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# GPCRs: An advanced technology for managing insect pests

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**Abstract**
Attempts to reduce the use of synthetic pesticides, especially broad-spectrum insecticides in plant protection and to use alternatives and novel methods for pest control or ("biorational control") are the challenges of pest control for the twenty- first century. Continued pest management by using insecticide is threatened by the development of insecticide resistance to commercial insecticides (Casida and Durkin, 2013). New mode-of-action chemistries are urgently sought for the management of arthropod pests that reduce the crop yield and transmit disease causing agents. Not only in humans, but also in insects, development, reproduction, metabolism and various behaviours are under the control of neuropeptides and biogenic amines that signal through G-protein coupled receptors (GPCRs) (Caers *et al*. 2012). Novel insecticide targets potentially exist among the arthropod GPCRs. These proteins comprise a large family of membrane-bound molecules that mediate critical biological processes such as neurotransmission, vision and hormonal regulation. More than 100 different GPCRs have been identified in the genomes of multiple insect species. The dopamine GPCR, AaDOP2, antagonist screen hits amitriptyline and doxepin caused significant lethality in the mosquito bioassay (Meyer *et al*. 2012). Serotonin GPCRs are involved in many key processes of insect life. No insecticides that target this receptor group are available at this moment. Serotonin receptor agonists/antagonist may form potential lead compound. The (1-[(4-aminophenyl) ethyl]-4-[3-(trifluoromethyl) phenyl] piperazine) PAPP scaffold was used to design and synthesize a series of compounds that were evaluated for biological activity against the armyworm *Pseudaletia separate* (Vleugels *et al*., 2015). The pyrokinin (PK) family of neuropeptides plays a multifunctional role in the physiology of insects. Incorporation of the dihydroimidazole motif into active core regions of the PK superfamily of peptides provides a unique strategy for the development of mimetic analogs that can serve either as selective agonists or antagonists of the broad class of PK GPCRs (Nachman, 2014). Such analogs provide leads in the development of novel insect-specific, environmentally favourable pest management agents capable of disrupting PK-regulated physiological systems. These ''proof-of-concept'' studies sets the stage for target-specific approaches for pest management. GPCRs are potential candidate targets for next generation insecticides and provide opportunities to discover new mode-of-action chemistries for pest management.

**Keywords:** Attempts, approaches, chemistries, regulated

## Introduction
The goal of agriculture is to make available nutritious and affordable food and fibre supply. The challenge of growing enough food to feed the world's expanding population, coupled with the changing dietary habits of an expanding middle class throughout Asia, has driven the need to improve crop yield and quality through the control of a wide range of insect pests. Additionally, there is also the strong desire to control a host of insect vectors of diseases such as malaria. There are a wide range of tools and approaches available to control pest insects, including insecticides, genetically modified plants, host plant resistance, cultural practices, biological control, and microbial control. Because all of these approaches have their own benefits and limitations, no one approach is suitable to all situations. As such, a variety of these tools/approaches are often employed to provide insect control for a particular crop or health need. The success of modern agriculture in achieving and maintaining high-yield crops strongly depends on controlling insect pests via the intensive utilization of insecticides. For many pest control problems, insecticides have and continue to provide growers and public health workers with the means to predictably, quickly and effectively address a specific pest problem. These highly attractive attributes have led to wide array of insecticidal chemistries (Sparks, 2013) [6]. The term biorational (biological + rational) pest control or approach can be defined as the use of selective means that are compatible with natural enemies and the environment, with minimal effect on non-target organisms. Biorational control is based on a diversity of chemical, biological and physical resources for controlling insect pests, which results in reduced risk to man and the environment, and in accordance with IPM concepts. Biorational agents and approaches will be the key for inspiring IPM strategies to meet our community challenges. This overview is based on the different chapters of this book, which deals with advanced and novel technologies for managing insect pests. These technologies focus on safer and environmentally friendly (biorational) approaches. One such approach is based on disrupting the activity of specific biochemical sites serving as targets for insecticide discovery; these sites include transcription factors belonging to the basic Helix-Loop-Helix (bHLH) family, antijuvenile hormone (AJH) agents that target JH biosynthetic enzymes, G protein-coupled receptors (GPCR) and bursicon as targets for insect pest control. Another section is related to screening potential insecticides by cell-based and other advanced screenings. The third segment deals with novel biotechnology control strategies ("the genetic

