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Targeting Isoprenoid Biosynthesis for Drug Discovery: Bench to Bedside

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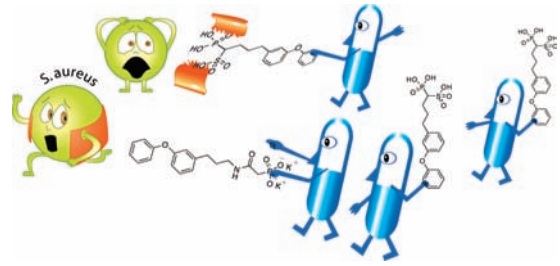

The isoprenoid biosynthesis pathways produce the largest class of small molecules in Nature: isoprenoids (also called terpenoids). Not surprisingly then, isoprenoid biosynthesis is a target for drug discovery, and many drugs—such as Lipitor (used to lower cholesterol), Fosamax (used to treat osteoporosis), and many anti-infectives—target isoprenoid biosynthesis. However, drug resistance in malaria, tuberculosis, and staph infections is rising, cheap and effective drugs for the neglected tropical diseases are lacking, and progress in the development of anticancer drugs is relatively slow. Isoprenoid biosynthesis is thus an attractive target, and in this Account, I describe developments in four areas, using in each case knowledge derived from one area of chemistry to guide the development of inhibitors (or drug leads) in another, seemingly unrelated, area.

First, I describe mechanistic studies of the enzyme IspH, which is present in malaria parasites and most pathogenic bacteria, but not in humans. IspH is a 4Fe−4S protein and produces the five-carbon (C5) isoprenoids IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate) from HMBPP (*E*-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate) via a $2H^+/2e^-$ reduction (of an allyl alcohol to an alkene). The mechanism is unusual in that it involves organometallic species: "metallacycles" (η^2-alkenes) and η^1/η^3-allyls. These observations lead to novel alkyne inhibitors, which also form metallacycles. Second, I describe structure−function−inhibition studies of FPP synthase, the macromolecule that condenses IPP and DMAPP to the sesquiterpene farnesyl diphosphate (FPP) in a "head-to-tail" manner. This enzyme uses a carbocation mechanism and is potently inhibited by bone resorption drugs (bisphosphonates), which I show are also antiparasitic agents that block sterol biosynthesis in protozoa. Moreover, "lipophilic" bisphosphonates inhibit protein prenylation and invasiveness in tumor cells, in addition to activating $\gamma\delta$ T-cells to kill tumor cells, and are important new leads in oncology.

Third, I describe structural and inhibition studies of a "head-to-head" triterpene synthase, dehydrosqualene synthase (CrtM), from *Staphylococcus aureus*. CrtM catalyzes the first committed step in biosynthesis of the carotenoid virulence factor staphyloxanthin: the condensation of two FPP molecules to produce a cyclopropane (presqualene diphosphate). The structure of CrtM is similar to that of human squalene synthase (SQS), and some SQS inhibitors (originally developed as cholesterol-lowering drugs) block staphyloxanthin biosynthesis. Treated bacteria are white and nonvirulent (because they lack the carotenoid shield that protects them from reactive oxygen species produced by neutrophils), rendering them susceptible to innate immune system clearance—a new therapeutic approach. And finally, I show that the heart drug amiodarone, also known to have antifungal activity, blocks ergosterol biosynthesis at the level of oxidosqualene cyclase in *Trypanosoma cruzi*, work that has led to its use in the clinic as a novel antiparasitic agent.

In each of these four examples, I use information from one area (organometallic chemistry, bone resorption drugs, cholesterol-lowering agents, heart disease) to develop drug leads in an unrelated area: a "knowledge-based" approach that represents an important advance in the search for new drugs.

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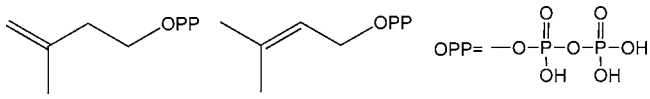
Introduction

Isoprenoids represent the largest class of small molecules on earth,[1] so it is not surprising that many of the enzymes that are involved in isoprenoid biosynthesis are drug targets. For example, the most widely prescribed drug, Lipitor, targets cholesterol biosynthesis at an early stage; bisphosphonates, such as Fosamax, used to treat bone resorption diseases, target the middle of the isoprenoid biosynthesis pathway, while anti-infectives such as terbinafine (Lamisil) target the later stages of sterol biosynthesis in fungi and yeasts. The early stages of isoprenoid biosynthesis involve formation of isopentenyl diphosphate (**1**, IPP) and dimethylallyl diphosphate (**2**, DMAPP):



1, IPP **2**, DMAPP

In most pathogenic bacteria, these molecules are produced in the Rohmer or nonmevalonate pathway,[2] but in humans and in bacteria such as *Staphylococcus aureus*, they are formed in the mevalonate pathway.[3] The last two enzymes in the nonmevalonate pathway, IspG and IspH, contain Fe_4S_4 clusters[4,5] and carry out $2H^+/2e^-$ reductions, converting 2-*C*-methyl-D-erythritol-2,4-cyclo-diphosphate (MEcPP, **3**) to HMBPP (*E*-1-hydroxy-2-methyl-but-2-enyl 4-diphosphate, **4**) and HMBPP to IPP and DMAPP, Scheme 1.

Once formed, IPP and DMAPP condense via a "head-to-tail" mechanism to form geranyl diphosphate (**5**) and farnesyl diphosphate (**6**) in reactions catalyzed by the enzyme farnesyl diphosphate synthase (FPPS), and further reaction with IPP catalyzed by the enzyme geranylgeranyl diphosphate synthase (GGPPS) yields the C_{20} species, geranylgeranyl diphosphate (GGPP, **7**),[6,7] Scheme 2.

Both FPP and GGPP are used in protein prenylation (of importance in cell survival and signaling pathways), and FPP can also condense in a "head-to-head" manner via presqualene diphosphate (PSPP, **8**)[8] to form triterpenes, Scheme 3.

