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Trisnorsqualene Cyclopropylamine: A Reversible, Tight-Binding Inhibitor of Squalene Epoxidase

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The epoxidation of squalene to (3*S*)-2,3-oxidosqualene by squalene epoxidase (SE) and its subsequent cyclization by vertebrate oxidosqualene cyclase (OSC) to lanosterol are the key steps in cholesterol biosynthesis.[1] The best hypocholesteremic drugs available at present (e.g., mevinolin and its congeners) decrease steroid levels by reducing mevalonate production via the inhibition of HMG-CoA reductase.[2] In contrast, our strategy has been the development of inhibitors of SE and OSC.[3] Recently, we[4] and others[5] described the inhibition of SE by compounds derived from modification of the terminal isopropylidene group of squalene. We now report the first example of a cyclopropylamine-containing squalene analogue which acts as a highly selective, slow tight-binding inhibitor[6] of pig liver squalene epoxidase.

Squalene analogues containing the cyclopropylamine moiety were synthesized as follows. Analogue **1** was prepared from trisnorsqualene aldehyde[4] and cyclopropylamine, by the procedure of Borch et al.[7] Reductive amination of **1** with aqueous formaldehyde, followed by hydrogen peroxide oxidation,[8] provided cyclopropylamine analogues **2** and **2b**. Cyclopropylamine **3** was prepared by reductive amination of tetranorsqualene aldehyde.[9]

Cyclopropylamine analogues **1**–**3** could function in three ways. First, in their protonated forms, the amines could simply interact ionically with either of the two enzymes. Second, the amines could undergo oxidation to the corresponding *N*-oxides, thus acting as prodrugs for a functionality known to be a transition-state mimic of OSC epoxide opening.[10] Third, the amines could be oxidized by a one-electron process and thus act as mechanism-based inactivators[11] to irreversibly inactivate SE or OSC. A variety of

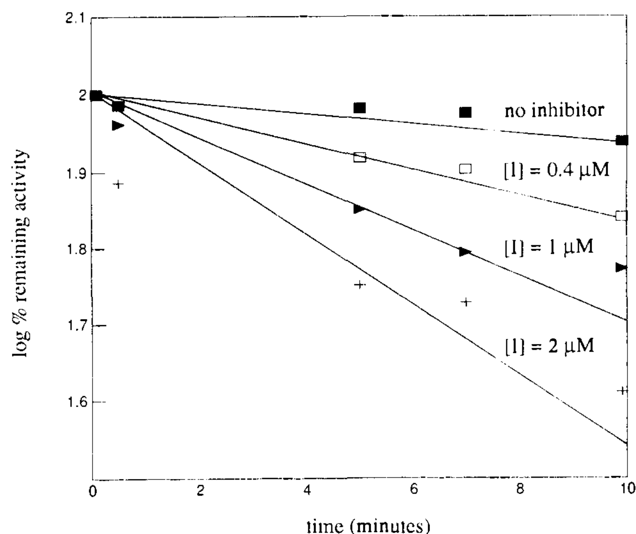

Figure 1. Time dependency of inactivation of trisnorsqualene cyclopropylamine (**1**). Incubations of 0.5, 5, 7, and 10 min were performed with inhibitor concentrations of 0, 0.4, 1, and 2 μM.

Table I. IC_{50} Values of SE and OSC Inhibition for Compounds **1**–**8**[a]

compd	X	IC_{50} (SE), μM	IC_{50} (OSC), μM
1	$CH_2NH\text{-c-}C_3H_5$	2	ni
2	$CH_2N(CH_3)\text{-c-}C_3H_5$	100	ni
2b	$CH_2N(O)(CH_3)\text{-c-}C_3H_5$	200	40
3	$NH\text{-c-}C_3H_5$	4	ni
4	CH_2NHEt	200	ni
5	$CH_2NH(i\text{-Pr})$	ni	ni
6	$CH_2N(CH_3)_2$	20	ni
7	CH_2OH	4	ni
8	CH_2NH_2	200	ni

[a] The abbreviation ni represents no inhibition at [I] = 400 μM. IC_{50} values for OSC were calculated by subtracting SE inhibitory effects from inhibition in mixed OSC + SE assays. 2-Aza-2,3-dihydrosqualene (**6**), trisnorsqualene alcohol (**7**), and trisnorsqualene amine (**8**) were previously reported as SE inhibitors (see ref 4a and 14).

cyclopropylamines are potent mechanism-based inactivators of mitochondrial monoamine oxidase, plasma amine oxidase, and cytochrome P-450 enzymes.[12] Although SE is believed to be an external flavoprotein monooxygenase,[13] the mechanism by which the delivery of one oxygen atom to squalene occurs is poorly understood. Squalenoid cyclopropylamines may address these mechanistic questions.

