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Meeting Review

Natural Product Drug Discovery: The Times Have Never Been Better

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The annual meeting of the Society for Industrial Microbiology (SIM) this year was held in Denver, Colorado, from July 29 to August 2, 2007. Nearly 200 oral and about 120 poster presentations kept the approximately 600 participants busy for more than 4 days. This report focuses on the 35 talks dedicated to natural products chemistry, biosynthesis, engineering, drug discovery and development, as well as neutraceuticals.

Introduction

On Sunday, July 29, the approximately 600 international participants of the Society for Industrial Microbiology (SIM) Annual Meeting 2007 came together at the Hyatt Regency Convention Center in Denver, CO, prepared to be informed about and to discuss the exciting news and advances in the sciences and technologies of industrial microbiology. The following 4 days were packed with nearly 200 oral and 120 poster presentations covering biocatalysis, environmental microbiology, fermentation and cell culture, industrial eukaryotic microbiology, metabolic engineering, and natural products, yet still left ample time for the participants to network and discover the local sights of Denver and its beautiful surroundings. Drs. Kevin Reynolds (Portland State University, Portland, OR) and Ben Shen (University of Wisconsin, Madison, WI) cochaired the scientific program on natural products, which featured 6 sessions with 35 oral and approximately 30 poster presentations. This year's SIM Annual meeting proved once again to be the premier venue for natural products chemistry, biosynthesis, engineering, drug discovery and development, and natural products as neutraceuticals. This review summarizes the highlights from the oral presentations covering the various aspects of natural products.

Natural Products as Anticancer Agents: From Discovery to Development

The first session featured six talks illustrating how natural products were discovered and developed into new anticancer drugs, with Drs. Guy Carter (Wyeth Research, Pearl River, NY) and Ray Lam (Nereus Pharmaceuticals, San Diego, CA) as cochairs of the session. Dr. Gregory Vite (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ) started the session with a presentation titled "Unnatural Selection and the Evolution of Anticancer Agent Ixabepilone: Optimizing the Fermentation and Drug-Like Properties of Epothilones" that described his team's effort to optimize epothilone B production by strain improvement, followed by a combination of semisynthetic modification and microbial biotransformation of epothilone B to generate analogs. Evaluation of the resultant analogs for their in vitro activity and metabolic stability as well as the in vivo activity in tumor models yielded ixabepilone (BMS-247550), a novel lactam analog, as a superior agent for human clinical trials (currently in phase III). In general, epothilones, first isolated from the myxobacterium *Sorangium cellulosum* in the mid 1990s, are very attractive anticancer drug leads, since their mode of action is identical to that of paclitaxel; epothilones also have the unique ability to overcome multiple drug resistance and possess other favorable drug-like properties. However, they are still far from being ideal, and production of analogs is a viable strategy for obtaining anticancer compounds with improved properties.

Opening with the statement that 20 marine-derived anticancer agents are currently in various stages of clinical trials, Dr. William Gerwick (University of California at San Diego, La Jolla, CA) presented "Anticancer Drug Discovery from Marine Cyanobacteria: New Molecules Working by New Mechanisms." Marine cyanobacteria represent a rich source of novel peptide and peptide-polyketide natural products (over 800 are known), many of which are highly cytotoxic to mammalian cells, serving as excellent anticancer drug leads. The continued effort by the Gerwick lab has resulted in the identification and structure elucidation of numerous marine natural products with new chemical structures and new mechanisms of action. For example, coibamide A, produced by a *Leptolyngbia* species, features an unprecedented extent of *O*- and *N*-methylation comprising methylated serine, valine, tyrosine, and threonine residues, is antiproliferative, and apparently shows a new mechanism of action. Another interesting example is somocystinamide A, a peptide-polyketide metabolite isolated from *Lyngbya majuscule*. A newly developed screen for caspase 8 activators facilitated the identification of somocystinamide's apoptotic activity. With these and other examples, Dr. Gerwick concluded that the tide for natural product research is rising and it is an exciting time for natural product discovery through the effective combination of chemistry and biology.

The next presentation, ‘‘From Sea Bed to Sick Bed: Discovery and Development of a Novel Proteasome Inhibitor from a Marine Actinomycete,’’ was given by the session’s cochair, Dr. Lam. He reported titer improvement, fermentation scale-up, and development of a defined salt fermentation medium for the commercial production of salinosporamide A at kilogram scale. Salinosporamide A (NPI-0052), produced by the marine organism *Salinispora tropica*, represents a novel inhibitor of the 20S proteasome and enhances the response to conventional therapy in colon cancer. However, access to a reliable supply of the compound for further clinical development represented a great obstacle, since it required the use of saline fermentation. The process of saline fermentation is challenging due to the commonly used ingredients for marine organisms, such as sea water and animal nutrients, and the damaging effects of high salt concentrations on the fermentation equipment. The process reported by Dr. Lam represents the first use of saline fermentation for production of salinosporamide A in quantities sufficient for human clinical application, and the compound has recently entered phase I clinical trials.