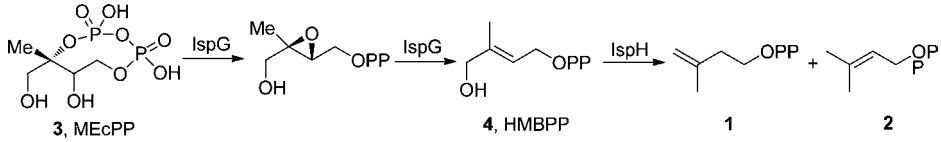
In humans, this condensation is accompanied by an NADPH reduction step and results in formation of squalene (**9**),[9] but in the bacterium *S. aureus*, the reduction step is missing and the enzyme CrtM converts FPP to *dehydro*squalene (**10**).[10] In many organisms, squalene is epoxidized to form oxidosqualene (**11**), which is then cyclized to form lanosterol (**12**), which after several additional steps, is transformed into cholesterol (**13**) in humans or ergosterol (**14**) or episterol (**15**) in yeasts, fungi, and parasitic protozoa. In *S. aureus,* **10** is also converted to a carotenoid pigment, staphyloxanthin (**16**),[10] an important virulence factor. The enzymes involved in these reactions are our targets, and I describe here our progress in understanding their structures, mechanisms of action, and inhibition, focusing on the use of a less-conventional, knowledge-based approach to inhibitor or drug discovery.

IspH (LytB), an Fe_4S_4-Cluster-Containing Enzyme

The IspH enzyme is found in the vast majority of pathogenic bacteria,[11] as well as in malaria parasites,[12] and since it is not found in humans and is essential for pathogen survival, it is an important target for anti-infective development. Working with Jomaa and Ermler, we reported[13] that the enzyme has a unique, trefoil-like structure, Figure 1A,B, with a central Fe_3S_4 cluster, and a similar structure was then reported by Grawert et al.[14] The observation that both proteins contained 3Fe and not 4Fe was inconsistent with the results of electron paramagnetic resonance (EPR),[5] chemical analysis,[5,15] and activity[5,15] results, which all pointed to an Fe_4S_4 cluster, so we next used computational methods to construct an Fe_4S_4 model, with the HMBPP substrate docking to the unique fourth Fe in oxidized IspH, via its 1-OH group, initially as an alkoxide,[13] Figure 1C.

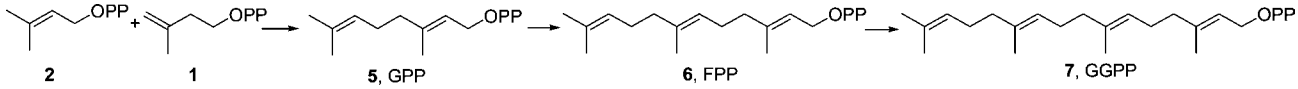
Interestingly, very recent X-ray crystallographic results[16] have shown that HMBPP does in fact bind to the 4Fe cluster

SCHEME 1. Formation of Isopentenyl Diphosphate (**1**) and Dimethyallyl Diphosphate (**2**) in the Nonmevalonate Pathway



