinuclidines have been identified, which are potent inhibitors of rat and human microsomal SQS. Carboxylic ester derivatives hydrolysed in rat blood plasma to equiactive carboxylic acids with both acids and esters affording oral ED_{50} values for the inhibition of rat cholesterol biosynthesis below 1 mg/kg. These novel quinuclidine SQS inhibitors may afford a new series of hypocholesterolaemic agents with potential for the treatment of coronary heart disease.

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(8) ¹ H-NMR of 1c : (CDCl₃) δ ppm 1.22 (t, 3H), 1.34-1.50 (m, 1H), 1.57-1.72 (m, 1H), 1.90-2.15 (m, 3H), 2.55-2.65 (t, 3H), 2.75-2.95 (m, 6H), 3.08 (d, 1H), 3.31 (d, 1H), 3.51 (d, 2H), 4.12 (q, 2H), 4.98-5.10 (m, 2H), 5.86-6.03 (m, 1H), 6.97-7.05 (m, 2H), 7.32 (d, 1H); EI-MS *m/z* 368 (M+H).

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