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SYMPOSIUM INTRODUCTION

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China: Forward to the Green Pesticides via a Basic Research Program†

The 973 Program is China's keystone national research program established to support basic research in natural and physical sciences. In addition to promoting the development of core technology and scientific infrastructure needed to enable China to meet the social and economic challenges of the 21st century, the training and mentoring of the new generation of China's young scientists are also important objectives of this national program. The green chemical pesticide research program is a part of the 973 Program. The main objectives of stage 1 of the green chemical pesticide research program (2003−2008) are to establish China's capability to conduct basic research in the discovery of "green" crop protection chemicals that are not only novel in mode of action and highly selective to pest species that are unique to China's agricultural situation but also possess favorable environmental and human hazard and risk potentials. The target-based discovery strategy was selected as the main discovery platform. This strategy not only provided this research program the best chance to discover new products but also provided members of this research team opportunities to establish core technologies in chemoinformatics/computation-aided pesticide design using QSAR, QAAR, sensitive and selective bioassay methodology, combinatorial synthesis, hit to lead optimization, and biological targets that were derived from resistance-AChE, IGR, nAChR, etc. On the basis of the learning from stage 1, stage 2 (2010−2014) of this program will focus on the molecular target-oriented innovation of green chemical pesticides. This commentary presents key learnings and accomplishments from the stage 1 of China's green chemical pesticide research program. It is hoped that this information will stimulate further constructive collaborations between pesticide scientists from China and abroad.

KEYWORDS: Green chemistry; pesticides; crop protection; molecular design; hit to lead optimization

1. PREFACE: ORIGIN OF THE CHINESE BASIC RESEARCH (973) PROGRAM AND STRATEGIC SIGNIFICANCE

The first stage of China's National 973 Basic Research Program, green pesticide research project, was recently completed. To celebrate this project milestone, we are pleased to present some of the significant scientific findings in this special issue of the *Journal of Agricultural and Food Chemistry* (*JAFC*). We wish to use this opportunity to stimulate scientific collaboration with the international pesticide research community. The purpose of this commentary is to introduce the Chinese 973 Green Pesticide Research Program and to highlight some of the key research projects and significant findings.

Since the early 1980s, China's new economic reform policy had promoted a rapid expansion of social and economic growth. This progress was fueled, in part, by unprecedented changes in China's educational and research systems. To stimulate the advancement of innovative basic and applied research, China implemented three major national science and technology plans: (1) 973, Key Basic Research; (2) 863, High Technology Development; and (3) R&D Support for Industrialization. The 973 Program is designed to build long-term (10+ years) discovery capability, whereas the 863 Program supports ongoing invention and R&D. These national research programs provided the Chinese scientific community a stable research funding system to promote scientific innovation across the vast natural and physical scientific disciplines.

The strategic objectives of the 973 Program are (1) to strengthen China's capabilities in independent and original innovations, (2) to address important scientific issues critical to national economic and social development, and (3) to establish scientific research infrastructure, training, and management and support systems for the future development of the country. One of the top research priorities of the 973 Program is to transform strategic basic research into industrial application in the future, thus overcoming the traditional bias of "emphasizing research while ignoring application". The 973 Program improved research focus on the importance of intellectual property rights and its social or economic effects/impacts. To be more specific, this program has four main tasks: (1) to prioritize and conduct multidisciplinary basic research to address fundamental scientific issues that are critical to the sustainable development of China's economical and social strategic plans; (2) to stimulate relevant frontier research with transparent and effective grant authorization, evaluation, and administrative systems; (3) to educate and mentor outstanding Chinese scientists to meet the challenges of the 21st century; and (4) to establish world-class scientific research centers in the different regions of China to promote and support basic research and development.

The implementation of strong financial support from the 973 Program stabilized China's scientific research community and the training of young scientists and midcareer professionals. This program also provided outstanding Chinese scientists living abroad the opportunity to return and conduct research in China.

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1.1. Introduction to the Green Pesticide Research Program. China has met the challenges to clothe and feed her 1.3 billion population; moreover, agriculture is the critical driving force sustaining China's phenomenal economic growth and development. The evolution of agriculture productivity is attributed not only to fundamental changes in China's farm policy and land reform but also to the broad adaptation of modern best agricultural practices and scientific innovations such as the use of fertilizers, crop protection chemicals, plant genetics, and biotechnology. Although China is the world's leading pesticide chemical producer and user, the infrastructure for research and development of pesticides in China is still in the early stages of development. Pesticide research efforts are carried out only in several chemical institutes and universities across the country. There is no large domestically based industrial sponsored pesticide research and development effort in China.

To maintain sustainable growth and to meet challenges from the domestic weather, pest pressure, and soil and agricultural conditions, China must establish herself as an important contributor to the global pesticide discovery effort via innovation and optimize the use of scientific capabilities and resources within the country. The National 973 Program recognizes the importance of chemistry, biology, and environmental stewardship to pesticide discovery. There were three basic research projects related to crop protection (2003−2008): (1) genomics and molecular mechanism of microbiological pesticides, (2) molecular biology and genetics of drought-resistance crops, and (3) green chemical pesticide research. The objective of the green chemical pesticide research program is to develop new approaches, methodologies, and basic research tools applicable to the discovery of chemical pesticides. Stage 1 (2003−2008) of the green chemical pesticide research program focused not only on establishing the infrastructure and research management system to conduct basic pesticide discovery research in China but also addressing the importance of research on pesticides that are novel in mode of action (MOA) and chemicals with favorable environmental and human hazard potential. Stage 2 (2010−2014) of the program will focus on the molecular target-oriented innovation of green chemical pesticides.

To better understand the scientific rationale behind the 973 green pesticide research program, we need to be familiar first with the current pesticide discovery being conducted by the multinational crop protection chemical companies. Historically, it is estimated that it will take about 8−10 years and a cost of approximately U.S. $400 million to develop a crop protection chemical from discovery to commercialization today. It took an average of screening of above 200,000 compounds to come up with one successful commercial product. Only a handful of multinational crop protection chemical companies have the resources and technical infrastructure to conduct pesticide research and development. There are only limited basic pesticide research programs carried out through governments, universities, and research institutes around the world. The basic discovery platform consists of a combination of several different approaches including (1) high-volume chemical screening, (2) chemistry/structure-based synthesis programs, (3) biological target-based synthesis programs, and (4) natural products research. The high-volume chemical screening and the natural products platforms yielded only limited successes and are unlikely to be sustainable. The structure-based synthesis approach, which is driven by the rapid exploration of biologically active structure scaffolds reported in the open literature and patents, is the most productive so far, and highly effective novel pesticides have been discovered. This platform had been adopted successfully in China. More than 25 pesticides have been discovered in the past 10 years and commercialized in China for domestic uses (*1*).

