A Novel Series of 4-Piperidinopyridine and 4-Piperidinopyrimidine Inhibitors of 2,3-Oxidosqualene Cyclase−Lanosterol Synthase

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A novel series of 4-piperidinopyridines and 4-piperidinopyrimidines showed potent and selective inhibition of rat 2,3-oxidosqualene cyclase−lanosterol synthase (OSC) (e.g. **26** IC_{50} rat = 398 ± 25 nM, human = 112 ± 25 nM) and gave selective oral inhibition of rat cholesterol biosynthesis (**26** ED_{80} = 1.2 ± 0.3 mg/kg, n = 5; HMGCoA reductase inhibitor simvastatin ED_{80} = 1.2 ± 0.3 mg/kg, n = 5). The piperidinopyrimidine OSC inhibitors have a significantly lower pK_a than the corresponding pyridine or the previously reported quinuclidine OSC inhibitor series. This indicates that other novel OSC inhibitors may be found in analogues of this series across a broader pK_a range (6.0−9.0). These series may yield novel hypocholesterolemic agents for the treatment of cardiovascular disease.

Introduction

The HMGCoA reductase inhibitory drugs (statins) are now well-established as agents for lowering plasma cholesterol levels and have widespread therapeutic use in the treatment of cardiovascular disease. Their use is strongly supported[1,2] by long-term clinical trials, which showed significant reductions in the mortality rates of patients with cardiovascular disease. This successful therapy with the statin class of hypocholesterolemic agents has led to a search for other novel inhibitors of the cholesterol biosynthesis pathway[3] that should also afford a lowering of plasma cholesterol levels. The area of greatest interest to date has been inhibition at the squalene synthase (SQS) step.[4] In addition to work on novel SQS inhibitors,[5,6] we have considered[7] the inhibition of other enzyme steps of the cholesterol biosynthesis pathway as potential targets for medicinal chemistry. We sought new inhibitors at later biosynthetic steps where the pathway is dedicated[3] to the biosynthesis of cholesterol, as others have previously reasoned[4,8] in the design of both SQS and 2,3-oxidosqualene cyclase (OSC; EC 5.4.99.7) inhibitors. The enzyme targets considered were thus beyond the formation of farnesyl pyrophosphate and before the formation of lanosterol. In this approach inhibition of steps before SQS is avoided because they are involved in protein prenylation and the synthesis of key biosynthetic precursors such as ubiquinone. Inhibition of steps after the formation of lanosterol is also avoided because it previously afforded[3] toxic inhibitors by accumulation of higher sterols such as desmosterol. This analysis leads to squalene oxidation and the subsequent cyclization (Scheme 1, **1**−**3**) of 2,3-oxidosqualene (OS, **1**) by OSC, as optimal steps for enzyme inhibition. Herein we describe a novel series of 4-piperidinopyridine and 4-piperidinopyrimidine inhibitors of OSC together with their in vivo effects on cholesterol biosynthesis as a whole.

A considerable number of OSC inhibitors have been reported,[8] and many of these compounds closely resemble the enzyme substrate **1** or the cyclization product lanosterol **3**. These mimics tend to have high lipophilicity (CLOGP[9] > 7) and molecular weight (>400). The synthesis of further analogues of these mostly weak inhibitors was rejected on the grounds that they represented a poor medicinal chemistry starting point for the discovery of an orally active inhibitor of cholesterol biosynthesis. Instead a compound was sought which would potentially interfere with the cyclization of **1** to the putative protolanosteryl cation **2** (Scheme 1). This cation is widely accepted[10] as the first tetracyclic intermediate in the cyclization (**1**−**3**) of oxidosqualene to lanosterol. In addition we sought, as part of our rationale, to mimic the cation formed in the acid-catalyzed opening of the 2,3-oxirane ring of **1** with a physiologically protonated amine. The structures of the similarly derived potent and orally active OSC inhibitors (Chart 1): **4a** (BIBB 515),[11] **5** (BIBX 79),[12] **6a** (Ro 48-8071),[13] and **6b** (Ro 44-2103)[14] were also considered in this analysis. The more rigid inhibitors **4a**, **5**, and **6b** were used as templates for directed 2D-searching of the >300000 compounds in the AstraZeneca Alderley Park compound collection. A requirement was included for the presence of a terminal nitrogen lone pair at the position potentially corresponding to the oxygen lone

Scheme 1. Oxidosqualene Cyclization to Lanosterol

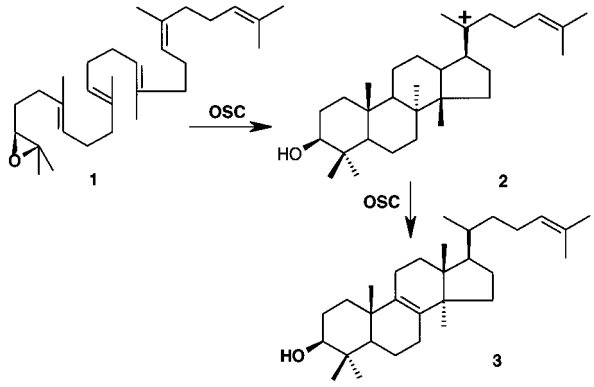

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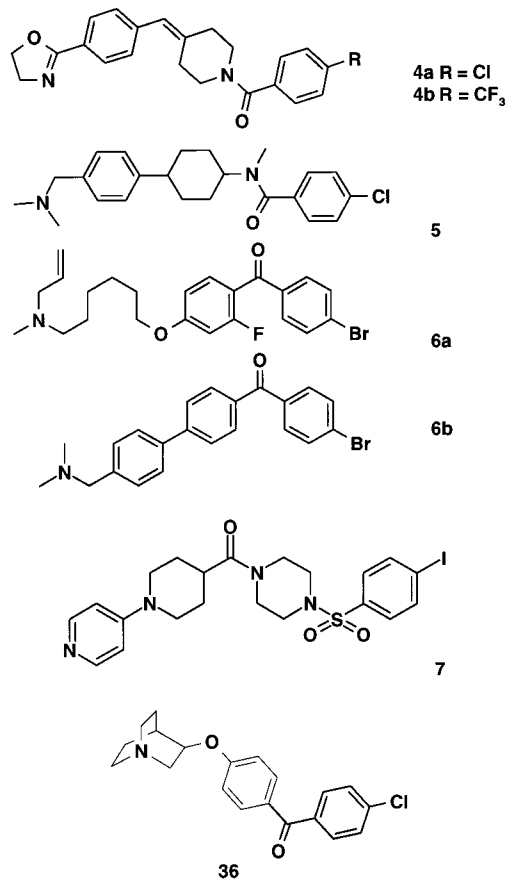
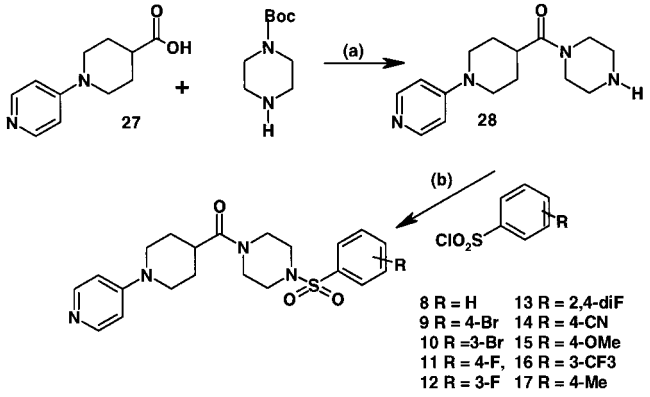
Chart 1



