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The Tiny but Marvelous Methyl Group in Insecticide Discovery: A Perspective

Qiu Liu, Xingjie Zhang, Tangbing Yang, Yuqin Luo, Runjiang Song *, Baoan Song *

State Key Laboratory of Green Pesticides, Center for R&D of Fine Chemicals, Guizhou University, Guiyang 550025, China

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ABSTRACT

As the most fundamental organic unit, the methyl group is ubiquitously present yet frequently overlooked in various insecticide architectures. Despite its simplicity, this moiety plays a pivotal role in insecticide discovery. This perspective highlights documented cases of popular insecticides in which methyl substitution increases target affinity and bioactivity, alongside an analysis of the underlying molecular mechanisms. We propose insights into currently unsolved issues and future directions for leveraging methyl incorporation to accelerate the discovery of new agrochemicals. To our knowledge, this constitutes the first comprehensive perspective on the functional significance of methyl groups in agricultural chemistry. We expect this work to inspire methyl-driven optimization strategies for next-generation insecticides, thereby contributing to sustainable pest management.

1. Introduction

At present, the dual challenges of increased pest and disease outbreaks driven by climate change and growing food demand due to population expansion have intensified global food security concerns [1–3]. According to the Food and Agriculture Organization of the United Nations, agricultural pests account for 20%–40% crop yield losses per year, inflicting approximately 300 billion USD in economic damages [4,5]. While pesticides play a crucial role in safeguarding food security and mitigating losses, the rapid evolution of pest resistance to insecticides has led to the management failures of numerous conventional agrochemicals [6]. Furthermore, the fact that sexual reproduction in most insect species facilitates extensive genetic recombination significantly increases the probability of resistance being transmitted to offspring [7,8]. The diminished bioavailability of existing pesticides has paradoxically increased agrochemical application rates, exacerbating environmental burdens. These unresolved issues underscore the urgent need for discovering and sustaining novel alternative insecticidal candidates.

The development of active ingredients remains an exceptionally costly and time-intensive process, typically requiring over a decade and hundreds of millions of dollars from lead discovery through regulatory evaluations [9,10]. The identification of bioactive entities represents the key step, traditionally accessed through random screening, natural product-based modification, "me-too/follow-on" chemistry, and intermediate derivatization [11–14]. Recent advances in computational chemistry have introduced transformative methodologies—particularly structure-based virtual screening, which has demonstrated promising improvements in efficiency and target specificity during early-stage discovery phases, although limitations in broad-spectrum bioactivity remain [15,16]. The "me-too" paradigm is one of the most popular methodologies; it usually involves the structural optimization of established skeletons via scaffold hopping (e.g., bioisosteric replacement, chain lengthening/shortening, and ring opening/closure) [17]. However, the feasibility of this strategy largely depends on the availability of privileged scaffolds and the extent to which they can be adjusted. The Insecticide Resistance Action Committee (IRAC) classification system recognizes 37 insecticide mode-of-action families, with most members of each family either having undergone exhaustive structural optimization or possessing structural peculiarities that make modification nearly impossible [18]. Furthermore, many intermediates may require *de novo* route design and synthesis during the pathway, presenting substantial process chemistry challenges. When novel scaffolds emerge, the molecular hybridization method often incorporates classical insecticidal pharmacophores (e.g., pyrimidine, chlorothiazole, chloropyridine, cyano, trifluoromethyl, and diaryl ether groups) to

* Corresponding authors.
E-mail addresses: songrj@gzu.edu.cn (R. Song), basong@gzu.edu.cn (B. Song).

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increase lethality to pests [19]. Nevertheless, the limited reservoir of available lead structures in insecticide discovery makes it imperative for innovative strategies to accelerate agrochemical development through the maximal utilization of existing chemical resources.

The methyl group, comprising a carbon atom bonded to three hydrogen atoms, is the most basic organic moiety. Despite its seemingly minor and easily overlooked appearance, it has been found to play significant roles in biology, genetics, and pharmacology [20,21]. For instance, the methylation modification of a genome serves as a precisely tuned switch to govern the dynamic expression of genes and thus holds substantial importance in epigenetics [22]. Moreover, in medicine, strategic methyl incorporation routinely enhances drug efficacy and pharmacokinetic characteristics [23]. This phenomenon, in which the introduction of a single methyl group leads to a remarkable and often unexpected improvement in drug properties, is commonly referred to as the "magic methyl" effect. Notably, to our knowledge, although this impact has been extensively reported and explained in the medical discipline [24], examples in agricultural chemistry—particularly in insecticide development—remain scarce yet genuinely exist. The systematic exploitation of the methyl effect in agrochemistry has lagged, for three possible reasons. First, the historical success of discovering novel, potent, and active ingredients through the high-throughput screening of diverse compound libraries has diverted attention from subtle, rational modifications such as methylation [25]. Second, the agrochemical industry's intense focus on cost-effectiveness and synthetic scalability has often prioritized the introduction of robust, high-value functional groups over seemingly minor changes. Finally, the lack of a consolidated perspective and a mechanistic understanding of how methyl incorporation alters the physicochemical properties, bioactivity, and environmental fate of agrochemicals has hindered the formulation of strategic design principles.

The relentless evolution of insecticide resistance poses an existential threat to global food security, necessitating innovative approaches in molecular design. While contemporary research prioritizes complex structural modifications, we posit that revisiting the most elementary organic unit—the methyl group—may yield unexpected solutions. This ubiquitous yet underappreciated substituent exhibits unique capabilities in fine-tuning molecular recognition. In this perspective, we summarize the observed "magic methyl" effects in insecticides, including both efficacy enhancement and target affinity improvement, while proposing strategic directions for methyl group implementation in agrochemical design. We anticipate that this discussion will inspire renewed attention to methyl-based optimization as a viable approach to alleviate candidate scarcity in insecticide development.

2. "Magic methyl" effects in insecticides

It is difficult to conceive that minor structural modifications of a single functional group can profoundly increase a compound's bioactivity, yet the methyl group has demonstrated this capability. This methyl-driven magnitude enhancement in bioactivity has been increasingly observed in several commercialized insecticides. However, the manifestation of this phenomenon often requires specific structural and electronic prerequisites in the parent molecule. This section focuses on delineating the "magic methyl" effect observed across diverse classes of insecticides.

2.1. Carbamates

Carbamates are a major class of insecticides characterized by a carbamate ester functional group (–OCONH–) that act as reversible inhibitors of acetylcholinesterase (AChE) [26]. Classified under IRAC Group 1A, these compounds disrupt neurotransmission by binding to the catalytic serine residue of AChE, preventing the hydrolysis of acetylcholine and leading to hyperexcitation, paralysis, and death in target organisms [27]. Carbamates, also termed *N*-methylcarbamates due to the universal presence of a methyl group on the carbamate nitrogen, derive their insecticidal efficacy from this critical structural feature [28]. Representative commercial agents include carbofuran, carbosulfan, and methomyl, all of which retain the *N*-methyl substitution essential for bioactivity (Fig. 1(a)). This methyl effect was visualized in structure–activity relationship (SAR) studies on substituted carbamates in the middle of the 20th century [29–31]. For example, Kolbezen et al. [32] found that the *N*-methyl derivative of 2-isopropyl-5-methylphenyl carbamate **1** exhibited potent activity against thrips, with a median lethal concentration (LC_{50}) of 3 $mg{\cdot}L^{-1}$. However, replacing the methyl group with an ethyl substituent (compound **2**) reduced efficacy by an order of magnitude (LC_{50} = 30 $mg{\cdot}L^{-1}$), while bulkier benzyl (compound **3**, LC_{50} > 100 $mg{\cdot}L^{-1}$) or phenyl (compound **4**, LC_{50} > 1000 $mg{\cdot}L^{-1}$) substitutions rendered the compounds nearly inactive. Although bioassay data for the *N*-unsubstituted carbamate are unavailable, its bioactivity is expected to be particularly unfavorable.