The target-based approach is the principal discovery platform for the pharmaceutical industry; however, the successful use of this approach in pesticide discovery has yet to be achieved. The pest targets (weeds, plant diseases, and insects), unlike most human disease targets, are much more complex and are not fully understood. The translation of laboratory in vitro activity to whole organisms, then to field efficacy, is much more difficult due to the contribution of potential host−target interactions, formulation selection, application methodology, environmental effects, and other biokinetic factors such as absorption/penetration, translocation, and metabolism. Although the success in adopting the biological target-based discovery strategy is questionable, from a basic research perspective, we believe this approach still holds the potential to identify new targets and modes of action for further exploration. Stage 1 of the green pesticide project focused on building up the core technology within China and attempted to discover innovative compounds by this research platform.

Approximately 40 principal investigators from the following major research institutes and universities were involved: Nankai University (Tianjin), East China University of Science and Technology (Shanghai), Central China Normal University (Wuhan), the China Agricultural University (Beijing), Shanghai Institute of Organic Chemistry, Shanghai Institute of Materia Medica, Shanghai Pesticide Research Institute, Northwest Agriculture and Forestry University (Yangling), Guizhou University (Guiyang), Dalian University of Technology (Dalian) and the Chinese Academy of Agricultural Sciences (Beijing).

2. SIGNIFICANT FINDINGS AND ACCOMPLISHMENTS

The design of the green pesticide research project fell into two main basic research strategies: "from gene via target to leads" (G2C) and "from chemistry via target to leads" (C2G), with the aim to identify novel lead structures and biological targets that will lead to the discovery of effective insect control compounds, herbicides, fungicides, antivirals, and elicitors. In addition, complementary technologies such as chemical−biological informatics for pesticide design and property estimations, combinatorial chemistry methodology, and in vivo high-throughput bioactivity microscreens were investigated.

In the past 5 years, greater than 600 papers have been published in the various SCI journals and about 40 have been published in *JAFC*. In addition, a selection of 22 papers are also published in this special issue of the *JAFC* (**Table 1**).

This commentary summarizes some of the key findings, which can be categorized into the following seven topics: (1) development chemoinformtics and bioinformatics decision support systems to aid hit generation, lead optimization, and virtual screening; (2) applications of combinatorial chemistry methodology and in vivo high-throughput bioactivity microscreens; (3) study on pesticide of resistance allosteric receptors and new methods of activity evaluation; (4) discovery of lead compounds with novel targets and new modes of action from natural products; (5) discovery of novel lead structures with insect growth regulator activity; (6) target-based design approach to discover lead herbicidal compounds; (7) novel lead compounds and targets of fungicides, antivirals, and elicitors.

2.1. Topic 1: Development of Chemoinformatics and Bioinformatics Decision Support Systems To Aid Hit Generation, Lead Optimization, and Virtual Screening. Computational technology has streamlined drug and pesticide discovery, design, development, and optimization, specifically expediting and facilitating hit generation/identification, hit-to-lead selection, and optimization processes. Computational tools are useful to optimize the physical chemical properties, absorption, distribution, metabolism, excretion (ADME), and toxicity profile and to provide an early safety assessment.

Table 1. Papers Published in This Special Issue of *Journal of Agricultural and Food Chemistry*

Fan, Zhijin	Synthesis and Biological Activity of Organotin 4-Methyl-1,2,3-thiadiazole-5-carboxylates and Benzo[1,2,3]thiadiazole-7-carboxylates
Fan, Zhijin	Synthesis, Crystal Structure, and Biological Activity of 4-Methyl-1,2,3-thiadiazole-Containing 1,2,4-Triazolo[3,4-*b*][1,3,4]thiadiazoles
Fan, Zhijin	Synthesis of 4-Methyl-1,2,3-thiadiazole Derivitaves via Ugi Reaction and Their Biological Activities
Huang, Qingchun	Comparable Susceptibilities of Human 293 Cells and Insect Tn-5B1-4 Cells to Photoactivated α-Terthienyl
Li, Zhong	Design, Multicomponent Synthesis, and Bioactivities of Novel Neonicotinoid Analogues with 1,4-Dihydropyridine Scaffold
Li, Zhong	Divalent and Oxabridged Neonicotinoids Constructed by Dialdehydes and Nitromethylene Analogues of Imidacloprid: Design, Synthesis, Crystal Structure, and Insecticidal Activities
Li, Zhong	Synthesis, Crystal Structure, and Insecticidal Activities of Highly Congested Hexahydroimidazo[1,2-*a*]pyridine Derivatives: Effect of Conformation on Activities
Liu, Changling	Synthesis and Biological Activity of New (*E*)-α-(Methoxyimino)benzeneacetate Derivatives Containing a Substituted Pyrazole Ring
Song, Baoan	Synthesis and Antiviral Bioactivities of 2-Cyano-3-substituted-amino(phenyl) Methylphosphonylacrylates (Acrylamides) Containing Alkoxyethyl Moieties
Cao, Song	Synthesis and Insecticidal Activity of Heptafluoroisopropyl-Containing Benzoylphenylurea Structures
Song, Gonghua	Design and Synthesis of Novel Insecticides Based on the Serotonergic Ligand 1-[(4-Aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (PAPP)
Wang, Daoquan	Synthesis, Fungicidal Activity, and Structure−Activity Relationship of Spiro-Compounds Containing Macrolactam (Macrolactone) and Thiadiazoline Rings
Wang, Daoquan	Primary Study on Mode of Action of Macrocyclic Fungicide Candidates (7B3, D-1) against *Rhizoctonia solani* Kühn
Wang, Qingmin	Synthesis and Antiviral Activities of Phenanthroindolizidine Alkaloids and Their Derivatives
Wang, Qingmin	Design, Synthesis, and Herbicidal Activities of Novel 2-Cyanoacrylates Containing Isoxazole Moieties
Xiang, Wensheng	Isolation and Identification of Novel Macrocyclic Lactones from *Streptomyces avermitilis* NEAU1069 with Acaricidal and Nematocidal Activity
Xu, Xiaoyong	Photodegradation of Novel *cis*-Configuration Nitromethylene Neonicotinoids with Tetrahydropyridine in Aqueous Solution
Wu, Wenjun	Synthesis of 1-Acyl-3-isopropenylbenzimidazolone Derivatives and Their Activity against *Botrytis cinerea*
Yang, Guangfu	Design, Synthesis, and 3D-QSAR Analysis of Novel 1,3,4-Oxadiazol-2(3*H*)-ones as Protoporphyrinogen Oxidase Inhibitors
Yang, Xinling	Synthesis, Biological Activity, and Hologram Quantitative Structure−Activity Relationships of Novel Allatostatin Analogues
Yao, Jianhua	Screening Rules of Leads of Fungicides, Herbicides, and Insecticides
Yuan, Huizhu	Study of Inhibitory Effects and Action Mechanism of the Novel Fungicide Pyrimorph against *Phytophthora capsici*

Commonly used computational approaches include ligand-based drug design (pharmacophore, a 3D spatial arrangement of chemical features essential for biological activity) and structure-based drug design (drug−target docking). Quantitative structure−activity and quantitative structure−property relationships (QSAR, QSPR) are actively utilized/supported by both global regulatory agencies and the pharmaceutical/pesticide industries. Because the training sets of most of these property predictive systems were based primarily on pharmaceutical molecules, their application to pesticide discovery might not be optimal. Three property prediction systems based mostly on highly diversified compounds as training sets hadve been developed: CISOC-PSCT for the prediction of carcinogenic toxicity, CISOC-PSMT for the prediction of mutagenic toxicity, and CISOC-LogP for the prediction of octanol−water partition coefficient (*2*,*3*). An in silico lead screening system (CISOC-LSS), which can assess property ranges such as LogP, NHD, NHA, MW, and PSA as screening rules of fungicides, insecticides, and herbicides, was also developed.

