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Novel 4-Piperidinopyridine Inhibitors of Oxidosqualene Cyclase-Lanosterol Synthase Derived by Consideration of Inhibitor pK_a

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Abstract—Potent inhibition of rat microsomal oxidosqualene cyclase-lanosterol synthase (OSC) was maintained after structural modification of the 4-piperidinopyridine OSC inhibitor series. These novel analogues with a much lower pK_a range (5.8–6.7) gave potent oral inhibition of rat cholesterol biosynthesis (**8** ED_{80} 0.7 mg/kg), and diminished effects on rat feeding after a 100 mg/kg oral dose. © 2001 Elsevier Science Ltd. All rights reserved.

The lowering of raised plasma cholesterol levels with the statin class of HMGCoA reductase inhibitor drugs has been very successfully used in the treatment of cardiovascular disease.[1,2] This widespread clinical use of the statin drugs has led to a search for other inhibitors of the cholesterol biosynthesis pathway, particularly at later steps of the biosynthetic cascade. In this respect, we[3] (and others[4]) have identified 2,3-oxidosqualene cyclase-lanosterol synthase (OSC, EC 5.4.99.7) as an appropriate step to seek novel cholesterol biosynthesis inhibitors. Recently we have described[3] in detail a novel series of *N*-pyridyl- and *N*-pyrimidinylpiperidines (e.g., **1**, **2**) as inhibitors of microsomal rat and human OSC. Due to this OSC inhibition, these compounds also showed inhibition of rat cholesterol biosynthesis to a similar extent to the statin drug simvastatin. When rats ($n = 5$) were given a higher oral dose of the pyridyl and pyrimidinyl derivatives **1** and **2**, however, there were significantly different effects on food intake that were typical of these series. A 50 mg/kg oral dose of pyridine **1** gave, after 24 h, a 64% reduction in rat food intake. By contrast, **2** gave only a 17% reduction at 100 mg/kg (rats dosed vehicle alone gave a 7% reduction). This reduction in food intake found for the pyridine **1** would prevent clinical development of compounds from the pyridine series, because safety testing at higher doses could not be completed. Herein we report the synthesis of novel pyridine OSC inhibitors designed to have a lower potential for feeding effects in rats (Fig. 1).

The mechanism of the effects of these compounds on feeding is not known, and could not readily be determined. Thus, a basis was sought for the discovery of pyridines with a limited effect on rat feeding, without the need for the synthesis of large numbers of random compounds supported by rat feeding tests. Examination of the pK_a values of the terminal pyridine and pyrimidine rings of **1** and **2** showed that as well as having different feeding effects, they had very different pK_a

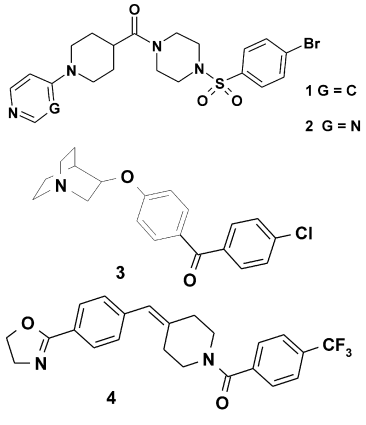

Figure 1.

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values (Table 1). The pyridine **1** and a previously described[5] quinuclidine OSC inhibitor **3** had a much higher pK_a (>9) than the pyrimidine **2**. In addition, a known standard OSC inhibitor compound **4** with a weak feeding effect in rats (**4** 15% reduction at 100 mg/kg) also showed a lower pK_a value. Thus two compounds from different OSC inhibitory series have marginal feeding effects and much lower pK_a values than the pyridine inhibitors. It was postulated that lowering of the pyridine ring pK_a by appropriate ring substitution might afford OSC inhibitory pyridines with a reduced potential for feeding effects. This hypothesis was approached in two ways. First the lead series (Fig. 2A) was halogenated to give the 3-halogen-substituted pyridines (e.g., B). This electron withdrawing halogen substituent should counteract the electron donating effect of the 4-piperidino ring nitrogen atom present in A (Table 3, **5**–**11**). A pyrimidine was also similarly substituted (e.g., C). In the second approach (e.g., D), the electron donating nitrogen atom in the piperidine ring was replaced by a carbon atom to give analogues **12**–**15** (Table 4).