‘‘What Do You Do with a Natural Product That Is Too Toxic’’ was the title Dr. Justin Moran (Wyeth Research, Pearl River, NY) used to chronicle the discovery and development of calicheamicin into a clinical anticancer drug (Mylotarg®). The calicheamicins, discovered in the mid 1980s from several *Micromonospora* species, represent some of the most potent cytotoxic molecules known to date. A combination of strain improvement, medium development and fermentation optimization resulted in a dramatic improvement of calicheamicin titer (by five orders of magnitude!), but the excitement of this discovery was dampened by the finding that improved compounds were too toxic for the safe use in humans. Selective tumor cell delivery, using monoclonal antibodies, emerged as a method to overcome the observed extreme toxicity. First, calicheamicin was attached to a hybrid amide-hydrazone linker that did not significantly reduce the potency of the compound and was stable in serum but hydrolyzed upon internalization where the pH is low. Next, the anti-CD33 antibody, which targets acute myeloid leukemia cells, was attached to the drug via this hydrazone linker. After surviving all clinical trials, the resulting complex chemotherapeutic agent was finally commercialized in 2000 as Mylotarg®, representing the first and only FDA approved antibody-conjugated chemotherapy to date. Calicheamicin, with several additional targeting opportunities, is currently undergoing preclinical evaluation.

Dr. Jim McAlpine (Thallion Pharmaceuticals Inc., Saint-Laurent, QC, Canada) presented a progress report on their anticancer drug lead titled ‘‘ECO-4601, a Novel Anticancer Pharmacophore, Development through Phase I.’’ ECO-4601 (diazepinomicin), a farnesylated dibenzodiazepinone produced by a *Micromonospora* strain, was discovered using Thallion’s DECIPHER technology, a genomics based platform for natural product drug discovery, by predicting the chemical structure of a compound from microbial genome sequences. ECO-4601 represents a new pharmacophore with a dual mode of action. It binds to the peripheral benzodiazepine receptor that plays a role in apoptosis and also inhibits the Ras signaling pathway. Production of ECO-4601 has now approached kilogram scale via strain improvement and fermentation optimization, and the recent phase I clinical trials concluded with a strong safety and tolerability profile without dose limiting toxicity. ECO-4601 is currently in phase I/II trials and could be viewed as a validation of the DECIPHER technology for natural product drug discovery.

Dr. Leonard McDonald (Wyeth Research, Pearl River, NY) ended the session by reporting Wyeth’s effort on ‘‘Pyranonaphthoquinone Inhibitors of AKT Kinase.’’ AKT, when activated, promotes cell proliferation and survival. Specific AKT inhibitors, therefore, could be important cancer chemotherapy drug leads. High throughput screening for AKT inhibition against Wyeth’s synthetic and natural product libraries resulted in several hits, one of which was an extract made from a *Streptomyces* species. The extract was active for AKT inhibition even after 1000-fold dilution, and natural product dereplication eventually resulted in the identification of the pyranonaphthoquinones (e.g., lactoquinomycin) as AKT-specific inhibitors. The pyranonaphthoquinones, which have been reported previously to be toxic to human tumor cells, and their semisynthetic analogs now serve as excellent small molecule probes to evaluate AKT biology.

Lipid II-Targeted Antibiotics: Chemistry, Biosynthesis, and Development

Drs. Stefano Donadio (KtedoGen, Malnate, Italy) and Jon Thorson (University of Wisconsin, Madison, WI) cochaired the Monday afternoon session with its six presentations focusing on lipid II as a common target for antibacterial agents. Dr. Frank Koehn (Wyeth Research, Pearl River, NY) started the session with a talk titled ‘‘Mannopeptimycins: Unique Lipid II-Targeted Anti-Infective Agents’’ to inform the audience about the discovery of the mannopeptimycins through Wyeth’s look-back program. The mannopeptimycins, a family of glycopeptides first isolated from a *Streptomyces* species in the 1950s, do not block lipid II synthesis, but rather inhibit the lipid II-dependent peptidoglycan maturation by binding to lipid II in a way that is different from other compounds. SAR analysis revealed the need for lipophilic side chains, and a combination of directed biosynthesis and semisynthetic modifications was applied to introduce these structural variations. Finally, an adamantyl substitution on the disaccharide and a cyclohexyl side chain on the mannopeptimycin core scaffold resulted in an analog with improved therapeutic properties. Dr. Koehn concluded that Lipid-II, a well-known target, still provides novel opportunities, microbial natural products continue to be a rich source of new drug leads, and Wyeth continues its natural product program for anti-infective agents. Through its look-back program, Wyeth continues to take advantage of its 50+ years of natural product collections, to incorporate combinatorial biosynthesis strategies to further expand natural product diversity, and to combine the strengths of its synthetic and natural product chemists, all of which lead to successful discovery of novel anti-infective agents.