2.2. Pyrethroids

Developed by FMC Corporation (USA), bifenthrin is a third-generation synthetic pyrethroid [33]. It is a potent insecticide and acaricide widely employed in agricultural, residential, and public health pest control [34]. Classified under IRAC Group 3A as a sodium channel modulator, bifenthrin disrupts voltage-gated sodium channels (VGSCs) in insect nerve membranes, prolonging depolarization and causing hyperexcitation, paralysis, and death [35,36]. Structurally, it bears a notable methyl group at the 2-position of its biphenyl moiety (Fig. 1(b)). SAR studies from FMC Corporation's patent revealed that this methyl substituent plays an indispensable role in acaricidal efficacy. In bioassays against *Tetranychus urticae* (*T. urticae*), bifenthrin showed 99% and 90% mortality rates at concentrations of 16 and 8 ppm (1 ppm = 1 $mg{\cdot}L^{-1}$), respectively. However, elongation of this methyl group to an ethyl substituent (compound **5**) drastically reduced efficacy, achieving only 32% control at 16 ppm. Complete removal of the methyl group (compound **6**) resulted in a complete loss of bioactivity, while substitution with electron-withdrawing halogen atoms (Cl in **7**; Br in **8**) at this position also led to significantly diminished acaricidal properties.

2.3. Sulfoximines

Sulfoxaflor, a sulfoximine-class insecticide developed by Dow AgroSciences (now Corteva Agriscience, USA), represents a novel class of neuroactive compounds classified under IRAC Group 4C as nicotinic acetylcholine receptor (nAChR) competitive modulators [37]. Unlike conventional neonicotinoids (Group 4A), which feature chloropyridinyl or chlorothiazolyl pharmacophores, sulfoxaflor incorporates a sulfoximine moiety (—N═S(O)—) and exhibits potent systemic activity against a broad spectrum of piercing-sucking insect pests [38,39]. A distinguishing feature of this insecticide is the presence of a methyl substitution at the benzyl position of its pyridine ring. Dow AgroSciences' patent showed the SAR by synthesizing a desmethyl derivative (compound **9**) and comparing its aphicidal activity with that of sulfoxaflor via foliar spray bioassays [40]. At concentrations of 3.125, 0.781, and 0.195 ppm, sulfoxaflor demonstrated exceptional efficacy against *Myzus persicae* (*M. persicae*), achieving >80% mortality across all tested doses. In stark contrast, compound **9** exhibited significantly

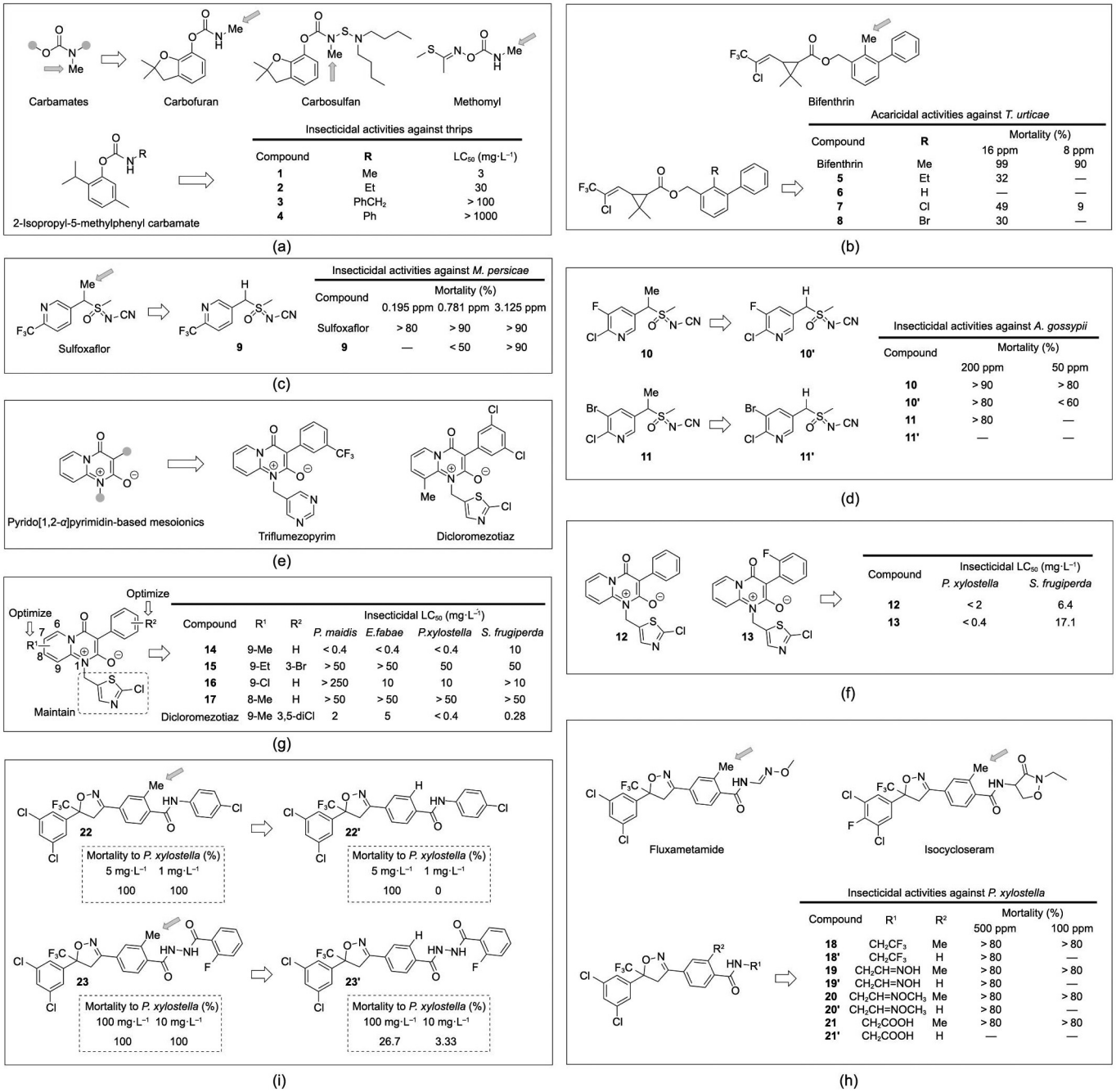

Fig. 1. Selected diverse classes of insecticides exhibiting "magic methyl" effects. (a) *N*-methylcarbamate insecticides; (b) bifenthrin and its derivatives; (c) sulfoxaflor and desmethyl sulfoxaflor; (d) sulfoxaflor analogues; (e) pyrido[1,2-*a*]pyrimidin-based insecticides: triflumezopyrim and dicloromezotiaz; (f) compounds without methyl substitution, containing only a 2-chlorothiazol-5-ylmethyl substituent at the 1-*N* position; (g) modified compounds at the R^1 and R^2 positions with the 2-chlorothiazol-5-ylmethyl substituent at the 1-*N* position retained; (h) fluxametamide, isocycloseram, and their derivatives; (i) isoxazoline insecticides.

reduced activity, with mortality dropping below 50% at 0.781 ppm and negligible insecticidal effects at 0.195 ppm (Fig. 1(c)). Further SAR investigations into analogs **10** and **10'**, targeting *Aphis gossypii* (*A. gossypii*), revealed that the absence of the methyl group in the analogs' desmethyl derivatives (**11** and **11'**) resulted in a drastic reduction or complete abolition of aphicidal activity (Fig. 1(d)) [41].