approach"), which exploit the huge development in arthropod genomics, gene silencing (RNA-interference) and Cry toxins (based on the crystal protein produced by Bacillus thuringiensis – Bt ) usage in insect control. The last section of the book covers various new aspects of pest control such as the usefulness of plant natural-product mixtures, optical manipulation for reducing sucking pests, recent progress in bed bug management, and insect pests in stored-product food and the utilization of nanotechnology for development of potent insecticides. Insecticides are principal defence against insect pest of crops, livestock and peoples since last one and half century.

**Present pesticide perspective**

Among the more than 25 Mode of Actions currently in the IRAC classification, 85% of the value of these MoAs is derived from insecticides that act on the insect nerve-muscle system. In contrast, insecticides altering growth and development account for only 9% of the total insecticides sales, while those disrupting energy production (respiration targets) account for only 4%. Because small perturbations in the nervous system are quickly amplified, the insect nervous system has been and remains a prime target for new insecticides. Within the nerve-muscle acting insecticides, the neonicotinoids predominate with 27% of the market, nearly as much as the current organophosphates, carbamates and pyrethroids combined (31%). Although a relatively new class of chemistry, the diamides acting on ryanodine receptors now account for about 8% of total global end-user insecticide sales, a number that has been steadily increasing, and that is certain to continue to increase with the potential addition of other new diamides to the marketplace (Sparks and Nauen, 2014) [7].