A series of quantum-chemical geometrical descriptors using the technique of density functional theory (DFT) for QSAR analysis was defined (*4*, *5*). This approach determined the bioactive conformations of several pesticides, and this strategy was applied in pesticide design research programs, such as the acetohydroxy-acid synthase inhibitors (AHAS).

Furthermore, a quantitative aggregation−activity relationship (QAAR) was established on the basis of the supermolecular view. On the basis of the structural information of dimers, the simplest model of the aggregation state, classical QSAR, was used for the investigation of the relationship between aggregation state and bioactivity. Aided by the template of the crystal structure of teflubenzuron, two dimer descriptors that could describe parts of the aggregation state characters were used to establish the QAAR model. The observed pesticide activity correlated strongly with the molecular aggregation state. The dimer descriptors showed better correlation with the bioactivity than the monomer descriptors (*6*).

2.2. Topic 2: Applications of Combinatorial Chemistry Methodology and in Vivo High-Throughput Bioactivity Microscreens. *2.2.1. Application of Ionic Liquid and a Combined Microwave/Ultrasound Irradiation Technique in Combinatorial Synthesis.* Combinatorial chemistry has been widely used in drugs, agrochemicals, new materials, and catalyst discovery processes. This application generates diverse molecular libraries for both hit generation and lead optimization purposes. The applications of ionic liquid and a combined microwave and ultrasound irradiation technique were evaluated. A parallel purification strategy based on "task-specific" (or functionalized) ionic liquids was developed. Amino- and carboxyl-functionalized ionic liquids were used as scavengers for the removal of excess acyl chloride, isothiocycanate, isocyanate, methanesulfonyl chloride, aniline, toluidine, and benzyl chloride reagents in combinatorial synthesis, such as in solution-phase reactions. These have several advantages, namely, higher load capacity, shorter sequestration duration, lower dosage, reusability, ease of scale-up, broader solvent selection, and higher chemical yields and purities of final products. Ionic liquids have proved to be efficient scavengers in the removal of excess reagents (*7*).

Furthermore, hydroxyl-functionalized ionic liquid has been used as a soluble support in solution-phase combinatorial synthesis of methyl 6-amino-5-cyano-4-aryl-2-methyl-4*H*-pyran-3-carboxylate. This process can be accelerated by microwave irradiation, which offered considerable advantages such as homogeneous reaction conditions, higher loading capacity, compatible with automatic manipulation, and high yields and purities without further chromatographic cleanup. A small library of 4-aminophenyl ether derivatives was constructed with high yields and purities via the Williamson reaction using the carboxyl-functionalized ionic liquid as soluble support. The recovered ionic liquid after cleavage can be reused in another cycle without losing its activity (*8*).

Microwave-assisted combinatorial chemistry has become increasingly popular due to its advantages in reducing reaction time, avoiding side products, and increasing yields. By utilizing the synergistic effect of microwave and ultrasound, the combined microwave and ultrasound irradiation (CMUI) technique was more efficient, inexpensive, and an energy-saving tool in parallel synthesis. The dramatic acceleration in reaction rate is ascribed to the simultaneous enhancement of heat and mass transfer across the interface of phases even in the absence of any auxiliary reagents such as phase transfer catalysts (*9*). The applicability

of CMUI methodology will be further evaluated under stage 2 of this project.

2.2.2. Establishment and Application of Highly Efficient in Vivo Biological Screening Models for Novel Agrochemicals. The novelty of the chemistry and a highly sensitive and selective whole organism biological screening system are two of the most important criteria for a successful pesticide discovery platform. The objective of this project was to establish highly efficient, miniaturized in vivo biological screening models (using insect pests, plants, and plant diseases that are of economic importance to China). The screening capacity for the primary insecticide, fungicide, and herbicide screen is approximately 10000 samples per year, and the total compound requirement is about 10 mg. Although this screening capability is still low, it provided Chinese pesticide researchers a standardized and sensitive bioassay platform (*10*).

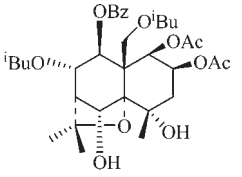
The primary insecticide screens include 11 insect pest species and 7 application/exposure models (compound requirement = 2−3 mg): topical application, leaf sandwich, Erlenmeyer flask, continuous immersion of host plant, dipping of eggs, immersion of insect, and spraying. During stage 1 of this project, the insecticidal activity of several novel insect growth regulators (IGRs) and neonicotinoids had been identified. Biological assay data also confirmed that the metamorphosis from the pupae to the adult stage was the MOA of several oxadiazole derivatives to Lepidopterans (*11*).

The primary fungicide screens target 23 important crop diseases in China (compound requirement = 1−2 mg). The microspraying system evaluates six different types of phytopathogens, which are air-spread or soilborne fungi, bacteria, and viruses at the same time. Crop pathogen collections include approximately 210 types of plant diseases and 5000 pathogen strains. Some of the tests plants used in the in vivo microscreening of fungicides include tomato, cucumber, potato, cereal, and rice (*10*). The primary herbicide screens use both the culture dish method and small plant method in 96-well plates and the greenhouse pot culture methods. The compound requirement is about 3 mg. A diverse collection of 33 types of weed species, 13 types of crop species, and 8 types of lawn seeds are used for herbicidal screening.

For elicitor screening for systemic acquired resistance, BTH and β-aminobutyric acid are chosen as positive controls; 1,2,3-benzothiadiazole-7-carboxylic acid and 2,4-dioxohexahydro-1,3,5-triazine are chosen as negative controls (*12*).

2.3. Topic 3: Novel High-Throughput System of Screening Pesticide Efficacy on Resistant Allosteric Target Site. Four mutations of acetylcholinesterase (AChE) confer insecticide resistance in *Drosophila melanogaster* natural populations, and these four mutations with different combinations are distributed around the world (*13*). It was also found that the resistance level increased and the resistant spectra widened with the combination number of mutations in the same allele. For a specific mutant, the mutated enzyme demonstrated a unique pesticide resistance spectrum. By testing the catalytic activity and protein thermostability of the engineered mutated proteins, researcher's findings indicated that alteration of enzymatic activity and protein stability resulted in the fitness cost associated with pesticide resistance (*14*). The resistant allosteric AChE library was established from series of engineering housefly (*Musca domestica*) AChE using PCR-based site-directed mutagenesis and baculovirus expression systems. Pesticide efficacy on the resistant allosteric target site using novel high-throughput screening system was evaluated. A DFT-based QSAR technique was used to design AChE inhibitors. The highly selective chemicals were synthesized on the basis of structural biology and computational chemistry. Biological activity was measured by high-throughput in vitro screening using highly purified engineering mutated AChE. With the assistance of enzyme-induced dynamic combinatorial chemical synthesis some polypyridine derivatives were obtained, which showed very strong inhibition activity to the resistant allosteric target site of AChE (Z. H. Tang, unpublished data).

