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Enyzmatic cyclization of squalene analogs*

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Abstract: Enzymatic cyclizations of squalene and oxidosqualene lead to sterols and other triterpenoids in bacteria, fungi, plants, and animals. The cyclases for these reactions catalyze formation and stabilization of polycyclic carbocations and direct the enzyme-specific, templated formation of new carbon-carbon bonds in regio- and stereochemically defined contexts. The development of mechanism-based irreversible inhibitors, photoactivatable inhibitors, and numerous substrate analogs have helped to unravel the stepwise events occurring in the catalytic sites of these enzymes by covalent modification of specific amino acid residues.

MECHANISM-BASED INHIBITORS

Affinity labeling of OSLC and SHC by 29-MOS and analogs

The first mechanism-based irreversible inactivator of mammalian oxidosqualene:lanosterol cyclase (OSLC) was 29-methylidene-2,3-oxidosqualene (29-MOS), which showed a K_i value of 4.4 μM and k_{inact} = 221 min^{-1} for pig OSLC [1]. The proposed mechanism of inhibition involves initial cyclization to the 21-methylidene-protosterol cation as proposed for lanosterol formation. However, this allylic cation can be trapped by an active-site nucleophile, resulting in covalent bond formation and concomitant irreversible inactivation (Fig. 1). We demonstrated that other 29-functionalized 2,3-oxidosqualene analogs such as 29-difluoromethylidene-2,3-oxidosqualene ($IC_{50} = 3.0$ μM, $K_i = 10.2$ μM for rat OSLC) were also irreversible inhibitors of OSLC, suggesting that initiation, not termination, of cyclization was rate-limiting for these suicide substrates [2]. In contrast, truncation of the side chain in 29-MOS analogs such as 29-difluoromethylidene-hexanor-2,3-oxidosqualene ($IC_{50} = 60$ μM for rat OSLC) resulted in reversible inhibitors [2].

Interestingly (3*S*)29-MOS was also a mechanism-based irreversible inhibitor of *Acidobacillus acidocaldarius* squalene:hopene cyclase (SHC) (Fig. 1) [3]. Inhibition kinetics revealed that the inhibition was non-competitive and time-dependent ($IC_{50} = 1.2$ μM, $K_I = 2.1$ μM, $k_{inact} = 0.06\ min^{-1}$). A C_{31} dammarene derivative with a 6.6.6.5 + 6 ring system was isolated from the incubation mixture as a major cyclization product of (3*S*)29-MOS [3]. This suggested that the presence of the methylidene residue interrupted the cyclization reaction by SHC at the tetracyclic dammarene C-20 cation; this cation could then undergo either final ring closure to yield the unnatural product or trapping by an active-site nucleophile to give covalent modification of the enzyme active site.

Recently (18*E*)-(3*S*)-29-MOS, the Δ^{18} regioisomer of the previously used (18*Z*)-(3*S*)-29-MOS, was also found to covalently modify the active site of rat OSLC. When (18*E*)-(3*S*)-29-MOS was incubated with *A. acidocaldarius* SHC, the same C_{31} dammarene derivative with a 6.6.6.5 + 6 ring system, as described above for (18*Z*)-(3*S*)-29-MOS, was obtained as a minor product, while a bicyclic product was obtained as a major product (Fig. 2).

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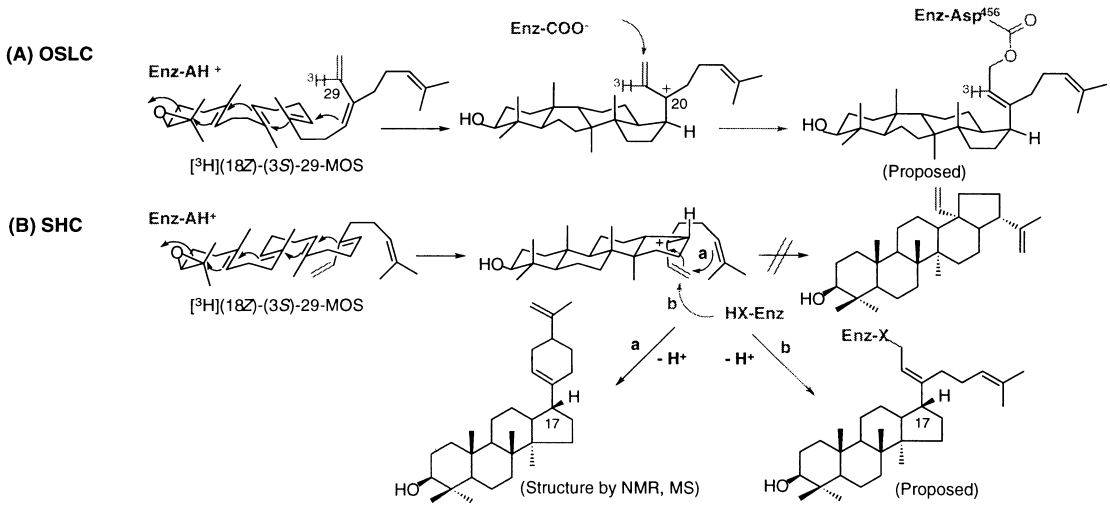