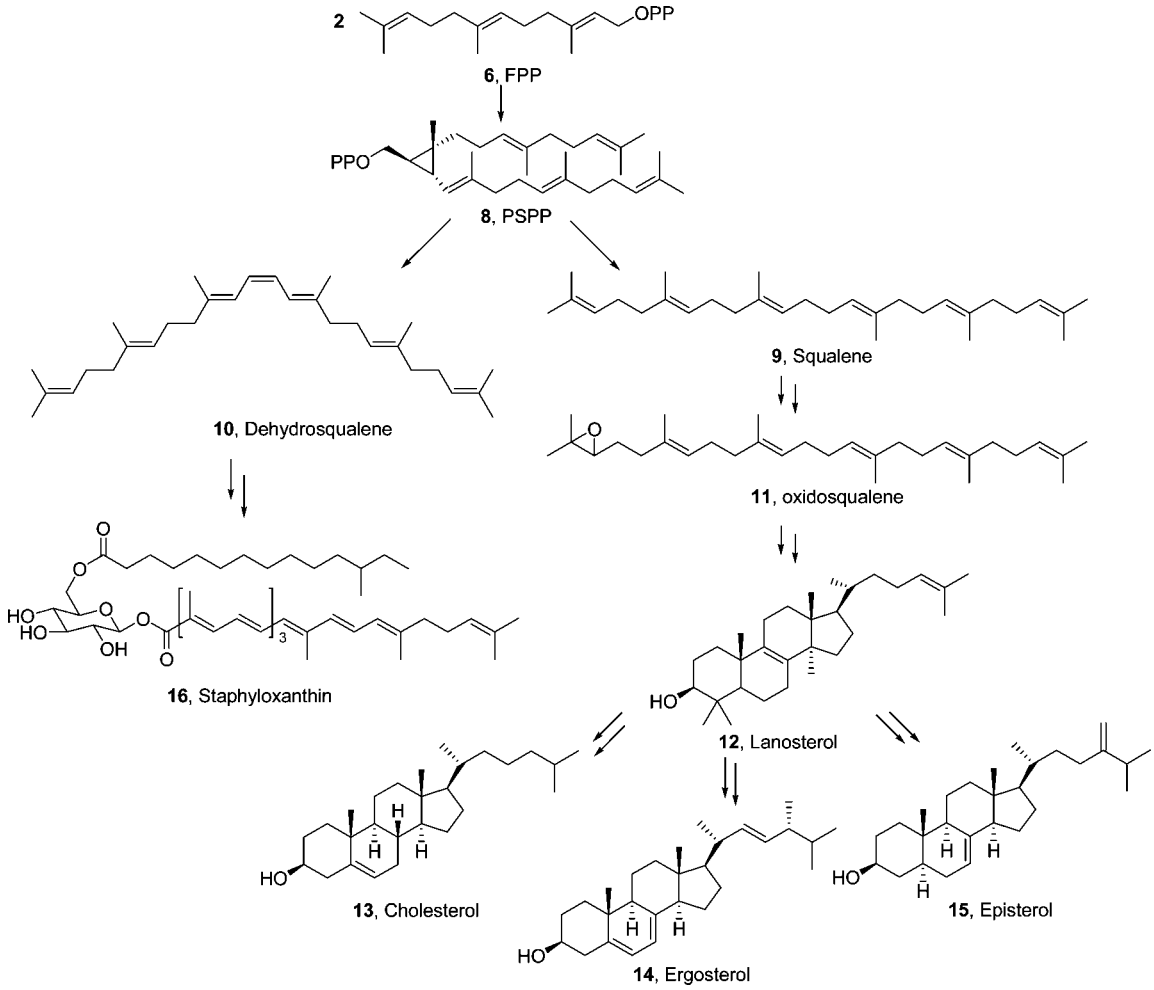
3, MEcPP **4**, HMBPP **1** **2**

SCHEME 2. Formation of Farnesyl Diphosphate (6) and Geranylgeranyl Diphosphate (7)



2 **1** **5**, GPP **6**, FPP **7**, GGPP

SCHEME 3. Formation of Triterpenes from Farnesyl Diphosphate (**6**)



in IspH via O-1 (as we proposed), and the structure of HMBPP bound to the Fe_4S_4 cluster that we deduced[13] from computational docking is very similar to that determined by crystallography, Figure 1D (a 0.3 Å ligand rmsd). Apparently then, the 4Fe cluster can be stabilized by ligands binding to the fourth Fe, although the reason for this is not yet known. But how does this Fe_4S_4 cluster catalyze the $2H^+/2e^-$ reduction, the removal of the 1-OH oxygen, to form the IPP and DMAPP products? Based on our crystallographic results and on bioinformatics, we proposed[13] that E126 was a key residue in catalysis, providing the H^+ needed for activity. The essential nature of E126 was then demonstrated in later work by others,[14] and we reasoned that by using an inactive IspH mutant (E126A), it might be possible to "trap" a reaction intermediate, which would give clues as to the catalytic mechanism, if its structure could be deduced. To do this, we used EPR and electron-nuclear double resonance (ENDOR) spectroscopy.[17]

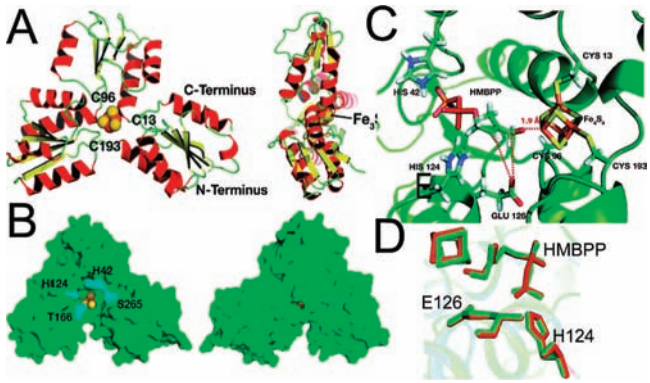


FIGURE 1. Structural results for IspH (LytB): (A,B) crystal structure results for *Aquifex aeolicus* IspH; (C) initial docking pose for HMBPP to oxidized IspH Fe_4S_4 cluster obtained by using the "open-form" structure; (D) Comparison of HMBPP bound to IspH from X-ray[16] (green) and docking[13] (red). From refs 13 and 16, with permission. Copyright 2008 American Chemical Society and 2010 National Academy of Sciences, U.S.A.

Simply adding HMBPP to reduced IspH yielded an EPR spectrum that was essentially the same as that obtained on adding the IPP product (Figure 2A). However, the EPR spectrum obtained when using the E126A mutant was very different, exhibiting *g*-values of 2.124, 1.999 and 1.958, and had

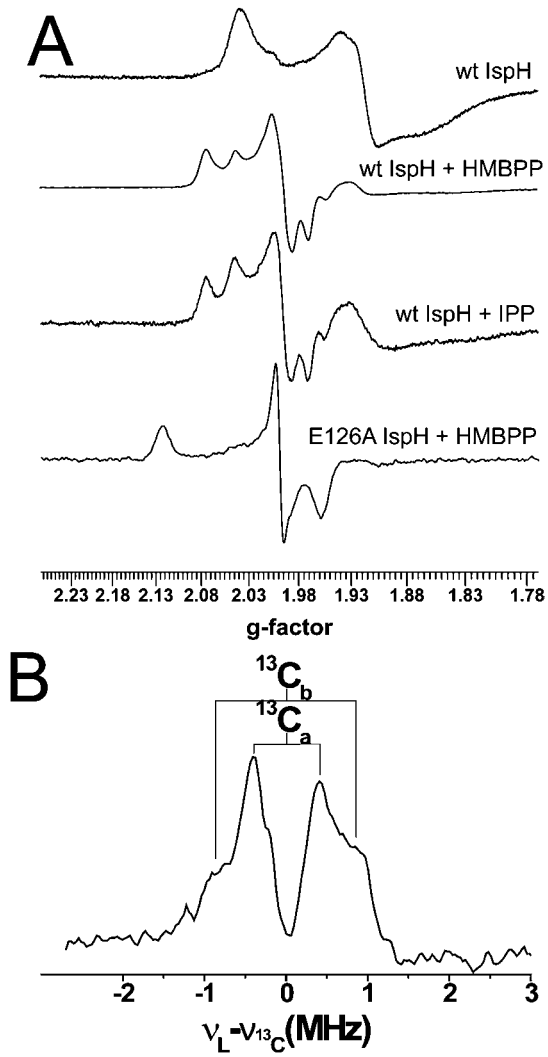


FIGURE 2. EPR and ENDOR results for IspH: (A) EPR spectra of IspH (and an E126A mutant) with and without ligands; (B) ENDOR spectrum with [u-^{13}C]-**26**. From ref 17 with permission. Copyright 2010 National Academy of Sciences.

similarities to the EPR spectra of the HMBPP "parent" molecules, ethylene (**17**) and allyl alcohol (**18**), when bound to a nitrogenase FeMO cofactor.[18,19]