Next, Dr. James Janc (Theravance, South San Francisco, CA) presented a talk titled "Telavancin, a Multifunctional Lipoglycopeptide Antibiotic, Disrupts Both Cell Wall Biosynthesis and Cell Membrane Integrity," highlighting their strategy to find novel antibacterial agents based on the vancomycin scaffold. The novel lipoglycopeptide antibiotic telavancin was specifically designed to overcome the emerging resistance mechanisms against vancomycin by attaching a lipophilic decylaminoethyl-side chain to the vancosamine sugar. Due to this structural modification, telavancin not only inhibits the transpeptidation reaction in peptidoglycan biosynthesis, as is the case for vancomycin, but it also inhibits the earlier transglycosylation step by virtue of its affinity for lipid II. As a second mechanism of action, telavancin disrupts the barrier function of the bacterial cell membrane. These features ensure that telavancin retains activity against strains with reduced susceptibility for vancomycin. Telavancin is currently in phase III clinical trials.

Dr. Wilfred van der Donk (University of Illinois, Urbana, IL) presented a progress report on their efforts focusing on the lantibiotics, titled "Biosynthesis of the Lantibiotics Nisin and Haloduracin." Lantibiotics, comprising a class of more than 60 compounds, are highly potent antibacterial agents. The mode of lantibiotics' action includes specific binding to lipid II followed by membrane permeabilization. Biosynthesis of class I lantibiotics requires three enzymes (dehydratase, cyclase, and protease) to generate the final compound from the peptide precursor. However, only two genes encoding the modifying enzymes are found in class II lantibiotic gene clusters. The van der Donk lab established that, in class II lantibiotic biosynthesis, both the dehydratase and cyclization activities reside within one protein, as exemplified by LctM for the lacticins. These characteristics were exploited to mine microbial genomes for the discovery of novel lantibiotics, as demonstrated for the haloduracins from *Bacillus halodurans*. The dehydratase domain of LctM was found to have a very relaxed substrate specificity. Thus, site-directed mutagenesis of the structural gene, incorporation of nonproteinogenic amino acids by in vitro peptide synthesis, and even an attachment of a completely different nonlantibiotic peptide to the lantibiotic leader peptide all resulted in dehydration activity, as deduced from the resulting enzymatic products. With these findings, Dr. van der Donk ended the talk with a great promise to engineer novel lantibiotics by manipulating their biosynthetic machinery.

In the talk titled "Lipid II Utilizing Enzymes as Antivirulence Targets," Dr. Dewey McCafferty (Duke University, Durham, NC) reported that the lipid II utilizing enzymes, sortases, might be good antivirulence targets for treatment of Gram-positive bacterial infections. *S. aureus* has two sortase isoforms: sortase A, essential for adhesion during infection onset, and sortase B, required for heme-iron acquisition in later stage of infection. The sortase gene knockouts displayed drastically reduces virulence. Screening and discovery of sortase inhibitors as a novel strategy for the identification of antibacterial agents were also discussed.

Dr. Dan Kahne (Harvard University, Cambridge, MA) illustrated how chemical, biochemical, and structural tools are combined to optimize a lipid II-targeting natural product lead in his talk titled "Dissecting the Structural Requirements for Moenomycin A Activity Using Semisynthetic Analogs with Modified Phosphoglycerate Lipids." Moenomycin, first discovered in the mid 1960s from a *Streptomyces* species, is the only known natural product that targets the peptidoglycan glycosyltransferase. While it is several orders of magnitude more potent than vancomycin against many Gram-positive pathogens, its poor pharmacokinetic properties have so far prevented its clinical application. The Kahne lab developed a modular synthesis that allowed them to make a variety of moenomycin analogs with shorter lipid chains and various other modifications for SAR studies. Analyses of their activity profiles revealed that the carboxylic acid and phosphate moieties are very important for enzyme binding, while shorter lipid chains did not significantly reduce transglycosylase binding activity. This information should guide production of analogs with improved biological activity.