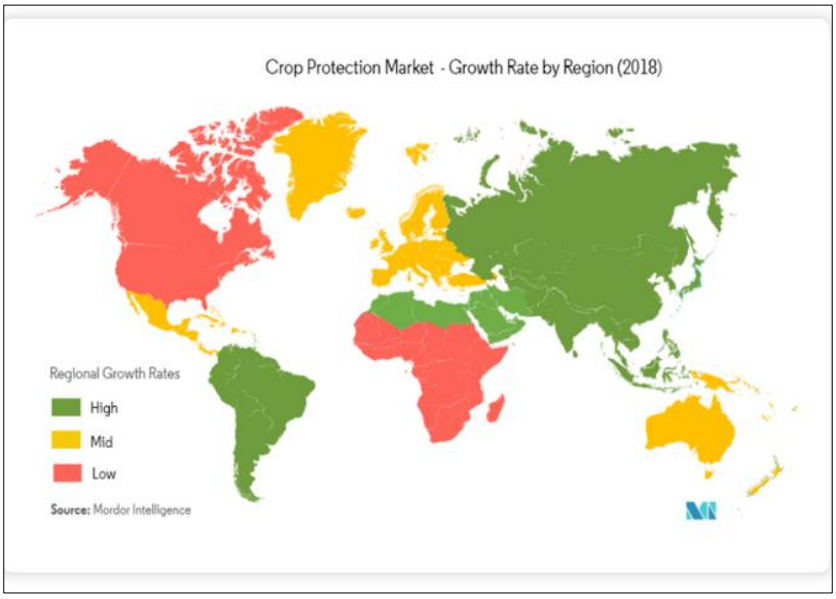


**Fig 1:** Crop protection market- growth rate by region (2018)

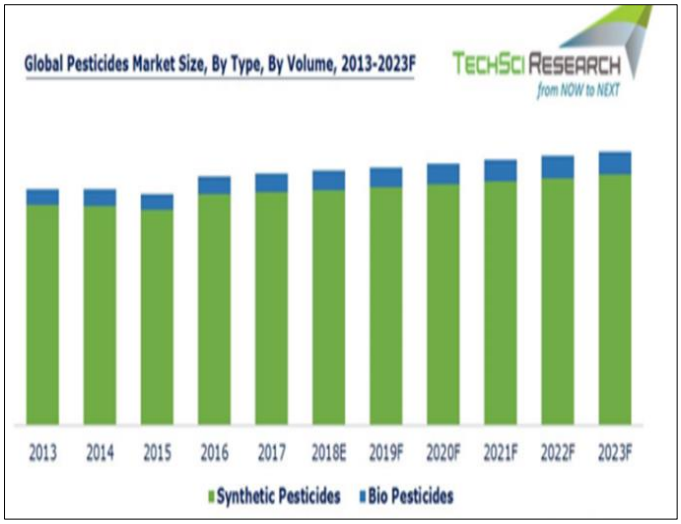


**Fig 2:** Global pesticide market size, 2013-2023.

**Insecticide resistance**

Continued pest and vector control is threatened by the development of pest populations with resistance to commercial insecticides. Insecticide resistance has been a major factor influencing insect control and pest management for more than half a century. The first paper documenting insecticide resistance was published 100 years ago and involved lime sulfur and the San Jose scale. Thereafter, a few sporadic cases of insecticide resistance were reported through the mid-1940s. The introduction of the synthetic organic insecticides (i.e. DDT, cyclodienes and organophosphorus insecticides) in the 1940s lead to great improvements in insecticidal efficacy and spectrum, with the consequent large scale, expanded use of these new tools for pest insect control (Casida and Durkin, 2013) [3]. Not surprisingly, there was also a rapid rise in the number of cases of resistance due to extensive, repeated use of these products. Since the late 1940s, the number of cases of insecticide resistance, and the number of

species and compounds involved has been continually increasing. Moreover, resistance has been documented to each of the major insecticide classes used in the past and present for pest management, including organochlorines, organophosphates and carbamates, and pyrethroids which target sodium channels, acetylcholinesterase, and GABA receptors, respectively, in the insect nervous system(Sparks and Nauen, 2014) [7].