2.4. Mesoionics

Mesoionic compounds are a distinctive class of heterocyclic organic molecules characterized by a delocalized electronic structure that confers both cationic and anionic charges within a single cyclic framework [42]. This unique dipolar nature, stabilized through resonance, distinguishes them from conventional aromatic or ionic compounds. Among mesoionic compounds, the notable commercial successes of pyrido[1,2-*a*]pyrimidin-based insecticides include triflumezopyrim and dicloromezotiaz (Fig. 1(e)), both developed by DuPont (USA), which target hemipteran and lepidopteran pests, respectively [43,44]. The two insecticides act as nAChR modulators, classified under IRAC Group 4E. In DuPont's story [45,46], mesoionics bearing the pyrido[1,2-*a*]pyrimidin structure typically exhibited potent activity—preferentially against sap-sucking insects—in initial tests. However, during

screening, derivatives **12** and **13**, which contained a 2-chlorothiazol-5-ylmethyl substituent at the 1-*N* position, exhibited promising lethality toward *Plutella xylostella* (*P. xylostella*) and *Spodoptera frugiperda* (*S. frugiperda*) (Fig. 1(f)). This discovery led to systematic optimization of the pyrido[1,2-*a*]pyrimidin scaffold, focusing on substituents at the R^1 and R^2 positions while retaining the 2-chlorothiazol-5-ylmethyl moiety (Fig. 1(g)). The "magic methyl" effect emerged when R^1 substitution with a 9-methyl group yielded derivative **14**, which displayed superior insecticidal activity against *Peregrinus maidis* (*P. maidis*), *Empoasca fabae* (*E. fabae*), and *P. xylostella* compared with derivative **12**. Strikingly, the 9-methyl group proved uniquely critical, as replacing it with ethyl or chlorine (derivatives **15** and **16**) drastically reduced efficacy across pest species. Furthermore, methylation at the 8-position (analog **17**) nearly abolished activity. Notably, the 9-methyl-driven enhancement was contingent on the presence of the 2-chlorothiazol-5-ylmethyl group at the 1-position, with alternative substituents at this position failing to replicate the effect. This SAR guided the subsequent optimization, which retained both the 2-chlorothiazol-5-ylmethyl and 9-methyl groups while modifying R^2. These efforts culminated in dicloromezotiaz, which exhibits specific efficacy against Lepidopteran pests.

2.5. Isoxazolines

Isoxazolines are a class of heterocyclic compounds structurally characterized by a five-membered aromatic ring bearing nitrogen and oxygen atoms [47,48]. In recent decades, isoxazoline derivatives have emerged as a revolutionary scaffold in agrochemical research, particularly for the development of insecticides with broad-spectrum activity and novel modes of action [49]. These compounds target the γ-aminobutyric acid (GABA)-gated chloride channels in insect nervous systems, disrupting neuronal signaling and leading to rapid paralysis and mortality [50,51]. Their unique mechanism circumvents the resistance mechanisms associated with traditional insecticides, such as pyrethroids and neonicotinoids. Fluxametamide and isocycloseram, members of IRAC Group 30, are representative commercial insecticides within the isoxazoline class, developed by Nissan Chemical (Japan) and Syngenta (Switzerland), respectively [51,52]. These compounds exhibit broad-spectrum activity against diverse agricultural pests, including Lepidopteran, Hemipteran, and Coleopteran species [53]. Notably, both molecules feature a methyl group at the 2-position of their benzamide aromatic ring. This methyl group can be "magic," as fluxametamide demonstrated > 80% mortality against *Spodoptera exigua* at 100 ppm in Nissan Chemical's patent, whereas replacing the methyl group with chlorine, bromine, iodine, ethyl, 2-$OCHF_2$, nitro, cyano, or phenyl substituents, while retaining all other structural features, significantly reduced activity [54]. Similarly, derivatives **18**, **19**, and **20**—all of which bear the 2-methyl group—achieved > 80% lethality against *P. xylostella* at both 500 and 100 ppm. In contrast, their methyl-deleted analogs (**18'**, **19'**, and **20'**) showed insecticidal activity only at 500 ppm [55]. The absence of the methyl group in derivative **21'** (vs **21**) nearly abolished insecticidal potency. Beyond patent evidence, extensive literature corroborates this SAR (Fig. 1(h)). For example, Gao et al. [56] reported that the methyl-containing isoxazoline analog **22** achieved 100% mortality against *P. xylostella* at 5 and 1 $mg{\cdot}L^{-1}$, whereas switching the 2-methyl with hydrogen (compound **22'**) resulted in negligible activity at 1 $mg{\cdot}L^{-1}$. Furthermore, in our prior work [57], the diacylhydrazine-containing isoxazoline derivative **23** exhibited 100% insecticidal activity at both 100 and 10 $mg{\cdot}L^{-1}$. Strikingly, removing the methyl group (compound **23'**) caused a dramatic decline in efficacy, with mortality dropping to 26.7% and 3.33% at the respective concentrations (Fig. 1(i)). These findings collectively indicate the indispensable role of the 2-methyl group in maintaining high insecticidal potency across diverse isoxazoline scaffolds and bioassay systems.

3. The molecular basis behind "magic methyl" effects

Despite recognition of the "magic methyl" effect in SAR analyses of insecticides, molecular-level interrogation of its mechanistic origins remains scarce. Barreiro et al. [24] comprehensively dissected the "methyl effect" in medicinal chemistry, elucidating its multifaceted roles in drug design (enhancing potency, selectivity, metabolic stability, and target affinity) through mechanisms such as hydrophobic pocket filling, conformational rigidification, and blockade of oxidative metabolism. This systematic framework provides a foundational paradigm for understanding analogous methyl-driven bioactivity in agricultural chemistry, which stems from interconnected physicochemical and biological mechanisms. The introduction of a methyl group primarily increases lipophilicity, thereby improving membrane permeability and tissue uptake. Concurrently, it exerts a subtle yet critical steric influence, which can pre-organize a molecule into its bioactive conformation or optimize its fit within a protein binding pocket through van der Waals interactions. Furthermore, the electron-donating character of the methyl group can alter the electronic landscape of the pharmacophore, fine-tuning binding affinity. Beyond these direct effects, methyl groups can shield metabolically labile sites from enzymatic oxidation, thereby improving metabolic stability and prolonging systemic activity. The following case studies, which focus on carbamates, pyrethroids, sulfoximines, mesoionics, and isoxazolines, exemplify how these intertwined physicochemical alterations collectively underpin the "magic methyl" effects observed.

