Oxidosqualene Cyclase Inhibitors as Antimicrobial Agents

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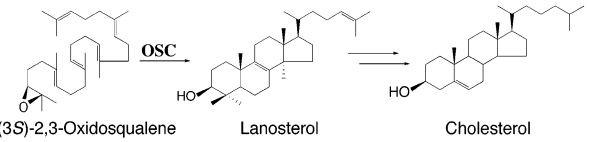
Abstract: Small-molecule oxidosqualene cyclase (OSC) inhibitors were found to be effective in assays against cloned OSC-like enzymes from human pathogens. A combinatorial library was prepared and used to identify lead compounds that inhibit the growth of *Trypanosoma cruzi*, *Leishmania mexicana amazonensis*, and *Pneumocystis carinii* in culture. Selectivity for the microorganisms in preference to mammalian cells was observed.

Introduction. The world's population continues to be seriously plagued by pathogenic organisms. In third world countries, millions are afflicted with diseases such as leishmaniasis and trypanosomiasis. Even in more developed societies, immunosuppression from organ transplants, cancer chemotherapy, and the AIDS epidemic has resulted in the increased appearance of grave opportunistic infections, such as *Pneumocystis* pneumonia and toxoplasmosis. New treatments for all these pathogens are desperately needed. Current drugs for these illnesses show toxicity, limited effectiveness, or pathogen resistance, which leads to considerable suffering and millions of deaths worldwide annually.

Sterol and triterpene biosynthetic pathways are widespread in living organisms because these polycyclic compounds are vital in cell membrane function and, in some cases, are hormonal effectors.[1,2] As a result, inhibitors of terpene biosynthesis have found successful applications as antifungals, herbicides, and drugs,[1] including the notable statin family of HMG-CoA reductase inhibitors, for controlling cholesterol levels in humans.[3−5] Oxidosqualene cyclases (OSCs) are important enzymes in triterpenoid biosynthesis.[6] These enzymes catalyze the cyclization of (3*S*)-2,3-oxidosqualene to lanosterol in fungi, mammals, and some protists and to cycloartenol, as well as several other pentacyclic triterpenes in plants[1] (Scheme 1).

Scheme 1. Cyclization of Oxidosqualene



(3*S*)-2,3-Oxidosqualene Lanosterol Cholesterol

Recent studies have revealed that a number of human pathogenic microorganisms synthesize sterols. These include *Pneumocystis carinii* (pneumonia),[7] *Trypanosoma brucei* (African sleeping sickness),[8] *Trypanosoma cruzi* (Chagas disease),[9] and *Leishmania* sp. (leishmaniasis).[10−12] Sterol biosynthetic routes have been examined as drug targets against Trypanosome[13,14] and *Pneumocystis*[15] organisms.

Because hypercholesterolemia is a major risk factor for the development of atherosclerosis in humans, considerable research and development have been directed toward the inhibition of pathways in cholesterol biosynthesis. OSC is a pivotal enzyme in the biosynthesis of cholesterol in humans.[1] A potential approach toward cholesterol level modulation would be the selective inhibition of OSC. Several small-molecule OSC inhibitors have been designed and synthesized for this purpose and are in various stages of preclinical and clinical evaluation.[2,16−22]

Exploiting this ongoing development effort, we envisaged that OSCs present in human pathogens might be inhibited by known OSC inhibitors, thus providing new leads to antibiotics and antiparasitic agents. Inhibition of the key sterol cyclization step in pathogens is attractive because it is downstream in the overall pathway. Therefore, microbes using either the mevalonate or methylerythritol phosphate pathways for isoprenoid product biosynthesis[23−25] would be targeted.