In nitrogenase, the results of both ENDOR[18,19] and DFT calculations[20] indicated that both of these species (**17**, **18**) bind to one of the Fe atoms in the FeMo cofactor cluster, forming π complexes, η^2-alkenyl "metallacycles" (**19**, **20**), Scheme 4, and it seemed possible that this might occur with the Fe_4S_4 cluster in IspH as well. A prediction of this binding mode is that there would be substantial hyperfine interactions in the ENDOR spectrum, and as shown in Figure 2B, this is clearly the case with [u-^{13}C]-HMBPP, with hyperfine couplings for ^{13}C being observed, consistent with the idea that HMBPP (**4**) binds to the $[Fe_4S_4]^+$ cluster as the metallacycle **21**. This opens up the possibility that this binding mode might in reduced IspH "activate" the molecule such that on protonation (by the E126 CO_2H), an η^1-allyl complex **22** or the η^3 π-allyl complex **23** can form, Figure 3. On reduction and protonation at C2, the IPP product forms, while protonation at C4 would form DMAPP, Figure 3A,B, an organometallic as opposed to a purely radical mechanism for catalysis.[17] These spectroscopic results suggest the likely importance of organometallic intermediates in IspH catalysis, which leads to a new idea for inhibitor design, based on organometallic precedent.

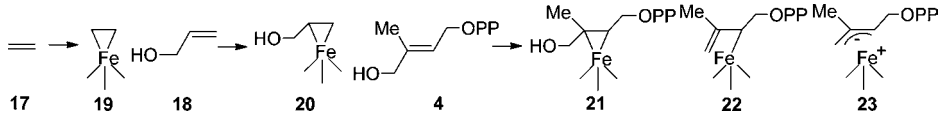
In previous work, several groups reported that alkynes could be *cis*-reduced by "model" Fe_4S_4 clusters such as $[Fe_4S_4(SPh)_4]^{2-/3-}$ to form olefins,[21,22] and it was proposed that binding might occur via an η^2-alkynyl species, another π or π/σ "metallacycle." These observations lead to the idea that alkynes might also bind to reduced IspH and would inhibit catalytic activity.

To test this idea, we obtained the EPR and ENDOR spectra of the alkynes **24** and **25**,[17] Scheme 5, bound to IspH. Both bound but were poor inhibitors. However, with propargyl diphosphate **26**, there were large changes in the EPR spectra, and the ENDOR spectra of [$^{13}C_3$]-**26** bound to IspH[17] (e.g., Figure 4A) exhibited large hyperfine couplings (~6 MHz for ^{13}C, ~10 MHz for ^{1}H).[17] Compound **26** was also a $K_i \approx 970$ nM IspH inhibitor (Figure 4B), ~1000× more active than previously reported inhibitors.[23] A likely explanation of this good inhibition is formation of the π/σ metallacycle **27**, in which the alkyne can bind to the unique fourth Fe, Figure 4C, opening up, potentially, a new route to anti-infective development.

FPPS (and GGPPS): Structure, Mechanism, and Inhibition by Lipophilic Bisphosphonates

The IPP and DMAPP produced by either the nonmevalonate or mevalonate pathways are next condensed by FPPS and GGPPS to form FPP and GGPP, Scheme 2. FPPS is the target of the bisphosphonate class of drugs used to treat bone resorption diseases, but for many years, their mechanism of action was unknown. Our interest in these systems arose from several chance observations. First, working with Urbina and Docampo, we found[24] that *Trypanosoma cruzi* contained very high levels of condensed phosphates, such as diphosphate **28**, Scheme 6. This led to the idea that nonhydrolyzable PPi analogs, bisphosphonates such as pamidronate (**29**) and risedronate (**30**, Actonel), might inhibit parasite cell growth. This turned out to be the case,[24,25] but the target was not known! The second chance observation was that we noticed that nitrogen-containing analogs of GPP such as **31**, known to be potent, low nanomolar inhibitors of terpene cyclases, looked

SCHEME 4. Schematic Illustration of π/σ Bioorganometallic Species in Nitrogenase and IspH



suspiciously like the bisphosphonate ibandronate, **32**, as did their electrostatic potential surfaces, Φ(r), Figure 5A,B.[26] This suggested that cationic bisphosphonates might act as carbocation/diphosphate isosteres, inhibiting isoprenoid biosynthesis, a view supported by the observation that bisphosphonates were reported to act in the mevalonate pathway.[27] The third observation was that bisphosphonates such as **33** had been developed by Zeneca as herbicides[28] and had been shown to be low nanomolar inhibitors of a daffodil FPPS.[28] Since we noticed that **33** had also been shown[29] to be active in bone resorption, we proposed[26] that the bone-resorption drugs might act by inhibiting FPPS, mimicking a carbocation reactive intermediate (**34**), Scheme 7, docking into the allylic site in FPPS, Figure 5C.[26] The FPPS target was soon confirmed,[30−32] and the allylic binding mode that we proposed was later confirmed crystallographically by Hosfield et al.[33] (Figure 5D). In later work, we also showed that pamidronate provided a parasitological cure of cutaneous leishmaniasis in mice, Figure 6A,B,[34] by blocking FPPS and, thus, ergosterol biosynthesis,[25] opening up the possibility of the clinical use of bisphosphonates as anti-infectives.[35]

In addition to their activity as bone resorption drugs and antiparasitics, bisphosphonates kill tumor cells,[36] and they activate $\gamma\delta$ T cells[37] to also kill tumor cells.[38] There is, therefore, interest in developing bisphosphonates as anticancer drugs, and the results of small clinical trials on pamidronate,[39] as well as zoledronate (+ interleukin-2),[40] have shown promise. More recently, the results of a much larger scale study, of 1803 patients with breast cancer, showed a 30% decrease in the recurrence of disease in patients treated postsurgery with an aromatase inhibitor plus zoledronate (**35**).[41] Conventional bisphosphonates are, however, rapidly removed from the circulation (in <1 h), binding to bone mineral. We reasoned that