Scheme 2. Synthetic Route A[a]



[a] (a) $SOCl_2$, Et_3N, TFA, CH_2Cl_2; (b) Et_3N, pyridine, 0 °C.

pair of the oxirane ring of **1** at the enzyme active site. This search led to recognition (Chart 1) that the substituted pyridine **7** resembled the known[11] inhibitor **4a** and was a suitable medicinal chemistry starting point for optimization of OSC inhibition. Novel analogues **8**−**26** (Tables 1, 3, and 4) of **7** were synthesized and examined for oral inhibition of rat cholesterol biosynthesis and as inhibitors of rat microsomal OSC. Inhibition of cholesterol biosynthesis was accepted as a surrogate for plasma cholesterol-lowering activity, as seen with the clinically used statin drugs.

Chemistry

Compounds **8**−**17** were prepared in multiparallel fashion by synthetic route A (Scheme 2) with the synthesis of **14** being described as a typical compound in the Experimental Section. This method involved the reaction of 4-carboxy-*N*-(4-pyridyl)piperidine[15] (**27**) with $SOCl_2$ and subsequent acylation of *N*-Boc-piperazine in the presence of Et_3N. The Boc protective group was removed with TFA to afford **28**. The substituted piperidine **28** was reacted with an appropriately substituted benzenesulfonyl chloride in the presence of Et_3N to give **8**−**17**. Compounds **18** and **19** (Table 3) were similarly obtained by reaction of **28** with 4-bromobenzyl bromide and 4-bromobenzoyl chloride, respectively, but for **18** the base used was NaH.

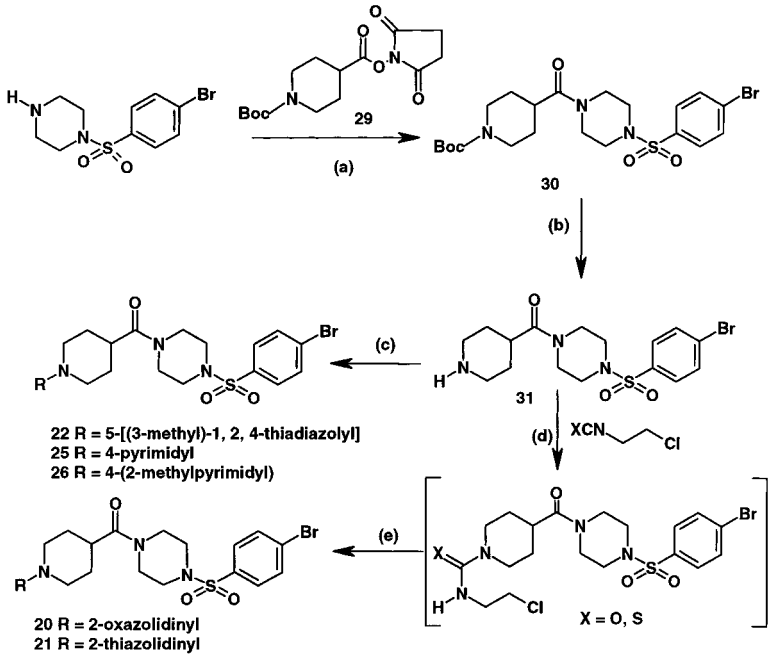
A second synthetic route B (Scheme 3) was used to prepare compounds **7**, **20**−**22**, **25**, and **26** (Table 4). **7** was synthesized by reaction of *N*-4-iodophenylsulfonylpiperazine with the acid chloride of **27** in the presence of Et_3N. In a second variation *N*-4-bromophenylsulfonylpiperazine was allowed to react with **29** in CH_2Cl_2 to afford **30** and the Boc group removed with TFA to give **31**. The final synthetic step for compounds **22**, **25**, and **26** was reaction of **31** with the appropriate chloro heterocycle in the presence of Et_3N in boiling EtOH or THF. For **20** and **21** the 2-chloroethyl isocyanate and 2-chloroethyl isothiocyanate, respectively, were allowed to react with **31** in THF before subsequent ring closure in boiling H_2O.

Synthetic route C (Scheme 4) was used for the synthesis of **23** and **24** (Table 4). Isonipecotic acid was reacted with CbzCl in the presence of Na_2CO_3 to give **32**, which was condensed with *N*-Boc-piperazine in the presence of HOBt and EDAC (1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride) to afford **33**. The Cbz group was removed by hydrogenation over 10% Pd/C at 25 °C to afford **34**. **34** was allowed to react with 2-chloropyrimidine in boiling EtOH containing Et_3N to give **35a**. The Boc protective group was removed from **35a** with TFA to give **35b**, which gave **23** after reaction with 4-bromophenylsulfonyl chloride in Et_3N/THF. For the pyridazine **24**, regioselective[16] alkylation with 3,4,5-trichloropyridazine gave the 5-substituted **35c** as the main product. The two additional 3- and 4-chlorine atoms were removed by catalytic reduction with hydrogen over 10% Pd/C to give **35d**. The Boc protecting group was removed from **35d** with TFA to afford **35e**, and reaction of **35e** with 4-bromophenylsulfonyl chloride in Et_3N/THF yielded **24**.

Biological Results and Discussion

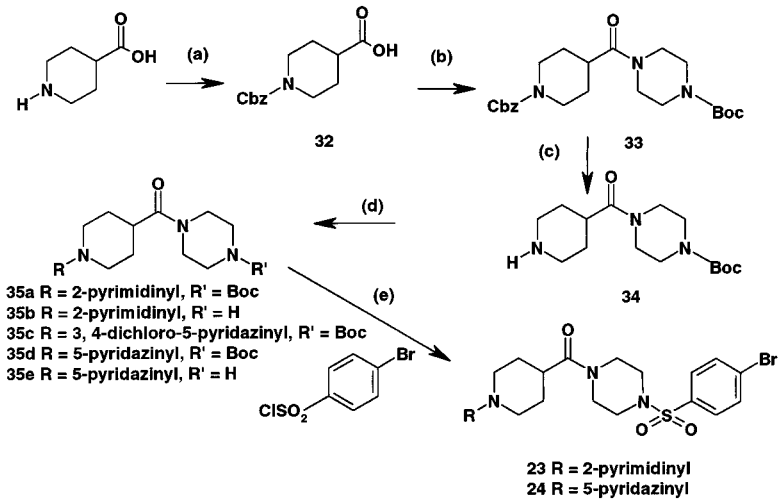
The strategy employed for evaluation of compounds as OSC inhibitors has been fully described in relation to our study of quinuclidine inhibitors of OSC.[7] Compounds were assessed first in oral cholesterol biosynthesis tests in rats before in vitro confirmation that the in vivo activity found was derived from inhibition of the target OSC enzyme. In summary, compounds were assessed for inhibition of cholesterol biosynthesis in rats 2 h after an oral dose of test compound. First the cholesterol biosynthetic precursor pattern found in the HPLC analysis of extracts of liver samples from orally dosed rats was compared with the same profile from control animals. These HPLC chromatograms showed whether accumulated substrates for cholesterol biosynthesis enzymes were present and thereby gave an indication of which steps of cholesterol biosynthesis had been inhibited: i.e. it enabled determination of whether