To explain why nearly all carbamate insecticides are *N*-methylcarbamates, we propose an analysis rooted in their mechanism of action. According to the model proposed by Fukuto et al. [27,58], carbamate insecticides act as inhibitors of pest AChE by undergoing a transesterification reaction with the serine hydroxyl group of the enzyme (Fig. 2). This process involves the formation of intermediate **I**, which adopts an imine-like structure. The *N*-methyl group plays a critical role in stabilizing this sp^2-hybridized amine intermediate through hyperconjugative effects ($\sigma \rightarrow p$ orbital interactions), thereby lowering its energy and increasing its thermodynamic stability. In contrast, the demethylated intermediate **I**, which lacks the methyl group, would lack such stabilization, rendering it both thermodynamically and kinetically unfavorable. Furthermore, the carbamylated AChE undergoes hydrolysis to regenerate active AChE, completing the detoxification cycle. However, the *N*-methyl group in *N*-methylcarbamylated AChE provides greater steric/electronic shielding to the positively charged moiety embedded within the molecular interior. This shielding impedes the nucleophilic attack of water molecules on the carbamyl group, significantly reducing the hydrolysis rate. Methyl-substituted carbamates exhibit hydrolysis rates >10 times slower than their non-methylated analogs, effectively prolonging AChE inhibition and increasing insecticidal efficacy [58]. Thus, the "magic methyl" effect in carbamates may arise from a synergistic interplay of thermodynamic stabilization (via hyperconjugation in the intermediate) and kinetic modulation (via steric/electronic shielding in the inhibited enzyme). These factors collectively optimize the insecticide's inhibitory potency and persistence.

The methyl impact of bifenthrin's ortho-methyl group in modulating VGSCs can be seen in its unique ability to mimic the spatial and functional roles of the α-cyano group in Type II pyrethroids. Gammon et al. [35] used electrophysiological data to show that bifenthrin exhibits its half maximal effective concentration (1–2 $\mu mol{\cdot}L^{-1}$) clusters with Type II agents (e.g., cypermethrin), but its tail current modulation resembles both Type I (amplitude) and Type II (prolonged deactivation) effects. Structural modeling

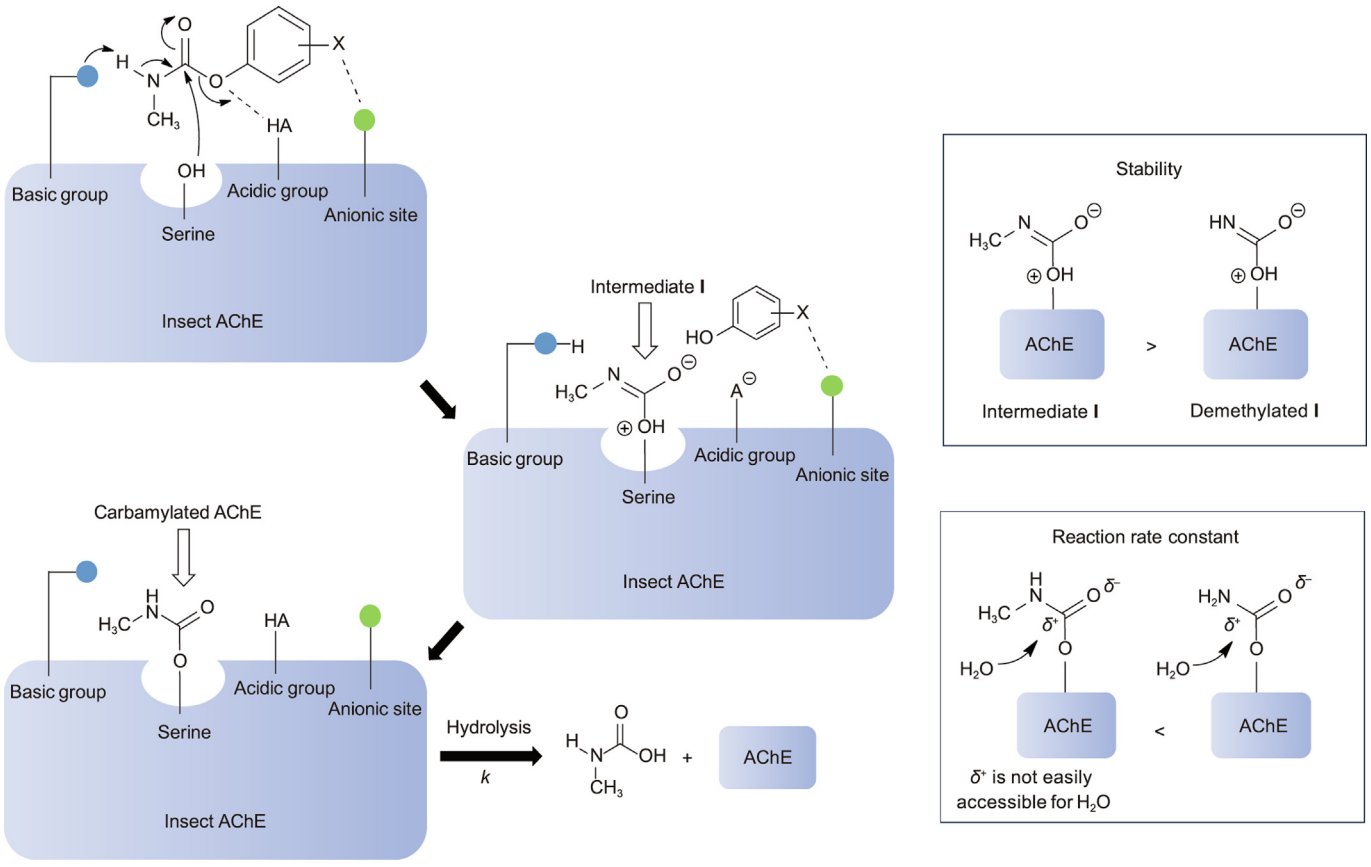

Fig. 2. General mode of action of carbamate insecticides. HA: acidic group (a proton donor). *k*: hydrolysis reaction rate constant, the magnitude of *k* directly reflects the speed of the hydrolysis reaction (a larger *k* corresponds to faster hydrolysis). δ^+/δ^-: partial positive charge and partial negative charge, respectively, used to describe the charge distribution on atoms within the molecule; X: substituent.

revealed that the methyl occupies a steric domain analogous to the α-cyano in Type II pyrethroids when folded into their thermodynamically stable conformations. This spatial mimicry may enable bifenthrin to engage receptor-binding pockets similarly to Type II pyrethroids (Fig. 3(a)), possibly facilitated by hydrophobic packing and torsional restraint.

The methyl substitution at the benzyl position of the pyridine ring in sulfoxaflor has been demonstrated to significantly increase sulfoxaflor's bioactivity, particularly by promoting affinity for insect nAChRs [59,60]. Loso et al. [61] proposed that the critical role of the methyl group may be based on multiple mechanisms: ① inducing a conformational preference favorable for nAChR binding, ② providing additional binding interactions at the target site, ③ inhibiting metabolic oxidation at the methylene bridge carbon, or ④ improving plant uptake and translocation properties. However, the relative contribution of these factors to the observed activity enhancement remains unresolved. Notably, sulfoxaflor possesses two asymmetric centers, theoretically presenting four stereoisomers (labeled A, B, C, and D in Fig. 3(b)). Enantiomerically pure isomers, however, undergo rapid epimerization in solution to form diastereomeric pairs (A/C and B/D), leading to the commercial formulation being a mixture of all four isomers [62]. Wang et al. [63] predicted differential nAChR binding affinities among these stereoisomers, revealing an approximately five-fold variation in predicted potency when transitioning from A to C or from B to D. This finding suggests that the configuration at chiral center 1 exerts a dominant influence on affinity, likely through steric or electronic modulation of the upper substituent.