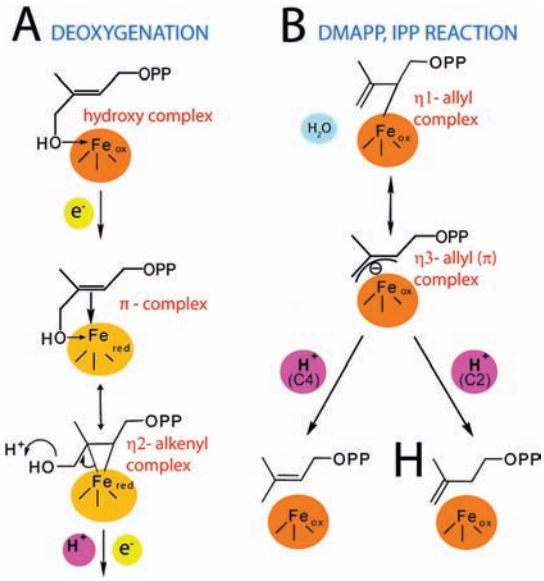
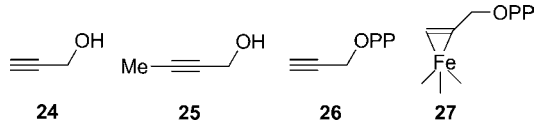


FIGURE 3. IspH mechanism proposal: (A) deoxygenation steps; (B) reductive cleavage forming IPP, DMAPP from allyl species. From ref 17 with permission. Copyright 2010 National Academy of Sciences.

SCHEME 5. Acetylene Inhibitors of IspH and Proposed Binding Mode



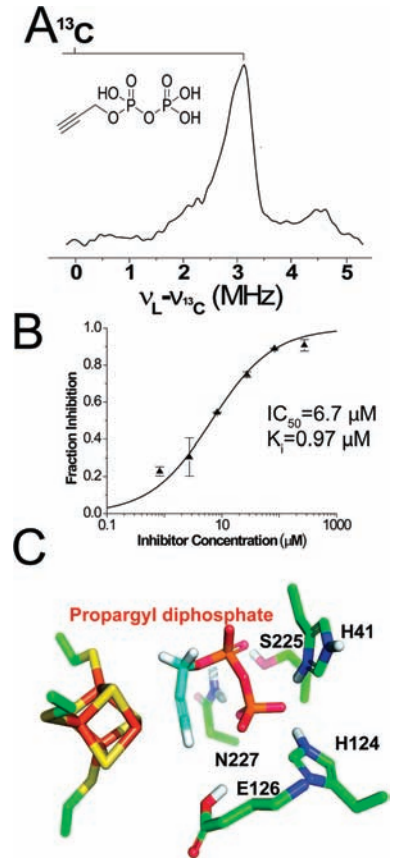


FIGURE 4. IspH inhibition by the alkyne diphosphate, **26**: (A) 9 GHz ENDOR spectrum of [u-^{13}C]-propargyl diphosphate (**26**) showing ~6 MHz ^{13}C hyperfine coupling; (B) dose−response curve showing IspH inhibition by **26**; (C) docking results showing close apposition of the alkyne group to the unique, fourth Fe in IspH. From ref 17 with permission. Copyright 2010 National Academy of Sciences.

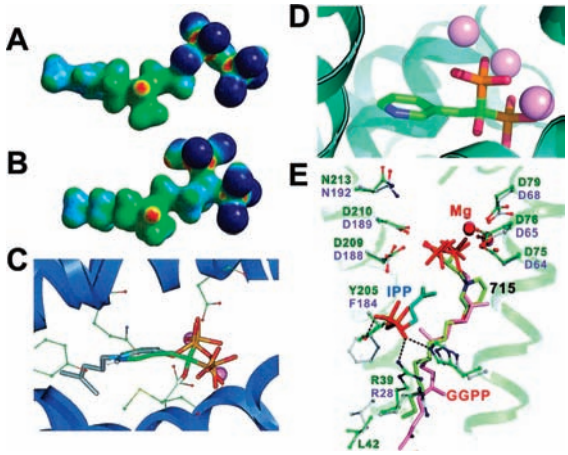
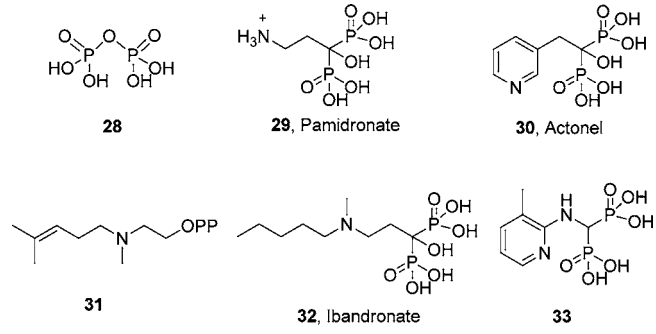
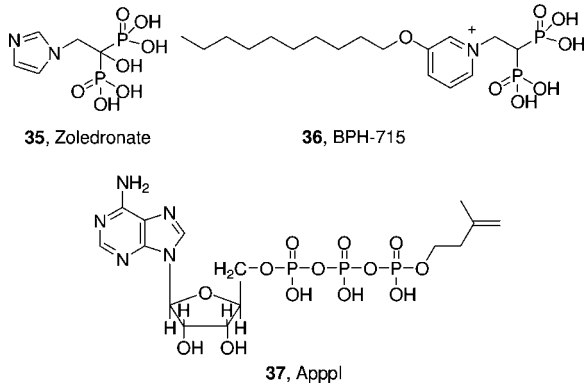


FIGURE 5. Cationic bisphosphonates as FPPS/GGPPS inhibitors. (A, B) $\Phi(r)$ electrostatic potential surfaces for an ammonium diphosphate-based terpene cyclase inhibitor (A) and ibandronate (B); (C) early model for bisphosphonate inhibition of FPPS;[26] (D) crystal structure showing a similar pose as in panel C; (E) BPH-715 bound to GGPPS.[42] From refs 26 and 42 with permission. Copyright 1999 Elsevier and Copyright 2009 American Chemical Society.

