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INHIBITION OF 2,3-OXIDOSQUALENE CYCLASE BY N-ALKYLPIPERIDINES

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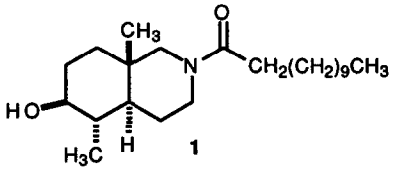
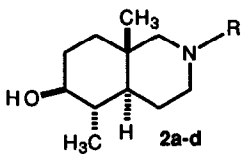
Abstract: A series of N-alkylpiperidines (3)-(6), truncated A-ring analogs of the azadecalins (2) were synthesized, to further elucidate the structural requirements of OSC inhibition. From the structure-activity data obtained with the "clipped" A-ring analogs (3) and piperidines (4)-(6), important interactions between these inhibitors and oxidosqualene cyclase, and an important structural feature of azadecalin (1) that is responsible for its potent OSC inhibition were deduced.

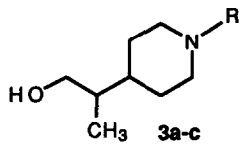
Enzymes subsequent to HMG-CoA reductase have attracted considerable attention as sites for cholesterol biosynthesis blockade. The enzyme 2,3-oxidosqualene cyclase (OSC) is an attractive target,[1] in part, due to the potential for regulation of HMG-CoA reductase by oxysterols formed from squalene diepoxide.[2] We recently reported that the azadecalin amide (1) is a potent, competitive inhibitor of OSC (K_i = 28 nM) that



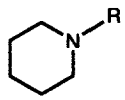
1



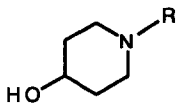
2a-d



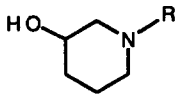
3a-c



4c-d



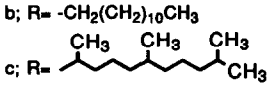
5b-d

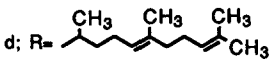


6b-d

a; R= $-CH_2C_6H_5$

b; R= $-CH_2(CH_2)_{10}CH_3$



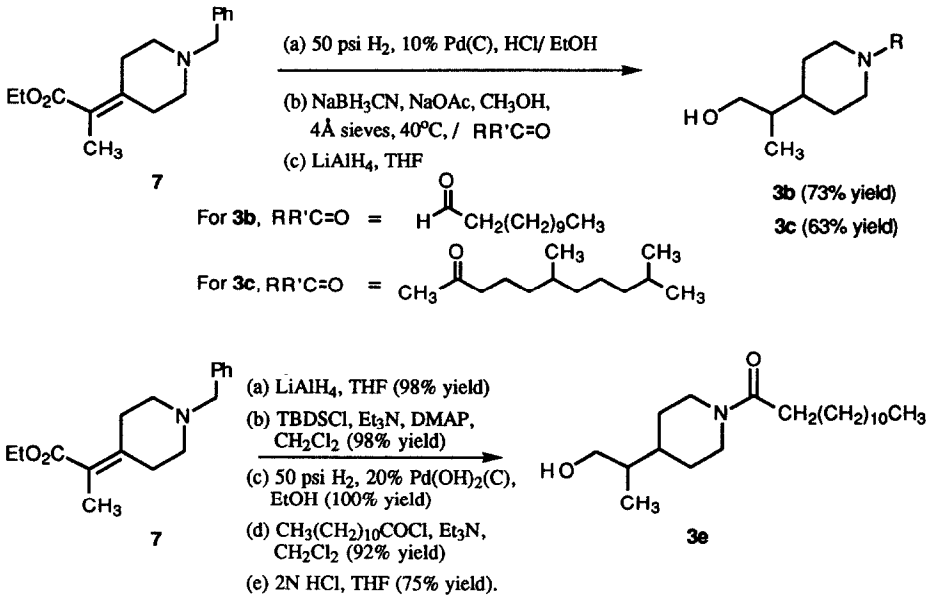


inhibits cholesterol biosynthesis (IC_{50} = 0.7 μM) in HepG2 cells by selectively blocking OSC.[3] In contrast, the amines (2b) and (2c) were shown to block cholesterol biosynthesis by inhibiting sterol Δ^{14}-reductase,

resulting in an abnormal accumulation of 5α-cholest-8(9),14(15)-dien-3β-ol.[3] This communication describes the synthesis and biological evaluation of a series of piperidines designed to inhibit OSC by mimicking the putative high-energy intermediate (HEI) cations formed during the cyclization of 2,3-oxidosqualene.[1]

Working on the hypothesis that the introduction of rotational freedom to the hydroxyl group of (2) might lead to increased enzyme affinity, C1, C2, and C19 (steroid numbering) were formally removed from the A-ring of (2) to give the piperidine (3). Piperidines (3b) and (3c) were synthesized[4] from (7)[5] in high yield by the sequence of reactions outlined in Scheme I. The N-benzyl analog (3a) was prepared by reduction of (7) with Mg in methanol[6] followed by $LiAlH_4$ reduction in THF (95 % overall yield).