2.4. Topic 4: Discovery of Lead Compounds with Novel Targets and New Modes of Action from Natural Products. Natural products continue to provide compounds with novel biological properties. Celangulin V (**1**), α,β-dihydroagrofuran sesquiterpene polyester, is one of the bioactive components in *Celastrus angulatus*. Insects affected by celangulin V exhibited typical symptoms of body fluid loss. Further investigation on the ultramicrostructure of the midgut of the intoxicant larvae revealed that the microvilli, mitochondria, and rough endoplasmic reticulum were affected. These results suggested that there might be a putative receptor targeted by celangulin V in the midguts of affected insects.



Celangulin V (1)

The aim of this investigation is to isolate the receptor protein, analyze the molecular structure, and develop the receptor model to design novel pesticides that could affect the insect digestive system.

The receptor localization of celangulin V in the midgut tissues of *Mythimna separata* was carried out by immune−electron−microscopy (IEM) using anti-celangulin V monoclonal antibodies (Mabs) as the primary antibody and coated anti-mouse/IgG labeled with colloidal gold as the secondary antibody. Immunohistochemical staining of the midgut sections revealed that there was no specific celangulin V staining detected in the tissue section of the control insects, whereas the most colloidal gold staining was located on the treated midgut epithelia of the insects (**Figure 1**). The results proved that there are receptors of celangulin V in the midgut tissues of several Lepidopteran species.

To isolate the target protein, fluorescein-labeled celangulin V was synthesized. Brush border membrane vesicles (BBMV) were prepared from the midgut of the larvae treated with fluorescein-labeled celangulin V. Two celangulin V receptor protein fractions were separated from BBMV by the techniques of DEAE 52 ion-exchange chromatography, C18 HPLC, and SDS-PAGE. The primary structures of the protein were characterized by means of MALDI-TOF-MS. Proteins I and II consisted of 776 and 349 amino acid residues, molecular masses of 85717 and 41489 Da, respectively. This potential target will be further explored as a novel insecticide lead (*15*, *16*).

Tautomycin, owing to a unique 2,3-dialkylmaleic anhydride or diacid moiety, has strong antifungal activity against *Sclerotinia sclerotiorum* and inhibitory activity to protein phosphatase of types 1 and 1A. In nature, products with maleic anhydride or maleic diacid structure showed good biological activities, such as antifungal agents, enzyme inhibitors, and herbicides. Systemic acquired resistance (SAR) with maleic anhydride as the core structure against fungi was investigated to determine whether the dialkylmaleic anhydride or diacid moiety is required for the biological activity in these compounds. Results showed tautomycin could be hydrolyzed into two fragments, including the maleic anhydride moiety, which could be bound with other compounds to form new compounds with maleic anhydride (*17*). In addition,

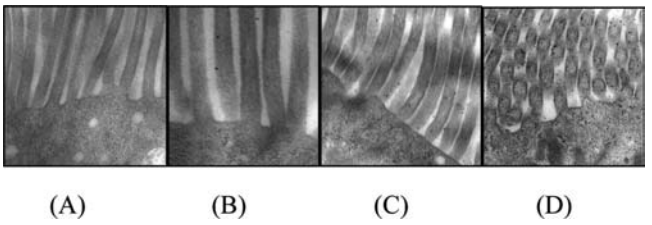
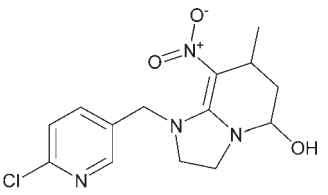


Figure 1. Immunohistochemical staining of microvilli in the insect midgut treated by celangulin V: immediately before treatment (**A**) and 0.5 h (**B**), 1.0 h (**C**), and 1.5 h (**D**) after treatment.

validamycin A is effective against sheath blight of rice plants and soilborne diseases, and its microbial transformation to produce valienamine as well as the C−N lyase has been explored (*18*, *19*).

2.5. Topic 5: Discovery of Novel Lead Structures with Insect Growth Regulator Activities. One alternative approach to pest management, other than acute/lethal insecticidal activity, is to control the target pest via nonlethal modes of action such as antifeeding or growth regular activity. We highlight the results of several projects that examined novel compounds with antifeeding and IGR properties.

2.5.1. Antifeeding Activity of a Novel Neonictinoid IPP-10 against Rhopalosiphum padi on Wheat. IPP-10 (**2**) is a novel neonicotinoid insecticide developed by East China University of Science and Technology. Primary bioassay data showed that IPP-10 had good activity against sucking insects, such as *Bemisia tabaci*, green aphid, wheat aphid, and rice brown plant hopper. Another interesting biological observation was that IPP-10 was also active against rice brown plant hoppers that were resistant to imidacloprid. This observation suggests that the MOA of IPP-10 might be different from that of imidacloprid and that this compound can be used to combat imidacloprid resistance.



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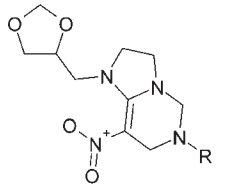
The LC_{50} values of IPP-10 against *Rhopalosiphum padi* by foliar-spray and root-systemic uptake methods were 2.5 and 4.3 mg L^{-1}, respectively. IPP-10 has good xylem and phloem mobility, and hence it has potential for seed treatment and soil application.

Compared to imidacloprid, after treatment of the wheat upper leaf with 1000 mg L^{-1} IPP-10, the mortalities of *R. padi* in lower wheat leafs were 14 and 33%, respectively, 24 and 48 h after treatment. Mortality was only 3 and 7% for imidacloprid. IPP-10 also affected the feeding behavior, growth rate, and reproduction of *R. padi*.

2.5.2. Evaluation of Nitroketene Neonicotinoids with Cis Configuration. Neonicotinoids, targeting insect nicotinic acetylcholine receptors (nAChRs), exhibited low mammalian toxicity and favorable environmental fate and effects. The nitro group in neonicotinoids played an important role in their insecticidal activities. The nitro group in all commercialized neonicotinoids is in the trans configuration, and the proposed MOA is based on the trans configuration. However, research on neonicotinoids with the nitro group in the cis configuration was very rare due to synthesis difficulties. The objective of this project was to synthesize neonicotinoids with the cis configuration and to explore the effect of configuration on activity and MOA. In addition to the search for novel compounds, this project evaluated strategies to combat the emergent insect resistance to the conventional *trans*-neonicotinoids. The initial molecular design involved the use of fused heterocyclic or bulky groups to direct the position of the nitro group.