Fig. 1 Proposed mechanism of (A) irreversible inactivation of OSLC and (B) cyclization and SHC inactivation by (3*S*)29-MOS.

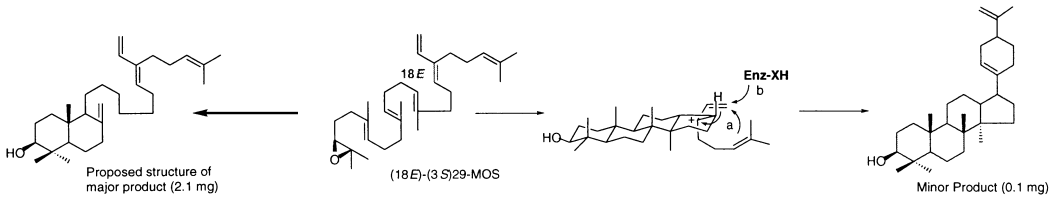


Fig. 2 Cyclization of (18*E*)-(3*S*)29-MOS by *A. acidocaldarius* SHC.

Affinity labeling of OSLC and SHC by sulfur OS analogs

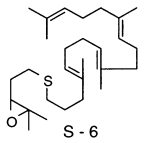
Sulfur-containing 2,3-OS analogs were synthesized by the Oehlschlager group at Simon Fraser University (British Columbia, Canada), and were shown to be extremely potent inhibitors of OSLCs [5–8]. In particular, OS analogs in which sulfur has replaced carbons C-18 and C-19 were reported to have subnanomolar IC_{50} values for both vertebrate and fungal OSCs [6,8]. Sulfoxides were generally much less active than corresponding thioethers. In our inhibition assays with purified enzymes, these unusually low IC_{50} values have been revised as indicated in Table 1 [8]. Inhibition kinetics with purified vertebrate OSLCs demonstrated that the S-18 compound was a time-dependent, irreversible inhibitor of *pig* OSLC ($K_I = 1.5\ \mu M$, $k_{inact} = 0.06\ min^{-1}$, partition ratio = 16), while the inhibition by S-19 was reversible and not time-dependent [8]. Surprisingly, the inhibition of *rat* OSLC by the S-18 compound was reversible. When tested with recombinant *A. acidocaldarius* SHC, these analogs, in which sulfur replaces carbons at C-6, C-10, C-14, C-18, or C-19 of OS, also showed inhibition of the enzyme at submicromolar level [9], Among them, S-18 was the most potent inhibitor and showed time-dependent inhibition (Table 1).

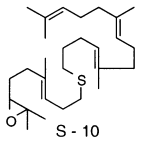
Both *pig* OSLC and *A. acidocaldarius* SHC could be covalently modified with [17-^{3}H]S-18 and [22-^{3}H]S-18, two tritium-labeled radioisotopomers of S-18 [9,10]. The covalent modification would require partial cyclization of S-18 at the active site of the enzyme with trapping of a cationic intermediate by an active-site nucleophile (Fig. 3). Retention of the tritium label for both radioisotopomers excluded the possibility of an attack at C-20 with transfer of the side chain to the active site; alternatively, nucleophilic trapping could occur on a bicyclic or tricyclic intermediate [10].

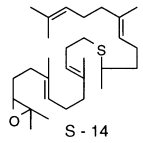
PHOTOAFFINITY LABELING

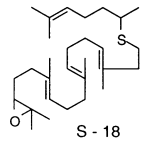
A new orally active OSLC inhibitor, Ro48-8071 [11], showed potent noncompetitive inhibition of *A. acidocaldarius* SHC ($IC_{50} = 9.0$ nM, $K_I = 6.6$ nM) and OSLC ($IC_{50} = 40$ nM, $K_I = 22$ nM for homogeneous rat liver OSLC) (Fig. 4). This non-terpenoid inhibitor included a benzophenone (BP) photophore, and

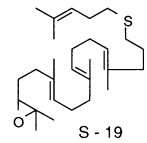
Table 1 Inhibitory studies of sulfur-substituted-OS as inhibitors of rat liver OSLC and recombinant *A. acidocaldarius* SHC