Dr. Wolfgang Wohlleben (University of Tübingen, Tübingen, Germany) concluded the afternoon session by presenting an overview on the biosynthetic pathway of the glycopeptide antibiotic balhimycin titled "Glycopeptide Antibiotics: Biosynthesis, Resistance and Regulation." After a brief review of the balhimycin biosynthetic machinery, various strategies to access balhimycin analogs were demonstrated. Precursor supply in balhimycin biosynthesis is ensured through one type III polyketide synthase (PKS) for dihydroxyphenylglycine and through an operon spanning three genes for β-hydroxytyrosine. The glycopeptide backbone is assembled using a nonribosomal peptide synthetase (NRPS) machinery, and methylation and glycosylation occur after the release of the peptide backbone from the NRPS. In contrast, the oxidative coupling reactions as well as the halogenation of β-hydroxytyrosine appear to be performed on NRPS-bound intermediates. Given the modular nature of the glycopeptide biosynthetic machinery, Dr. Wohlleben ended his talk by posing a question on whether combinatorial biosynthesis occurs in nature and what the requirements to access glycopeptide structural diversity by combinatorial biosynthetic methods are.

Discovery and Development of Natural Products: New Applications and New Targets

The Tuesday morning session, cochaired by Drs. Hiro Osada (RIKEN, Saitama, Japan) and Kevin Reynolds, consisted of five talks covering several new aspects on natural product drug discovery. As a starter, Dr. Steve Kendrew (Biotica, Little Chesterford, UK) reported on "Biosynthesis of the Angiogenesis Inhibitor Borrelidin: Precursor-Directed Biosynthesis of Novel Analogs." Borrelidin, first discovered in the 1940s from a *Streptomyces* species, exhibited dual cytotoxicity and anti-angiogenic activity. After a brief review of the cloning and characterization of the borrelidin biosynthetic machinery, Dr. Kendrew discussed various strategies to modify the borrelidin scaffold with the goal of separating its anti-angiogenic activity from its cytotoxicity. Through directed biosynthesis, a library of borrelidin analogs was produced, and one of the analogs, a C17-cyclobutyl-substituted borrelidin, showed a significantly improved profile of angiogenesis inhibition versus cytotoxicity, demonstrating once again the power of natural products as drug leads.

Dr. Hiroshi Tomoda (Kitasato University, Tokyo, Japan) next presented the results on using silkworms to screen natural products, titled “Antimicrobial Screen Using Silkworms as an In Vivo Infection Model.” Like mice, silkworm larvae have a natural immune system, but they are easier to handle, available at fairly low cost, and have low space requirements. Screening more than 5000 culture broths from actinomycete and fungal strains in silkworm larvae infected with methicillin-resistant *Staphylococcus aureus* (MRSA) or *Candida albicans* resulted in the discovery of the nosokomycins, a family of glycopeptides. The nosokomycins are active against MRSA using a mechanism similar to that of moenomycin, demonstrating that silkworms can be used as an effective and selective in vivo infection model for antimicrobial screening.

With a title of “Metabolomic and Genomic Analyses of *Streptomyces reveromyceticus*, a Producer of an Anti-Osteoclast Compound,” Dr. Hiro Osada’s presentation summarized his group’s studies of chemistry, biology, and biosynthesis of the reveromycins. Reveromycin A, a polyketide with a unique spiroketal moiety first discovered in the early 1990s, induces apoptosis by isoleucyl-tRNA inhibition specifically in osteoclasts and can therefore be a useful compound in treating osteoporosis and tumor bone metastasis. These new findings initially prompted the Osada lab to improve reveromycin production by strain improvement in order to obtain enough material for futher biological studies. Next, they cloned the reveromycin biosynthetic gene cluster, studied its biosynthetic machinery, and generated analogs by combinatorial biosynthetic methods. Reveromycin A was found to be assembled by a modular PKS with extra domains accounting for the formation of the reveromycin A2 shunt metabolite. So far no candidate for the formation of the spiroketal moiety of reveromycin A has been identified. Finally, the Osada lab sequenced the *Streptomyces reveromyceticus* genome, opening up many additional opportunities for studying and engineering reveromycin biosynthesis.