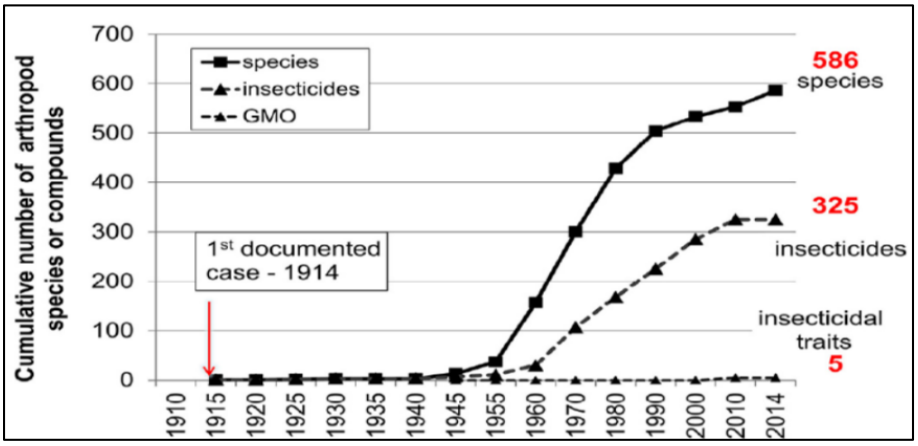


**Fig 3:** Cumulative increase the number of species resistant to one or more insecticides

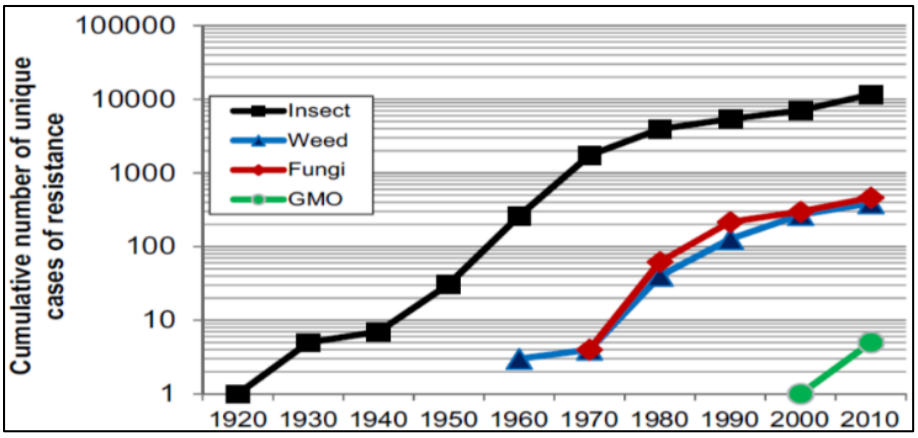


**Fig 4:** Cumulative increase in the number of individual cases of resistance for insecticides

**Discovery of new mode-of-action chemistries**

New mode-of-action chemistries are urgently sought for the management of arthropods pest that reduces the crop yield and transmit disease-causing agents impacting human health.

Novel insecticide targets potentially exist among the arthropod G protein-coupled receptors (GPCRs) (Meyer *et al*, 2012). These proteins comprise a large family of membrane-bound molecules that mediate critical biological processes such as neurotransmission, vision, and hormonal regulation, among others. GPCRs are extensively targeted for drug development in humans - approximately 40% of prescription pharmaceuticals interact with these receptors and more recently, Gamo *et al*. reported multiple GPCR interacting chemistries as promising anti-malarial leads. Also, the mode-of-action of amitraz, a chemistry registered for tick and insect control, is presumed to have partial agonistic activity at an octopamine sensitive GPCR. These studies have provided a basis for the functional characterization of GPCRs and their prioritization as potential subjects for insecticide development.

**G-protein**

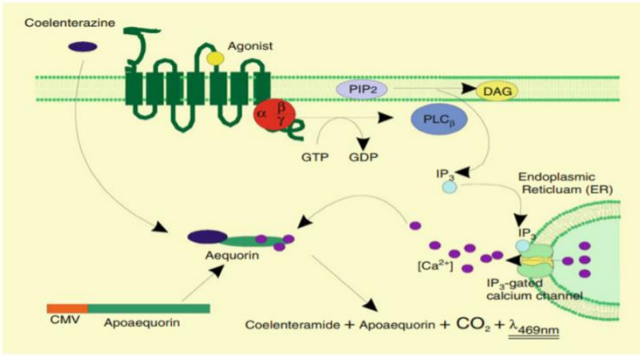


**Fig 5:** Mechanism of action of G- protein coupled receptors

G-proteins, also known as guanine nucleotide-binding proteins, are a family of proteins that act as molecular switches inside cells, and are involved in transmitting signals from a variety of stimuli outside a cell to the inside. Their activity is regulated by

factors that control their ability to bind to and hydrolyze guanosine triphosphate (GTP) to guanosine diphosphate(GDP). When they bind GTP, they are 'on', and, when they bind GDP, they are 'off'. G proteins belong to the larger group of enzymes called GTPases (Caers *et al*., 2012) [2].

G proteins located within the cell are activated by G protein-coupled receptors (GPCRs) that span the cell membrane. Signaling molecules bind to a domain of the GPCR located outside the cell, and an intracellular GPCR domain then in turn activates a particular G protein. Some inactive-state GPCRs have also been shown to be "pre-coupled" with G proteins. The G protein activates a cascade of further signaling events that finally results in a change in cell function. G protein-coupled receptor and G proteins working together transmit signals from many hormones, neurotransmitters, and other signaling factors. G proteins regulate metabolic enzymes, ion channels, transporter, and other parts of the cell machinery, controlling transcription, motility, contractility, and secretion, which in turn regulate diverse systemic functions such as embryonic development, learning and memory, and homeostasis.

**GPCRs**

GPCRs constitute the largest superfamily of cell surface proteins. They can interact with a wide diversity of ligands and are involved in all major processes taking place in arthropods. It is remarkable that GPCRs mediate a diverse array of important biological activities, such as the regulation of vision, smell, taste, behavioural and mood regulation, immune defence and processes controlled by the nervous and endocrine systems. This is also why GPCRs have drawn the utmost attention of the pharmaceutical industry. Nowadays, nearly half of all medications achieve their effect through GPCRs. The 2012 Nobel Prize in chemistry awarded to Lefkowitz and Kobilka for their work on GPCRs emphasised even more how important these signal transducing proteins are.