Scheme I:



IC_{50} values[3] for the inhibition of purified[7] rat liver OSC for compounds (3), along with previously reported values for the azadecalins (2) are given in Table I. The N-benzylpiperidine (3a) is a weak inhibitor; therefore, no additional benzyl analogs were prepared. However, both (3b) and (3c) are potent inhibitors of OSC; activity was not sacrificed by the removal of the A-ring, thereby indicating that the hydroxypropyl side chain of (3) is able to obtain a favorable orientation for binding with the enzyme.[8]

Table I. IC_{50} Values (μM)[a] for OSC Inhibition by compounds 2-6.

Structure	R= a	b	c	d
2	NI[b]	0.55	2.0	4[c]
3	57	0.42	2.0	-[d]
4	-[d]	-[d]	1.5	40
5	-[d]	9.0	1.6	10
6	-[d]	5.0	5.0	40

[a]Standard error for all IC_{50} values determined in this work is ≤ ±10%.
[b]No OSC Inhibition; see reference 10. [c]IC_{50} for inhibition of oxidosqualene-cycloartenol cyclase; see reference 11. [d]Compound not prepared for this study.

We also prepared (3e) (Scheme I), an amide analog of the *n*-dodecylpiperidine (3b), to compare its activity with (1). The IC_{50} value observed for (3e) (10 μM) is 100-fold less than that observed for the azadecalin (1) (IC_{50} = 0.1 μM) but only 24-fold less than that observed for the amine (3b); thus, rigidity contributes significantly to the unique activity of (1). Furthermore, OSC inhibition by (1) and (3e) demonstrates that a carbocation mimic (i.e. an amine) is not necessary to achieve enzyme inhibition.

Next, to investigate the effect of completely removing the A-ring of (2) on OSC inhibition, we prepared a series of N-alkylpiperidines (4)-(6). These inhibitors were prepared by reductive amination of piperidine or *t*-butyldimethylsilyl protected 3- and 4-hydroxypiperidine with the appropriate aldehyde or ketone, followed by deprotection with 2N HCl/ THF.

From the the data in Table I, it is apparent that compounds containing the branched trimethyldecyl chain (R = c) have similar affinity for OSC, whereas multiple unsaturation in the trimethyldecyl chain (R = d) results in less potent enzyme inhibition. In contrast, it has been observed recently that a polyunsaturated N-alkyl-4-hydroxypiperidine was a 10-fold more potent inhibitor of rat liver OSC than the fully saturated derivative.[9] Our data, however, are consistent with that observed for the azadecalin series where unsaturation in the trimethyldecyl chain leads to less potent inhibition of oxidosqualene-cycloartenol cyclase.[11,12] Finally, for structures (5) and (6), when R is saturated but unbranched (R = b), activity is diminished relative to structures (2) and (3)- presumably due to missing interactions afforded by the A-ring of (2) and the hydroxypropyl side chain of (3).

As described previously,[3,13] we tested (3b), (4c), and (3e) for their ability to inhibit cholesterol biosynthesis in HepG2 cells. The amines (3b) and (4c) are significantly more potent inhibitors of cholesterol synthesis than of

purified OSC having IC_{50} values of 40 nM and 30 nM, respectively. In contrast, the amide (3e), inhibits cholesterol biosynthesis (IC_{50} = 12 μM) with a potency nearly equal to that observed for the purified enzyme- a result also observed for (1).[3] The similarity observed between the IC_{50} values for (3e) and (1) and the disparity between values obtained for (3b) and (4c) suggests that, in HepG2 cells, OSC is the enzyme blocked by (3e) and (1) and that additional enzymes are blocked by (3b) and (4c).

In summary, a series of piperidines that are truncated A-ring analogs of the azadecalin (2) have prepared. Some of these compounds((3b),(3c), (4c) and (5c)) inhibit OSC with a potency equal to or greater than the corresponding azadecalins (2b) and (2c). The importance of the contribution of the A-ring conformation to OSC inhibition by (1) is demonstrated by the substantial decrease in potency of OSC inhibition by (3e). Finally, potent OSC inhibitors have been synthesized that are simplified analogs of azadecalin (2).

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4. All new compounds where characterized by NMR, MS, IR, and combustion analysis.
5. Prepared in 95% yield by the addition of N-benzyl-4-piperidinone to a THF solution of the anion of triethylphosphonopropiate generated at -78 °C with 1 equivalent of *n*-butyllithium.
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8. The effect of the asymmetric center in (3) is probably insignificant; if one isomer were completely inactive, the IC_{50} would be decreased, at most, by a factor of two.
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