Scheme 3. Synthetic Route B[a]



[a] (a) CH_2Cl_2; (b) TFA, Na_2CO_3; (c) chloro heterocycle, Et_3N, EtOH; (d) THF; (e) H_2O, 100 °C.

Scheme 4. Synthetic Route C[a]

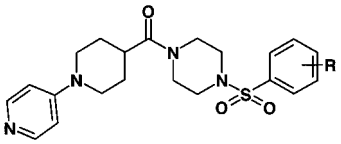


[a] (a) CbzCl, Na_2CO_3, 2 M HCl; (b) EDAC, HOBt, DMF, *N*-Boc-piperazine; (c) H_2, Pd/C, EtOH; (d) chloro heterocycle, Et_3N, THF, TFA, CH_2Cl_2, 25 °C; (e) Et_3N, THF.

the enzyme inhibition was selective for the OSC step. This analysis of the in vivo mode of action was seen as an important process for rejecting compounds with inhibitory actions at other biosynthetic steps outside of our interest. In addition it alleviated the need for much in vitro selectivity testing at the other cholesterol biosynthetic steps. Second, the extent of the inhibition of cholesterol biosynthesis was also measured from the same HPLC chromatograms and initially expressed as percent inhibition after a 2 mg/kg (Table 1) oral dose of test compound ($n = 2$). Compounds were initially tested in rats at this relatively low dose for two reasons: (a) **7** and **8** gave >70% inhibition of cholesterol biosynthesis when tested at 5 mg/kg ($n = 2$), making this dose level potentially unsuitable for differentiating between test compounds in this series, and (b) the aim of the study was to discover inhibitors of the cholesterol biosynthesis pathway with at least similar inhibitory potency to that found for the widely prescribed HMGCoA reductase inhibitors, (e.g. simvastatin $ED_{80} = 1.2 \pm 0.3$ mg/kg in this test). Key compounds were retested to obtain an IC_{50} for OSC inhibition and a more detailed confirmatory oral ED_{50} or ED_{80} for inhibition of rat cholesterol biosynthesis. The trifluoromethyl-substituted oxazolidine **4b**[17] was used as a standard OSC inhibitor, and the HMGCoA reductase inhibitor simvastatin was the standard compound used for cholesterol synthesis inhibition.

Compound **7** was examined in this manner and it afforded an IC_{50} of 82 ± 14 nM for the inhibition of rat OSC with a 52% inhibition of cholesterol biosynthesis at 2 mg/kg (Tables 1 and 2). The subsequent approximate ED_{80} found for **7** (shallow dose response) was <20 mg/kg and the ED_{50} 2.0 ± 0.3 mg/kg. Thus overall **7** was a potent inhibitor of cholesterol biosynthesis by inhibition at the OSC step. This level of inhibition of

Table 1. Inhibition of Rat Cholesterol Biosynthesis after a 2 mg/kg Oral Dose



compd	R	formula	mp, °C	% inhib[a]
7	4-I	$C_{21}H_{25}IN_4O_3S$	211−213	52
8	H	$C_{21}H_{26}N_4O_3S$	143−146	53
9	4-Br	$C_{21}H_{25}BrN_4O_3S$	188−189	69
10	3-Br	$C_{21}H_{25}BrN_4O_3S$	gum	43
11	4-F	$C_{21}H_{25}FN_4O_3S$	130−133	46
12	3-F	$C_{21}H_{25}FN_4O_3S$	144−146	52
13	2,4-di-F	$C_{21}H_{24}F_2N_4O_3S$	133−135	10
14	4-CN	$C_{22}H_{25}N_5O_3S$	168−169	12
15	4-OMe	$C_{22}H_{28}N_4O_4S$	64−66	38
16	3-CF_3	$C_{21}H_{25}F_3N_4O_3S$	160−164	7
17	4-Me	$C_{22}H_{28}N_4O_3S$	201−203	66
4b		$C_{23}H_{21}F_3N_2O_2$	151−152	93

[a] ±9.0%, $n = 2$; inhibition of cholesterol biosynthesis after an oral dose.

Table 2. Inhibition of Rat and Human OSC by 4-*N*-Piperazinopyridines

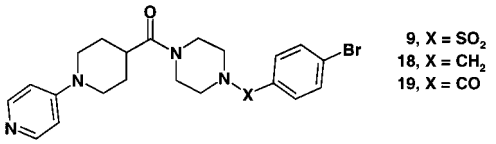
	IC_{50}, nM[a]	
compd	rat	human
7	82[b]	
8	326[c]	143 ± 33
9	228[c]	161 ± 39
4b	90[b]	

[a] $n = 5$. [b] Error = ±14 nM. [c] Error = ±25 nM.

cholesterol biosynthesis, however, compared unfavorably with the superior ED_{80} of 1.2 ± 0.3 mg/kg found for the clinically used simvastatin, indicating that an improved in vivo biosynthesis inhibition was desirable. The 4-iodo atom in the phenyl ring of **7** was considered to be potentially unstable in vivo; it contributed significantly to the molecular weight as well as added over 1 unit to the CLOGP value of **7** compared to hydrogen substitution at the same position. Hence using a parallel synthesis procedure, synthetic analogues of **7** were prepared (Table 1, **8**−**17**). Preliminary structure−activity information for phenyl ring substitution was obtained from the determination of the inhibition of rat cholesterol biosynthesis following oral dosing. The inhibitory results obtained after a 2 mg/kg oral dose indicated that inhibition of cholesterol biosynthesis comparable to that found with **4b** and **7** was only achievable at this dose with a very limited group of lipophilic substituents. Confirmatory IC_{50} data for inhibition of rat and human OSC (human IC_{50} data were obtained in the same manner as described in the Experimental Section for rat IC_{50} data) were determined for compounds **7**−**9** (Table 2); the inhibitory potency was greater at the human OSC enzyme. On the basis of the preliminary structure−activity information derived from in vitro and in vivo testing, **9** was selected as a lead structure for further structural variation.

