Quinuclidine Inhibitors of 2,3-Oxidosqualene Cyclase-Lanosterol Synthase: Optimization from Lipid Profiles[1]

George R. Brown,* David M. Hollinshead, Elaine S. E. Stokes, David S. Clarke, M. Allan Eakin, Alan J. Foubister, Steven C. Glossop, David Griffiths, Michael C. Johnson, Fergus McTaggart, Donald J. Mirrlees, Graham J. Smith, and Robin Wood

Cardiovascular Metabolism & Muscoskeletal Research Department, Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.

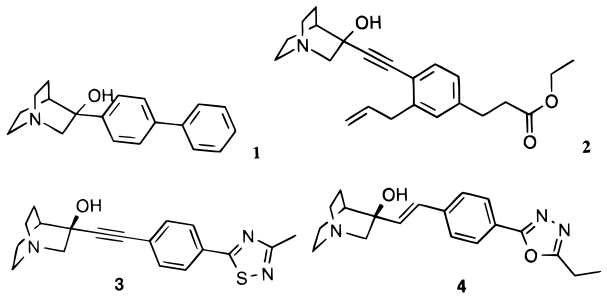
Received January 22, 1999

Novel 3-substituted quinuclidine inhibitors of cholesterol biosynthesis are reported. Compounds were optimized against oxidosqualene cyclase-lanosterol synthase (OSC) inhibition in vivo, rather than by the conventional optimization of structure−activity relationship information based on in vitro OSC inhibition. Thus, examination of HPLC lipid profiles from orally dosed rats showed cholesterol biosynthetic intermediates and whether cholesterol levels were reduced. A new substituted quinuclidine pharmacophore **18a**−**c** was rapidly found for the inhibition of OSC, and the most promising inhibitors were validated by the confirmation of potent OSC inhibition. Compound **16** gave an IC_{50} value of 83 ± 11 nM for human and an IC_{50} value of 124 ± 14 nM, for rat, coupled with oral and selective inhibition of cholesterol biosynthesis derived from OSC inhibition (rat, ED_{50} = 1.3 ± 0.7 mg/kg, n = 5; marmoset, 15 mg/kg dose, n = 3, caused complete inhibition). These 3-substituted quinuclidines, which were derived from a quinuclidine series previously known to inhibit cholesterol biosynthesis at the squalene synthase step, may afford a novel series of hypocholesterolemic agents acting by the inhibition of OSC.

Introduction

Recent 5 year clinical trials of the cholesterol-lowering HMGCoA reductase inhibitor drugs (HMGCoARIs) pravastatin[2] and simvastatin[3] have shown significant reductions in patient mortality rates for both hypercholesterolemic patients[2] without known coronary disease and for those with existing coronary heart disease.[3] These landmark trials have thus established cholesterol-lowering drugs as an important treatment for coronary disease and have stimulated the search for new cholesterol-lowering agents with other mechanisms of action. In seeking alternative approaches to the discovery of novel hypocholesterolemic agents, we considered the inhibition of other cholesterol biosynthesis enzymes.[4] The very widespread clinical use of the HMGCoARIs indicates that they are well-tolerated drugs, despite their interruption of cholesterol biosynthesis before the pathway becomes dedicated to the biosynthesis of cholesterol. Nevertheless as a target mechanism for new inhibitors, we chose to reject the steps before the pathway becomes dedicated to the biosynthesis of cholesterol. Potentially dose-limiting toxicity might arise from the consequent reduced levels of essential isoprenoid precursors, the antioxidant ubiquinone, or the dolichols. Similarly, inhibition at enzyme steps after the formation of lanosterol has the potential[4] to afford toxicity arising from the utilization (in place of cholesterol) of biosynthetic intermediates containing the full sterol ring system. From this analysis, the enzyme steps of squalene synthase (SQS), squalene epoxidase, and oxidosqualene cyclase-lanosterol synthase (OSC, EC 5.4.99.7) remained for further consideration as enzyme inhibition targets.

Considerable effort has been directed to the search[5] for novel inhibitors of SQS, and 3-substituted quinuclidines have been reported[6,7] as an inhibitor class. Recently we further described[8,9] 3-substituted quinuclidine SQS inhibitors (e.g., **1** and **2**) which were potent inhibitors of rat microsomal SQS and orally active inhibitors of rat cholesterol biosynthesis. A small group of these quinuclidines (which had been optimized in the rat as SQS inhibitors) was tested orally at 20 mg/kg in the dog for the inhibition of cholesterol biosynthesis from mevalonate using a test protocol identical to that described below for rats. Liver extracts from dosed animals were examined by HPLC, and unexpectedly a few compounds, e.g., **3** and **4**[10] (**3**, *S*(−), oral ED_{50} for inhibition of rat cholesterol biosyntheis = 4.7 ± 0.7 mg/kg and **4**, *S*(−), oral ED_{50} = 3.6 ± 0.9 mg/kg), gave HPLC chromatograms with a main peak corresponding



to oxidosqualene, the substrate for OSC. This finding indicated that in the dog **3** and **4** inhibited OSC rather than SQS as the mechanism of cholesterol biosynthesis

* To whom correspondence should be addressed. Phone: 01625-515918. Fax: 01625-516667. E-mail: George.Brown@alderley.zeneca.com.