Because oxidation of squalene might be initiated at either the C-2 or C-3 position, both the N-2 (trisnorsqualene) analogue **1** and the N-3 (tetranorsqualene) cyclopropylamine analogue **3** were required. The *N*-methyl analogue **2** and *N*-methyl *N*-oxide **2b** test for procyclase inhibitory activity (secondary amines **1** and **3** would be converted to hydroxylamines).

Compounds **1**–**3** and **2b** were tested for pig liver SE and OSC inhibition. The results are presented in Table I. Secondary amine **1** is one of the most potent inhibitors of vertebrate SE known, with IC_{50} = 2 μM.[14] Interestingly, amine **3**, bearing one less methylene

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(1) Mulheirn, L. J.; Ramm, P. J. *Chem. Soc. Rev.* **1972**, 259–291.
(2) Endo, A. *J. Med. Chem.* **1985**, *28*, 401–410.
(3) Attempts have previously been made to develop inhibitors of SE and OSC. See: Cattel, L.; Ceruti, M.; Viola, F.; Delprino, L.; Balliano, G.; Duriatti, A.; Bouvier-Navé, P. *Lipids* **1986**, *21*, 31–38 and references therein.
(4) (a) Sen, S. E.; Prestwich, G. D. *J. Am. Chem. Soc.* **1989**, *111*, 1508–1510. (b) Sen, S. E.; Prestwich, G. D. *J. Med. Chem.* **1989**, *32*, 2152–2158.
(5) Ceruti, M.; Viola, F.; Grosa, G.; Balliano, G.; Delprino, L.; Cattel, L. *J. Chem. Res., Synop.* **1988**, 18–19; *J. Chem. Res., Miniprint* **1988**, 239–260.
(6) Schloss, J. V. *Acc. Chem. Res.* **1988**, *21*, 348–353.
(7) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897–2904.
(8) Ceruti, M.; Balliano, G.; Viola, F.; Cattel, L.; Gerst, N.; Schuber, F. *Eur. J. Med. Chem.—Chim. Ther.* **1987**, *22*, 199–208.
(9) van Tamelen, E. E.; Pedlar, A. D.; Li, E.; James, D. R. *J. Am. Chem. Soc.* **1977**, *99*, 6778–6780.
(10) Ceruti, M.; Delprino, L.; Cattel, L.; Bouvier-Navé, P.; Duriatti, A.; Schuber, F.; Benveniste, P. *J. Chem. Soc., Chem. Commun.* **1985**, 1054–1055.
(11) Silverman, R. B. *Mechanism-Based Enzyme Inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, 1988; Vol. 2.
(12) In each case, one-electron oxidation of the nitrogen center can produce a cyclopropylamine radical cation which rapidly rearranges to give a homoallyl iminium radical. See: (a) Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048–2050. (b) Macdonald, T. L.; Zirvi, K.; Burka, L. T.; Peyman, P.; Guengerich, F. P. *J. Am. Chem. Soc.* **1982**, *104*, 2050–2052. (c) Silverman, R. B.; Hoffman, S. J.; Catus W. B., III *J. Am. Chem. Soc.* **1980**, *102*, 7126–7128. (d) Silverman, R. B. *J. Biol. Chem.* **1983**, *208*, 14766–14769.
(13) Ono, T.; Ozasa, S.; Hasegawa, F.; Imai, Y. *Biochim. Biophys. Acta* **1977**, *486*, 401–407.

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unit, was only slightly less potent (IC_{50} = 4 μM).[15] *N*-Methylcyclopropylamine **2** was a poor inhibitor of SE and showed only a moderate decrease in [^{14}C]lanosterol formation at 100 μM. Although not as potent as several related compounds for OSC and SE mixtures,[10] *N*-oxide **2b** was a modest inhibitor of OSC (IC_{50} = 40 μM), as indicated by an accumulation of [^{14}C]squalene epoxide. These results suggest that oxidation of the nitrogen atom to the corresponding *N*-oxide does not occur in vitro. The difference in activity between secondary and tertiary amines **1** and **2** can be attributed to steric perturbation.

The time dependency and irreversibility of inhibition of SE by cyclopropylamine **1** was investigated. Figure 1 shows a time dependency of inactivation with K_i = 2.4 μM and k_{inact} = 0.055 min^{-1}.[16] Addition of excess squalene (ca. 400 μM) to a mixture of **1** (2 μM) and pig liver microsomes caused complete recovery of SE activity, illustrating substrate protection.