Changing the sulfonyl link of **9** to methylene (**18**) and carbonyl (**19**) afforded OSC inhibitors (Table 3), but **9** and **18** were slightly more potent inhibitors of cholesterol biosynthesis in vivo than **19**. Interestingly **9**, **18**, and **19** were all inhibitors of OSC even though the linked phenylsulfonyl and benzyl groups of **9** and **18**

Table 3. Comparison of Percent Inhibition for Phenylpiperazine Link Groups in the Rat



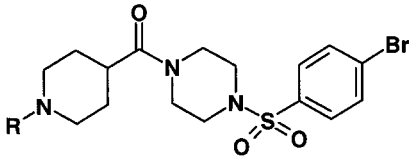
		% inhib		
compd	formula	OSC at 1 μM[a]	cholesterol biosynth at 2 mg/kg[b]	ED_{50} cholesterol biosynth inhib, mg/kg[c]
9	$C_{21}H_{25}BrN_4O_3S$	100	69	2.0
18	$C_{21}H_{27}BrN_4O$	100	48	1.8
19	$C_{22}H_{27}BrN_4O_2$	96	28	

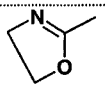
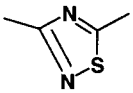
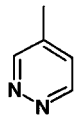
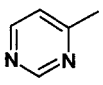
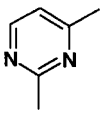
[a] $n = 2$, ±9%. [b] $n = 2$, ±9.0%, oral dose. [c] $n = 5$, ±0.3%, oral dose.

would have a different conformational orientation in relation to the piperazino ring, compared to the benzoyl group of **19**. These in vitro findings contrast with the drug design paradigm used for the similarly shaped benzophenone class of OSC inhibitors: e.g. Ro 44-2103 (**6b**).[13] In this inhibitor series an electrophilic carbonyl group was incorporated at a structural position remote from the terminal basic group (in a similar position to group X in **9**, **18**, and **19**, Table 3). The intention here was to interact with OSC at both the intermediate protosteryl cation site and the 2,3-oxirane ring of **1**. The good inhibitory activity found for the benzyl-linked **18** suggests that the piperidinopyridine series of inhibitors does not have the bifunctional interaction with the OSC enzyme postulated for Ro 44-2103 (**6b**).

Structural variation of the lead structure **9** involving the synthesis of compounds containing other heterocycles in place of the pyridine ring led to several potent inhibitors of rat cholesterol biosynthesis (Table 4). For example the oxazoline **20** and thiazoline **21** were very potent inhibitors of rat cholesterol biosynthesis, whereas the thiadiazole **22** gave no inhibition of rat cholesterol biosynthesis at a 2 mg/kg oral dose. Examination of the HPLC chromatograms derived from the extraction of liver samples of rats dosed with **20** and **21** showed, however, that both compounds were nonselective inhibitors blocking a number of cholesterol biosynthetic steps. Particularly the conversion of desmosterol to cholesterol was inhibited. Thus, despite their inhibitory potency **20** and **21** were not examined further because of this potential to accumulate higher sterols, for which our design hypothesis predicted toxic effects. The 2-substituted pyrimidine **23**, which did not have the terminal nitrogen atom present in the 4-pyridyl and 4-pyrimidyl compounds (Table 4), did not inhibit rat OSC at a 1 μM concentration or rat cholesterol biosynthesis at an oral dose of 2 mg/kg. By contrast the pyridazine **24** did inhibit cholesterol biosynthesis, but much more potent cholesterol biosynthesis inhibition was found for the 4-substituted pyrimidine **25** and its 2-methyl analogue **26**. The inhibitory activity of both of these compounds was explored in detail (Table 5) against rat and human OSC (human IC_{50} data were obtained in the same manner as described in the Experimental Section for rat IC_{50} data) and cholesterol biosynthesis. The rat oral ED_{80} values for **25** and **26** were similar to the result for simvastatin (**25**, 1.4 ± 0.3 mg/kg; **26**, 1.2 ±0.3 mg/kg; simvastatin, 1.2 ± 0.3 mg/kg).

Table 4. Inhibition of Rat OSC and Rat Cholesterol Biosynthesis for Pyridine Ring Analogues



compd	R	formula	mp	[a] % inhib rat OSC at 1 μM	[b] % inhib rat cholesterol biosyn , at 2 mg/kg
20		$C_{19}H_{25}BrN_4O_4S$	210-212	87	82
21		$C_{19}H_{25}BrN_4O_3S_2$	208-210	100	81
22		$C_{19}H_{24}BrN_5O_3S_2$	173-174	3	9
23		$C_{20}H_{24}BrN_5O_3S$	211-213	8	23
24		$C_{20}H_{24}BrN_5O_3S$	85-86		54
25		$C_{20}H_{24}BrN_5O_3S$	197-198	100	81
26		$C_{21}H_{26}BrN_5O_3S$	200-202	76	83
4b				98	93

[a] % Inhib ± 15%, $n = 2$. [b] % Inhib ± 9%, $n = 2$, after an oral dose.

Table 5. Comparison of Inhibitory Activities of Pyridino- and Pyrimidinopiperidines

	IC_{50}, nM[a]		rat inhib cholesterol biosynth[b]	
compd	rat	human	ED_{50}, mg/kg	ED_{80}, mg/kg
9	228[c]	161 ± 39	2.0 ± 0.3	>2.0
25	116[c]	37 ± 9	0.5 ± 0.2	1.4 ± 0.3
26	398[c]	112 ± 25	0.3 ± 0.2	1.2 ± 0.3
4b	90[d]		0. 4 ± 0.2	1.0 ± 0.3
36	58[d]	35 ± 9	2.0 ± 0.3	

[a] $n = 5$. [b] $n = 5$, inhibition after an oral dose. [c] Error = ±25 nM. [d] Error = ±14 nM.

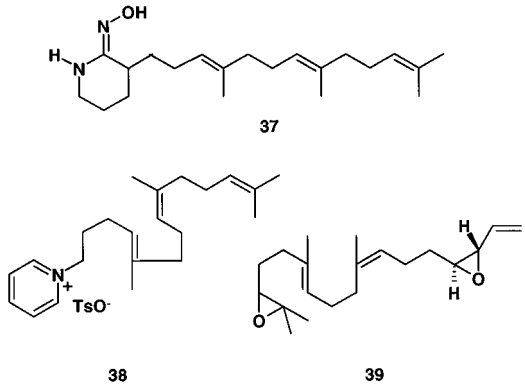
Comparison of the inhibitory properties of the pyridino- and pyrimidino-substituted piperazines (Table 5) indicated that substitution with pyrimidine afforded compounds with significant in vitro OSC inhibitory potency and with superior in vivo inhibition of rat cholesterol biosynthesis compared with the corresponding pyridine **9**. These pyrimidines were more potent in vivo than the standard quinuclidine OSC inhibitor **36** (Table 5) but were similar in potency (both in vitro and in vivo) to the reported[11−13] lipophilic amine OSC inhibitors **4a**, **4b**, **5**, **6a**, and **6b**, e.g. **4b** in Table 5.