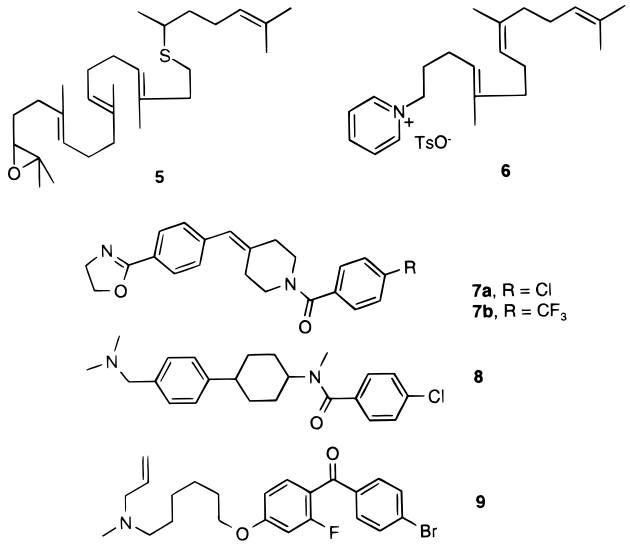
10.1021/jm990038q CCC: $18.00
Published on Web 03/23/1999

Table 1. Selective Quinuclidine SQS Inhibitors

compd	mp °C	IC_{50} SQS ($n = 2$) rat microsomes, in nM	IC_{50} OSC ($n = 2$) rat microsomes, in nM
1	174−175	16	>100
2	55−56	6	>100

inhibition. Quinuclidines **1** and **2** did not exhibit in vitro inhibitory activity against rat microsomal OSC (Table 1), and this led to an investigation of the scope of the OSC inhibitory activity of 3-substituted quinuclidines. More particularly, novel, selective, and more potent quinuclidine OSC inhibitors were sought as potential new hypocholesterolemic agents.

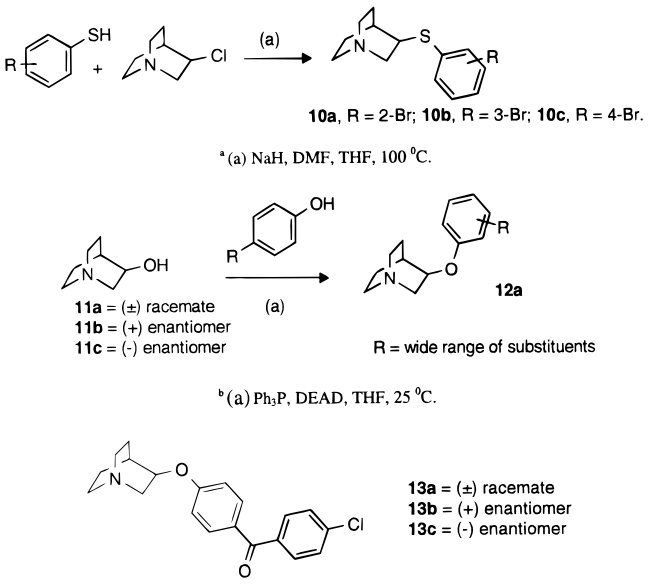
Whereas quinuclidines have not previously been reported as inhibitors of OSC, many inhibitors of the OSC step of cholesterol biosynthesis have been described[11] by academic groups, e.g., **5**[12] and **6**.[13] However, most of these inhibitors have high lipophilicities, which may not be consistent with oral drug delivery in vivo. The mechanism of oxidosqualene cyclization has been studied in detail,[14] and potent orally active OSC inhibitors have been reported, e.g., **7a** (BIBB 515),[15] **8** (BIBX 79),[16] and **9** (Ro 48-8071).[17] The trifluoromethyl analogue **7b**[18] of **7a** (BIBB 515) was used as a standard OSC inhibitor before **7a** had been reported[15] as the OSC inhibitor of choice in this series.



Chemistry

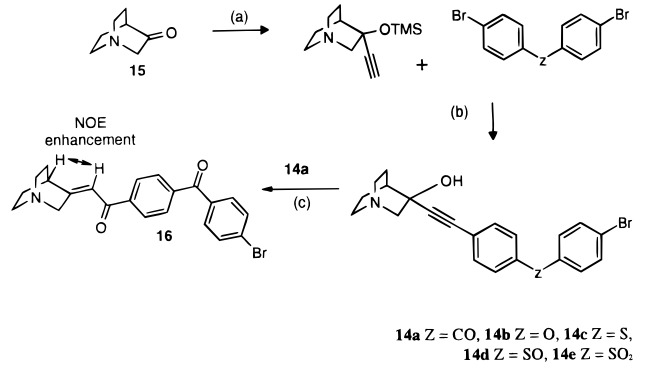
Reaction of 3-chloroquinuclidine with bromophenylthiols, in the presence of NaH/DMF at 100 °C, gave the 3-phenylthio derivatives **10a**−**c** in 36−76% yields (Scheme 1). Mitsunobu reaction of quinuclidin-3-ol **11a**, and 40 variously substituted phenols (Scheme 1) in the presence of Ph_3P/DEAD using a multiple-parallel procedure, gave a 20−80% yield of quinuclidines **12a**. The enantiomers **13b** and **13c** were also made by identical Mitsunobu reactions, but with the quinuclidin-3-ol enantiomers **11b** and **11c** as the starting materials (**11b** and **11c** were obtained by resolution[19] of **11a**, using (+)camphor sulfonic acid as the resolving agent). The general published[9,10] procedure (Scheme 2) for preparing ethyne-linked quinuclidines, e.g., **14a**−**e**, involved reaction of quinuclidin-3-one **15** and lithium trimethylsilylacetylide in THF at −70 °C. A subsequent[9,10] palladium(II) catalyzed coupling reaction with appropriately

Scheme 1. Synthesis of Sulfur- and Oxygen-Linked Quinuclidines[a,b]



[a] Reagents and conditions: (a) NaH, DMF, THF, 100 °C. [b] Reagents and conditions: (a) Ph_3P, DEAD, THF, 25 °C.