SCHEME 6. Structures of Diphosphate, Several Bisphosphonates, and a Terpene Cyclase Inhibitor



removing the 1-OH group would reduce bone-binding, and adding more hydrophobic substituents would enhance cell or tissue penetration, so that species such as BPH-715 (**36**) would be more potent inhibitors of tumor cell growth.



This turned out to be the case,[42] with **36** killing tumor cell lines with an IC_{50} of ~100 nM, at least 100× lower than that found with the bisphosphonate zoledronate (**35**). Compound **36** also blocked tumor cell invasion,[42] and it was a potent activator of $\gamma\delta$ T cells,[43] in addition to having good activity, *in vivo*.[42]

The enhanced activity of **36** is likely due to several factors. First, it inhibits FPPS,[42] which results in blocking protein (e.g., K-ras) prenylation. Second, since it is lipophilic, it gets into cells more readily than do more polar analogs. Third, when FPPS is inhibited, the substrates IPP and DMAPP build up, and these are converted to toxic ATP analogs[44] such as Apppl (**37**).[44] Fourth, the buildup of IPP (and DMAPP) in tumor cells on FPPS inhibition leads to activation of $\gamma\delta$ T cells, since both IPP and DMAPP are so-called "phosphoantigens".[45] Fifth, lipophilic bisphosphonates inhibit GGPPS by docking into the product binding site (Figure 5E),[42,46] and the combined effects of FPPS and GGPPS inhibition are likely synergistic (preventing cross-prenylation). When combined with poor bone-binding, this leads to potent *in vivo* activity.

Dehydrosqualene Synthase (CrtM) and Staphyloxanthin: An Anti-Virulence Approach to Staph Infections

In humans, most FPP is converted via the "head-to-head" triterpene synthase, squalene synthase (SQS), to squalene **9**. While involved in some "recreational" reading, I noticed an

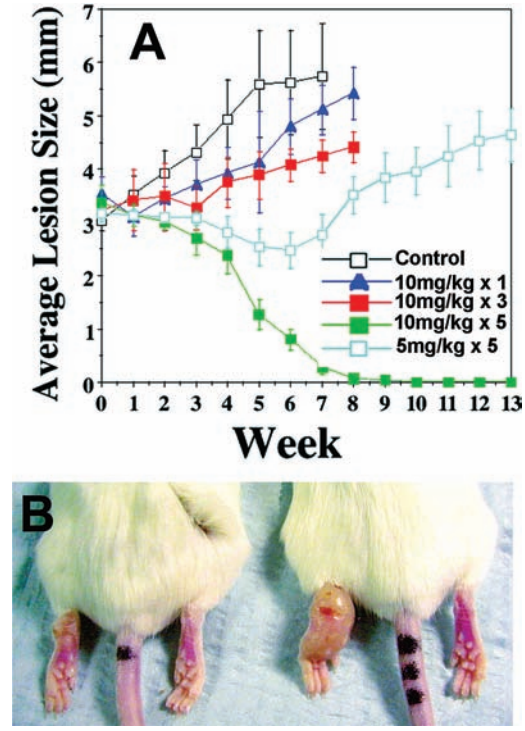
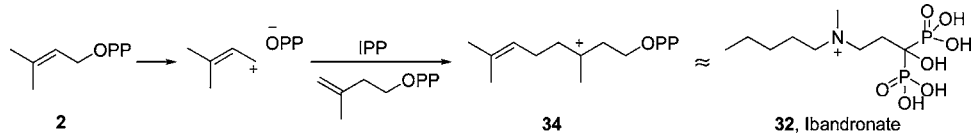
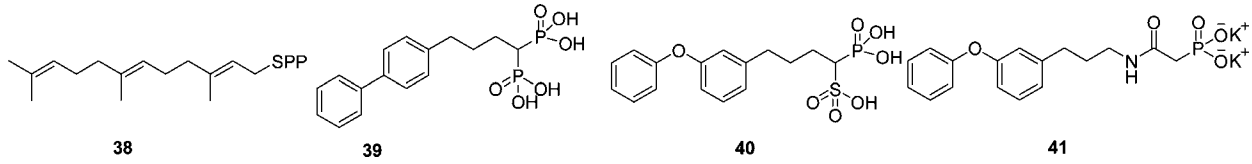


FIGURE 6. Effects of the bisphosphonate pamidronate (**29**) on cutaneous leishmaniasis (*Leishmania mexicana*) in mice: (A) effects of pamidronate dose on lesion progression; (B) cure of infection in treated mouse (on the left). From ref 34 with permission. Copyright 2002 Infectious Diseases Society of America.

SCHEME 7. Proposed Carbocation Mechanism for FPPS Catalysis and Similarity between a Transition State/Reactive Intermediate and the Bisphosphonate Drug Ibandronate