Series of neonicotinoids (**3** and **4**) with tetrahydropyrimidine in the cis configuration were synthesized. Some of these compounds showed only moderate activity against cowpea aphids (*20*). By introducing the tetrahydropyridine substructure, a series of nitromethylene neonicotinoids with the cis configuration (**5**) were synthesized. Meanwhile, small substituent groups were introduced into the 5-, 6-, and 7-positions of hexahydroimidazo[1,2-α]pyridine to generate analogues (**6**). Most of the compounds in this series were active against cowpea aphids and brown plant hopper. Furthermore, some compounds also showed higher insecticidal activity against imidacloprid-resistant brown plant hoppers. Radioligand displacement and electrophysiology experiments showed that these compounds had different MOA from imidacloprid (*21*).



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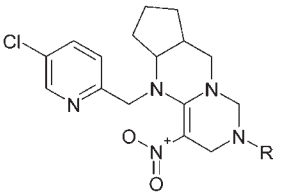

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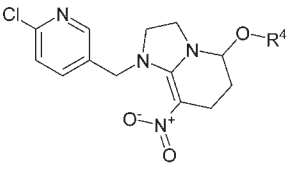

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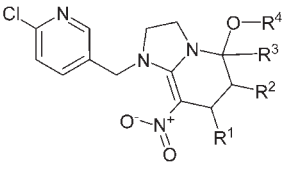

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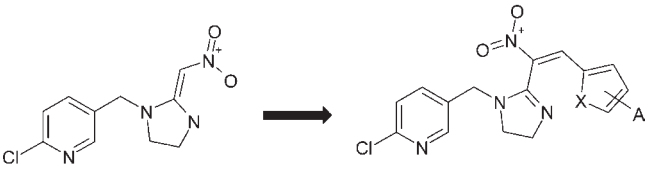
R^1 =methyl, ethyl, n-propyl, i-propy, n-butyl, Phenyl;
R^2 = methyl, ethyl; R^3 = methyl ;
R^4 = methyl, ethyl, n-propyl, i-propy, n-butyl, chloroethyl, benzyl

A series of novel neonicotinoids analogues with bulky groups fixed at the cis configuration (**7**) were synthesized (*22*). Some of the synthesized compounds exhibited high insecticidal activities against cowpea aphids (*Aphis craccivora*), armyworm (*Pseudaletia separate*), *Nephotettix bipunctatus*, small brown rice plant hopper (*Laodelphasx striatellus*), and imidacloprid-resistant brown plant hopper. The activity levels of some of these new compounds were comparable to that of imidacloprid. Preliminary research on MOA revealed that these *cis*-neonicotinoids are potent antagonists of the nAChRs. The potential use of the *cis*-neonicotinoids in resistance management is now under further investigation.



X = O, S, NH (7)

2.5.3. Actions between Neonicotinoids and Receptor: Hydrogen Bonding and π−π Interaction. Neonicotinoid insecticides show selective action on insect nicotinic acetylcholine receptor (nAChR). Two key residues (Trp and Arg/Lys) have been

identified as contributors to the neonicotinic binding. To investigate the selective mechanism, a computational model was developed to simulate the interaction between residues (Trp and Arg) of insect nAChR and neonicotinoids by quantum chemistry methodology. Three analogues of neonicotinoid derivatives without the chloropyridinyl moiety, 3-methylindole (3MI), and guanidinium (Gua) were used to mimic the neonicotinoids and the side chain of key residues Trp and Arg accordingly. Interaction features of the 3MI-analogue, analogue-Gua, and 3MI-analogie−Gua complexes were simulated and compared. Hydrogen bonding between the nitro group of the analogues and Gua was found to be the most important for binding. Moreover, the cooperative $\pi-\pi$ interaction between the analogue and the indole ring, which is strengthened by the presence of Gua, also contributed to the binding (**Figure 2**). The alternative binding model of neonicotinoid involving hydrogen-bond-induced $\pi-\pi$ interaction was proposed (*23*).

2.5.4. Discovery of New IGR Leads and Receptor with Peptidomimetics Approach. IGRs are good examples of green pesticides because of their highly selective potency and minimal impact on the environment and nontarget organisms. The potentials of insect corpora allata (CA) and cockroach-type allatostatins (ASTs) as the target of IGRs were investigated. A novel series of AST analogues were discovered as IGR candidates by the peptidomimetics approach (PA). PA strategy can be used to discover new drugs or bioactive compounds through mimicking the structure of peptides while overcoming the shortage of natural peptides.

Conformation analyses by 2D NMR and molecular dynamics (MD) showed native Dippu-AST I (LYDFGLa, belonging to type A ASTs that shared the common C-terminal sequence Y/FXFGLa) formed a type I′β-turn conformation in DMSO. The low-energy conformations (in water) of Dippu-AST I and six other peptides (also containing β-turn) were studied using MD. The results showed that X in the C-terminal sequence played an important role in the turn conformation, which is ubiquitous in cockroach-type allatostatins (*24*).

The common C-terminus Y/FXFGLa, a pentapeptide sequence as the minimal sequence required for functional inhibition of JH in vitro biosynthesis, is generally regarded as the "active core" region responsible for direct receptor interaction. Thus, this core pentapeptide region was corroborated as the ideal lead in the search for new IGRs (*25*). A series of novel AST analogues, which mimicked each amino acid of the core region pentapeptide, were designed with different aromatic acids, fatty acids, and dicarboxylic acids to replace the Y/FX region. The effect of the AST analogues on JH biosynthesis by cockroach CA in vitro was carried out. The activities of K15 and K24 (IC_{50} = 1.79 and 5.32 nM, respectively) were better than that of a natural AST (Dippu-AST 1, IC_{50} = 8 nM) on in vitro JH biosynthesis by cockroach CA. Particularly, K15 was more active than most natural Dippu-ASTs. The preliminary SAR results suggested that the terminal sequence FGLa was more important than Y/FX to bioactivity. A predictable and statistically meaningful Hologram Quantitative Structure−Activity Relationship (HQSAR) model of 32 AST analogues was obtained. The final model showed that a potent AST analogue should contain an aromatic group, an appropriate linker in the Y/FX region, and the FGLa portion. This result is useful in the design of new AST analogues that are structurally related to the training set compounds.

On the basis of the effects on JH biosynthesis inhibition by the tripeptide (FGLa) and its mimics incorporating the interaction between the analogues and target, a new hypothesis of a shorter core region (Phe-Gly-Leu-NH_2) was proposed, and additional IGR candidates for cockroach control are under further evaluation.

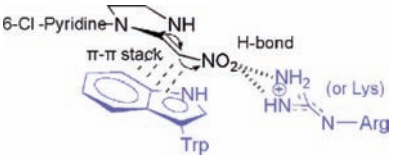
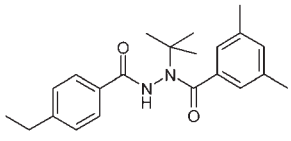


Figure 2. H-bond-induced $\pi-\pi$ interaction between neonicotinoids and nAChR.