Montgomery et al. [64] showed and analyzed the co-crystal structures of several insecticides from IRAC Group 4 in complex with their binding sites on the AChBP, clarifying the methyl's significance in mediating drug–receptor interactions. In both isomers **B** and **C**, the indole moiety of sulfoxaflor engages in tight contact with Trp143. Intriguingly, while the methyl group at the pyridine benzyl position in isomer **B** points toward Trp53, its counterpart in isomer **C** directs toward Tyr192. Despite these divergent orientations, strong electron-withdrawing effects from adjacent substituents increase the dispersed CH–π interactions in both cases. This observation implies that the remarkable methyl effect may not be chirality driven but rather arises from electronic modulation of local bonding networks. Furthermore, in the researchers' reported binding pattern of dicloromezotiaz to AChBP (Fig. 3(c)), the 9-methyl substituent on the mesoionic core induces a distortion in the bicyclic structure to alleviate steric clashes with the adjacent CH_2-thiazole group [64]. This distortion forces partial sp^3 hybridization of the nitrogen atom, generating chiral helicity in the mesoionic ring. The resulting charge separation (via cross-conjugated mesomeric betaine) strengthens π–π interactions with Arg55 and CH–π interactions with Trp53/Trp143. Additionally, this methyl group directly engages in a CH–π interaction with Tyr192 and a CH–O electrostatic contact with the backbone of Trp142, stabilizing hydrophobic complementarity at the binding site.

Over 3000 isoxazoline derivatives incorporate a methyl group at the ortho position of the aromatic ring adjacent to the carboxyl group [65]. The "magic methyl" effect in isoxazolines, exemplified by fluralaner and lotilaner, is potentially driven by conformational pre-organization and steric complementarity. The 2-methyl sub-

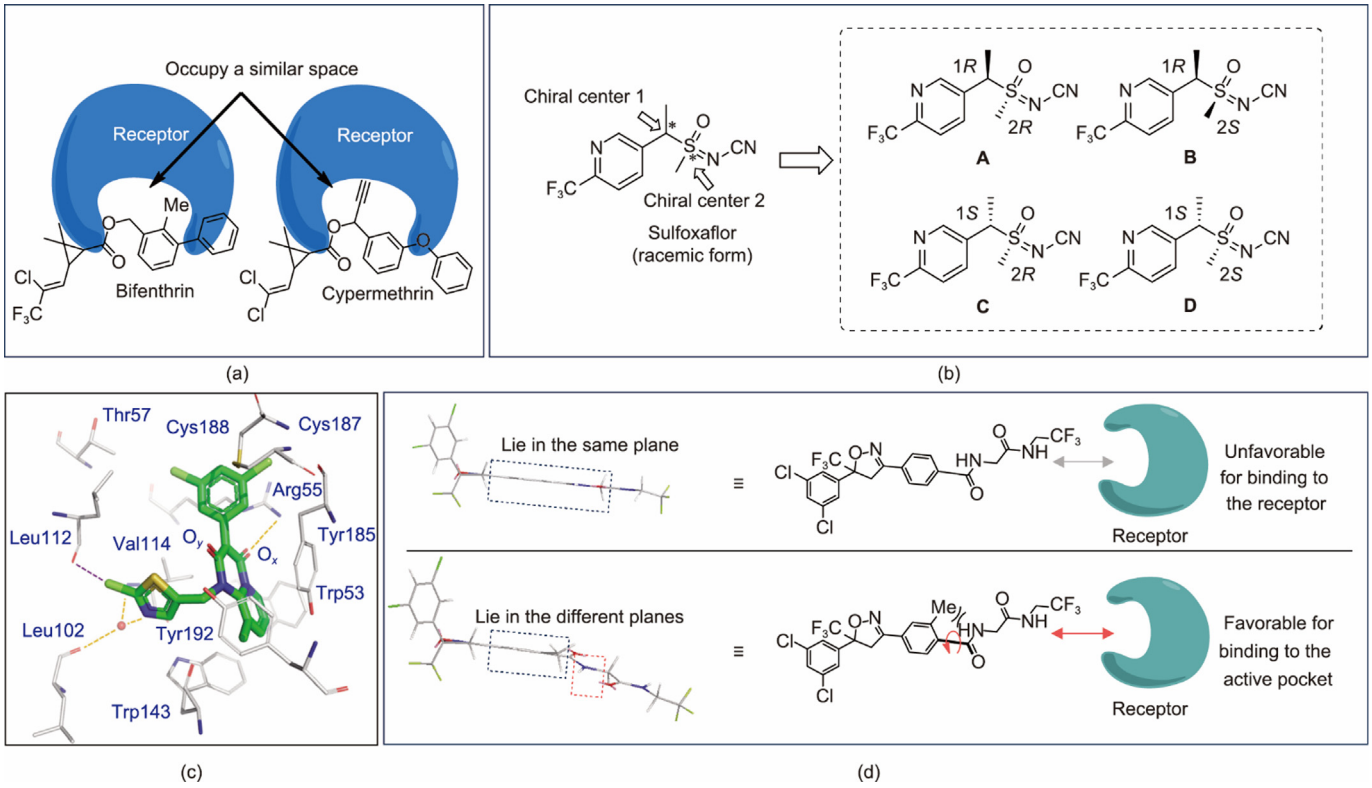

Fig. 3. (a) The methyl group in bifenthrin and the cyano group in cypermethrin occupy analogous spatial positions; (b) four stereoisomers of sulfoxaflor, * denotes a chiral center, *S* represents the *S*-configuration of enantiomers, *R* denotes the *R*-configuration of enantiomers; (c) binding mode of dicloromezotiaz [64]; (d) conformational differentiation in amide-containing isoxazolines induced by methyl substitution [65,66].

stituent adjacent to the amide may induce an approximately 40°–50° rotation of the amide group out of the aromatic plane, enforcing a bioactive conformation (as confirmed by molecular modeling) critical for target engagement (Fig. 3(d)) [65,66]. Cassayre et al. [67] further compared the bioactivities of two pyridyl-substituted isoxazoline isomers using matched molecular pair analysis. The pronounced differences between these two series provided additional evidence supporting the conformation preference.

4. Perspective

The methyl group has emerged as a pivotal structural motif in agrochemical design, facilitating the discovery of novel crop-protection agents. We have summarized the "magic methyl" phenomenon across five major insecticide classes (carbamates, pyrethroids, sulfoximines, mesoionics, and isoxazolines) and elucidated potential mechanisms underpinning its effects. Methyl incorporation typically enhances target binding by enforcing conformational restriction or optimizing geometric alignment. The *N*-methyl group in carbamates confers thermodynamic and kinetic advantages during AChE inhibition. However, whether additional mechanisms beyond these established factors collectively mediate methyl's role in increasing pest lethality remains underexplored.

Current evidence suggests that methyl acts as a highly precise molecular "tweak" to fine-tune properties toward optimal bioactivity, with its steric and electronic tolerances being exceptionally narrow. For example, while the methyl group on the methylene bridge of sulfoxaflor is critical for nAChR binding, introducing a gem-dimethyl derivative at this position drastically reduces receptor affinity and insecticidal efficacy [59]. Similarly, installing a second ortho-methyl group on the aryl ring adjacent to the amide in isoxazolines forces the amide into a vertical orientation, destabilizing the bioactive conformation and diminishing potency [67]. These instances underscore the methyl group's role as a conformational "lock" that balances steric demand and molecular flexibility—a duality often disrupted by even minor modifications. In addition to optimizing efficacy, strategic methyl incorporation holds promise for enhancing environmental and human safety profiles. When compared with historically persistent and bioaccumulative organochlorine pesticides (e.g., dichlorodiphenyltrichloroethane), which often feature fully halogenated and highly stable carbon backbones, pesticides containing a placed methyl group could exhibit more predictable degradation into non-toxic metabolites in the environment, thus showing reduced persistence and potential for long-range transport [68,69].