Results and Discussion. To test this hypothesis, we evaluated two known OSC inhibitors[16,22] (Figure 1) against several organisms in cell assays. For our initial experiments, we examined human pathogenic microorganisms from three groups. The first group included organisms known or suspected of utilizing OSC-like enzymes (*Pneumocystis* sp., *Leishmania* sp., *Trypanosoma* sp.).[7,9,13] In the second group was *Toxoplasma gondii*, which is now believed to rely minimally, if at all, on de novo sterol synthesis.[26,27] *Giardia lamblia*,[28] known not to biosynthesize sterols, was examined as a representative of group three. In these experiments, Chinese hamster ovary (CHO) cells provided a benchmark for effects on mammalian cells. Activities were noted to be consistent with what is known about sterol synthesis in these organisms. As shown in Figure 2, microorganisms with known sterol synthesis pathways are affected by the two OSC inhibitors with varying sensitivities. Thus, *T. cruzi* was more sensitive to **II**, while *P. carinii* was more sensitive to **I**. These two OSC

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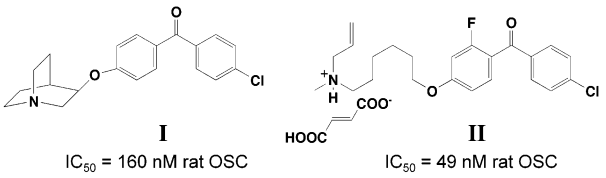


Figure 1. OSC inhibitors.

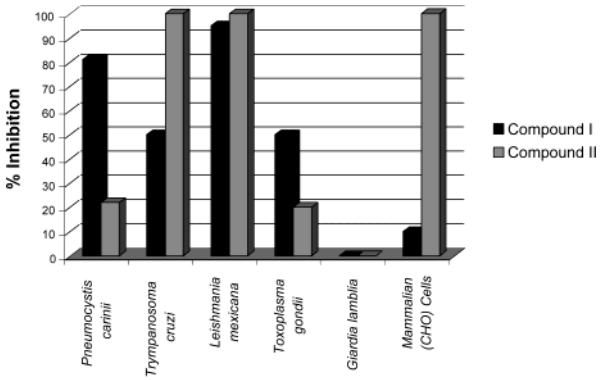


Figure 2. Effects of **I** and **II** on cell cultures at 10 μM.

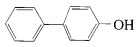
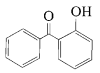
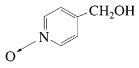
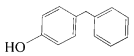
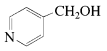
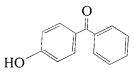
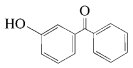
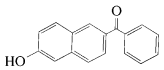
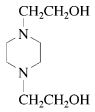
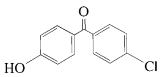
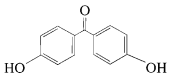
inhibitors did not affect the growth of *G. lamblia*, which does not synthesize sterols. The intermediate sensitivity of *Toxoplasma gondii* to either compound is more difficult to rationalize. This organism is cultured in CHO cells in order to provide its cholesterol requirements, and the observed effects may be the result of the influence of **I** or **II** on cholesterol availability to the organism from the mammalian cells. Alternatively, the data could reflect background sterol biosynthesis in the organism or interference with an unanticipated biochemical pathway. In any case, overall the results are consistent with inhibition of organism growth by way of inhibition of the parasite OSCs. The selectivity of **I** for some of the organisms compared to the mammalian CHO cells was encouraging.

Encouraged by these preliminary data, a small indexed combinatorial library of potential OSC inhibitors was prepared. Following the general structural features of **I** and **II** in which a tertiary amine functionality is linked to a hydrophobic partner, several commercially available amino alcohols were combined with a number of available phenols (Table 1) using Mitsunobu chemistry[29,30] (Scheme 2).

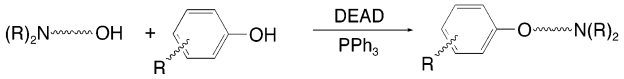
Each amino alcohol was coupled separately with an equimolar mixture of all the phenols, giving eight pools. Likewise, each phenol was coupled separately with an equimolar mixture of all the amines to give an additional eight pools. To avoid polymer formation, phenol **8** was not used with diol **G**. After reaction, pool components were readily separated from the reaction byproducts via aqueous acid extraction. ESI-MS analysis indicated the presence of the anticipated individual compounds within a given pool. The library of compounds was evaluated in enzyme assays in vitro against recombinant *P. carinii*, *T. brucei*, and *T. cruzi* OSCs,[8,9,31] and activity against purified rat OSC[32] was used to determine selective toxicity. Figure 3 summarizes the results.