SCHEME 8. Some Inhibitors of the CrtM Enzyme from *S. aureus*



article[47] reviewing work[48] by Nizet and Liu on the role of the carotenoid virulence factor, staphyloxanthin (**16**), in *S. aureus*. This compound is a golden carotenoid pigment found only in *S. aureus*, the causative agent of staph infections. These workers showed that the pigment acts as a "protective shield", preventing the organism from being killed by host immune cells that generate reactive oxygen species (such as O_2^-, ClO•, H_2O_2), which are thought to be "deactivated" by reacting with the polyene. What caught my attention was that the initial step in staphyloxanthin biosynthesis involved exactly the same first step as in cholesterol/ergosterol biosynthesis: FPP (**6**) → PSPP (**8**). I knew from my work with Urbina that many drug leads targeting SQS had been developed by the pharmaceutical industry as cholesterol-lowering agents, and after an examination of the amino acid sequences of the *S. aureus* dehydrosqualene synthase (called CrtM) and human squalene synthase, it seemed that both enzymes would have similar three-dimensional structures. I posited that the bacterial enzyme would be inhibited by the compounds that had already been developed as cholesterol-lowering drugs. As anticipated, we found (with Liu and Wang) that the 3D structure of CrtM[49] was very similar to that found with human SQS (Figure 7A), and using a nonreactive, sulfur-containing analog of FPP, *S-thiolo*-FSPP (**38**), Scheme 8, we found two "FPP" binding sites, as hoped (Figure 7B). We then synthesized a range of potential CrtM inhibitors, compounds that had all been developed as SQS inhibitors, and tested them for CrtM inhibition. The most potent inhibitors were bisphosphonates such as **39**. However, they did not block staphyloxanthin (**16**) formation in cells. On the other hand, phosphonosulfonates (**40**) and phosphonoacetamides (**41**) inhibited both CrtM activity *in vitro* and staphyloxanthin biosynthesis in *S. aureus*, with the crystallographic results showing that they bound to one or the other FPP sites, Figure 7C.[49] When *S. aureus* is stripped of its protective carotenoid shield, cells grow normally *in vitro* since virulence factors are not essential for cell growth. However, the cells are white (Figure 8A), and when the cells are exposed to reactive oxygen species, either from H_2O_2 or by adding neutrophils, cell growth is greatly inhibited (Figures 8B).[49−51] Moreover, in mice (Figure 8C), we found a 98% decrease in *S. aureus* in the kidneys[49] on treatment with **40**. These results are of interest since they represent a potentially new, highly selective approach to blocking staph infections in which cells are made highly susceptible to killing by the host's

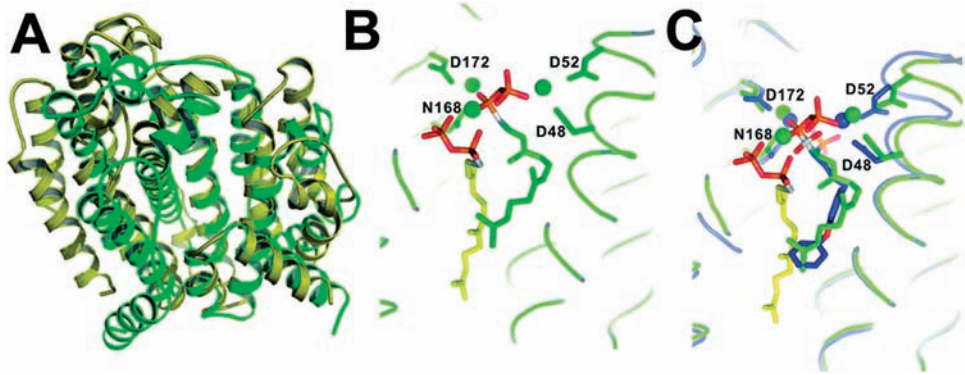


FIGURE 7. CrtM as a target for antivirulence therapy: (A) comparison between CrtM (green) and SQS (yellow) structures; (B) FSPP (two molecules) bound to CrtM; (C) BPH-652 (**40**, in blue) bound to CrtM. The two FSPP molecules (green, yellow) are also shown. From ref 49 with permission. Copyright 2008 AAAS.

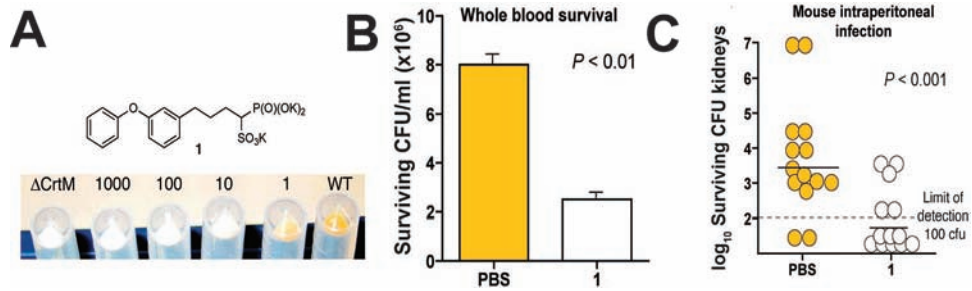
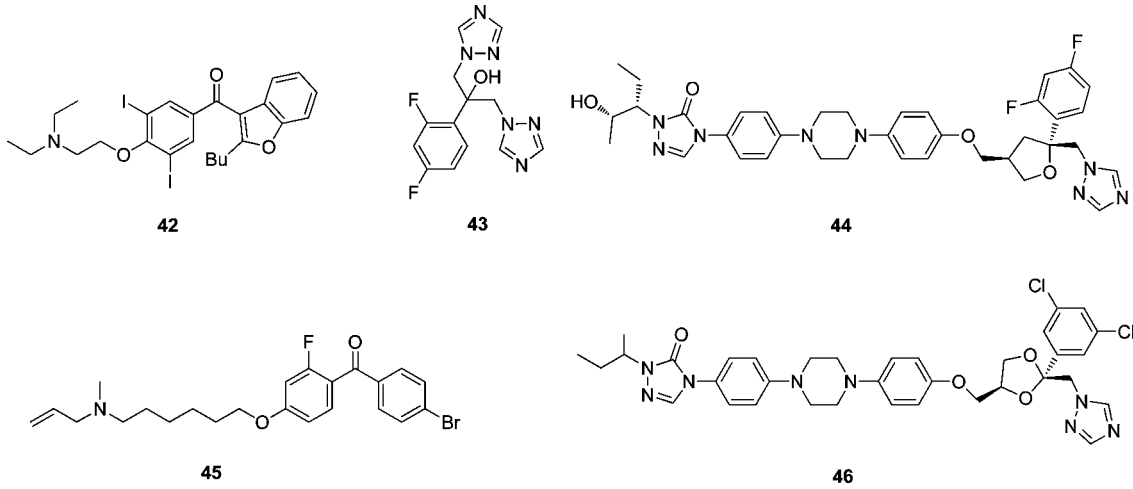


FIGURE 8. Effects of BPH-652 (**40**) on staphyloxanthin biosynthesis and *S. aureus* infection: (A) BPH-652 blocks staphyloxanthin biosynthesis in cells; BPH-652 (B) renders staph susceptible to killing by neutrophils in blood and (C) reduces infectivity in mice by 98%. From ref 49 with permission. Copyright 2008 AAAS.