2.5.5. Novel β-N-Acetyl-D-hexosaminidase from the Asian Corn Borer Ostrinia furnacalis (Guenee) as Potential IGR Target. A novel β-*N*-acetyld-hexosaminidase (OfHex1) from the fifth instar larva integument of the Asian corn borer, *Ostrinia furnacalis* (Guenee) was isolated, purified, characterized and evaluated as a potential insecticide target. OfHex1 had been shown to be an exosplitting enzyme, acted by cleaving one β-GlcNAc unit from the nonreducing end of substrates. Sequence analysis indicated that it was different from the reported β-*N*-acetyl-D-hexosaminidase with *N*-glycan hydrolytic activity. Real-time PCR determined that its transcriptional level increased dramatically before the molting stage (*26*).

According to its hydrolytic activity, substrate spectrum, cDNA sequence and mRNA transcriptional level, it is postulated that OfHex1 could only be involved in the insect chitin catabolism. Its species specificity and narrow substrate spectrum made it a potentially specific target for the development of novel pesticides, and to further our knowledge about glycosyl hydrolases enzyme (*27*, *28*).

2.5.6. Discovery of Novel N-Sulfenate Derivatives of N, N′-Diacylhydrazines as New Insect Growth Regulators. The MOA of *N*-*tert*-butyl-*N*,*N*′-diacylhydrazines (for example, tebufenozide (**8**) discovered by Rohm and Haas) was identified as a nonsteroidal inducer (MOA as ecdysone agonists), especially in *Lepidoptera*. However, the low water and organic solvent solubility limited the biological efficacy of the diacylhydraines. This study explored the hypothesis if the introduction of a substituted phenoxysulfenyl or alkoxysulfenyl (**9**) substituent into *N*′-*tert*-butyl-*N*,*N*′-diacylhydrazines could improve physical, chemical, and biological properties.



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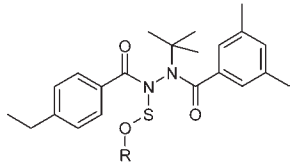

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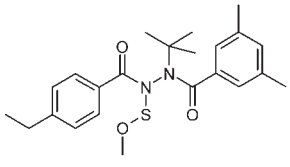

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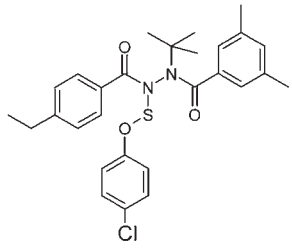

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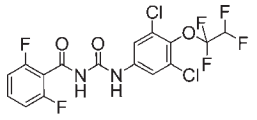
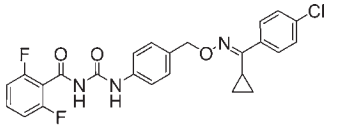
Analogues based on compound **9** had significantly improved solubility compared with compound **8** in organic solvents such as methylene dichloride, chloroform, toluene, xylene, petroleum ether, which made them easier to formulate for field application. Analogues based on compound **9** showed both oral and contact toxicities higher than the corresponding values for **8**. In particular,

N-methoxysufenyl-*N*′-*tert*-butyl-*N*-4-ethylbenzoyl-*N*′-3,5-dimethylbenzoylhydrazide (**10**) and *N*-(4-chlorophenoxy)sulfenyl-*N*′-*tert*-butyl-*N*-4-ethylbenzoyl-*N*′-3,5-dimethylbenzoylhydrazide (**11**) showed higher oral toxicities against oriental armyworm (2×), beet armyworm (3−5×), and tobacco cutworm (2×) than tebufenozide. Furthermore, compounds **10** and **11** exhibited higher contact activities against Asian corn borer (2×), tobacco cutworm (2−33×), and cotton bollworm (18×) than tebufenozide. The observed high contact toxicities of **10** and **11** warrant the further field evaluation of these compounds (*29*).

2.5.7. Design, Synthesis, Bioactivity, and SAR Studies of Novel Benzoylphenylureas (BPUs) Containing the Oxime Ether Moiety. BPUs had been developed as chitin synthesis inhibitor since diflubenzuron (Dimilin) was first introduced. A unique MOA coupled with a high degree of activity on targeted pests and low toxicity to nontarget organisms (including many beneficial arthropods) made BPUs a useful tool for integrated pest management (IPM). Hexaflumuron (**12**) is highly effective to control termites.



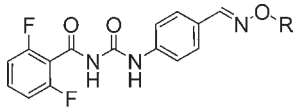
hexaflumuron (12)



flucycloxuron (13)

The oxime ether moiety is widely used in pesticide and drug molecular design. For example, flucycloxuron (**13**) discovered by Solvay-Duphar B.V. is an IGR containing the oxime ether moiety.

The synthesis route for BPUs containing the oxime ether moiety (**14**) involved the condensation of *p*-nitrobenzaldehyde with hydroxylamine hydrochloride to yield (*E*)-4-nitrobenzaldehyde oxime,and subsequent reaction with RX or TsOR, reduction using iron powder, and finally combination with 2,6-difluorobenzoyl isocyanate to yield the target compounds (**14**).



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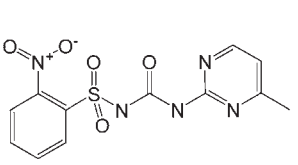
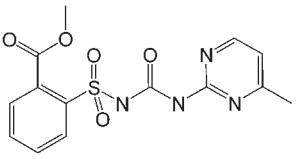
The results of bioassays showed that most compounds (**14** series) exhibited excellent larvicidal activities against oriental armyworm and mosquito. SAR indicated that the larger size of the *O*-alkyl target compounds increased the larvicidal activity. Several new analogues, which are more active than hexaflumuron and flucycloxuron, are under further field evaluation (*30*).

2.6. Topic 6: Target-Based Design Approach To Discover Lead Herbicidal Compounds. *2.6.1. Discovery of Highly Active Herbicidal Compounds Targeting Acetolactate Synthase (ALS)*. ALS (EC 2.2.1.6) is a key enzyme that catalyzes the synthesis of the critical branched-chain amino acids (valine, leucine, and isoleucine) in higher plants. These enzymes are highly plant specific, and adverse effect to insects, mammals, and the environment have not been reported.

There are more than 30 commercial sulfonylurea herbicides on the market today. All of them contain a disubstituted heterocyclic (trazine, pyrimidine) moiety. This is the first report that a monosubstituted pyrimidine sulfonylurea showed superior herbicidal activity. Following this lead, more than 900 novel monosubstituted sulfonylurea compounds have been synthesized. Two candidate compounds were selected for commercial development: monosulfuron (**15**) and monosulfuron-ester (**16**) (*31*).



monosulfuron (15)

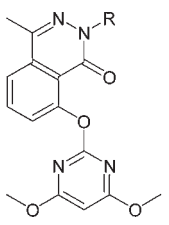


monosulfuron-ester (16)

Monosulfuron and monosulfuron-ester are selective in controlling economically important weeds such as goosefoot and climbing bindweed, which are difficult to control by other herbicides in millet and wheat in northern China. These compounds are safe for rotational crops such corn, soybean, and peanut. Monosulfuron is considered to be the first official registered herbicide invented, developed, and commercialized in China.