Several of the pools showed activity, notably pools based on amines **A**, **G**, and **H**, as well as the pool from phenol **7**. However, significant activity against the mammalian enzyme is also evident. Interestingly, pools

Table 1. Combinatorial Library Building Blocks

Amines		Phenols	
(A)	$(CH_3)_2NCH_2CH_2OH$	(1)	
(B)		(2)	
(C)		(3)	
(D)		(4)	
(E)		(5)	
(F)	$(Et)_2NCH_2CH_2OH$	(6)	
(G)		(7)	
(H)	$(CH_3)_2NCH_2CH_2CH_2OH$	(8)	

Scheme 2. Combinatorial Pool Preparation



F1−**F8** and **A1**−**H1** also showed some activity against microbial enzymes with minimal effect on the mammalian OSC. On the basis of these results, selected individual compounds from each of the active pools were prepared and evaluated in the same enzyme assay. As a check, **B1** from a pool with minimal activity was also evaluated. The results are summarized in Figure 4.

F1, **G7**, **H1**, and **H7** showed potent inhibition at 2 μM. However, only **H1** exhibited appreciable microbial OSC selectivity relative to the mammalian enzyme. Therefore, the inhibitory effects of **H1** along with **I** and **II** on individual OSC enzymes were studied in more detail. As summarized in Table 2, **I** and **II** showed a 2- to 10-fold range of selectivity toward *T. brucei* and *T. cruzi* OSCs in this enzyme assay relative to rat OSC. **H1**, while less potent overall, still exhibited a 3- to 10-fold range of selectivity toward *Trypanosoma* OSCs in preference to rat OSC.

Next, we evaluated our library and compounds in pathogen cell cultures (Tables 3 and 4). Included in these experiments were *T. gondii* and *L. mexicana amazonensis* (organisms for which cyclase enzymes have

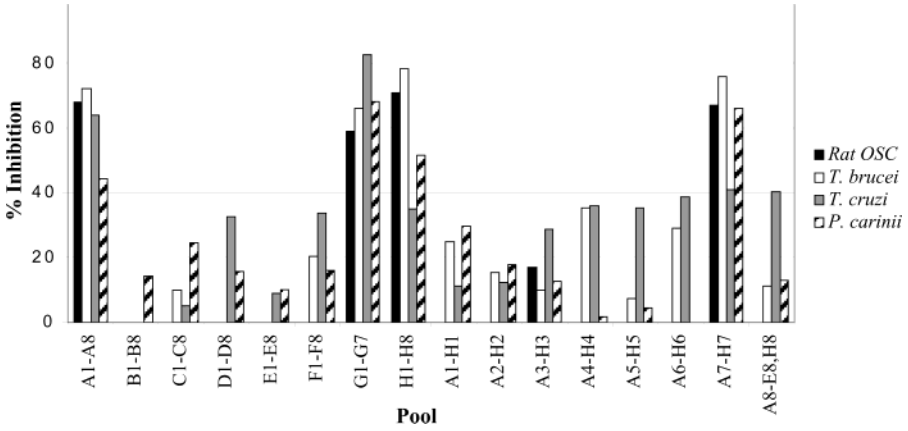


Figure 3. Inhibition of OSC enzymes by combinatorial pools, 2 μM.

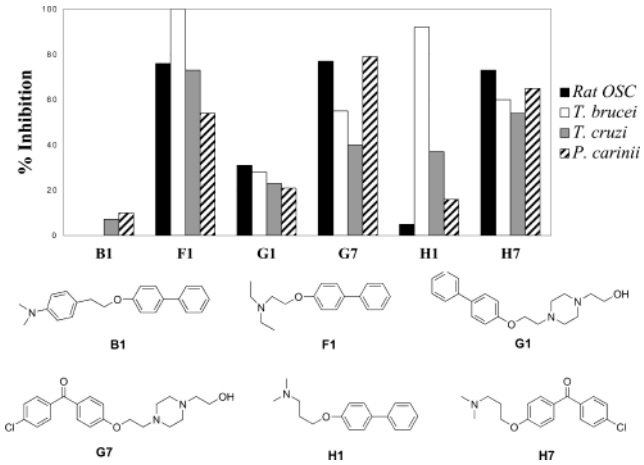


Figure 4. Inhibition of OSCs by selected compounds at 2 μM.