Table 1. Comparison of physical properties in OSC inhibitory series

Compd	pK_a	IC_{50} nM inhib. rat OSC
1	9.2	228±49
2	6.1	116±21
3	9.1	58±19
4	4.7	90±14

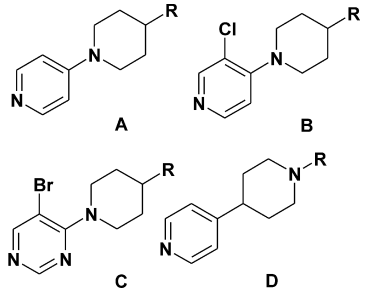
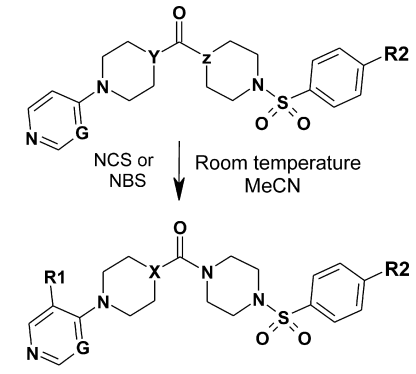
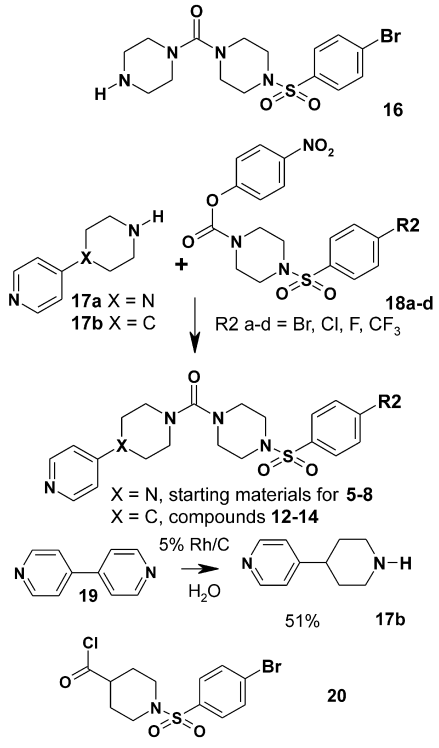


Figure 2.



Scheme 1.



Scheme 2.

Chemistry

The chloro- and bromo-substituted compounds **5**–**9** and **11** were prepared by halogenation (Scheme 1) of the unsubstituted pyridine/pyrimidine ring with the appropriate *N*-halosuccinimide in MeCN at room temperature. Compound **10** was synthesised from the known[6] 3-fluoro-4-iodopyridine and the piperidine[3] **16** by the reported[3] general method. The starting materials for **9**, and **11** are known.[3] For **5**–**8** the starting materials were synthesised by reaction of **17a** in DMF at 100 °C (Scheme 2) with the 4-nitrophenylcarbamate derivative of the known[3] phenylsulphonylpiperazines of general structure **18**. Compounds **12**–**14** were prepared from **17b** in a similar manner. Selective reduction of 4,4′-dipyridyl **19** by hydrogenation at 50 °C in H_2O over 5% Rh/C (5 atm, 7 h) gave the piperidine intermediate **17b**. Compound **15** was prepared by reaction of **17a** and **20** (Scheme 2).

Biological Results and Discussion

Inhibitor pK_a was determined for examples of the two approaches to pK_a lowering, using a Beckman DU-7 spectrophotometer. Much lower pK_a values were confirmed for the new compounds (Table 2), compared with the pyridine **1**. An unexpectedly high pK_a value of 6.7 was found for the fluoro-substituted **10**, compared to the chloro derivative **5** (pK_a 5.8). This higher pK_a was attributed to the absence of a twisting effect on the

Table 2. pK_a measurements of typical pyridines

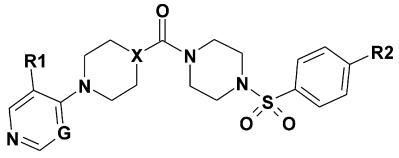
Compd	pK_a	CLOGP
1	9.2	3.4
5	5.8	3.7
10	6.7	3.7
12	5.8	3.2

piperidine ring from the fluoro atom of **10**. Thereby a greater electron donation into the pyridine ring would occur from the piperidine ring nitrogen atom.