2.6.2. Rational Design of a Protoporphyrinogen Oxidase (PPO) Inhibitor Herbicide Based on Bioactive Conformation Analysis. The potential of novel PPO and AHAS inhibitor as herbicidal candidates was evaluated using an integrated technique of molecular simulations, organic synthesis, and molecular biology. The experimental approach included (1) the definition of a series of quantum-chemical geometrical descriptor at density functional theory (DFT) level for QSAR analysis, (2) the use of a new DFT-QSAR strategy by combining DFT calculation and QSAR to determine the bioactive conformations of some pesticide molecules, and (3) the determination of the ability of small molecules to adopt the conformational change of the binding pocket upon site mutation of the target protein. This project developed a novel strategy of conformational flexibility analysis based rational design to guide the molecular design of lead structure with low resistance risk.

QSAR models for a series of PPO inhibitors, including diverse cyclic imides and phenyl triazolinone derivatives, were developed. The results indicated that DFT-based models always achieve better correlation than the PM3-based models. It should be pointed out that this is the first example of DFT application in the QSAR study of agrochemicals. The application of the DFT-based steric descriptors in the QSAR analysis of sulfonylurea analogues resulted in excellent QSAR models, thus leading to a better understanding of the MOA at the electronic structural level. Using the DFT-QSAR strategy, the bioactive conformation of a series of cyclic imide derivatives as PPO inhibitors and pyrimidinylthiobenzoates as AHAS inhibitors was confirmed. Lead optimization based on 8-(4,6-dimethoxypyrimidin-2-yloxy)-4-methylphthalazin-1-one derivatives (**17**) showed good AHAS inhibition activity and interesting herbicidal activity. Furthermore, on the basis of the strategy of conformational flexibility analysis, a series of 2-aroxyl-1,2,4-triazolo[1,5-*c*]pyrimidines (**18**) as conformation-flexible AHAS inhibitors were also synthesized. Due to similar inhibition effects against the wild-type and W574L mutant AHAS, these compounds could be used as new leads for future antiresistant herbicide development (*32*, *33*).



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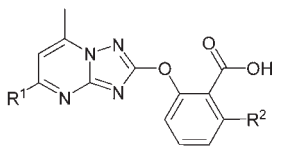

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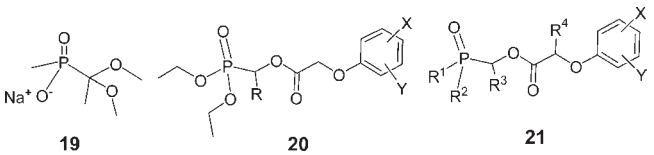
2.6.3. O,O-(Substituted-phenoxyacetoxy)alkylphosphonates as Novel Pyruvate Dehydrogenase Complex Inhibitors (PDHc). PDHc is a target enzyme acted on by some organophosphorus

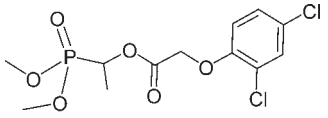
compounds, such as acetylphosphonate M (**19**). Although they exhibited good activity, 80−100% inhibitory effect against weeds at the rate of 2.8 kg ha^{-1}, these OP compounds were not candidates for commercial development due to unacceptable phytotoxicity to the crops at rates that gave good weed control. Furthermore, some PDHc E1 inhibitors also exhibited adverse mammalian toxicity.



19 **20** **21**

The synthesis strategy focused on the phosphonate scaffold; an aryl or a heterocyclic group was introduced to form α-oxophosphonic acid derivatives. *O*,*O*-Diethyl (substituted-phenoxyacetoxy)alkylphosphonates (**20**) and (**21**) were identified as scaffolds for lead structures.

Several analogues of compound **21** showed notable herbicidal activity against dicotyledons and were also effective in vitro inhibitors of PDHc (*34*). SAR analyses indicated that both the inhibitory potency against PDHc and herbicidal activity were affected by the chemical modification of R^1, R^2, R^3, R^4, X, and Y. Optimal inhibitory potency against PDHc and herbicidal activity could be achieved by introducing smaller groups such as MeO or NaO as R^1 and R^2 in the phosphonate moiety and Cl or F atoms as X and Y at the 2- and 4-positions on the phenoxy ring. This SAR was further explained by the molecular docking studies on the binding modes of compounds **21** to PDHc E1. Electronegative groups (F or Cl) at the 2- and 4-positions enhanced the interaction of inhibitor and the active site of PDHc E1 (*35*). *O*,*O*-Dimethyl 1-(2,4-dichlorophenoxyacetoxy)ethylphosphonate (HW02, **22**) was found to have good selectivity between monocotyledonous crops and dicotyledonous weeds. More than 40 field trials in different regions of China showed that HW02 controlled a broad spectrum of broadleaved and sedge weeds at the rate of 150−450 g ha^{-1} for postemergence in lawn, wheat, and maize fields. HW02 has low acute toxicity against rats and low toxicity to bees, birds, fishes, and silkworm. HW02 has received temporary registration recently in China.



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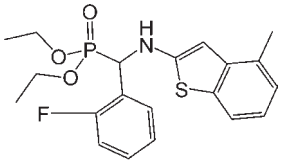
2.7. Topic 7: Target-Based Design To Discover Lead Fungicides, Antivirals, and Elicitor Compounds. *2.7.1. Discovery and Application of Lead Compounds as Potent Antiplant Virus Agent Based on Natural Products and Novel Molecular Targets.* This project investigated antiplant virus compounds from wild plants and animals in Karst areas of western China using SAR as molecular target (*36*).

Five different types of potent antiviral compounds including α-aminophosphonate derivatives, chiral α-aminophosphonates, chiral cyanoacrylates, pyrazole and quinazoline heterocyclic derivatives, and chiral thiourea derivatives were synthesized by structural modification of natural compounds derived from wild plants and animals in the Karst area of western China.

Key molecular targets of the SAR signal pathway, such as the PR-1a gene, PR-5 gene, SOD, POD, PAL, and chlorophyll, were examined using real-time PCR, semiquantitative PCR, and other biochemical procedures.

The confirmation of dufulin (*O*,*O*′-diethyl-α-(4-methylbenzothiazole-2-ylamino)-(2-fluorophenyl) phosphonate, **23**) as an effective antiviral agent is a breakthrough achieved in this project. Results showed dufulin induced PR gene up-regulation in tobacco, increasing the activity of the tobacco defense enzyme and chlorophyll content. Plastid-lipid-associated protein and chloroplast cysteine synthase 1 were also related with SA content. SAR was activated by SA signal molecule (*37*).



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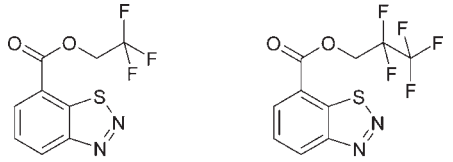
Dufulin was granted registration as a new chemical entity with high anti-TMV activity by the Ministry of Agriculture of China. It is the first antiplant viral agent to meet the environmentally friendly criteria defined in China (*38*, *39*).