Amine **1** was not readily removed from the crude inactivated enzyme mixtures. However, incubation of ^{3}H-labeled cyclopropylamine **1** (prepared from reductive amination of [^{3}H]trisnorsqualene aldehyde)[4a] with crude pig liver homogenate, followed by anion-exchange chromatography, resulted in complete recovery of the radiolabeled amine **1** (detected by radio-TLC) and full restoration of SE activity.[17] Thus, cyclopropylamine **1** cannot be a mechanism-based inactivator of SE. Instead, it is probable that there exists a strong electrostatic interaction between enzyme and inhibitor. The protonated amine may mimic the transient positive charge which develops at the C-3 and/or C-2 positions during the epoxidation of squalene.

Secondary amines **4** and **5** analogous to cyclopropylamine **1** are less potent inhibitors of SE by more than 2 orders of magnitude (IC_{50} = 200 μM and >400 μM, respectively). Replacement of alkyl substituents by the cyclopropyl moiety can result in increased inhibition.[18] The reasons for the increased activity of many cyclopropane-containing analogues are poorly understood and involve a combination of steric and electronic effects.[19]

In conclusion, trisnorsqualene cyclopropylamine (**1**) is a potent inhibitor of pig liver SE. Because **1** does not appear to be a mechanism-based inactivator of SE, interaction between enzyme and inhibitor is most probably electrostatic in nature. Structural studies of cyclopropylamine variants suggest that there is considerable flexibility for the position of the nitrogen atom. However, the strong requirement of the cyclopropyl moiety for SE inhibition suggests that there also exists a specific, but as yet undefined, interaction between the cyclopropyl ring and SE.

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Supplementary Material Available: Experimental details for syntheses of **1**, **2**, **2b**, **3**, and eight other compounds and for enzyme experiments (8 pages). Ordering information is given on any current masthead page.

(14) 2-Aza-2,3-dihydrosqualene (**6**), the most potent inhibitor of vertebrate squalene epoxidase thus far described in the literature (Ryder, N. S.; Dupont, M.-C.; Frank, I. *FEBS Lett.* **1986**, *204*, 239–242), was tested for pig liver SE inhibition. Under our assay conditions, this analogue was less potent than **1**, with IC_{50} = 20 μM.

(15) In contrast, the trisnorsqualene alcohol is 10-fold more potent than its truncated tetranor analogue. See: Sen, S. E.; Prestwich, G. D. *J. Med. Chem.*, submitted.

(16) Cyclopropylamine **1** was incubated for 0.5, 5, 7, and 10 min at inhibitor concentrations of 0, 0.4, 1, and 2 μM.

(17) Sedimentation of nonsolubilized, inactivated microsomes, dialysis, and ultrafiltration of an inactivated enzyme mixture all failed to restore SE activity. However, an independent observation (M. Bai, unpublished results) showed that a variety of amine-containing inhibitors of SE could be removed from reversibly inhibited SE preparations by using ion-exchange chromatography on DEAE-Sephacel.

(18) O'Leary, M. H.; DeGrooyer, N. J.; Dougherty, T. M.; Anderson, V. *Biochem. Biophys. Res. Commun.* **1981**, *100*, 1320–1325.

(19) (a) Ariens, E. J., Ed. *Drug Design*; Academic Press: New York, 1976; Vol. 7, pp 165–207. (b) Suckling, C. J. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 537–552. (c) March, J. *Advanced Organic Chemistry*; McGraw-Hill: New York, 1978; pp 141–142. (d) Clark, R. A.; Fiato, R. A. *J. Am. Chem. Soc.* **1970**, *92*, 4736–4738.

A Trigonal Planar $[Zn(SR)_3]^{1-}$ Complex. A Possible New Coordination Mode for Zinc–Cysteine Centers

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The recent discovery of the "zinc finger" structural motif and its wide occurrence in nucleic acid binding proteins has greatly increased interest in zinc–cysteine coordination.[1-3] We have been studying the coordination chemistry of zinc and spectroscopically observable metals with similar structural requirements to serve as models for zinc–cysteine metalloproteins.[4-7] Herein, we report the synthesis and structure of the first example of a trigonal planar $[M(SR)_3]^{1-}$ complex of zinc.

The reactions of $ZnSO_4{\cdot}7H_2O$ with 5 equiv of lithium 2,3,5,6-tetramethylbenzenethiolate (LiS-2,3,5,6-Me_4C_6H) in CH_3CN and 1 equiv of $(n\text{-}Pr_4N)Br$ gave a white crystalline compound, which was recrystallized from CH_3CN. The ^{1}H NMR spectrum of the product indicated the empirical ratio of one $(n\text{-}Pr)_4N$ cation per three thiolate ligands and no resonance for CH_3CN. An X-ray crystal structure established the monomeric, three-coordinate nature of the anion in $[(n\text{-}Pr)_4N][Zn(S\text{-}2,3,5,6\text{-}Me_4C_6H)_3]$ (Figure 1).[8] The sum of the three S–Zn–S angles equals 360.0°, indicative of the planar ZnS_3 coordination. The distortions from 3-fold symmetry (S1–Zn–S2, 110.11 (7)°; S1–Zn–S3, 134.10 (8)°; S2–Zn–S3, 115.78 (7)°) result from the stacking interactions between two of the aromatic rings.