Our previous work[7] in the quinuclidine series of OSC inhibitors suggested that an inhibitor with a pK_a close to 9 might be necessary for inhibition of OSC (e.g. **36**, pK_a = 9.1), which is similar to that found (pK_a values were measured spectrophotometrically using a Beckman DU-7 spectrophotometer) for the pyridine-substituted inhibitors **7** and **9** (Table 6, pK_a = 9.2). This contrasts with the much lower pK_a of 4.7 found for the weakly basic phenyloxazoline inhibitor **4b** used as a standard OSC inhibitor and the wide pK_a range of the other OSC inhibitors cited[11−13] above. In addition some recently described polyprenylated mimics (Chart 2, **37**−**39**)[18−20] of the putative carbocation intermediates of OS cyclization with a wide pK_a range have also shown good in vitro

Table 6. Comparison of Physical Properties in the OSC Inhibitory Series

compd	pK_a	CLOGP[9]
7	9.2	3.7
9	9.2	3.4
25	6.1	2.6
26	7.2	3.2
36	9.1	4.7
4b	4.7	5.3

Chart 2



OSC inhibitory activity (IC_{50} values in the nanomolar and low-micromolar ranges). Our replacement of the pyridine ring by pyrimidine (e.g. in **25** and **26**) reduced the inhibitor pK_a to 6 and 7, respectively: i.e. in this series compounds in a lower pK_a range were also able to give potent inhibition at the OSC enzyme site. Use of information from the recently described[21] photoaffinity labeling of OSC (aimed at locating binding sites for **6a**, Ro 48-8071) should allow a better definition of binding sites in this series. Hence the relevance of inhibitor pK_a to OSC inhibitor binding should be clarified.

Conclusions

A novel series of 4-piperidinopyridines and 4-piperidinopyrimidines was synthesized, which afforded potent inhibitors of both rat and human 2,3-oxidosqualene cyclase−lanosterol synthase, and was shown in rats to give (after oral dosing) selective inhibition of cholesterol biosynthesis at the OSC step. This oral inhibition of rat cholesterol biosynthesis for compounds **25** and **26** was comparable with that found for the clinically used HMGCoA reductase inhibitor simvastatin and the cited standard orally active lipophilic amine inhibitors of OSC. In line with the wide pK_a range of known OSC inhibitors, the piperidinopyrimidine inhibitors had a significantly lower pK_a than the corresponding pyridine series or the previously reported quinuclidine OSC inhibitor series. This indicated that further novel OSC inhibitors may be found in either series by exploring a broader range of inhibitor pK_a (5.0−9.0). A more detailed pharmacological and toxicological investigation of the pyrimidines **25** and **26** may afford novel hypocholesterolemic agents for the treatment of cardiovascular disease.

Experimental Section

A. Chemistry. Melting points were determined with a Buchi apparatus and are uncorrected. The ^{1}H NMR spectra were determined with a Bruker AM (200 MHZ) spectrometer (with $SiMe_4$ as an internal standard) and mass spectra were measured on a MS902 Kratos (AEI) instrument. Elemental analyses were determined on a Perkin-Elmer series II-2400 analyzer. Reactions were carried out under an atmosphere of argon and column chromatography was on E. Merck silica gel (Kieselgel 60, 230−400 mesh) or neutral alumina N (ICN Biomedicals). Bond Elut columns were silica Mega Bond Elut (Varian). Solvents were dried over $MgSO_4$ before evaporation.

Example of Synthetic Route A. 1-(1-(4-Pyridyl)piperidin-4-ylcarbonyl)piperazine (28). **27**[15] (4.12 g, 20 mmol) was suspended in dry CH_2Cl_2 (30 mL) and treated with $SOCl_2$ (3 mL, 41 mmol) dropwise with cooling at 0 °C. The mixture was stirred for 1 h followed by the removal by evaporation of the solvent and the excess $SOCl_2$. The resulting gum was dissolved in CH_2Cl_2 (80 mL) and added slowly, with cooling to a solution of *N*-Boc-piperazine (3.72 g, 20 mmol) in CH_2Cl_2 (100 mL) and Et_3N (15 mL, 107 mmol). The reaction mixture was stirred for 2 h and the solvent removed by evaporation. The residue was crystallized from EtOAc to give, as a waxy yellow solid, 4-(*tert*-butoxycarbonyl)-1-(4-pyridylpiperidin-4-ylcarbonyl)piperazine (3.74 g, 50%): ^{1}H NMR (DMSO-d_6) δ 1.38(s, 9H), 1.58(m, 4H), 2.90(m, 3H), 3.36(m, 6H), 3.50(bs, 2H), 3.91-(d, J = 12.5 Hz, 2H), 6.79(d, J = 6.4 Hz, 2H), 8.09(d, J = 6.4 Hz, 2H); EI-MS *m*/*z* 374 (M + H).

This product was dissolved in dry CH_2Cl_2 (50 mL), treated with TFA (5.3 mL, 68 mmol) and stirred under an argon atmosphere at room temperature for 3 h. The CH_2Cl_2 solvent was removed by evaporation to afford a brown oil which slowly solidified after treatment with an excess of saturated $NaHCO_3$ solution. This solid was taken up in CH_2Cl_2, and the solution washed with H_2O, brine and dried. The resultant solution was evaporated to dryness to yield an oil, which slowly crystallized to give, as a yellow solid, **28** (2.45 g, 90%): mp 131−132 °C; ^{1}H NMR (DMSO-d_6) δ 1.53(m, 2H), 1.78(m, 2H), 3.03(m, 5H), 3.241(m, 2H), 3.74(m, 4H), 4.20(d, J = 12.5 Hz, 2H), 7.17(d, J = 6.4 Hz, 2H), 8.18(d, J = 6.4 Hz, 2H), exchangeable proton not observed; EI-MS *m*/*z* 274 (M + H).

1-(4-Cyanophenylsulfonyl)-4-(1-(4-pyridyl)piperidin-4-ylcarbonyl)piperazine (14). **28** (0.411 g, 1.5 mmol) was dissolved in dry CH_2Cl_2 (20 mL) and stirred at 0 °C. Et_3N (0.56 mL, 4.0 mmol) was added to the resulting solution followed by the dropwise addition of a solution of 4-cyanobenzenesulfonyl chloride (0.33 g, 1.6 mmol) in dry pyridine (20 mL). The reaction mixture was stirred at 0 °C for 10 min and allowed to warm to room temperature and stirred for a further 1 h. The CH_2Cl_2 and pyridine were evaporated, the residue dissolved in H_2O (60 mL) and the solution extracted with EtOAc (3 × 50 mL). The organic extracts were washed with H_2O, brine, dried and evaporated to dryness. The product was recrystallized from isohexane/EtOAc to give, as a colorless, hygroscopic solid, **14** (512 mg, 78%): mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 1.83(m, 4H), 2.64(m, 1H), 2.88(m, 2H), 3.10(s, 4H), 3.68(bs, 4H), 3.88(m, 2H), 6.64(d, J = 7.4 Hz, 2H), 7.86(d, J = 8.2 Hz, 2H), 8.03(d, J = 7.4 Hz, 2H), 8.14(d, J = 8.2 Hz, 2H). Anal. ($C_{22}H_{25}N_5O_3S\cdot0.5H_2O$) C, H, N.