Scheme 2. Synthesis of Acetylene-Linked Quinuclidines[a]



[a] Reagents and conditions: (a) Li trimethylsilylacetylide, THF, −10 °C; (b) $(Ph_3P)_2PdCl_2$, CuI, Et_3N, DMF, 70 °C; (c) H_2SO_4 or HCO_2H.

substituted bromobenzenes gave **14a**−**e** in 20−80% overall yield. Acid-catalyzed (H_2SO_4 or HCO_2H) rearrangement[20] of the hydroxy propyne group present in **14a** gave only the Meyer−Schuster 1,3-rearrangement product **16** (50% yield). The assigned structure of **16** (Scheme 2) was supported by ^1H NMR NOE experiments, in which irradiation of the olefinic proton at δ 7.09 showed signal enhancement to the quinuclidine C-4 proton at δ 2.67 (14%, 250 MHz), there being no signal enhancement to the C-2 quinuclidine protons at δ 3.90.

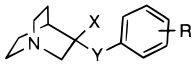
The quinuclidine starting materials, e.g., **11a** and **15**, are commercially available, as are the bromophenylthiol precursors for **10a**−**c**. All of the 4,4′-dihalodiphenyl reagents required to prepare **13a**−**c** and **14a**−**e** can be purchased, except for the known[21] 4,4′-dibromodiphenyl sulfide and its sulfoxide derivative. The reported quinuclidine structures were consistent with the observed ^1H NMR and MS spectra.

Results and Discussion

The optimization strategy for quinuclidines as OSC inhibitors (which is detailed below) was not developed

in the conventional manner of gathering detailed in vitro data. It involved the collection of lipid profile data from orally dosed rats, which showed cholesterol biosynthetic intermediates and whether cholesterol levels were reduced. This process avoided the risk of optimizing cholesterol biosynthesis inhibition, resulting from the inhibition of steps other than OSC. Thus selective OSC inhibitors could have been sought among synthetic analogues of the primary OSC inhibitor lead compounds **3** and **4**, but this tactic was rejected because of the large number of compounds of this series known[10] to be SQS inhibitors in rats and the nonselectivity of these leads. Instead, alternative structures were sought by examining the OSC inhibitory activity of the >700 available quinuclidines in the Zeneca company collection. This quinuclidine set contained diverse quinuclidines with 2- or 3-substitution by aryl or heteroaryl rings linked directly to the quinuclidine ring carbons or via commonly used medicinal chemistry linking groups such as O, S, CH_2, CH=CH, etc. A conventional approach to identifying selective OSC inhibitors among these quinuclidines would be first to seek in vitro OSC inhibitory activity against microsomal OSC. However, in addition to the current lead compounds **3** and **4**, there are several known[11] cases where in vitro OSC inhibitors have subsequently been shown to also inhibit other cholesterol biosynthesis enzymes. Following an in vitro test strategy would thus necessitate in vitro selectivity testing at the relatively large number[4] of other cholesterol biosynthesis steps, and subsequently orally active compounds would need to be found from these selective in vitro inhibitors. As an alternative, an in vivo screen was developed which gave an indication of potency (in terms of cholesterol biosynthesis inhibition) and selectivity for the OSC step. All 700 quinuclidines were tested orally in the rat (50 mg/kg, $n = 1$) for the inhibition of cholesterol biosynthesis from tritiated mevalonate. Extracts from test animal livers were analyzed by HPLC using a radiochemical detector to give radiochromatograms as lipid profiles showing the intermediates of cholesterol biosynthesis and cholesterol levels. The HPLC peaks obtained were identified by comparison with the retention times of the well-established[4] intermediates of cholesterol biosynthesis. The risk of poor reliability in single-animal testing was accepted, as pilot testing suggested this risk was negligible. OSC inhibitors with poor oral availability would not have been detected, but this issue was considered minimal due to the good oral bioavailability previously found[8,9,10] in the rat of this set of quinuclidines. The identification of characteristic biosynthetic substrate peaks in the radiochromatograms enabled test compounds to be classed as 49% inactive (cholesterol peak unchanged), 18% SQS inhibitors (farnesyl pyrophosphate peak present and cholesterol peak reduced by >50%), 12% nonselective inhibitors (cholesterol peak reduced, but several peaks observed), and 21% (i.e., 147 quinuclidines) selective OSC inhibitors (cholesterol peak reduced by >50% and oxidosqualene peak present).