The strategy employed for evaluation of compounds as OSC inhibitors has been fully described.[3,5] Compounds were assessed first in oral cholesterol biosynthesis tests in rats before confirmation in vitro that the in vivo activity found was derived from inhibition of the target OSC enzyme. Compounds were assessed for inhibition of cholesterol biosynthesis in rats dosed with tritiated mevalonate. At 1 h later an oral dose of test compound was administered. First, the cholesterol biosynthetic precursor pattern was found from an HPLC analysis (radiochemical detector) of extracts of saponified liver samples. This lipid profile was compared with the same profile from control animals. These HPLC chromatograms indicated which steps of cholesterol biosynthesis had been inhibited, i.e., whether the enzyme inhibition was selective for the OSC step. Second, the level of cholesterol present was also measured from the same HPLC chromatograms, and initially expressed as an oral ED_{50} or ED_{80} for the inhibition ($n=5$). Confirmation of OSC inhibition was carried out in a rat microsomal OSC assay[3] at compound concentrations of 100 nanomolar. Food intake was determined 24 h after an oral dose of test compound to chow pellet fed rats ($n=5$). The percentage reductions in food eaten were found by comparison with the food intake in the same animals dosed the previous day with vehicle alone.

The effective doses for the inhibition of rat cholesterol biosynthesis shown in Table 3 indicate that a considerable lowering of pyridine pK_a had not diminished the very potent oral inhibition of rat cholesterol biosynthesis.[3]

Table 3. Oral inhibition of rat cholesterol biosynthesis and rat OSC in vitro after halogen substitution

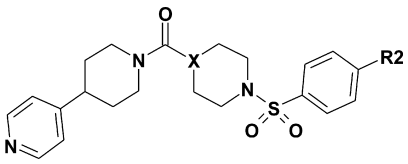


Compd	R1	R2	X	ED_{50} (mg/kg)	ED_{80} (mg/kg)	OSC% inhibition (100 nM)
5[a]	Cl	Cl	N	0.6	1.4	87
6[a]	Cl	Br	N	1.1	2.3	100
7[a]	Cl	F	N	1.1	2.3	100
8[a]	Cl	CF_3	N	0.2	0.7	100
9[a]	Br	Br	C	0.5	—	89
10[a]	F	CF_3	C	0.4	1.0	100
11[a]	Br	Br	C	>2.0	—	47
1				2.0	>2.0	100
2				0.4	1.4	100

[a]**5**–**10**, G = C; **11**, G = N.

Good in vitro inhibition of rat microsomal OSC was also found. In particular pyridine **8** showed an ED_{80} of 0.7 mg/kg for cholesterol biosynthesis inhibition, which was similar to that for pyrimidine **2** (ED_{80} 1.4 mg/kg). The bromo-substituted pyrimidine **11**, however, was not inhibitory in rats at the 2 mg/kg test dose. The second approach (Table 4), gave similar results for rat OSC and cholesterol biosynthesis inhibition. Overall the inhibitory potency of **12**–**15** for cholesterol biosynthesis was lower than with the halogen-substituted pyridines in Table 3. Here **13** was the best oral inhibitor of rat cholesterol biosynthesis with an ED_{80} of 1.8 mg/kg. Compounds **5** and **13** were tested in the rat feeding test as examples of pyridines from the two pK_a lowering approaches followed. Both compounds gave an improved feeding effect at oral doses of 100 mg/kg (**5** = 14%; **13** = 23%; vehicle dosed control = 7%). Thus, structural modification in the pyridine series of OSC inhibitors has led to a significant widening of the ratio of oral inhibition of cholesterol biosynthesis and feeding inhibition.

Table 4. Oral inhibition of rat cholesterol biosynthesis and rat OSC in vitro after carbon substitution



Compd	R2	X	ED_{50} (mg/kg)	ED_{80} (mg/kg)	OSC% inhibition (100 nM)
12	4-Cl	N	1.7	3.6	91
13	4-Br	N	0.4	1.8	94
14	4-F	N	0.5	—	97
15	4-Br	C	0.5	4.7	100

In summary the pyridine OSC inhibitors have been modified to give analogues with a lower pK_a. These novel pyridines have similar inhibitory potency to the pyrimidine **2** in terms of the oral inhibition of rat cholesterol biosynthesis and rat microsomal OSC in vitro. In addition the effect on rat feeding seen with the original pyridine series was considerably alleviated. The new pyridine analogues with a lower pK_a were candidates for development as novel cholesterol lowering agents.

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