Not only in humans, but also in insects, development, reproduction, metabolism and various behaviours, such as feeding, are under the control of neuropeptides and biogenic amines that signal through GPCRs (Caers *et al*., 2012) [2]. Disrupting or over-activating GPCRs (or their pathways) that mediate vital biological functions may lead to dramatic mortality, developmental arrest, defects in growth and reproduction, or reduced fitness of insect pests. In addition, protein/peptide ligands and their receptors are gene products and therefore subjected to (co)evolution, often rendering them to be species-specific. Therefore, insect GPCRs constitute an interesting group of potential pharmacological targets for developing novel and selective agents for insect pest control. More than 100 different GPCRs have been identified in the genomes of multiple insect species, including malaria- and yellow fever-transmitting mosquitoes. Following are some important GPCRs found in the insects, regulating critical physiological processes.

- Chromophores GPCRs
- Amino acid GPCRs
- Biogenic amine GPCRs
- Neuropeptides GPCRs
- Olfactory and taste GPCRs
- Glycoprotein hormones GPCRs
- Prostaglandins GPCRs
- Nucleotides GPCRs

**Biogenic amines GPCRs**

There are six well-established aminergic neurotransmitters in insects: themonophenolic amines (derived from the amino acid tyrosine) octopamine and tyramine; the catecholamine (derived from tyrosine via L-DOPA) dopamine; the indolamine (derived from the amino acid tryptophan) serotonin (5-HT); histamine (derived from the amino acid histidine) and acetylcholine. In terms of synthesis, packaging, release, degradation and mode of action, the amine neurotransmitters can be classified in terms of properties somewhere in between other small-molecule neurotransmitters (amino acids) and neuropeptides. Biogenic amines, which are derivatives of aromatic amino acids, can regulate a variety of behavioural and physiological processes, including locomotion, aggression, circadian rhythms, cardio-vascular control, learning and memory. All biogenic amine GPCRs belong to the rhodopsin GPCR family. Several amines can also interact with specific receptors that belong to the category of ligand-gated ion channels. These also include a number of interesting targets for insecticides (e.g. nicotinic acetylcholine receptors, glutamate and A-type GABA receptors).

**Dopamine receptor agonist/antagonist**

The dopamine receptors are classified as either D1- or D2-like based on their differential functional roles. Ligand binding to the D1-like dopamine receptors causes Gas mediated stimulation of adenylyl cyclase (AC) production of cAMP. Dopamine and its receptors are essential for complex behavioral mechanisms in arthropods such as locomotion, arousal and olfactory learning. The importance of dopaminergic-related functions has stimulated research to understand these processes in mosquitoes. Dopamine and serotonin have been tied to salivary gland functioning of vectors and may have an impact on pathogen acquisition and transmission during blood feeding. Much attention has been given to the role of dopamine in the melanization pathway of mosquitoes and other insects, as well as the effect of dopamine on development, pigmentation, reproduction, immune responses to parasites, wound healing, and Wolbachia infection. Putative D1-like and D2-like dopamine receptors have been identified in the genomes of the mosquitoes *A. aegypti* and *Anopheles gambiae*. Moreover, due to their presumed significance in mosquito neurobiology, these dopamine receptors are attractive candidates to explore as new targets for chemical control (Meyer *et al*, 2012).

Typically, insects possess three different dopamine receptors including two D1-like receptors and a single D2-like receptor. Here, RT-PCR data were used to validate the two mosquito D1-like dopamine receptor gene models; this enabled confirmation of intron/exon boundaries and prediction of the complete protein coding regions needed prior to heterologous expression studies. A putative D2-like dopamine receptor gene (AaDOP3) was also identified in Ae. aegypti although this receptor has not yet been functionally characterized.