The 147 selective OSC inhibitors were further optimized by examining the 50 most potent OSC inhibitors identified from the HPLC radiochromatogram peaks (7% of the original 700 compounds screened at 50 mg/kg, $n = 1$) in orally dosed rats (20 mg/kg, $n = 5$). The lipid profiles from these lower dose studies confirmed the selective OSC inhibitory activity found in the primary screening at 50 mg/kg and defined a smaller group of compounds (cholesterol synthesis inhibition >50% at 20 mg/kg) from which a provisional quinuclidine OSC inhibitory pharmacophore covered by **17a,b** could be described. This pharmacophore embraced a



17a, X = H, Y = O, S
R = varied groups

17b, X = OH, Y = ethynyl
R = varied groups

3-substituted quinuclidine ring linked (only by X = oxygen or sulfur, Y = H; or X = ethynyl, Y = OH) to a substituted phenyl ring. Additional in vivo optimization of the pharmacophore **17a,b** was based on newly synthesized quinuclidine analogues, in order to find the scope of substitution in the 3-linked phenyl ring. Compounds were also confirmed as OSC inhibitors by showing >50% inhibition of rat microsomal OSC in vitro at 1 μM. The optimum substitution position on the phenyl ring was examined by synthesis of the thio-linked bromo derivatives **10a**−**c**, (Table 2). Only the para bromo substitution in **10c** afforded potent selective OSC inhibitory activity in the rat (oral dose at 20 mg/kg gave 47% inhibition). The corresponding 2- and 3-bromo analogues were much less active (<30% inhibition). Results from in vivo testing (oral rat, 50 mg/kg, $n = 1$) of 40 substituted 3-phenoxyquinuclidines **12a** confirmed the requirement for para substitution. In this multiple-parallel synthesis, the phenyl ring substituents included alkyl, alkoxy, halogens, CN, COMe, CF_3, *N*-morpholino, *N*-imidazolinyl, NO_2, PhO, $PhCH_2$, and PhCO, and the phenyl ring was replaced by 5-benzodioxalanyl and 6-quinolinyl. These phenoxyquinuclidines also indicated that the most potent selective OSC inhibitory activity would be found in quinuclidines having a link group Z (O, CH_2, CO) to a second phenyl ring (as in **13a**−**c**, Z = CO). The benzophenone moiety present in **13a**−**c** has also been independently identified in the Roche OSC inhibitor series,[17] e.g., **9**. A range of these second link groups Z (**14a**−**e**, Z = CO, O, S, SO, SO_2) in the ethyne-linked quinuclidines was examined in rats (oral, 20 mg/kg, $n = 5$, Table 2). Selective inhibition of cholesterol biosynthesis derived from OSC inhibition was found for link groups with and without hydrogen bond acceptor properties. The enantiomers **13b** and **13c** of **13a** showed identical activity in both in

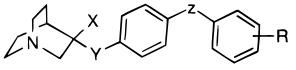
Table 2. Oral Inhibition of Rat Cholesterol Biosynthesis

compd	formula	mp °C	% yield	% inhibition 20 mg/kg, $n = 5$
10a	$C_{13}H_{16}BrNSHCl$[a]	214−215[b]	52	<5
10b	$C_{13}H_{16}BrNS$	oil	38	<5
10c	$C_{13}H_{16}BrNS{\cdot}HCl$	180−182[b]	76	47 ± 12
13a	$C_{20}H_{20}ClNO_2$	147−149	22	92 ± 3 at 5 mg/kg
14a	$C_{22}H_{20}BrNO_2$	214−216	20	59 ± 5
14b	$C_{21}H_{20}BrNO_2$	178−180	39	55 ± 12
14c	$C_{21}H_{20}BrNOS$	291−292	63	79 ± 10
14d	$C_{21}H_{20}BrNO_2S$	223−224	28	83 ± 10
14e	$C_{21}H_{20}BrNO_3S$	218−219	25	94 ± 10
16	$C_{22}H_{20}BrNO_2$	159−161	50	84 ± 3 at 5 mg/kg
7b	$C_{23}H_{21}F_3N_2O_2$	151−152		98 ± 2 at 5 mg/kg

[a] All new compounds were analyzed correctly (±0.4%) for C, H, and N and afforded ^{1}H NMR data consistent with the structures assigned. [b] HCl salt.

vitro and orally dosed in vivo tests in the rat (**13b**, 60% inhibition at 100 nM ($n = 2$) against rat microsomal OSC, and 60% inhibition of rat cholesterol biosynthesis at 2 mg/kg $n = 5$; **13c**, 58% inhibition at 100 nM against rat microsomal OSC, and 57% inhibition of rat cholesterol biosynthesis at 2 mg/kg).

Separate from the above iterative optimization process, the ethyne-linked compound **14a** was rearranged under Meyer−Schuster reaction conditions[20] to give the enone **16**. This purely chemical ploy afforded another linking group for inclusion in the quinuclidine OSC pharmacophore **17a,b** that was not present in the original set of 700 quinuclidines. On the basis of the above in vivo optimization process (involving the reduction of cholesterol peaks and the appearance of oxidosqualene peaks in the plasma profiles) and the confirmatory data (shown below) on quinuclidines **13a** and **16**, the provisional quinuclidine OSC inhibitory pharmacophore **17a,b** was modified to **18a**−**c**. The benzo-