4-(4-Bromophenylmethyl)-1-(1-(4-pyridyl)piperidin-4-ylcarbonyl)piperazine (18). **28** (0.722 g, 2.64 mmol) was dissolved in dry DMF (22 mL) and treated with NaH (0.19 g, 50% dispersion in mineral oil, 4.0 mmol) under an argon atmosphere. The resultant mixture was allowed to stir for 30 min before the addition of 4-bromobenzyl bromide (0.66 g, 2.64 mmol). The reaction was stirred at room temperature for 2 h and quenched by pouring into H_2O, basifying with saturated $NaHCO_3$ solution and extracting with Et_2O. The combined organic extracts were washed with H_2O, brine, dried and evaporated to afford a cream solid which was recrystallized twice from EtOAc/isohexane to give, as a colorless solid, **18** (945 mg, 83%): mp 148−149 °C; ^{1}H NMR (DMSO-d_6) δ 1.58-(m, 4H), 2.28(m, 4H), 2.92(t, J = 12.0 Hz, 3H), 3.48(m, 4H), 3.89(d, J = 13.0 Hz, 2H), 6.75(d, J = 6.4 Hz, 2H), 7.26(d, J = 8.2 Hz, 2H); 7.46(d, J = 8,2 Hz, 2H), 8.13(d, J = 6.4 Hz, 2H); EI-MS *m*/*z* 430 (M + H). Anal. ($C_{21}H_{27}BrN_4O$) C, H, N.

4-(4-Bromobenzoyl)-1-(1-(4-pyridyl)piperidin-4-ylcarbonyl)piperazine (19). Compound **19** was prepared in a

manner similar to **14** but using 4-bromobenzoyl chloride as starting material to give, as a colorless solid, **19** (74%): mp 214−215 °C; ^{1}H NMR (DMSO-d_6) δ 1.60(m, 4H), 2.86(t, J = 12.5 Hz, 3H), 3.50(m, 8H), 3.92(d, J = 12.5 Hz, 2H), 6.80(d, J = 7.4 Hz, 2H), 7.37(d, J = 8.3 Hz, 2H), 7.61(d, J = 8.3 Hz, 2H), 8.13(d, J = 7.4 Hz, 2H); EI-MS m/z 457 (M + H). Anal. ($C_{22}H_{25}BrN_4O_2$) C, H, N.

Examples of Synthetic Route B. 1-(4-Iodophenylsulfonyl)-4-(1-(4-pyridyl)piperidin-4-ylcarbonyl)piperazine (**7**). Thionyl chloride (0.21 mL, 2.86 mmol) was added to a suspension of **27** (0.29 g, 1.42 mmol) in CH_2Cl_2 (5 mL) and the mixture stirred for 0.5 h. The solvent was evaporated to a give a solid, which was suspended in CH_2Cl_2 (5 mL) and added to a suspension of *N*-4-iodophenylsulfonylpiperazine (0.5 g, 1.42 mmol) in CH_2Cl_2 (5 mL) and Et_3N (1.4 mL, 10 mmol). The mixture was stirred for 18 h and the organic phase washed with H_2O, dried and evaporated to give a solid. The solid was purified by chromatography on silica gel in 5% MeOH/CH_2Cl_2 to give, as a colorless solid, **7** (0.235 g, 31%): mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 1.52(m, 4H), 2.82(m, 1H), 2.95(m, 6H), 3.55(m, 4H), 3.92(d, J = 13.1 Hz, 2H), 6.75(d, J = 5.7 Hz, 2H), 7.48(d, J = 8.3 Hz, 2H), 8.04(d, J = 8.3 Hz, 2H), 8.11(d, J = 3.8 Hz, 2H); EI-MS m/z 541 (M + H). Anal. ($C_{21}H_{25}IN_4O_3S$) C, H, N.

4-(1-(4-Bromophenylsulfonyl)piperazin-4-ylcarbonyl)-piperidine (31). 29[18] (1.30 g, 4 mmol) was added to 4-(4-bromobenzenesulfonyl)piperazine (1.23 g, 4 mmol) in CH_2Cl_2 (50 mL) and the mixture stirred for 40 h. The CH_2Cl_2 solution was washed with H_2O, dried and evaporated to a foam which crystallized on trituration with EtOAc. The solid was purified by chromatography on a silica Bond Elut column, eluting with 2% MeOH/ CH_2Cl_2 to give, as a colorless solid, **30** (1.54 g, 75%): mp 209−210 °C; ^{1}H NMR ($CDCl_3$) δ 1.34(s, 9H), 1.65-(m, 4H), 2.51(m, 1H), 2.72(m, 2H), 3.03(t, 4H), 3.63(m, 4H), 4.10(d, 2H), 7.58(d, 2H), 7.68(d, 2H); EI-MS m/z 516 (M + H).

30 (1.53 g, 2.96 mmol) was stirred in TFA (10 mL, 130 mmol) for 1 h, and the TFA evaporated. H_2O (25 mL) was added and the mixture made basic with excess of Na_2CO_3 to pH 11. The aqueous was extracted with EtOAc and the organic phase washed with brine, dried and evaporated to give, as a colorless solid, **31** (693 mg, 57%): mp 158−162 °C; ^{1}H NMR (DMSO-d_6) δ 1.43(m, 4H), 2.47(m, 2H), 2.62(m, 1H), 2.90(m, 6H), 3.53-(m, 4H), 7.65(d, 2H), 7.87(d, 2H), exchangeable protons not observed; EI-MS m/z 416 (M + H). Anal. ($C_{16}H_{22}BrN_3O_3S\cdot 0.75H_2O$) C, H, N.

1-(2-Oxazolinyl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (20). **31** (1.01 g, 2.4 mmol) was dissolved in dry THF (50 mL) and 2-chloroethyl isocyanate (0.225 mL, 2.6 mmol) was added. The reaction mixture was stirred for 2 h. The solution was diluted with EtOAc, filtered through a plug of neutral alumina and evaporated to dryness to afford a colorless, waxy solid. This solid was dissolved in H_2O (100 mL) and heated to 100 °C for 0.5 h. The clear solution was basified with excess of NH_4OH (d = 0.880) and extracted with CH_2Cl_2 (3 × 30 mL). The extracts were washed with H_2O, brine and dried. The organic phase was evaporated to dryness and the residue recrystallized from EtOAc/isohexane to give, as a colorless solid, **20** (448 mg, 48%): mp 210−212 °C; ^{1}H NMR (DMSO-d_6) δ 1.41(m, 4H), 2.70(m, 3H), 2.90(m, 4H), 3.55-(m, 6H), 3.72(d, J = 12.4 Hz, 2H), 4.18(t, J = 7.9 Hz, 2H), 7.62(d, J = 8.2 Hz, 2H), 7.83(d, J = 8.2 Hz, 2H); EI-MS m/z 485 (M + H). Anal. ($C_{19}H_{25}BrN_4O_4S$) C, H, N.