Table 2. Selective Inhibition of Human Pathogen OSCs

	IC_{50} (μM)[a]		
OSC	**I**	**II**	**H1**
rat	0.160 ± 0.052	0.0490 ± 0.021	2.50 ± 1.0
P. carinii	0.092 ± 0.042	0.0046 ± 0.0028	16.00 ± 5.0
T. brucei	0.022 ± 0.0035	0.0043 ± 0.0010	0.28 ± 0.15
T. cruzi	0.400 ± 0.13	0.0710 ± 0.014	0.75 ± 0.20

[a] IC_{50} values for purified enzymes.

not yet been isolated) and, for the mammalian comparison, NIH 3T3 fibroblasts. For *T. cruzi* and *P. carinii*, the results of the combinatorial pools and selected single compounds generally mirrored the enzyme study, while **I** and **F1** were especially effective against *L. m. amazonensis*. The same compounds were similarly active against *P. carinii* with effects comparable to the effects of the currently available drug pentamidine. Remarkably, minimal toxicity (>10 μM) was noted for all of the materials toward the murine fibroblasts. For *T. gondii*, no pools were especially effective, although differences in activity were observed. This result likely reflects the unimportance of de novo sterol biosynthesis by this organism.[26,27] None of the compounds were effective against *T. brucei*, consistent with the fact that the mammalian-stage form examined does not synthesize sterols.[33]

Conclusions. We have explored a new target for developing drugs to treat several important human pathogens. Small-molecule inhibitors of human OSC can also effectively inhibit microorganism OSC-like enzymes in vitro. Using an indexed combinatorial library approach, we identified several leads that were effective against *L. m. amazonensis*, *T. cruzi*, and *P. carinii* in cell culture assays with some selectivity for the patho-

Table 3. Combinatorial Pools and Selected Compounds Evaluated in Microorganism Assays

	ED_{50} (μM)			
compd	*T. brucei*	*T. cruzi*	*L. mexicana*	NIH 3T3
A1−A8	>10	1−10	2−10	>10
B1−B8	>10	>10	>10	>10
D1−D8	>10	>10	>10	>10
E1−E8	>10	10	10	>10
F1−F8	>10	>10	10	>10
G1−G8	>10	1−10	0.4−2	>10
H1−H8	>10	1−10	0.4−2	>10
A2−H2	>10	>10	>10	>10
A3−H3	>10	10	2	>10
A4−H4	>10	>10	2−10	>10
A5−H5	>10	10	>10	>10
A7−H7	>10	1−10	0.4−2	>10
I	>10	1−10	0.08−0.4	>10
H1	>10	1−10	0.4	>10
G1	>10	10	0.4−2	>10
G7	>10	10	0.4−2	>10
H7	>10	1−10	0.4−2	>10
F1	>10	10	0.08−0.4	>10

Table 4. Combinatorial Pools and Selected Compounds Evaluated in Microorganism Assays (nt = Not Tested)

	% inhibition (10 μM)	
compd	*Toxoplasma*	*P. carinii*
A1−A8	15	88
B1−B8	15	<1
C1−C8	2	51
D1−D8	nt	45
E1−E8	1	33
F1−F8	<1	95
G1−G8	28	95
H1−H8	17	95
A2−H2	11	17
A3−H3	<1	4
A4−H4	11	21
A5−H5	9	4
A6−H6	8	13
A7−H7	30	92
A8−E8, H8	12	52
I	48	81
H1	nt	57
G1	nt	48
G7	nt	20
H7	nt	4
F1	nt	92
pentamidine	nt	90

gen over mammalian cells. As expected, organisms without an active sterol biosynthetic pathway were not targeted by this strategy. It is clear that the approach we have identified provides a new molecular target for the development of novel antimicrobial agents for human infections.

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Supporting Information Available: Experimental details for compound synthesis and assays are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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