18a, X = H, Y = O, S,
Z = CO, O, S, SO, SO2
R = varied groups

18b, X = OH, Y = ethyne,
Z = CO, O, S, SO, SO2
R = varied groups

18c, X = no substituent, Y = =CHCO
Z = CO, O, S, SO, SO2
R = varied groups

phenone substituted derivatives **13a** and **16** were selected for more detailed study as examples of the derived pharmacophore **18a**−**c**. Quinuclidine **13a** caused oral inhibition of rat cholesterol biosynthesis ($ED_{50} = 2 \pm 0.3$ mg/kg, $n = 5$), with corresponding IC_{50} values for the inhibition of microsomal OSC of 58 ± 19 (rat) and 35 ± 9 nM (human). Quinuclidine **16** also showed potent in vitro inhibitory activity (microsomal OSC: human $IC_{50} = 83 \pm 11$ nM; rat $IC_{50} = 124 \pm 14$ nM) and oral inhibition of rat cholesterol biosynthesis ($ED_{50} = 1.3 \pm 0.7$ mg/kg, $n = 5$); oral dosing of marmosets at 15 mg/kg, $n = 3$ (employing the test protocol described above for rat oral dosing) caused complete inhibition of cholesterol synthesis and the generation of oxidosqualene.

The factors influencing whether 3-substituted quinuclidines selectively inhibit cholesterol biosynthesis at the SQS or OSC steps were considered by comparing a typical selective SQS inhibitor **1** with a typical selective OSC inhibitor **13a**. The mechanism by which SQS converts farnesyl pyrophosphate to squalene, and subsequently in the pathway oxidosqualene is converted by OSC to lanosterol, is widely accepted[5,14] to proceed through carbocationic intermediates. The quinuclidine ring nitrogen atoms of **1** (SQS inhibitor) and **13a** (optimized OSC inhibitor) are in similar chemical environments with both potentially being able to act (when protonated) as carbocation mimics of the postulated[5,14] high-energy enzymatic intermediates of SQS or OSC. The similar physical properties of **1** and **13a** (CLOGP[22] **1**, = 5.0; **13a**, = 4.7, and pK_a **1**, = 9.0; **13a**, = 9.1) suggested no reason for the different inhibitory actions observed; however, both the selective OSC and SQS inhibitory quinuclidines have lipophilic 3-substituents. The different selective pharmacological actions found for **1** and **13a** were attributed to the different molecular size and orientation of the 3-substituent in relation to the quinuclidine ring. In the selective SQS inhibitory quinuclidines, potent activity is known[8] to be conferred by a linear side chain, which may mimic the farnesyl group present in the substrate and products of SQS. By contrast, potent and selective quinuclidine OSC inhibitors such as **13a** are distinguished from the quinuclidine SQS inhibitors by having longer angular side chains containing a link group to a phenyl ring and a second linking group to another phenyl ring.

Conclusions

Substituted quinuclidine cholesterol biosynthesis inhibitors were optimized by examination of rat lipid profiles of cholesterol biosynthetic intermediates. This gave the potent new quinuclidine OSC inhibitors **13a** and **16** for further study and, in addition, a quinuclidine pharmacophore **18a**−**c**, which described other orally active OSC inhibitors. Since 1960, lead optimization has commonly relied on in vitro structure−activity relationship (SAR) data obtained from interactions at enzyme and receptor sites. Here optimization via HPLC lipid analysis gave a measure of inhibitory potency and enabled selective OSC inhibitors to be identified as potential hypocholesterolemic agents. For success, the process relied on the known[8−10] good oral bioavailability in rats of 3-substituted quinuclidines and, with this limitation of oral availability, may have wider utility for the examination of enzyme inhibitors in other multiple-step pathways. In this approach, the gathering of only limited in vitro data prevents the establishment of conventional SAR analyses and the consequent consideration of the expression of in vitro OSC inhibitory potency in vivo. This drawback was counterbalanced by the identification of very potent, orally active, and selective OSC inhibitors within a quinuclidine set which had previously been a source of SQS inhibitors. The optimization approach was further validated by the demonstration of the inhibition of cholesterol biosynthesis in orally dosed rats and marmosets. More detailed evaluation of 3-substituted quinuclidines may thus afford a novel series of hypocholesterolemic agents.

Experimental Section

Melting points were determined with a Buchi apparatus and are uncorrected. The ^{1}H NMR spectra were determined with a Bruker AM (300 MHZ) spectrometer (with $SiMe_4$ as an internal standard), and mass spectra were measured on a Platform Micromass (electrospray) instrument. Reactions were carried out under an atmosphere of argon, and column chromatography was on E. Merck silica gel (Kieselgel 60, 230−400 mesh). Solvents were dried over $MgSO_4$ before evaporation.