1-(2-Thiazolinyl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (21). Compound **21** was prepared in a manner similar to **20** by using 2-chloroethyl isothiocyanate as the starting material to give **21**, as a colorless solid, (71%): mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ 1.40(m, 2H), 1.49(m, 2H), 2.72(m, 1H), 2.95(m, 6H), 3.22(t, J = 7.9 Hz, 2H), 3.52(m, 4H), 3.72(d, J = 12.3 Hz, 2H), 3.82(t, J = 7.9 Hz, 2H), 7.62(d, J = 8.2 Hz, 2H), 7.83(d, J = 8.2 Hz, 2H); EI-MS m/z 501 (M + H). Anal. ($C_{19}H_{25}BrN_4O_3S_2$) C, H, N.

1-(3-Methyl-1,2,4-thiadiazin-5-yl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (**22**). Compound **22** was synthesized as for **25** below, but starting with 5-chloro-3-methyl-1,2,4-thiadiazole, to give, as a colorless solid after crystallization from EtOH, **22** (64%): mp 173−174 °C; ^{1}H NMR ($CDCl_3$) δ 1.73(m, 2H), 1.85(m, 2H), 2.39(s, 3H), 3.02-(m, 4H), 3.12(t, J = 11.7 Hz, 2H), 3.27(m, 1H), 3.65(m, 4H), 3.92(d, J = 11.7 Hz, 2H), 7.58(d, J = 7.9 Hz, 2H), 7.68(d, J = 7.9 Hz, 2H); EI-MS m/z 514 (M + H). Anal. ($C_{19}H_{24}BrN_5O_3S_2$) C, H, N.

1-(4-Pyrimidinyl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (**25**). **31** (170 mg, 0.4 mmol) and 4-chloropyrimidine·2HCl (78 mg. 0.41 mmol) in absolute EtOH (10 mL) and Et_3N (0.5 mL, 3.5 mmol) were heated under reflux for 2 h. The solution was evaporated under vacuum and H_2O (50 mL) added. The aqueous mixture was extracted with EtOAc (2 × 50 mL), washed with water, brine and dried. The EtOAc was evaporated under vacuum to give an oil which was dissolved in EtOAc and purified by flash column chromatography on neutral alumina (ICN Alumina N 32-63) eluting with an increasing concentration of MeOH/EtOAc (0−10%). The resulting solid was recrystallized from a mixture of EtOAc/THF/isohexane and then from MeCN to give, as a solid, **25** (155 mg, 75%): mp 197−198 °C; ^{1}H NMR (DMSO-d_6) δ 1.80-(m, 4H), 2.70(m, 1H), 3.05(m, 6H), 3.65(bs, 4H), 4.40(m, 2H), 6.50(d, J = 6.4 Hz, 1H), 7.65(d, J = 8.0 Hz, 2H), 7.76(d, J = 8.0 Hz, 2H), 8.20(d, J = 6.4 Hz, 1H), 8.60(s, 1H); EI-MS m/z 494 (M + H). Anal. ($C_{20}H_{24}BrN_5O_3S$) C, H, N.

1-(2-Methyl-4-pyrimidinyl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (**26**). 4-Chloro-2-methylpyrimidine (135 mg, 1.07 mmol) was added to a solution of **31** (415 mg, 1.0 mmol) in THF (15 mL) containing Et_3N (0.2 mL, 1.5 mmol). The mixture was heated at reflux for 16 h, allowed to cool and the THF evaporated. The residue was treated with H_2O (20 mL) and the aqueous phase extracted with EtOAc (3 × 20 mL). The combined organic phases were washed with saturated brine (1 × 20 mL) dried and evaporated to give an oil which was purified by column chromatography on silica gel. Elution with CH_2Cl_2/MeOH/0.88 NH_4OH (96:3:1) gave an oil on evaporation. Trituration with Et_2O (10 mL) gave, as a colorless solid, **26** (152 mg, 30%): mp 200−202 °C; ^{1}H NMR ($CDCl_3$) δ 1.70(m, 4H), 2.50(s, 3H), 2.67(m, 1H), 2.94-(m, 2H), 3.05(m, 4H), 3.68(m, 4H), 4.41(d, J = 12.4 Hz, 2H), 6.30(d, J = 6.1 Hz, 1H), 7.62(d, J = 8.3 Hz, 2H), 7.70(d, J = 8.3 Hz, 2H), 8.10(d, J = 6.1 Hz, 1H); EI-MS m/z 508 (M + H). Anal. ($C_{21}H_{26}BrN_5O_3S$) C, H, N.

Examples of Synthetic Route C. 4-(*N*-Boc-piperazin-4-ylcarbonyl)piperidine (**34**). Na_2CO_3 (4.52 g, 42.6 mmol) was stirred with isonipecotic acid (5.0 g, 38.7 mmol) in H_2O (80 mL) and benzyl chloroformate (6.82 g, 5.71 mL, 40 mmol) added. The mixture was stirred for 18 h and acidified with 2 M HCl, before extraction with EtOAc. The organic phase was washed with H_2O, dried and evaporated to an oil. The oil was purified by column chromatography on silica gel in CH_2Cl_2, eluting with 5% MeOH/CH_2Cl_2 to give after evaporation, as a colorless oil **32** (4.45 g, 44%): ^{1}H NMR ($CDCl_3$) δ 1.67(m, 2H), 1.92(m, 2H), 2.51(m, 1H), 2.96(m, 2H), 4.09(m, 2H), 5.13(s, 2H), 7.33(m, 5H),), exchangeable protons not observed; EI-MS m/z 264 (M + H).

1-Hydroxybenzotriazole (4.67 g, 34.5 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (4.79 g, 25.3 mmol) were added to a solution of **32** (6.0 g, 23 mmol) and Boc-piperazine (4.24 g, 23 mmol) in DMF (75 mL). The mixture was stirred for 18 h and evaporated. Et_2O was added to the residue, and the organic layer washed with 2 M NaOH, saturated citric acid and H_2O. The organic phase was dried and evaporated to give, as a colorless waxy solid, **33** (7.4 g, 76%): ^{1}H NMR ($CDCl_3$) δ 1.48(s, 9H), 1.73(m, 4H), 2.64(m, 1H), 2.87(m, 2H), 3.48(m, 8H), 4.23(d, 2H), 5.12(s, 2H), 7.33-(m, 5H); EI-MS m/z 432 (M + H).

33 (3.71 g, 8.6 mmol) was hydrogenated over 5% Pd/C (120 mg) in EtOH (100 mL) at atmospheric pressure for 18 h. The catalyst was filtered and the EtOH evaporated. Trituration of the residue with Et_2O gave, as a colorless solid, **34** (1.81 g, 71%): ^{1}H NMR ($CDCl_3$) δ 1.47(s, 9H), 1.67(m, 4H), 2.64(m, 3H), 3.14(m, 2H), 3.50(m, 8H); EI-MS m/z 298 (M + H). Anal. ($C_{15}H_{27}N_3O_3$) C, H, N.