From a toxicological perspective, the precision of methyl-driven optimization may improve target selectivity. By fine-tuning molecular shape and electronics to perfectly fit insect-specific binding sites, methyl-substituted insecticides can potentially achieve heightened activity against pests while minimizing interactions with off-target organisms [23,70,71]. Methyl groups can also serve as prodrug modifiers. For instance, broflanilide functions as a pro-insecticide that is converted into its demethylated form upon entering the pest organism [72]. This metabolite then binds to the GABA-gated chloride channel and exerts insecticidal activity. In this case, although the methyl group does not directly participate in receptor binding, it ensures the stability and targeted delivery of broflanilide prior to reaching the site of action. In other words, the structural properties that enable a molecule to optimally interact with its insecticidal target are often distinct from those required for effective translocation and metabolic activation within the organism [73]. This explains why the treatment of pests with demethylated broflanilide results in unsatisfactory insecticidal efficacy [74].

Thus, deeper mechanistic studies are urgently required to dissect methyl's multifaceted contributions—particularly its interplay with entropic/enthalpic trade-offs and target-specific steric landscapes. Computational approaches paired with high-resolution structural biology (via cryo-electron microscopy or the X-ray free electron laser technique) may delineate how methyl groups modulate binding kinetics and thermodynamic driving forces in agrochemical-target complexes [75–78]. However, the majority of insecticides exhibiting the methyl effect are neurotoxic, and stable *in vitro* expression and purification of their target neuroreceptors in specific pests (e.g., nAChR and GABA receptors) remain technically challenging [79,80]. These receptors often exist as membrane–bound complexes with low stability and high conformational heterogeneity, complicating structural and functional studies [81]. Alternatively, researchers from Badische Anilin- und Soda-Fabrik (BASF, Germany) conducted a site-directed mutagenesis to convert the AChBP template from *Aplysia californica* into a soluble, insect-like AChBP featuring aphid nAChR subunit sequences, followed by structure-based drug design, which ultimately led to the discovery of the third commercialized mesoionic insecticide, fenmezoditiaz [82].

Novel chemical entities with new scaffolds often fail to exhibit promising bioactivity during early-stage evaluations, but this does not necessarily indicate inherent inefficacy. Strategically introducing methyl groups could significantly increase the bioavailability of such entities. Moreover, it is worth exploring whether the "methyl effect" widely observed in insecticides also exists in other agrochemicals, such as herbicides and fungicides. For example, Zhu et al. [83] and Wu et al. [84] demonstrated that replacing the hydrogen at the 1-position of a pyrimidine-based herbicide with a methyl group increased its activity against barnyard grass by approximately 45.6-fold, whereas replacing the methyl group on the 1-position nitrogen of a carboxamide fungicide with an aryl group markedly reduced antifungal efficacy. Recently, late-stage functionalization has emerged as a focal point in synthetic methodology, emphasizing the post-synthesis optimization of drug-like molecular scaffolds to improve their properties [85–87]. While these approaches have garnered significant attention and demonstrated considerable potential in pharmaceutical applications, their implementation for modifying active ingredients in agrochemistry remains underexplored.

Overall, with the rapid advancement of artificial intelligence (AI), AI-assisted drug design has shown immense potential in agrochemical lead discovery. By investigating the molecular mechanisms underlying the methyl effect in pesticides, we may transform methyl from a serendipitously discovered "magic bullet" into a rationally engineered tool for sustainable pest control.

CRediT authorship contribution statement

Qiu Liu: Writing – original draft, Visualization, Software, Resources, Data curation. **Xingjie Zhang:** Visualization, Data curation. **Tangbing Yang:** Methodology, Data curation. **Yuqin Luo:** Resources, Data curation. **Runjiang Song:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition. **Baoan Song:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Anderson WB, Seager R, Baethgen W, Cane M, You L. Synchronous crop failures and climate-forced production variability. Sci Adv 2019;5(7):eaaw1976.
[2] Carvajal-Yepes M, Cardwell K, Nelson A, Garrett KA, Giovani B, Saunders DGO, et al. A global surveillance system for crop diseases. Science 2019;364(6447):1237–9.
[3] Wang XY, Yang WJ, Wu S, Li XY, Chi YR, Song BA, et al. Carbene-catalyzed phthalide ether functionalization for discovering chiral phytovirucide that specifically targets viral Nia protein to inhibit proliferation. Research 2025;8:0637.
[4] Raman R. The impact of genetically modified (GM) crops in modern agriculture: a review. GM Crops Food 2017;8(4):195–208.
[5] Food and Agriculture Organization of the United Nations. The future of food and agriculture [Internet]. Rome: Food and Agriculture Organization (FAO); undated [cited 2025 Apr 23]. Available from: http://www.fao.org/publications/fofa/en/.
[6] Metcalf RL. Insect resistance to insecticides. Pestic Sci 1989;26(4):333–58.
[7] Guillemaud T, Brun A, Anthony N, Sauge MH, Boll R, Delorme R, et al. Incidence of insecticide resistance alleles in sexually-reproducing populations of the peach-potato aphid *Myzus persicae* (Hemiptera: aphididae) from southern France. Bull Entomol Res 2003;93(4):289–97.
[8] Zamoum T, Simon JC, Crochard D, Ballanger Y, Lapchin L, Vanlerberghe-Masutti F, et al. Does insecticide resistance alone account for the low genetic variability of asexually reproducing populations of the peach-potato aphid *Myzus persicae*? Heredity 2005;94(6):630–9.
[9] Sparks TC, Lorsbach BA. Perspectives on the agrochemical industry and agrochemical discovery. Pest Manag Sci 2017;73(4):672–7.
[10] Liu CL, Guan AY, Yang JD, Chai BS, Li M, Li HC, et al. Efficient approach to discover novel agrochemical candidates: intermediate derivatization method. J Agric Food Chem 2016;64(1):45–51.
[11] Walter MW. Structure-based design of agrochemicals. Nat Prod Rep 2002;19(3):278–91.
[12] Liu ZL, Xiong ZJ, Yang SF, Fan K, Jiang L, Mao YL, et al. Strained heterojunction enables high-performance, fully textured perovskite/silicon tandem solar cells. Joule 2024;8(10):2834–50.
[13] Pillmoor JB, Wright K, Terry AS. Natural products as a source of agrochemicals and leads for chemical synthesis. Pestic Sci 1993;39(2):131–40.
[14] Baker DR, Fenyes JG, Moberg WK, Cross B. Overview of agrochemical development. In: Baker DR, Fenyes JG, Moberg WK, Cross B, editors. Synthesis and chemistry of agrochemicals. Washington, DC: American Chemical Society; 1987. p. 1–8.
[15] Guan AY, Liu CL, Yang XP, Dekeyser M. Application of the intermediate derivatization approach in agrochemical discovery. Chem Rev 2014;114(14):7079–107.
[16] Kong YJ, Zhou C, Tan D, Xu XY, Li Z, Cheng JG. Discovery of potential neonicotinoid insecticides by an artificial intelligence generative model and structure-based virtual screening. J Agric Food Chem 2024;72(10):5145–52.
[17] Lamberth C. Agrochemical lead optimization by scaffold hopping. Pest Manag Sci 2018;74(2):282–92.
[18] Insecticide Resistance Action Committee (IRAC). Mode of Action classification structures poster [poster]. Toronto: IRAC International; 2025.
[19] Yang YC, Wu S, Zhao CN, He HF, Wu ZX, Zhang J, et al. Design, synthesis, and insecticidal activity of pyridino[1,2-*a*]pyrimidines containing indole moieties at the 1-position. J Agric Food Chem 2024;72(20):11331–40.
[20] Zhou YJ, Kong Y, Fan WG, Tao T, Xiao Q, Li N, et al. Principles of RNA methylation and their implications for biology and medicine. Biomed Pharmacother 2020;131:110731.
[21] Szyf M. DNA methylation properties: consequences for pharmacology. Trends Pharmacol Sci 1994;15(7):233–8.
[22] He PC, He C. m^6A RNA methylation: from mechanisms to therapeutic potential. EMBO J 2021;40(3):e105977.
[23] Pinheiro PSM, Franco LS, Fraga CAM. The magic methyl and its tricks in drug discovery and development. Pharmaceuticals 2023;16(8):1157.
[24] Barreiro EJ, Kümmerle AE, Fraga CAM. The methylation effect in medicinal chemistry. Chem Rev 2011;111(9):5215–46.
[25] Tietjen K, Drewes M, Stenzel K. High-throughput screening in agrochemical research. Comb Chem High Throughput Screen 2005;8(7):589–94.
[26] Locke RK. Carbamate insecticides: chemistry, biochemistry, and toxicology. J Assoc Off Anal Chem 1977;60(6):1441.
[27] Fukuto TR. Mechanism of action of organophosphorus and carbamate insecticides. Environ Health Perspect 1990;87:245–54.
[28] Matolcsy G, Nádasy M, Andriska V. Anti-insect agents. In: Doyle P, Fujita T, editors. Studies in environmental science. Amsterdam: Elsevier; 1988. p. 21–239.