A. Chemistry. Typical Synthesis of a 3-Thio Linked Quinuclidine. 3-(2-Bromophenylthio)quinuclidine (10a). NaH (0.96 g, 60% dispersion in mineral oil, 24 mmol) was added over 5 min to a stirred solution of 2-bromobenzene thiol (2.27 g, 12 mmol) in DMF (20 mL) at 20 °C. The mixture was stirred for 15 min, and 3-chloroquinuclidine hydrochloride (1.82 g, 10 mmol) was added over 5 min when the mixture was stirred for 15 min. The mixture was heated at 100 °C for 18 h and then cooled, and H_2O (200 mL) added. The aqueous phase was extracted with EtOAc (3 × 50 mL), and this extract was extracted with 2 M HCl (3 × 25 mL). The acid extract was basified to pH 12 with 10 M NaOH and extracted with EtOAc (3 × 40 mL). These EtOAc extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give an oil, which was purified by chromatography on an IST Bond Elut silica column (25 g) eluting with CH_2Cl_2 and CH_2Cl_2/MeOH/ammonia (200:

3:1) to give an oil **10a** (1.6 g, 52%): ^{1}H NMR ($CDCl_3$) δ 1.44 (m, 1H), 1.59 (m, 1H), 1.76 (m, 1H), 1.91 (m, 1H), 2.12 (m, 1H), 2.81 (m, 4H), 2.96 (m, 1H), 3.48 (m, 2H), 6.02 (m, 1H), 7.26 (m, 2H), 7.56 (d, 1H); EI-MS *m*/*z* 298 (M + H). The oil (300 mg) was dissolved in ^{i}PrOH (2 mL), and 11 M HCl (0.25 mL) and Et_2O were added to give a colorless hydrochloride (210 mg): mp 214−215 °C. Anal. ($C_{13}H_{16}BrNS$·HCl) C, H, N.

Typical Synthesis of a 3-Oxy Linked Quinuclidine (Multiple-Parallel Synthesis). 3-[(4-Chlorobenzoyl)-4-phenoxy]quinuclidine (13a). Compound **11a**, (0.64 g, 5 mmol) in THF (4.5 mL) and DMF (2.5 mL) was added to a stirred solution of 4-chloro-4′-hydroxybenzophenone (1.29 g, 5.5 mmol), PPh_3 (1.70 g, 6.5 mmol), and DEAD (0.94 mL, 6.0 mmol) in THF (4 mL) at 5−10 °C, and the mixture was stirred for 18 h. The solvent was evaporated and the residue dissolved in MTBE (8 mL); the solution was extracted with 2 M HCl, and the extracts were washed with MTBE. The extract was made basic with 4 M NaOH to pH = 11, and the mixture was stirred for 1 h and filtered to give a solid, which was triturated in EtOH to give, on crystallization from, EtOH **13a** (0.37 g, 22%): mp 147−149 °C; ^{1}H NMR ($CDCl_3$) δ 1.4 (m, 1H), 1.67 (m, 2H), 2.0 (m, 1H), 2.20 (m, 1H), 2.95 (m, 5H), 3.32 (m, 1H), 4.47 (m, 1H), 6.90 (d, 2H), 7.34 (d, 2H), 7.75 (q, 4H); EI-MS *m*/*z* 342 (M + H). Anal. ($C_{20}H_{20}ClNO_2$) C, H, N.

(+) and (−) 3-[(4-Chlorobenzoyl)-4-phenoxy]quinuclidine (13b) and (13c). These compounds were prepared in a manner similar to that of **13a**, but starting from (+) and (−)quinuclidin-3-ols **11b** and **11c**, respectively, to give **13b** and **13c** as colorless solids. **13b** (22%): mp 124−125 °C; $[\alpha_D^{25}]$ = +32.5° (*c* = 0.49, EtOH). Anal. ($C_{20}H_{20}ClNO_2$) C, H, N. **13c** (20%): mp 124−125 °C; $[\alpha_D^{25}]$ = −33.5° (*c* = 0.49, EtOH). Anal. ($C_{20}H_{20}ClNO_2$) C, H, N. The ^{1}H NMR data found for these enantiomers were identical to that found for **13a**.

Typical Synthesis of a 3-Ethynyl Linked Quinuclidine. 3-[4-(4-Bromophenylsulfonyl)phenylethynyl]quinuclidin-3-ol (14e). Bis(4-bromophenyl)sulfone (1.5 g, 4 mmol) and dichlorobis(triphenylphosphine) palladium(II) (60 mg, 0.085 mmol) were added to a solution of 3-ethynyl-3-trimethylsilyloxyquinuclidine (0.444 g, 1.995 mmol) in NEt_3 (6 mL) and DMF (2 mL), and the mixture was heated under reflux at 70 °C for 4 h. The cooled reaction mixture was added to H_2O (20 mL), and the mixture was extracted with EtOAc/Et_2O (1:1). The extract was washed with H_2O, dried, and evaporated. The residue was dissolved in EtOAc and filtered through alumina, eluting with EtOAc/MeOH (1:1) to give, after crystallization from THF-*n*-hexane, **14e** as a colorless solid (254 mg, 25%): mp 218−219 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (m, 1H), 1.63 (m, 2H), 1.91 (m, 2H), 2.03 (m, 1H), 2.84 (m, 3H), 3.05 (d, 1H), 3.29 (d, 1H), 7.53 (d, 2H), 7.55 (d, 2H), 7.78 (d, 2H), 7.88 (d, 2H); EI-MS *m*/*z* 445 (M^+). Anal. ($C_{21}H_{20}BrNO_3S$) C, H, N.

