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NOVEL OPTIMISED QUINUCLIDINE SQUALENE SYNTHASE INHIBITORS

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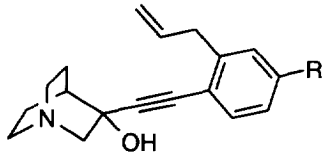
Abstract: Optimised quinuclidine squalene synthase (SQS) inhibitors are reported; 3-[2-(2-allyl-4-(2-ethoxy carbonylethyl)phenyl)ethynyl]quinuclidin-3-ol **1c**, is a potent inhibitor of rat (KI = 6 nM) and human (KI = 43 nM) microsomal SQS; the oral ED_{50} of **1c**, for the inhibition of rat cholesterol 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.

Recent clinical trials of the cholesterol lowering, HMGCoA reductase inhibitor drugs (HMGCoARIs) have shown significant reductions in patient mortality rates for both hypercholesterolaemic[1] patients and those with existing coronary heart disease[2]. 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 pursued[3], leading to the polyanionic natural product and phosphonate SQS inhibitors[4]. We have recently described[5a, 5b] novel series of 3-substituted quinuclidines as inhibitors of rat microsomal SQS, and now report their optimisation.



1a, R = CO_2Et
1b, R = CH_2CO_2Et
1c, R = $(CH_2)_2CO_2Et$

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±2.0 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

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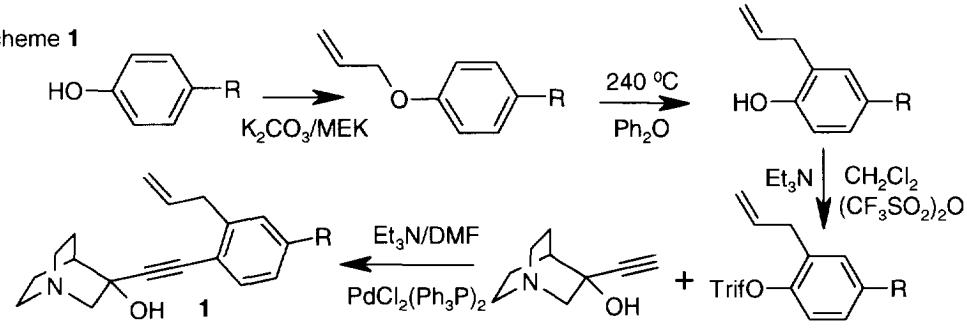
cholesterol lowering effect than that attainable with an HMGCoARI, and despite the good inhibitory activity of **1a**, we sought a compound with an oral ED_{50} <1 mg/kg for the inhibition of rat cholesterol biosynthesis. The choice of synthetic targets was based on how quinuclidine SQS inhibitors might act at the enzyme site. SQS assembles two molecules of farnesyl pyrophosphate (FPP) into squalene in two distinct steps[6]; both steps have been envisaged[7] 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 ^{0}C (Scheme 1). New

Scheme **1**



compounds analysed correctly (± 0.4%) for C, H, and N, and gave ^{1}H-NMR spectral data consistent with the structures assigned[8].

Placing a single CH_2 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 ED_{50} values for the inhibition of rat cholesterol biosynthesis were in the range 1.3-3.0 mg/kg, and in particular the ED_{50} 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**.

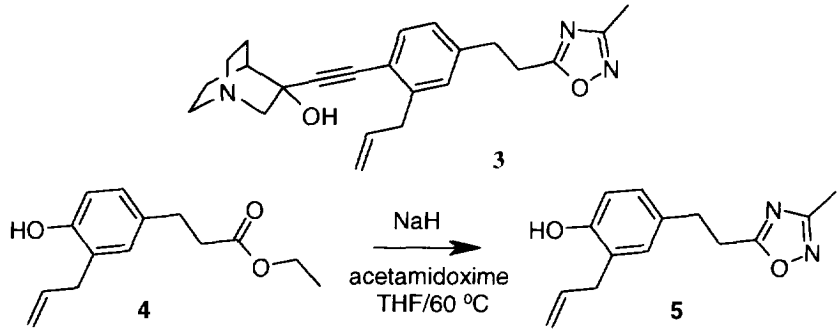
The ester **1c**, was selected for further study and structural variation as an example of the new compounds **1c-g**, because of its relative ease of synthesis; yields from the palladium coupling reaction fell as the methylene

TABLE 1: Inhibition of rat microsomal SQS

Compound	R	mp ^{0}C	KI nM, (n = 2)
1b	CH_2CO_2Et	84-86	100
1c	$(CH_2)_2CO_2Et$	55-56	6
1d	$(CH_2)_2CO_2Me$	63-65	20
1e	$(CH_2)_3CO_2Me$	34-35	2
1f	$(CH_2)_4CO_2Me$	35-37	8
1g	$(CH_2)_5CO_2Me$	oil	20
1h	$(CH_2)_2CONH_2$	137-137.5	>250
1i	$(CH_2)_2CONHMe$	115-115.5	>250
1j	$(CH_2)_2CONEt_2$	oil	>250
1k	$(CH_2)_2CN$	123-124	>250
2	Squibb 32377	oil	30 μM

(Squibb 32377 was used as the standard SQS inhibitor, lit.[9] IC_{50} = 9 μM.)

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% ee (HPLC, n-hexane/EtOH/Et_3N, 99:5:0.2; Chiralcel OD column) to give as oils **1l**, (S-enantiomer, $[\alpha]^{25}_D$= -21.6^0; c = 0.314, EtOH) and **1m**, (R-enantiomer, $[\alpha]^{25}_D$ = +21.8^0; 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 ED_{50} 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 ^{0}C); and benzyl **1o**, = 1.6 mg/kg (mp 47-49 ^{0}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 ≤ 5 min, indicating that the active species *in vivo* might be the corresponding carboxylic acid. This acid **6**, (mp 41-44 ^{0}C, HCl salt) was derived from the ester **1c**, by hydrolysis with KOH/EtOH, and shown to have an oral ED_{50} for the inhibition of rat cholesterol biosynthesis of 1.8±0.6 mg/kg; the corresponding R-enantiomer **6a**, (mp 161-163 ^{0}C, HCl salt) gave an ED_{50} 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** : ($CDCl_3$) 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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