This result is surprising with respect to the known coordination chemistry of zinc[9] and particularly its coordination with thiolate ligands.[10] Three coordination is extremely rare for Zn(II) and Cd(II),[11-13] although it is more commonly observed for other d^{10} transition metals, Hg(II), Cu(I), Ag(I), and Au(I).[14] The structure of **1** must be considered in relationship to other structures that would have been suggested by precedent. It is not a $[(RS)_2Zn(\mu_2\text{-}SR)_2Zn(SR)_2]^{2-}$ dimer as has been characterized for SR = SEt and SPh or a $Zn_4(\mu_2\text{-}SR)_6L_4$ adamantane cluster.[10,15,16] It has previously been demonstrated with a number of different metals that ortho-disubstituted aromatic thiolates have a reduced tendency to bridge metal centers.[4,17] This effect is not

(1) Klug, A.; Rhodes, D. *Trends Biochem. Sci.* **1987**, *12*, 464.

(2) Evans, R. M.; Hollenberg, S. M. *Cell* **1988**, *52*, 1.

(3) Berg, J. M. *Science* **1986**, *232*, 485.

(4) Corwin, D. T., Jr.; Fikar, R.; Koch, S. A. *Inorg. Chem.* **1987**, *26*, 3079.

(5) Corwin, D. T., Jr.; Gruff, E. S.; Koch, S. A. *J. Chem. Soc., Chem. Commun.* **1987**, 966.

(6) Corwin, D. T., Jr.; Koch, S. A. *Inorg. Chem.* **1988**, *27*, 493.

(7) Corwin, D. T., Jr.; Gruff, E. S.; Koch, S. A. *Inorg. Chim. Acta* **1988**, *151*, 5.

(8) $[n\text{-}Pr_4N][Zn(S\text{-}2,3,5,6\text{-}Me_4C_6H)_3]$, $ZnS_3NC_{42}H_{67}$, crystallizes in the monoclinic space group $P2_1/n$ with a = 11.029 (2) Å, b = 18.499 (3) Å, c = 21.588 (5) Å, β = 96.05 (2)°, V = 4380 (3) Å^3, Z = 4. Final least-squares refinement using 2947 unique reflections with $I > 3\sigma(I)$ gave $R(R_w)$ = 0.045(0.056).

(9) Prince, R. H. In *Comprehensive Coordination Chemistry*; Wilkinson, G., Gillard, R. D., McCleverty, J. A., Eds.; Pergamon: Oxford, 1987; Vol. 5, Chapter 56.1.

(10) (a) Dance, I. *Polyhedron* **1986**, *5*, 1037. (b) Blower, P. J.; Dilworth, J. R. *Coord. Chem. Rev.* **1987**, *76*, 121.

(11) (a) Al-Juaid, S. S.; Buttrus, N. H.; Eaborn, C.; Hitchcock, P. B.; Roberts, A. T. L.; Smith, J. D.; Sullivan, A. C. *J. Chem. Soc., Chem. Commun.* **1986**, 908. (b) Bell, N. A.; Shearer, H. M. M.; Spencer, C. B. *Acta Crystallogr.* **1983**, *C39*, 1182.

(12) Petersen, R. B.; Ragosta, J. M.; Whitwell, G. E., II; Burlitch, J. M. *Inorg. Chem.* **1983**, *22*, 3407.

(13) Benac, B. L.; Cowley, A. H.; Jones, R. A.; Nunn, C. M.; Wright, T. C. *J. Am. Chem. Soc.* **1989**, *111*, 4986.

(14) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; John Wiley & Sons: New York, 1988.

(15) Watson, A. D.; Rao, C. P.; Dorfman, J. R.; Holm, R. H. *Inorg. Chem.* **1985**, *24*, 2820.

(16) Abrahams, I. L.; Garner, C. D.; Clegg, W. *J. Chem. Soc., Dalton Trans.* **1987**, 1577.

(17) (a) Koch, S. A.; Fikar, R.; Millar, M.; O'Sullivan, T. *Inorg. Chem.* **1984**, *23*, 121. (b) Millar, M. M.; O'Sullivan, T.; de Vries, N.; Koch, S. A. *J. Am. Chem. Soc.* **1985**, *107*, 3714. (c) Soong, S.-L.; Chebolu, V.; Koch, S. A.; O'Sullivan, T.; Millar, M. *Inorg. Chem.* **1986**, *25*, 4067.

0002-7863/89/1511-8762$01.50/0 © 1989 American Chemical Society