Meyer−Schuster Rearrangement Reaction. (*Z*)-3-[4-(4-Bromobenzoyl)phenacylidene]quinuclidine (16). 3-[2-(4-Bromobenzophenone)ethynyl]quinuclidin-3-ol (0.5 g, 1.2 mmol) was added to 98% H_2SO_4 (5 mL) with stirring. The reaction mixture was stirred at ambient temperature for 18 h, and H_2O (10 mL) was added cautiously. The diluted solution was poured onto ice/10 M NaOH to precipitate a solid, and the solid was extracted into CH_2Cl_2 (2 × 50 mL). The extracts were combined, washed with brine, dried, and evaporated. The residue was purified by flash chromatography on silica gel, eluting with EtOH/EtOAc/Et_3N (80:20:3) to give **16** as a colorless solid (0.25 g, 50%): mp 182−183 °C; ^{1}H NMR (DMSO-d_6) δ 1.6−1.7 (m, 2H), 1.8−1.95 (m, 2H), 2.7 (m, 1H), 2.7−2.95 (m, 4H), 3.95 (s, 2H), 7.1 (m, 1H), 7.6−7.9 (m, 6H), 8.1 (m, 2H); EI-MS *m*/*z* 410 (M + H). Anal. (C_{22} $H_{20}BrNO_2$·0.25H_2O) C, H, N.

B. Biological Assays. In Vitro Assay for Inhibition of Rat Microsomal OSC. Rat microsomes (1 mL) containing protein (15−20 mg/mL) were diluted with 50 mM phosphate buffer (722 μL, pH 7.4), and tween (0.1 g) was added to 50 mM phosphate buffer (100 mL). A solution of "cold" oxidosqualene in ethanol (1 mL, 0.65 mg/mL) was added to an ethanolic solution (1 mL) of radiolabeled oxidosqualene (stock solution 18 μL, 1 mCi/mL), and compounds were dissolved in DMSO as follows: molecular weight/1000 × 7.875 = mg/mL required to give a 10^{-4} M stock solution. Dilutions were made from the stock to give 10^{-5}, 10^{-6} M, etc. concentrations of test compound. Phosphate-buffered tween (281 μL) was placed in 5 mL disposable vials, and compound solution (4 μL) was added and mixed well. Microsomes (14.6 μL) were added and the vials preincubated for 10 min at 37 °C. Oxidosqualene mix (15 μL) was added and the mixture incubated for another 1 h. The reaction was terminated by the addition of 16% KOH in 20% aqueous ethanol (315 μL). The samples were heated at 80 °C for 2 h, hexane (2 × 5 mL) was added, and the samples were "whirl-mixed" for 10 s. The hexane phases were separated and blown down with N_2, and the residue was dissolved in CH_3CN/^{i}PrOH (300 μL, 4:1). The samples were chromatographed using a Hichrom 3ODS1 column with an isocratic elution using CH_3CN/^{i}PrOH (95:5, flow rate 1 mL/min). The output from the UV detector was connected to a radiochemical detector to visualize radiolabeled sterol intermediates. The reaction rate was measured as the conversion of oxidosqualene to lanosterol, and the effects of compounds were expressed as an inhibition of this process. IC_{50} values were obtained using the "Origin" curve-fitting program supplied by MicroCal Software Inc., with a tight binding background.

In Vivo Assay for Inhibition of Cholesterol Biosynthesis in Rats. Female rats (130 ± 20 g) were acclimatized to reverse lighting and compounds orally dosed (*n* = 1) in DMSO/HPMC. One hour later, tritiated mevalonate (2.5 μCi) was administered, and after another 1 h the rats were sacrificed. A weighed piece of liver (ca. 0.5 g) was saponified in KOH/EtOH (2 mL, 1:9 w/v) at 80 °C for 2 h, and the mixture was diluted (2 mL) before extraction with isohexane (5 mL). The solvent was evaporated under N_2 at 40 °C, and the residue was dissolved in ^{i}PrOH/CH_3CN (300 μL, 1:4). Aliquots of this solution (100 μL) were examined by HPLC (Spherisorb column S3ODS1-1590; 10 cm × 4.6 mm) and the column eluates monitored by an on-line radiochemical detector. In this way an HPLC lipid profile was obtained for each compound relating radioactivity counts to elution times. Chromatographic peaks obtained at different retention times were identified by comparison to the retention time of standard intermediates in the cholesterol biosynthesis pathway (e.g., oxidosqualene 4.8 min; squalene 7.0 min; farnesyl pyrophosphate 2.0 min; cholesterol 13.1 min), so that a selectivity profile could be obtained in addition to the extent of the inhibition of the biosynthesis of cholesterol from peak area.

References

(1) These results were reported, in part, orally at the Royal Society of Chemistry 8th Symposium on Medicinal Chemistry in Eastern England, Hatfield, U.K., April 17, 1997.

(2) Shepherd, J.; Cobbe, S. M.; Ford, I.; Isles, C. G.; Lorimer, A. R.; Macfarlane, P. W.; McKillop, J. H.; Packard, C. J. Prevention of Coronary Heart Disease with Pravastatin in Men with Hypercholesterolemia. *New Engl. J. Med.* **1995**, *333*, 1301−1307.

(3) Pedersen, T. R.; Kjekshus, J.; Berg, K.; Haghfelt, T.; Fargeman, O.; Thorgeirsson, G.; Pyorala, K.; Mertinen, T.; Olsson, A. G.; Wedel, H.; Wilhelmsmen, L. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* **1994**, *344*, 1383−1389.

(4) Adams, J. L.; Metcalf, B. Therapeutic Consequences of the Inhibition of Sterol Metabolism. *Comprehensive Medicinal Chemistry*; Sammes, P. G., Taylor, J. B., Eds.; Pergamon Press: Oxford, 1990; Vol. 2, pp 333−364.