[29] Metcalf RL. Structure–activity relationships for insecticidal carbamates. Bull World Health Organ 1971;44(1–3):43–78.
[30] Haworth RD, Lamberton AH, Woodcock D. Investigations on the influence of chemical constitution upon toxicity. Part I. Compounds related to "Doryl". J Chem Soc 1947:176–82.
[31] Stevens JR, Beutel RH. Physostigmine substitutes. J Am Chem Soc 1941;63(1):308–11.
[32] Kolbezen MJ, Metcalf RL, Fukuto TR. Insecticide structure and activity, insecticidal activity of carbamate cholinesterase inhibitors. J Agric Food Chem 1954;2(17):864–70.
[33] Sarkar A, Khupse R. Bifenthrin. In: Wexler P, editor. Encyclopedia of toxicology. Amsterdam: Academic Press; 2024. p. 47–52.
[34] Hougard JM, Duchon S, Zaim M, Guillet P. Bifenthrin: a useful pyrethroid insecticide for treatment of mosquito nets. J Med Entomol 2002;39(3):526–33.
[35] Gammon DW, Liu ZW, Chandrasekaran A, El-Naggar SF, Kuryshev YA, Jackson S. Pyrethroid neurotoxicity studies with bifenthrin indicate a mixed Type I/II mode of action. Pest Manag Sci 2019;75(4):1190–7.
[36] Lund AE, Narahashi T. Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs. Pestic Biochem Physiol 1983;20(2):203–16.
[37] Watson GB, Siebert MW, Wang NX, Loso MR, Sparks TC. Sulfoxaflor—a sulfoximine insecticide: review and analysis of mode of action, resistance and cross-resistance. Pestic Biochem Physiol 2021;178:104924.
[38] Babcock JM, Gerwick CB, Huang JX, Loso MR, Nakamura G, Nolting SP, et al. Biological characterization of sulfoxaflor, a novel insecticide. Pest Manag Sci 2011;67(3):328–34.
[39] Bacci L, Convertini S, Rossaro B. A review of sulfoxaflor, a derivative of biological acting substances as a class of insecticides with a broad range of action against many insect pests. J Entomol Acarol Res 2018;50(3):51–71.
[40] Loso MR, Nugent BM, Huang JX, Rogers RB, Zhu Y, Renga JM, et al., inventors; Dow AgroSciences LLC, assignee. Insecticidal *N*-substituted (6-haloalkylpyridin-3-yl)alkyl sulfoximines. World Intellectual Property Organization patent WO 2007/095229 A2. 2007 Aug 23.
[41] Zhu Y, Loso MR, Nugent BM, Huang JX, Rogers RB., inventors; Dow AgroSciences LLC, assignee. Multi-substituted pyridyl sulfoximines and their use as insecticides. United States patent US 20080108667 A1. 2008 May 8.
[42] Ollis WD, Ramsden CA. Meso-ionic compounds. Adv Heterocycl Chem 1976;19:1–122.
[43] Liu ZJ, Li QX, Song BA. Recent research progress in and perspectives of mesoionic insecticides: nicotinic acetylcholine receptor inhibitors. J Agric Food Chem 2020;68(40):11039–53.
[44] Zhang WM, Lahm GP, Pahutski TF, Hughes KA. Applying a bioisosteric replacement strategy in the discovery and optimization of mesoionic pyrido[1,2-*a*]pyrimidinone insecticides: a review. J Agric Food Chem 2022;70(36):11056–62.
[45] Zhang WM. Mesoionic pyrido[1,2-*a*]pyrimidinone insecticides: from discovery to triflumezopyrim and dicloromezotiaz. Acc Chem Res 2017;50(9):2381–8.
[46] Zhang WM, Holyoke CW Jr, Hughes KA, Lahm GP, Pahutski TF Jr, Tong MT, et al., inventors; E. I. du Pont de Nemours and Company, assignee. Mesoionic pesticides. World Intellectual Property Organization patent WO 2011017342 A2. 2011 Feb 10.
[47] Wang XY, Hu QY, Tang H, Pan XH. Isoxazole/isoxazoline skeleton in the structural modification of natural products: a review. Pharmaceuticals 2023;16(2):228.
[48] Pandhurnekar CP, Pandhurnekar HC, Mungole AJ, Butoliya SS, Yadao BG. A review of recent synthetic strategies and biological activities of isoxazole. J Heterocycl Chem 2023;60(4):565–7.
[49] Kong LJ, Cao XY, Sun NB, Min LJ, Duke SO, Wu HK, et al. Isoxazoline: an emerging scaffold in pesticide discovery. J Agric Food Chem 2025;73(15):8678–93.
[50] Casida JE. Golden age of RyR and GABA-R diamide and isoxazoline insecticides: common genesis, serendipity, surprises, selectivity, and safety. Chem Res Toxicol 2015;28(4):560–6.
[51] Blythe J, Earley FGP, Piekarska-Hack K, Firth L, Bristow J, Hirst EA, et al. The mode of action of isocycloseram: a novel isoxazoline insecticide. Pestic Biochem Physiol 2022;187:105217.
[52] Asahi M, Kobayashi M, Kagami T, Nakahira K, Furukawa Y, Ozoe Y. Fluxametamide: a novel isoxazoline insecticide that acts via distinctive antagonism of insect ligand-gated chloride channels. Pestic Biochem Physiol 2018;151:67–72.
[53] Saran RK, Hoppé M, Mayor S, Long C, Blakely B, Eppler L, et al. Efficacy and utility of isocycloseram a novel isoxazoline insecticide against urban pests and public health disease vectors. Pest Manag Sci 2025;81(2):978–89.
[54] Mita T, Kikuchi T, Mizutani M, Kajimoto T., inventors; Nissan Chemical Industries, Ltd., assignee. Isoxazoline-substituted benzamide compound and pest control agent. World Intellectual Property Organization patent WO 2007/026965 A1. 2007 Mar 8.
[55] Mita T, Kikuchi T, Mizukoshi T, Yaosaka M, Komoda M., inventors; Nissan Chemical Industries, Ltd., assignee. Isoxazoline-substituted benzamide compound and noxious organism control agent. World Intellectual Property Organization patent WO 2005085216 A1. 2005 Sep 15.
[56] Gao YC, Song XM, Jia TH, Zhao C, Yao GK, Xu HH. Discovery of new *N*-phenylamide isoxazoline derivatives with high insecticidal activity and reduced honeybee toxicity. Pestic Biochem Physiol 2024;200:105843.
[57] Li YH, Zhang WB, Wu ZX, Song BA, Song RJ. Design, synthesis, and insecticidal activity of novel isoxazoline diacylhydrazine compounds as GABA receptor inhibitors. J Agric Food Chem 2023;71(17):6561–9.