2.7.2. Plant Activator of Benzothiadiazoles with Polyfluoro Groups as Potential Green Pesticides. SAR occurs during stress environment stimuli, including addition of elicitors, and induces a set of defense responses in signal transduction. On the basis of the mechanism of elicitors related with H_2O_2 burst and PAL enzyme activity, novel fluoro-substituent derivatives (trifluoroethyl and pentafluoro moieties) to the ester part of BTH were synthesized on the basis of the first commercial SAR activator of plants. The target compounds of 2,2,2-trifluoroethylbenzo-1,2,3-thiadiazole-7-carboxylate (**24**) and the related pentafluoro analogue (**25**) induced defense in the field for *Erysiphe cichoracearam* of 86%, higher than that of BTH, which was 66% at the same concentration of 100 mg L^{-1} (Y. F. Xu, unpublished data). Furthermore, two early and important events in plant defense responses, oxidative burst and activation of PAL, were also evaluated. Results showed that the H_2O_2 level and PAL activity were induced during the eliciting process; these compounds could induce the defense responses in signal transduction (*40*).



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2.7.3. Perylenequinonoid Photosensitizers (PQPs) as Potential Agricultural Antibiotics. The objectives of this project were to explore the potential of PQPs as novel agricultural antibiotics to control phytopathogens and to elucidate their photodynamic mechanisms and SAR. PQPs, including hypocrellin A (HA), hypocrellin B (HB), elsinochromes A, B, and C (EA, EB, EC), hypericin (HYP), and cercosporin (CP), are naturally occurring pigments, long used as folk medicines.

PQPs (e.g., HA, EA, EB, EC), when dispersed into micellar systems, were very effective in inhibiting the growth of various fungi (such as *Alternaria mali*, *Physalospora piricola*, *Glomerella cingulata*, *Fusarium oxysporum*, *Botrytis cinerea*). Light exposure further enhanced their activity. Reactive oxygen species (ROS) had been identified to be responsible for the antifungal activity of PQPs. Because the photodynamic and antifungal activities of HA, EA, EB, and EC are similar to each other, this implied that

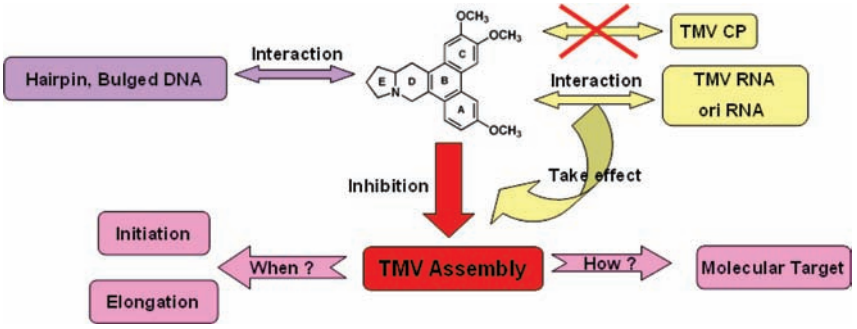
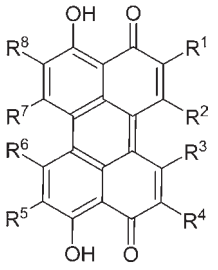


Figure 3. Molecular events for TMV assembly begin with specific recognition by the coat protein aggregate of oriRNA.

4,9-dihydroxy-3,10-perylenequinone (**26**) is the active moiety of PQPs (*41*).



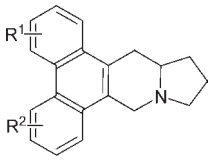
Tigecycline 26

To fully evaluate the potential of PQPs as photoactive antibiotics, the photobleaching behaviors of some PQPs (e.g., HA and EC) were studied in two organic solvent systems (ethanol and DMSO). Although HA and EC possess comparable photosensitizing activities, EC is more stable to photobleaching than HA. Both pigments exhibited higher photostability in ethanol than in DMSO. Therefore, it seems that EC has more developmental potential than HA, and its photobleaching behavior can be modified by changing media polarity (*42*).

Various physicochemical properties of PQPs (e.g., HA, EA, and HYP) were calculated by density functional theory (DFT) and time-dependent density functional theory (TD-DFT) methods. It was found that although PQPs can photogenerate $^1O_2$ through energy transfer in both nonpolar and polar solvents, they yielded $O_2^{\bullet -}$ only in polar solvents, and it is the PQP anions (generated from autoionization) that are responsible for the $O_2^{\bullet -}$ generation (*43*). The different photodynamic behaviors of HA and HYP could be elucidated by their different proton dissociation abilities (*44*).

In conclusion, PQPs have great potential as fungicides, and 4,9-dihydroxy-3,10-perylenequinone is a novel fungicidal lead. Moreover, the unique ROS-based antifungal mode of PQPs has the advantage of multiplying action targets on microbes and thus is promising to retard the resistance of pathogenic fungi.

2.7.4. Novel Antiviral Agent Tylophorine B and Its Mode of Action. Tylophorine B has remarkable biological activity against tobacco mosaic virus (TMV); it exhibited 60% inhibition against TMV at a concentration of 1×10^{-6} g mL^{-1}. In contrast, a commercial antiviral compound, DHT, provided 50% inhibition at the concentration of 5×10^{-4} g mL^{-1} under the same experimental condition. However, tylophorine B is not photostable; new derivatives, for example, NK-007 (**27**), were synthesized, which showed excellent photostability and antiviral activity to TMV (*45*).



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High affinity for TMV RNA and the assembly origin of TMV RNA (oriRNA) was revealed, accompanied by the conformational change of RNA. The molecular events for TMV assembly beginning with the specific recognition by the coat protein aggregate of oriRNA are illustrated in **Figure 3**. Tylophorine B has favorable interaction with oriRNA, likely exerting its virus inhibition by binding to oriRNA and interfering with virus assembly initiation. This exploratory study may shed light on the possible molecular inhibition mechanism against TMV by tylophorine B and provide clues in rational design of sequence-specific RNA-binding antiviral drugs (*46*).

3. CLOSING REMARKS

Stage 1 of the 973 green chemical pesticide research program has provided the Chinese research community valuable experiences in conducting basic pesticide discovery research. Several novel compounds with commercial level of activity were identified and are currently under further field evaluation. It is the goal of this program to establish China as a major scientific contributor in agricultural chemistry and pesticide sciences. Looking forward to stage 2 of this program, we will focus on the investigation of the relationship between small molecules as leads or pesticides and biological macromolecules as targets, for example, regulation and recognition. It is important to stress that the discovery of pesticides must be based on well-understood molecular targets. The design of lead chemistry and target specificity should be considered along with the understanding of the environmental and toxicity potential at the earliest stage of molecular design.

Finally, the support of and interest in our efforts expressed by the *Journal of Agricultural and Food Chemistry* and its Editor-in-Chief, Professor James N. Seiber, are greatly appreciated.

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