Dr. Ho Jeong Kwon (Yonsei University, Seoul, South Korea) illustrated that natural products can not only represent drug candidates but can also serve as bioprobes/biomarkers to identify new targets and functions in a talk titled “Small Natural Molecules Targeting Angiogenesis.” Using the fungal metabolite terpestacin as an example, Dr. Kwon noted that terpestacin is known to inhibit angiogenesis in vitro and in vivo, but its mechanism of action remains poorly understood. The Kwon lab then used a biotinylated terpestacin for biopanning and thereby identified the generation of reactive oxygen species and the expression of vascular endothelial growth factor as mechanisms by which terpestacin exerts its bioactivity.

Dr. Kevin Reynolds presented the last talk for the morning session titled “Generation and Analysis Of Novel Serine/Threonine Protein Phosphatase II Inhibitors.” In general, natural products that act as protein phosphatase inhibitors are known, and one of them, fostriecin, entered phase I clinical trials but was withdrawn partly due to drug stability and purity. Phoslactomycin B, first discovered in the late 1980s from several *Streptomyces* species, is an inhibitor of phosphatase 2A, an enzyme considered to be a new target for antitumor compounds. The Reynolds lab has cloned and characterized the phoslactomycin biosynthetic pathway and generated a library of phoslactomycin analogs through in vitro enzymatic esterification of pathway intermediates and in vivo-directed biosynthesis. Preliminary evaluation of this library showed that some of the analogs are as potent as the parent natural product. Phoslactomycin biosynthesis also revealed new insight into the mechanism of *cis*-double bond formation in natural products. Dr. Reynolds concluded that a rich diversity of new analogs can be generated if all the methods available (e.g., generating pathway intermediates, in vitro enzymatic modifications, hybrid pathways, and precursor directed biosynthesis) are combined and utilized.

Status and Prospects for Using Natural and Engineered Compounds as Nutraceuticals

Tuesday afternoon’s session, chaired by Dr. Adam Burja (Ocean Nutrition Canada, Dartmouth, NS, Canada), was devoted to nutritionally important natural products and featured six talks. Dr. Mark Scaife (University of Sheffield, Sheffield, UK) opened the session with a presentation titled “Exploitation of Cyanobacterial Genetics in the Development of an *E. coli*-Based Astaxanthin Production System.” The astaxanthins are currently produced by chemical synthesis, yet the genes encoding astaxanthin biosynthesis in several cyanobacteria are known. By cloning several beta-carotene ketolase and hydroxylase genes from different cyanobacteria species and expressing them in *E. coli*, Dr. Scaife showed that beta-carotene can be efficiently transformed into astaxanthin in the recombinant *E. coli* strain in yields up to 4 mg l^{-1} day^{-1}, demonstrating the feasibility of astaxanthin production by fermentation.

Plants are also known to produce astaxanthins, and Dr. Francis Cunningham (University of Maryland, College Park, MD) described advances in creating a plant-based biological production process for astaxanthins. In a talk titled “A Study in Scarlet: Using Color Complementation in *E. coli* to Unravel the Biochemical Pathway Leading to the Valuable Red Ketocarotenoid Astaxanthin in the Flowers of *Adonis aestivalis*,” Dr. Cunningham reported the identification of two genes from *Adonis aestivalis* responsible for conversion of beta-carotene into astaxanthin, setting the stage to improve astaxanthin production in plants.

Polyunsaturated fatty acids (PUFAs) are another family of neutraceutically important natural products, and at least two different pathways for PUFA biosynthesis are known: de novo biosynthesis by PUFA synthases and elongation of endogenous fatty acids by elongases and desaturases. Dr. Helia Radianingtyas (Ocean Nutrition Canada, Dartmouth, NS, Canada) reported her group's findings related to PUFA biosynthesis in a marine fungoid protist in a talk titled "Affecting Specific Fatty Acid Production within *Thraustochytrium* sp. ONC-T18." In this microorganism, PUFAs are made by fatty acid elongation and desaturation. By adding certain enzyme inhibitors to the fermentation process, it is possible to direct the formation of specific PUFAs, thereby allowing better control of the chain length and chemical properties of the PUFA.

Production of PUFAs in plants was discussed in Dr. Xiao Qiu's (University of Saskatchewan, Saskatoon, SK, Canada) presention "Metabolic Engineering of Microbial Biosynthetic Pathways of Very Long Chain Polyunsaturated Fatty Acids in Plants: Accomplishment and Challenge." By stepwise introduction of increasing numbers of genes of microbial origin, encoding various steps of PUFA biosynthesis, into *Brassica juncea*, the Qiu lab succeeded in the production of arachidonic, eicosapentaenoic, and docosahexaenoic acids in this plant. These findings demonstrated the feasibility of transgenic plants as alternative sources of PUFAs.