1-(2-Pyrimidinyl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (**23**). 2-Chloropyrimidine (230 mg, 2.0 mmol) and Et_3N (0.4 mL, 2.9 mmol) were added to a solution of **34** (594 mg, 2.0 mmol) in EtOH (20 mL), and the mixture heated under reflux for 2 h. The EtOH was evaporated and the residue shaken with EtOAc (80 mL) and H_2O (40 mL). The organic phase was separated, washed with brine, dried and evaporated. The residue was chromatographed on a silica Bond Elut column in 10% EtOAc/isohexane eluting with 50% EtOAc/isohexane to give, as a colorless solid, **35a** (390 mg, 52%): mp 149−150 °C; ^{1}H NMR (DMSO-d_6) δ 1.35(m. 4H), 1.37(s, 9H), 2.94(m, 3H), 3.32(m, 4H), 3.50(m, 4H), 4.62(d, J = 12.5 Hz, 2H), 5.46(t, J = 5.5 Hz, 1H), 8.33(d, J = 4.6 Hz, 2H); EI-MS m/z 376 (M + H). Anal. ($C_{19}H_{29}N_5O_3$) C, H, N.

A solution of TFA (0.7 mL 9.0 mmol) and **35a** (355 mg, 0.9 mmol) in CH_2Cl_2 (20 mL) was stirred at 25 °C for 16 h. The mixture was evaporated and triturated in EtOAc (25 mL) and saturated $NaHCO_3$ solution (15 mL). The EtOAc phase was dried and evaporated to an oil, which solidified to give **35b** (175 mg, 64%): mp 109−111 °C; ^{1}H NMR (DMSO-d_6) δ 1.45-(m, 2H), 1.63(m, 2H), 2.63(m, 4H), 2.91(m, 3H), 3.37(m, 4H), 4.62(d, J = 12.0 Hz, 2H), 6.55(t, J = 4.4 Hz, 1H), 8.32(d, J = 4.4 Hz, 2H); EI-MS m/z 276 (M + H).

4-Bromophenylsulfonyl chloride (130 mg, 0.5 mmol) was added to a solution of **35b** (137.5 mg, 0.5 mmol) and Et_3N (0.9 mL, 6.5 mmol) in THF (10 mL), and the mixture stirred for 18 h. The mixture was evaporated and EtOAc (15 mL) and H_2O (25 mL) added. The insoluble solid was collected and combined with the dried and evaporated EtOAc phase to give, as a colorless solid, **23** (206 mg, 83%): mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 1.38(m, 2H), 1.62(m, 2H), 2.90(m, 7H), 3.61-(m, 4H), 4.61(d, J = 12.0 Hz, 2H), 6.57(t, J = 4.4 Hz, 1H), 7.66(d, J = 8.4 Hz, 2H), 7.87(d, J = 8.4 Hz, 2H), 8.31(d, J = 4.4 Hz, 2H); EI-MS m/z 494 (M + H). Anal. ($C_{20}H_{24}BrN_5O_3S$) C, H, N.

1-(5-Pyridazinyl)-4-(1-(4-bromophenylsulfonyl)piperazin-4-ylcarbonyl)piperidine (**24**). Compound **24** was prepared by the same method as **23**, but 3,4,5-trichloropyridazine was used in the reaction with **34** (Scheme 4) to give, as a colorless solid, **35c** (42%): mp 180−182 °C; ^{1}H NMR (DMSO-d_6) δ 1.46(s, 9H), 1.81(m, 2H), 2.08(m, 2H), 2.76(m, 1H), 3.05-(m, 2H), 3.51(m, 8H), 3.81(m, 2H), 8.68(s, 1H); EI-MS m/z 444 (M + H). Anal. ($C_{19}H_{27}Cl_2N_5O_3$) C, H, N.

35c (360 mg, 0.75 mmol) and Et_3N (0.2 mL, 2.2 mmol) were hydrogenated in EtOH (40 mL) over 10% Pd/C (50 mg) at 1 atm for 16 h. The catalyst was filtered and the mixture evaporated. The residue was partitioned between H_2O and CH_2Cl_2 and the organic phase dried and evaporated to give, as a foam, **35d** (264 mg, 93%): ^{1}H NMR (DMSO-d_6) δ 1.48(s, 9H), 1.92(m, 4H), 2.78(m, 1H), 3.05(m, 2H), 3.51(m, 8H), 3.93-(m, 2H), 6.67(dd, J = 6.3 Hz and J = 3.3 Hz, 1H), 8.67(d, J = 6.3 Hz, 1H), 8.82(d, J = 3.3 Hz, 1H); EI-MS m/z 376 (M + H).

TFA (1 mL, 12 mmol) was added to **35d** (250 mg, 0.6 mmol) in CH_2Cl_2 (5 mL), and after 2 h the mixture was evaporated to give an oil. The oil was dissolved in saturated brine and 10M NaOH added. The mixture was extracted with CH_2Cl_2 and the solvent dried and evaporated to afford a gum **35e** (93 mg, 52%): ^{1}H NMR ($CDCl_3$) δ 1.90(m, 4H), 2.77(m, 1H), 2.90-(s, 4H), 3.02(m, 2H), 3.55(m, 4H), 3.95(m, 2H), 6.63(dd, J = 6.3 Hz and J = 3.3 Hz, 1H), 8.67(d, J = 6.3 Hz, 1H), 8.82(d, J = 3.3 Hz, 1H); EI-MS m/z 276 (M + H).

4-Bromophenylsulfonyl chloride (78 mg, 0.3 mmol) was added to a solution of **35e** (84 mg, 0.3 mmol) and Et_3N (0.063 mL, 0.45 mmol) in THF (4 mL) at ambient temperature, and after 1 h THF was evaporated. The residue was partitioned in H_2O (10 mL) and EtOAc (10 mL), and the insoluble product collected. The product was crystallized from MeOH to give, as a cream solid, **24** (32 mg, 22%): mp 83−86 °C; EI-MS m/z 494 (M + H). Anal. ($C_{20}H_{24}BrN_5O_3S$) 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} M, 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) 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 a further 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, 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_3-CN/*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 = 2) in DMSO/HPMC (hydroxypropylmethylcellulose). 1 h later tritiated mevalonate (2.5 μCi) was administered, and after a further 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 diluted (2 mL) before extraction with isohexane (5 mL). The solvent was evaporated under N_2 at 40 °C and the residue 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; flow rate 1 mL/min; eluants CH_3CN/H_2O 95:5, CH_3CN/*i*-PrOH 95:5, CH_3CN/*i*-PrOH 80:20). The column eluates were 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.

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Supporting Information Available: Spectral data and elemental analyses for compounds in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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