[58] Fukuto TR, Fahmy MAH, Metcalf RL. Alkaline hydrolysis, anticholinesterase, and insecticidal properties of some nitro-substituted phenyl carbamates. J Agric Food Chem 1967;15(2):273–81.
[59] Sparks TC, Watson GB, Loso MR, Geng C, Babcock JM, Thomas JD. Sulfoxaflor and the sulfoximine insecticides: chemistry, mode of action and basis for efficacy on resistant insects. Pestic Biochem Physiol 2013;107(1):1–7.
[60] Watson GB, Olson MB, Beavers KW, Loso MR, Sparks TC. Characterization of a nicotinic acetylcholine receptor binding site for sulfoxaflor, a new sulfoximine insecticide for the control of sap-feeding insect pests. Pestic Biochem Physiol 2017;143:90–4.
[61] Loso MR, Benko Z, Buysse A, Johnson TC, Nugent BM, Rogers RB, et al. SAR studies directed toward the pyridine moiety of the sap-feeding insecticide sulfoxaflor (Isoclast™ active). Bioorg Med Chem 2016;24(3):378–82.
[62] Jeschke P. Current status of chirality in agrochemicals. Pest Manag Sci 2018;74(11):2389–404.
[63] Wang NX, Watson GB, Loso MR, Sparks TC. Molecular modeling of sulfoxaflor and neonicotinoid binding in insect nicotinic acetylcholine receptors: impact of the *Myzus* β1 R81T mutation. Pest Manag Sci 2016;72(8):1467–74.
[64] Montgomery M, Rendine S, Zimmer CT, Elias J, Schaetzer J, Pitterna T, et al. Structural biology-guided design, synthesis, and biological evaluation of novel insect nicotinic acetylcholine receptor orthosteric modulators. J Med Chem 2022;65(3):2297–312.
[65] Long A. Isoxazolines: preeminent ectoparasiticides of the early twenty-first century. In: Meng CQ, Sluder AE, editors. Ectoparasites: drug discovery against moving targets. Weinheim: Wiley; 2018. p. 319–33.
[66] Weber T, Selzer PM. Isoxazolines: a novel chemotype highly effective on ectoparasites. ChemMedChem 2016;11(3):270–6.
[67] Cassayre J, Smejkal T, Blythe J, Hoegger P, Renold P, Pitterna T. The discovery of isocycloseram: a novel isoxazoline insecticide. In: Nauen R, Jeschke P, editors. Recent highlights in crop protection. Amsterdam: Academic Press; 2021. p. 145–65.
[68] Tang FHM, Wyckhuys KAG, Li ZJ, Maggi F, Silva V. Transboundary impacts of pesticide use in food production. Nat Rev Earth Environ 2025;6(6):383–400.
[69] Huang YB, Li ZJ. Global mapping of freshwater contamination by pesticides and implications for agriculture and water resource protection. iScience 2025;28(7):112861.
[70] Snøve Jr OJ, Rossi JJ. Chemical modifications rescue off-target effects of RNAi. ACS Chem Biol 2006;1(5):274–6.
[71] Casida JE, Durkin KA. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu Rev Entomol 2013;58(1):99–117.
[72] Nakao T, Banba S. Broflanilide: a meta-diamide insecticide with a novel mode of action. Bioorg Med Chem 2016;24(3):372–7.
[73] Salgado VL, David MD. Chance and design in proinsecticide discovery. Pest Manag Sci 2017;73(4):723–30.
[74] Kobayashi Y, Daedo H, Katsuta H, Nomura M, Sukada H, Hirabayashi A, et al., inventors; Mitsui Chemicals Agro, Inc., assignee. Amide derivative, pest control agent containing the amide derivative and use of the pest control agent. World Intellectual Property Organization patent WO 2010/018714 A1. 2010 Feb 18.
[75] Spence JCH. XFELs for structure and dynamics in biology. IUCrJ 2017;4(4):322–39.
[76] Yarla NS, Bishayee A, Sethi G, Reddanna P, Kalle AM, Dhananjaya BL, et al. Targeting arachidonic acid pathway by natural products for cancer prevention and therapy. Semin Cancer Biol 2016;40–41:48–81.
[77] Heerde T, Schütz D, Lin YJ, Münch J, Schmidt M, Fändrich M. Cryo-EM structure and polymorphic maturation of a viral transduction enhancing amyloid fibril. Nat Commun 2023;14(1):4293.
[78] Pan AC, Borhani DW, Dror RO, Shaw DE. Molecular determinants of drug–receptor binding kinetics. Drug Discov Today 2013;18(13–14):667–73.
[79] Hadiatullah H, Zhang Y, Samurkas A, Xie Y, Sundarraj R, Zuilhof H, et al. Recent progress in the structural study of ion channels as insecticide targets. Insect Sci 2022;29(6):1522–51.
[80] Tomizawa M, Casida JE. Structure and diversity of insect nicotinic acetylcholine receptors. Pest Manag Sci 2001;57(10):914–22.
[81] Zorman S, Botte M, Jiang Q, Collinson I, Schaffitzel C. Advances and challenges of membrane-protein complex production. Curr Opin Struct Biol 2015;32:123–30.
[82] Huang HZ, Dickhaut J, Weisel M, Mao LX, Rankl N, Takeda H, et al. Discovery and biological characterization of a novel mesoionic insecticide fenmezoditiaz. Pest Manag Sci 2025;81(5):2535–52.
[83] Zhu YQ, Zou XM, Li GC, Yao CS, Si XK, Yang HZ. Synthesis and herbicidal evaluation of 3-substituted benzyl-6-(trifluoromethyl) pyrimidine-2,4(1*H*,3*H*)-Dione derivatives. Chin J Org Chem 2007;27(6):753–7. Chinese.
[84] Wu ZB, Hu DY, Kuang JQ, Cai H, Wu SX, Xue W. Synthesis and antifungal activity of *N*-(substituted pyridinyl)-1-methyl(phenyl)-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide derivatives. Molecules 2012;17(12):14205–18.
[85] Aynetdinova D, Callens MC, Hicks HB, Poh CYX, Shennan BDA, Boyd AM, et al. Installing the "magic methyl" C–H methylation in synthesis. Chem Soc Rev 2021;50(9):5517–63.
[86] Schönherr H, Cernak T. Profound methyl effects in drug discovery and a call for new C–H methylation reactions. Angew Chem Int Ed 2013;52(47):12256–67.
[87] Gisbert Y, Fellert M, Stindt CN, Gerstner A, Feringa BL. Molecular motors' magic methyl and its pivotal influence on rotation. J Am Chem Soc 2024;146(18):12609–19.