The last group of nutraceutically important natural products discussed were the flavonoids. Isoflavones and their aglycones are both accumulated and extensively biotransformed in soy-based industrial fermentations. In his talk titled "Isolation of Isoflavones as Coproducts of the Erythromycin Fermentation," Dr. Mark Weber (Fermalogic, Inc., Chicago, IL) illustrated the development of a procedure for the recovery of isoflavone aglycones by acid treatment of the rhamnosylated coproducts, generated during soy-based fermentations of *Saccharopolyspora erythraea* used for erythromycin production.

Dr. Mark Koffas (The State University of New York at Buffalo, Buffalo, NY) presented a talk titled "A Systems Biology Approach for Plant Secondary Metabolite Optimization in Microorganisms." Over 500 anthocyanines are known, and the Koffas lab aims to generate nonnatural anthocyanines as food coloring agents and potential drugs for obesity and diabetes. They first reconstructed the plant flavonoid pathway in *E. coli* and *Saccharomyces cerevisiae* by combining enzymes derived from different plant species. They then optimized the precursor availability and fermentation conditions. Finally they combined directed biosynthesis with organic synthesis and demonstrated a new approach to produce novel flavanones and dihydroflavonols.

Antibacterial Antibiotics: Discovery, Development and Resistance

Drs. Ben Shen (author of this report) and Sheo Singh (Merck Research Laboratories, Rahway, NJ) cochaired the Wednesday morning session with six presentations addressing the pressing needs for new antibiotics and the great challenges of their discovery and development. Opening the session with a talk titled "Antibiotic Resistance in Environmental Organisms," Dr. Gerard Wright (McMaster University, Hamilton, ON, Canada) stressed the prevalence of antibiotic resistance in environmental organisms. A survey of the resistance profile of 480 soil-derived bacterial isolates against 21 commonly used antibiotics, both synthetic and natural-product based, showed that soil-derived bacteria are resistant to 7.5 antibiotics on average, with some demonstrating resistance to up to 15 antibiotics! This is a very sobering finding, and multidrug resistance appears to be the rule rather than the exception for environmental organisms. Moreover, many resistance genes isolated from environmental sources were found to be clustered by their mobilization elements and often encoded proteins highly similar to their counterparts isolated from clinical samples. Antibiotic resistance is inevitable, and investigation of resistance genes from environmental samples should help predict the potential for clinical resistance mechanisms.

Dr. David Pompliano (Merck Research Laboratories, Rahway, NJ) then presented a talk titled "Antibacterial Discovery: It's Not about the Target," summarizing GlaxoSmithKline's sobering experience in a genomics-derived, target-based approach to screen for new classes of drugs with novel modes of action. Over a period of 7 years starting in 1995, this approach resulted in the identification of more than 360 potential targets, 160 validated targets, and only 26 hit compounds for these targets. Of the 26 hits, only 5 became lead compounds and, eventually, none were suitable as drug candidates! These frustrating results prompted them to consider that a good, established target is better than a new target. With this new rationale in mind, they focused on well-established targets and increased the molecular diversity of compounds in their screens. So far, this new strategy has shown promise, resulting in six drug candidates entering clinical development.

With the controversial title "Natural Product Discovery: The Worst of Times and the Best of Times," Dr. Steve Projan (Wyeth Research, Cambridge, MA) delivered a provocative analysis of the good, the bad, and the ugly sides of anti-infective discovery. The challenges the industry is facing today are not only scientific and financial, but also social and governmental policy related. Target-based antibacterial discovery has not been successful. While many pharmaceutical companies have drastically reduced or even eliminated their effort to develop natural products as drug candidates, natural products have done well at Wyeth, and Wyeth remains committed to natural product drug discovery. Natural products remain an excellent source for structural diversity that cannot be matched by any other small molecules. Furthermore, the technology for natural product discovery and development has improved dramatically in recent times. It is Dr Projan's belief that there is no better time to be doing natural product drug discovery research and that Wyeth is a great place to do it.

Dr. James Balkovec (Merck Research Laboratories, Rahway, NJ) talked about the discovery and development of the first β-1,3-glucan synthesis inhibitor, caspofungin (Cancidas®), with the title "From Bugs to Drugs: The

Discovery of Caspofungin.'' The original natural product, known as pneumocandin, was first discovered in the mid 1980s and considered to be a good antifungal lead because of its few off-target activities and new mechanism of action. However, it suffered from a limited spectrum, lack of water solubility, and poor oral bioavailability. Successive strain improvement to increase the titer and produce the desired analogs, followed by extensive medicinal chemistry optimization, improved its activity spectrum, solubility, and pharmacokinetic properties. Caspofungin acetate (Cancidas®) received FDA approval in 2001 and recently became the number one intravenous agent for antifungal treatment in the US and Europe. While the discovery research was completed in 2 years, it took nearly 10 years of development effort to bring caspofungin to the market.