(5) Biller, S. A.; Neuenschwander, K.; Ponpipom, M. M.; Poulter, C. D. Squalene Synthase Inhibitors. *Curr. Pharm. Des.* **1996**, *2*, 1−40.

(6) Alberts, A. W.; Berger, G. D.; Meade, B.; Bergstrom, J. D. Squalene Synthase Inhibitors. US Appl. 5135935. *Chem. Abstr.* **1992**, *117*, 245613.

(7) Amin, D.; Morris, R. L.; Neuenschwander, K.; Scotese, A. C. Preparation of Azabicyclooctyloxymethylbenzenes and Related Compounds as Squalene Synthase Inhibitors. PCT Int. Appl. WO 9215579 AI 920917. *Chem. Abstr.* **1993**, *118*, 124402.

(8) Brown, G. R.; Clarke, D. S.; Foubister, A. J.; Freeman, S.; Harrison, P. J.; Johnson, M. C.; Mallion, K. B.; McCormick, J.; McTaggart, F.; Reid, A. C.; Smith, G. J.; Taylor, M. J. Synthesis and Activity of a Novel Series of 3-Biarylquinuclidine Squalene Synthase Inhibitors. *J. Med. Chem.* **1996**, *39*, 2971−2979.

(9) Brown, G. R.; Foubister, A. J.; Freeman, S.; McTaggart, F.; Mirrlees, D. R.; Reid, A. C.; Smith, G. J.; Taylor, M. J.; Thomason, D. A.; Whittamore, P. R. O. Novel Optimized Quinuclidine Squalene Synthase Inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 597−600.
(10) Brittain, D. R.; Brown, G. R.; Mallion, K. B.; Whittamore, P. R. O. Quinuclidine derivatives useful as squalene synthase inhibitors and their preparation. Canada Pat. Appl. CA 2104981; *Chem. Abstr.* **1995**, *122*, 81124f.
(11) Abe, I.; Tomesch, J. C.; Wattanasim, S.; Prestwich, G. D. Inhibitors of Squalene Biosynthesis and Metabolism. *Nat. Prod. Rep.* **1994,** *11*, 279−302.
(12) Stach, D.; Zheng, Y. F.; Perez, A. L.; Oehlschlager, A. C.; Abe, I.; Prestwich, G. D.; Hartman, P. G. Synthesis and Inhibition Studies of Sulfur-Substituted Squalene Oxide Analogues as Mechanism-Based Inhibitors of 2,3-Oxidosqualene-Lanosterol Cyclase. *J. Med. Chem.* **1997**, *40*, 201−209.
(13) Rose, I. G.; Sharpe, B. A.; Lee, R. C.; Griffin, J. H. Design Synthesis and In Vitro Evaluation of Pyridinium Ion Based Cyclase Inhibitors and Antifungal Agents. *Bioorg. Med. Chem.* **1996**, *4*, 97−103.
(14) Abe, I.; Rhomer, M.; Prestwich, G. D. Enzymatic Cyclisation of Squalene and Oxidosqualene to Sterols and Triterpenes. *Chem. Rev.* **1993**, *93*, 2109−2206.
(15) Eisele, B.; Budzinski, R.; Muller, P.; Maier, R.; Mark, M. Effects of a Novel 2,3-Oxidosqualene Cyclase Inhibitor on Cholesterol Biosynthesis and Lipid Metabolism In Vivo. *J. Lipid Res.* **1997**, *38*, 564−575.
(16) Eisele, B.; Muller, P.; Maier, R.; Mark, M. Effects of a Novel 2,3-Oxidosqualene Cyclase Inhibitor on the Regulation of Cholesterol Biosynthesis in HepG2 Cells. *J. Lipid Res.* **1996**, *37*, 148−158.
(17) Morand, O. H.; Aebi, J. D.; Dehmlow, H.; Ji, Yu-H.; Cairns, N.; Lengfeld, H.; Himber, J. Ro 48-8071 a New 2,3-Oxidosqualene-Lanosterol Cyclase Inhibitor Lowering Plasma Cholesterol in Hamsters, Squirrrel Monkeys and Minipigs: Comparison to Simvastatin. *J. Lipid Res.* **1997**, *38*, 373−390.
(18) Maier, R.; Mueller, P.; Woitun, E.; Hurnaus, R.; Mark, M.; Eisele, B.; Budzinski, R. M.; Hallermayer, G. Preparation and cholesterol biosynthesis inhibition activity of arylideneazacycloalkanes and their salts. Ger. Appl. DE 4325590 *Chem. Abstr.* **1994**, *121*, 108807.
(19) Sternbach, L. H.; Kaiser, S. Antispasmodics 1. Bicyclic Basic Alcohols. *J. Am. Chem. Soc.* **1952**, *74*, 2215−2219.
(20) Narayanan, K. V.; Swaminthan, S. The Rupe and Meyer-Schuster Rearrangements. *Chem. Rev.* **1971**, *71*, 429−438.
(21) Ho, T.-L.; Wong, C. M. Cerric ammonium nitrate oxidation of diaryl sulphides. *Synthesis* **1972**, 561−562.
(22) CLOGP software for calculating log*P* was from Daylight Chemical Information Systems Inc., Daylight v 4.42.

JM990038Q