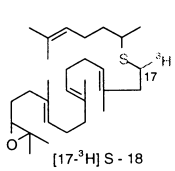


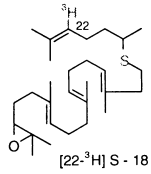


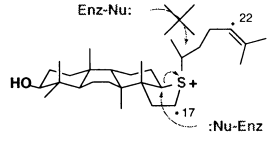




	Rat liver OSLC			*A. acidocaldarius* SHC		
	IC_{50} (nM)	K_I (nM)	k_{inact} (min^{-1})	IC_{50} (nM)	K_I (nM)	k_{inact} (min^{-1})
S-6	500	520	0.085	150	127	0.0001
S-10	1000	2100	0.037	570	971	0.0001
S-14	11000	4200	0.042	86	109	0.058
S-18	50	37	0.0001	60	31	0.071
S-19	260	180	0.0001	78	83	0.054







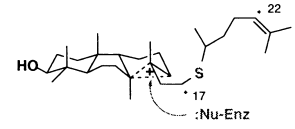
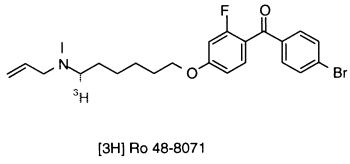


Fig. 3 Substrate analog S-18 and potential modes of active-site labeling.



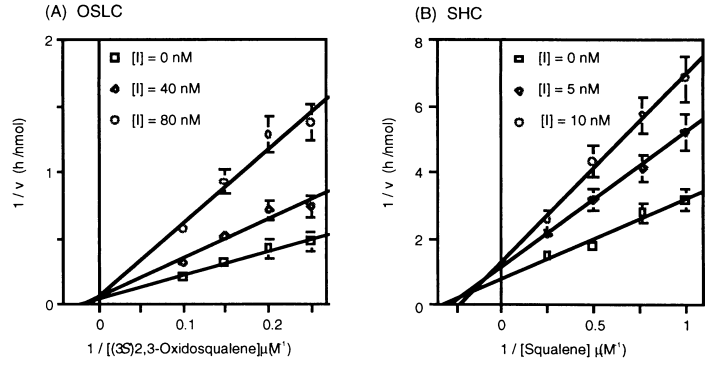


Fig. 4 Kinetics of inhibition of OSLC and SHC by Ro48-8071.

both unlabeled and tritium-labeled Ro48-8071 (18.8 Ci/mmol) were chemically synthesized at Utah as a photoaffinity label. BP derivatives have been employed in many biochemical systems as excellent photoaffinity probes with remarkable site specificity [12,13]. As expected, specific, efficient covalent modification of both OSLC and SHC enzymes was observed after UV irradiation at 360 nm. Labeling of both OSLC and SHC by [^{3}H]Ro48-8071 was competitively displaced by co-incubation with a 1000-fold molar excess of S-18 or the non-terpenoid inhibitor BIBX79. Displacement of labeling of OSLC was also achieved with the suicide substrate (3*S*)29-MOS. Thus, the non-substrate Ro48-8071 and both terpenoid and non-terpenoid inhibitors of these enzymes appear to share a common binding site [14].

CYCLIZATION OF FLUORO ANALOGS

In order to test the effect of fluorine atom displacement on the enzymatic cyclization reaction (3*S*)11-fluoro-2,3-oxidosqualene (FOS) and 14-FOS were synthesized [15,16]. The convergent synthesis of 11-FOS is illustrated in Fig. 5. The 14-FOS was obtained in an analogous route in which the fluorine substituent was on the farnesyl rather than the epoxyfarnesyl synthon [16].

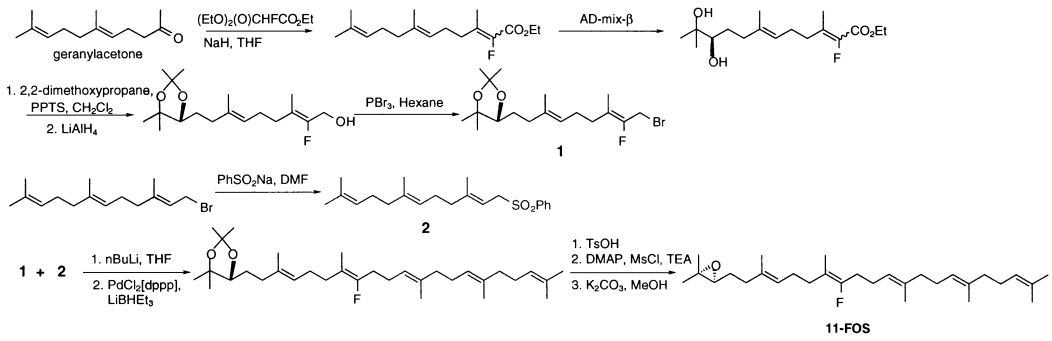


Fig. 5 Synthesis of (3*S*)-11-FOS.