SCHEME 9. Some Sterol Biosynthesis Inhibitors



innate immune system. And of course the fact that **40** has already been tested for safety in clinical trials (as a cholesterol lowering agent)[52] makes it of particular interest.

Using the Heart Drug Amiodarone as an Anti-infective against Chagas Disease and Leishmaniasis

After condensing FPP to squalene, humans, plants, fungi, and yeasts, as well as the pathogenic protozoa *T. cruzi* and *Leishmania mexicana*, carry out an epoxidation to form oxidosqualene (**11**), which is then cyclized to form lanosterol (**12**). Again while perusing one of the more populist journals, my attention was drawn to an article[53] reporting observations by Courchesne[54] and Gupta et al.[55] that the class III antiarrhythmia drug amiodarone (**42**), Scheme 9, had unexpected activity against baker's yeast. An effect on Ca^{2+} channels was shown, but what was more surprising was that cell growth inhibition activity was synergistic with the azole antibiotics that are commonly used to treat yeast or fungal infections, drugs such as such as fluconazole (**43**). It seemed likely to me that ergosterol biosynthesis might be involved. I e-mailed Urbina to see whether we should try amiodarone in *T. cruzi*. His response was encouraging: "We are going to pursue vigorously this lead against trypanosomatid parasites, especially because amiodarone is relatively cheap and non-toxic and, most interestingly, is frequently prescribed to Chagas disease patients to control their cardiac arrythmias!!!"

We screened amiodarone in *T. cruzi* finding that[56] (i) it killed *T. cruzi*, (ii) it blocked ergosterol biosynthesis, (iii) it acted at the level of oxidosqualene cyclase, OSC, (iv) it synergized with the azole posaconazole (**44**), (v) it blocked Ca^{2+} channels in *T. cruzi* much more effectively than in host cells, (vi) posaconazole, which blocks ergosterol biosynthesis at the lanosterol 14-α demethylase level, also blocked the parasites' Ca^{2+} channels, (vii) there were very good parasitological cures of mice treated with the combination therapy of amiodarone and posaconazole, and (viii) in addition, molecular docking results for lanosterol and a known OSC inhibitor (Ro48-8071, **45**) docked to an OSC showed good accord with the known crystallographic structures and amiodarone bound into the same site.

There was then an apparent lull in activity, but in very recent work, Serrano-Martin et al.[57] have reported that

amiodarone has similar effects in *L. mexicana*,[57] blocking ergosterol biosynthesis and inhibiting cell growth. More importantly, Paniz-Mondolfi et al.[58] have begun to report the results of small clinical trials of amiodarone (with and without itraconazole, **46**). In one case, a patient had concurrent Chagas disease and cutaneous leishmaniasis. This is a difficult combination to treat since the standard drugs used to treat leishmaniasis are antimonials, which are problematic in patients with cardiac arrythmias (as in Chagas disease). The patient was treated with amiodarone to stabilize the heart condition, but remarkably, the cutaneous lesions also healed, without use of any specific antileishmanial therapy.[58] In a second study,[59] the combination amiodarone and itraconazole was used to treat a Chagas disease patient, with a parasitological cure of the *T. cruzi* infection being reported.[59] In addition, a 100% cure rate has now been found in 11 patients with cutaneous leishmaniasis.[60] This efficacy is very high and is thought to be due, at least in part, to the very unusual excretion mechanism for amiodarone, through the skin![61]

Concluding Remarks and Perspectives

The results described above give a brief summary of the last 10 years work in our laboratory on isoprenoid biosynthesis enzymes, which has focused on discovering new drug targets, mechanisms, and inhibitors. The results with the Fe_4S_4 cluster-containing protein IspH seem radical but are simply based on precedent (ethylene, allyl alcohol nitrogenase ENDOR, and DFT) and have led to the first micromolar IspH inhibitors and a new proposal for catalysis, involving organometallic species. With the head-to-tail synthases FPPS (and GGPPS), there are now ~60 crystallographic structures reported, including some with the novel, lipophilic bisphosphonates, which now await more extensive preclinical testing. With CrtM, we have the first structure of a head-to-head triterpene synthase containing bound substrate analogs, together with novel inhibitors. These block *S. aureus* proliferation *in vivo*, and one has already been tested for safety in humans (in the context of its role as a cholesterol-lowering drug). And finally, we discovered another drug "repurposing": the use of the antiarrythmia drug, amiodarone, as an agent against both Chagas disease and cutaneous leishmaniasis. Since Chagas disease affects ~10 000 000 individuals in South America and there is no cure for the chronic stage of the disease (the leading cause of sudden death on the subcontinent), the combination of amiodarone plus an azole is of considerable interest, as is its use alone in treating some forms of cutaneous leishmaniasis.

In each of the examples described above, we have used a knowledge-based approach, rather than purely screening-based methods, to find new leads in which we use information from one area of research to suggest drug (or inhibitor) leads in another, seemingly unrelated area. Since terpenes or isoprenoids are the largest class of small molecules known and their biosynthesis is already the target for many current drugs, it seems likely that many new drugs will be found that target their formation, but as Pasteur famously said: "Chance favors only the prepared mind".

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BIOGRAPHICAL INFORMATION

Eric Oldfield was born in London, England, in 1948. He obtained a B.Sc. degree from Bristol University in 1969 and a Ph.D. degree from Sheffield University in 1972, with Dennis Chapman. After postdoctoral work with Adam Allerhand at Indiana University and with John S. Waugh at MIT, he joined the Chemistry Department at the University of Illinois at Urbana−Champaign in 1975, where he is currently the Alumni Research Scholar Professor of Chemistry. He has been the recipient of ACS's Award in Pure Chemistry, RSC's Meldola Medal, the Biochemical Society's Colworth Medal, the American Heart Association's Katz Basic Science Research Prize, and the RSC Awards in Spectroscopy and in Soft Matter and Biophysical Chemistry.

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