Dr. Sheo Singh next presented the recent discovery of platensimycin and platencin in the talk titled ''Novel Approaches for Antibiotic Discovery'' to showcase how innovation in screen design has helped Merck discover novel antibacterial leads. Platensimycin and platencin, specific inhibitors of FabF/FabH, two key enzymes in the fatty acid biosynthetic pathway in bacteria, were discovered from an innovative natural product screening program using a novel RNA silencing technology. The expression of *fabF* antisense RNA in *Staphylococcus aureus* leads to a decrease in FabF levels and, hence, an increase in sensitivity to FabF-inhibiting compounds. This assay was used to screen 250,000 extracts made from more than 80,000 strains, and the resulting hits were further confirmed by cell-free assay against FabF. Among the FabF inhibitors identified were platensimycin and platencin, both of which were produced by several isolates of *Streptomyces platensis*. Platensimycin and platencin feature an unprecedented molecular scaffold. Both were validated to be potent FabF inhibitors, with platencin also having a potent FabH inhibitory activity. The dual FabF and FabH inhibitory property of platencin should be advantageous in preventing resistance. Although development of these leads into therapeutic agents still faces many challenges, including poor pharmacokinetics and the emergence of resistance, Dr. Singh concluded his presentation on an optimistic note that more antibiotics with novel structures and new modes of action can be discovered by applying this RNA silencing technology.

Dr. Ben Shen ended the session by a progress report on his group's effort to clone, characterize, and engineer platensimycin biosynthesis, titled ''Biosynthesis of Platensimycin and Opportunities of Engineering Novel Platensimycin Analogs.'' The structures of platensimycin and platencin were reported in May 2006 and 2007, respectively, and the Shen lab obtained the platensimycin producing *S. platensis* in October 2006. The platensimycin biosynthetic gene cluster has now been cloned and characterized, unveiling many unusual features including (1) a rare chemistry for 3-amino-4-hydroxybenzoic acid biosynthesis, (2) a novel pathway for terpenoid biosynthesis in bacteria, (3) a common biosynthetic machinery shared by both platensimycin and platencin, the divergence for which most likely results from an unprecedented diterpene synthase, and (4) candidates for possible resistance mechanisms. These findings now set the stage to engineer the biosynthetic machinery to direct either platensimycin or platencin production or produce novel analogs. Dr. Shen pointed out how fast a natural product biosynthetic gene cluster can be accessed nowadays. Rapid accessibility of DNA, solid understanding of underlying biochemistry for many pathways, and availability of modern biotechnological techniques puts us in an excellant position to apply combinatorial biosynthetic strategies to natural product structural diversity and drug discovery.

Fungal Polyketides: Biosynthesis, Pathogenesis, and Regulation

The last session on Thursday afternoon, cochaired by Drs. Liangcheng Du (University of Nebraska, Lincoln, NE) and Isao Fujii (Iwate Medical University, Iwate, Japan), featured six presentations dedicated to fungal natural products. Dr. Russell Cox (University of Bristol, Bristol, United Kingdom) started the session with a talk titled ''Investigations of Fungal Pyridine Biosynthesis: A PKS-NRPS Megasynthetase.'' Using xenovulene, squalestatin, and tenellin as three examples, Dr. Cox first gave an overview on polyketide biosynthesis in fungi, which features a family of architecturally very similar iterative type I PKSs. He then explained how these fungal PKSs can generate an amazing array of structural diversity by repeatedly using their single PKS module with all or only some of its domains, depending on the elongation cycle.

Dr. Liangcheng Du then reported his group's efforts to dissect fungal PKS mechanisms in a talk titled ''Biosynthesis of Mycotoxin Fumonisins in Corn Pathogen *Fusarium verticillioides*.'' Dr. Du noted that the fungal PKSs for fumonisin (a nonaketide resulting from 8 cycles of PKS-catalyzed condensation) and T-toxin (a heneicosaketide resulting from 20 cycles of PKS-catalyzed condensation) are highly homologous in spite of the difference in the number of condensations during their biosynthesis. By swapping the fumonisin ketosynthase domain with the T-toxin ketosynthase domain in the fumonisin producer *F. verticilloides*, the Du lab showed that the resulting mutant strain still produced fumonisins. This finding suggests that the ketosynthase domain alone does not control the polyketide structure of fumonisins. The Du lab also unveiled an unprecedented PLP-dependent chain release mechanism in polyketide biosynthesis that involves a novel enzyme, Fum8p, and identified candidates that may confer mycotoxin resistance.