No cyclization of either 11-FOS or 14-FOS was detected with purified rat liver OSLC. The OSLC enzyme is particularly sensitive to structural changes on the pro-β-face and thus fails to bind (3*S*)11-FOS or 14-FOS. In contrast, recombinant *A. acidocaldarius* SHC converted both (3*S*)11-FOS into a monocarbocyclic compound with a bridged ether (Fig. 6). The presence of the fluorine atom apparently interrupted the cyclization reaction at the monocyclic cationic intermediate stage. Similarly, the corresponding bicyclic ether was obtained as a minor product from (3*S*)-14-FOS cyclization by SHC (Fig. 6). The (3*S*)-14-FOS substrate also provided a 2:3 ratio of two bicyclic alcohols as the major product, in analogy to the bicyclic compounds identified from the (18*E*)-29-MOS cyclization described above.

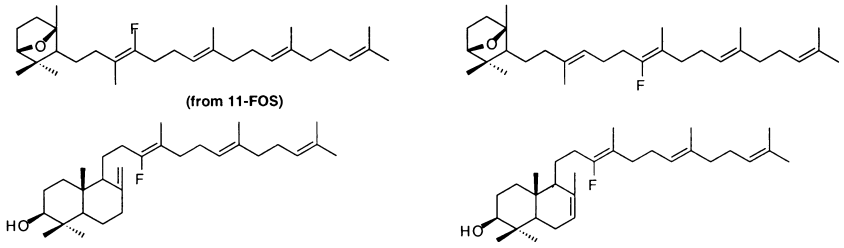


Fig. 6 Cyclization products of (3*S*)-11-FOS and (3*S*)-14-FOS.

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REFERENCES

1 X.-y. Xiao, G. D. Prestwich. *J. Am. Chem. Soc.* **13**, 9673–9674 (1991).

2 B. A. Madden, G. D. Prestwich. *Bioorg. Med. Chem. Lett.* **7**, 309–314 (1997).

3 I. Abe, T. Dang, Y. F. Zheng, B. A. Madden, C. Feil, K. Poralla, G. D. Prestwich. *J. Am. Chem. Soc.* **119**, 11 333–11 334 (1997).

4 Y. F. Zheng I. Abe, G. D. Prestwich. *J. Org. Chem.* **63**, 4872–4873 (1998).

5 Y. F. Zheng, A. C. Oehlschlager, P. G. Hartman. *J. Org. Chem.* **59**, 5803–5809 (1994).

6 Y. F. Zheng, A. C. Oehlschlager, N. H. Georgopapadakou, P. G. Hartman, P. Scheliga. *J. Am. Chem. Soc.* **117**, 670–680 (1995).

7 Y. F. Zheng, D. S. Dodd, A. C. Oehlschlager, P. G. Hartman. *Tetrahedron* **51**, 5255–5276 (1995).

8 D. Stack, Y. F. Zheng, A. L. Perez, A. C. Oehlschlager, I. Abe, G. D. Prestwich, P. G. Hartman. *J. Med. Chem.* **40**, 201–209 (1997).

9 Y. F. Zheng. I. Abe, G. D. Prestwich. *Biochemistry* **37**, 5981–5987 (1998).

10 I. Abe, W. Liu, A. C. Oehlschlager, G. D. Prestwich. *J. Am. Chem. Soc.* **118**, 9180–9181 (1996).

11 O. H. Morand, J. D. Aebi, H. Dehmlow. Y.-H. Ji, N. Gains, H. Lengsfeld, J. Himber. *J. Lipid Res.* **38**, 373–390 (1997).

12 G. Dormán, G. D. Prestwich. *Biochemistry* **33**, 5661–5673 (1994).

13 G. D. Prestwich, G. Dormán, J. T. Elliott, D. M. Marecak, A. Chaudhary. *Photochem. Photobiol.* **65**, 222–234 (1997).

14 I. Abe, Y. F. Zheng, G. D. Prestwich. *Biochemistry* **37**, 5779–5784 (1998).

15 B. Robustell, I. Abe, G. D. Prestwich. *Tetrahedron Lett.* **39**, 957–960 (1998).

16 B. J. Robustell, I. Abe, G. D. Prestwich. *Tetrahedron Lett.* **39**, 9385–9388 (1998).