The statins represent one of the most celebrated classes of natural product drugs, and Dr. John Vederas (University of Alberta, Edmonton, AB, Canada) provided an overview of lovastatin biosynthesis titled ''Recent Advances in Understanding Lovastatin Biosynthesis in *Aspergillus terreus*.'' While the polyketide side chain of lovastatin is formed by the LovF PKS, the LovB and LovC PKS complex has to catalyze minimally 35 reactions in order to biosynthesize the lovastatin precursor dihydromonacolin L from short acyl CoA substrates. Dr. Vederas pointed out that LovC was indispensable for dihydromonacolin L biosynthesis, suggesting that the enoylreductase domain of LovB is not fully functional and there is intimate protein-protein interaction between the LovB and LovC subunits. He also revealed the structure of LovC, which is monomeric and shares the same fold as the medium chain fatty acid dehydrogenase. LovB has been overproduced and assayed; solving the structure of LovB would be a tremendous accomplishment. Biotransformation using LovD has also resulted in the production of a library of lovastatin analogs, including simvastatin, a very significant feat given its current commercial value.

Aflatoxin is biosynthesized by a complex of two fatty acid synthase subunits (HexA and HexB) and one iterative type I PKS (PksA). Dr. Craig Townsend (The Johns Hopkins University, Baltimore, MD) disclosed the most recent findings from his group's efforts on aflatoxin biosynthesis in a talk titled "Understanding Programming in Iterative Type I Polyketide Synthases." Dr. Townsend discussed the occurrence of a previously unrecognized N-terminal domain in PksA and provided evidence that its mysterious function is to transfer a C_6 starter unit formed by HexA and HexB from the HexA to the PksA acyl carrier protein, ultimately resulting in the production of the aflatoxin precursor norsolorinic acid. This newly defined domain, SAT, for starter unit-ACP transacylase, will greatly aid the understanding of fungal PKSs.

In a presentation titled "Polyketide Biosynthesis in Phytopathogenic Fungus *Alternaria solani*," Dr. Isao Fujii presented the identification of seven reduced-type polyketide gene clusters from *A. solani* coding for the biosynthesis of compounds such as solanapyrone and alternaric acid. While heterologous expression in *Aspergillus oryzae* of PksN comprising a methyltransferase domain resulted in the production of a new octa-methylated decaketide (alternapyrone), expression of PksF lacking a methyltransferase domain led to the formation of eleven new compounds, the two main products of which were characterized as aslanipyrone and aslaniol. These findings underscore once again the flexibility and versatility of fungal PKSs in synthesizing polyketide natural products.

To conclude the session, Dr. Eric Schmidt (University of Utah, Salt Lake City, UT) presented his group's efforts on the equisetin biosynthetic pathway in a talk titled "Functional Analysis of Equisetin Biosynthetic Proteins." Equisetin is a hybrid polyketide-peptide natural product featuring a tetramic acid moiety. The equisetin synthetase represents a hybrid PKS-NRPS with similarity to the lovastatin PKS, except that it contains an additional NRPS module with a terminal reductase domain. The Schmidt lab expressed the peptide synthetase portion of this enzyme in *E. coli* and demonstrated its tetramic acid synthetase activity. Taken together, these findings shed light into iterative type I PKS-NRPS for hybrid peptide-polyketide natural product biosynthesis in fungi.

Concluding Remark

SIM 07 has rejuvenated natural product discovery once again, and the participants left Denver excited, inspired, and greatly optimistic; thus, it can be concluded that in "natural product drug discovery: the times have never been better." Finally, let us conclude with several key points that emerged during this meeting: (1) natural products continue to provide unmatched structural diversity for drug discovery. (2) The track record for natural product-based drugs and drug leads remains excellent, particularly for anticancer and anti-infective agents. (3) Advances in natural product biosynthesis and engineering have made it practical to apply the principles of combinatorial biosynthesis to strain improvement, novel natural product discovery, and generation of natural product analogs. (4) Advances in genomics and associated technologies to access biodiversity, innovation in assay and screen design, and improvement to existing and emergence of new technologies and instrumentations for detection, dereplication, isolation, and structural elucidation promise rapid access to an ever increasing pool of natural products. (5) Integration of natural products chemistry with synthetic chemistry, medicinal chemistry, pharmacology, biology, and associated disciplines ensures a greater chance to advance natural product leads into therapeutic drugs.

We look forward to the SIM 08 in San Diego next summer.