**Neuropeptide binding GPCR**

Neuropeptides and other peptide hormones are secreted from brain and endocrine glands in the nervous system or in the periphery, respectively. They are involved in many crucial physiological processes, for example feeding behaviour, learning and memory, fluid secretion, muscle activity/locomotion, ecdysis behaviour, metabolism, growth, metamorphosis and

reproduction. Most of the neuropeptides interact with GPCRs belonging to the rhodopsin GPCR (A) superfamily, while others, such as diuretic hormone and pigment dispersing factor (PDF) receptors, belong to secretin receptor-related GPCR (B) family. Other (neuro)peptides, such as ILPs, PTTH and EH, can interact with non-GPCR receptors that contain only one single transmembrane segment and signal through an intrinsic cytoplasmic domain possessing a receptor tyrosine kinase (RTK) or a guanylyl cyclase (GC) enzymatic activity (Caers *et al*, 2012) [2].

**Specific neuropeptide GPCRs**

- Adipokinetic hormone GPCRs
- Allatostatin GPCRs
- Allatotropin GPCRs
- Diuretic hormone GPCRs
- Ecdysis triggering hormone GPCRs
- Bursicon GPCRs
- Proctolin GPCRs
- Sex peptide GPCRs
- Pyrokinin / Pheromone Biosynthesis Activating Neuropeptide GPCRs

**Pyrokinin/pheromone biosynthesis activating neuropeptide binding GPCRs**

Pest trapping and mating disruption using pheromones are already an established and biologically-sound part of present-day integrated pest management. In these techniques, the insects' inherent allocrine signalling mechanisms are used to trap the animal or disrupt mate-finding by exogenous administration of pheromone-analogues. Disruption of mate-finding could also be achieved by inhibiting homogenous pheromone production by interference with the pyrokinin/pheromone biosynthesis-activating peptide (PK/PBAN) signalling pathway.

The pyrokinin (PK) family of peptides plays a multifunctional role in the physiology of insects. In 1986 the first member of the family, leucopyrokinin (LPK), was isolated from the cockroach *Leucophaea maderae* with over 100 members of this peptide class identified thereafter, in large measure *via* recent peptidomic studies that directly analyze neural tissues. Further, PK peptides are encoded in two genes (PK and CAPA) in most insect species studied so far; and a single *Schistocerca* species can process up to 10 pyrokinin sequences. All family members share the common *C*-terminal pentapeptide FXPRLamide (X = S, T, G or V), although rare modifications to the core pentapeptide have been observed in the cockroach (Y for F) and in stink bugs (T for P; and M for R) and include subfamilies such as PKs, myotropins (MTs), PBAN, diapause hormone (DH), melanization and reddish coloration hormone (MRCH), pheromonotropin (PT), as well as pheromonotropic and peptides derived from the cDNA of moths. The PK family has been shown to stimulate sex pheromone biosynthesis in moths, and mediate critical functions associated with feeding (gut contractions), development (egg diapause, pupal diapause and pupariation) and defense.

**PK/PBAN agonist/antagonist**

Although neuropeptide-based antagonists carry a high potential for insect management their application in pest control has not so far been implemented because of two major limitations. The pk is the linear peptidic nature of neuropeptides, which renders them non-selective, highly susceptible to proteolytic degradation, and impenetrable through biological tissues. The second is the lack of an approach for antagonists design because of lack of information regarding the 3-D structure of the receptor agonist complex and the mechanism of receptor activation.

Insect neuropeptides (Nps) are a prime target for the development of novel insecticides, as they regulate many physiological and behavioral processes during development, reproduction, and senescence. Their blockers (antagonists) disrupt and interfere with the normal growth, development, and behavior of insects and can yield therefore receptor selective, insect-specific insecticides (Altstein, 2003). Such antagonists are derived from and resemble natural peptides but have to be of a peptidomimetic nature. The chemical nature of Nps enables them to be used as the basis for the design of a generic group of insectspecific and nontoxic insecticides. Asimilar approach has recently been applied to human Nps as a novel direction in the drug industry.

Although neuropeptides of the PK family are potent regulators of physiological processes critical to insect survival, they hold little promise as pest management agents because they are subject to rapid degradation by peptidases in the hemolymph, tissues and gut of pest insects. Members of the PK family are hydrolyzed, and therefore inactivated, by tissue-bound peptidases of insects. Specifically, the PKs are hydrolyzed by tissue-bound peptidases at a primary susceptibility site between the P and R residues within the general C-terminal pentapeptide sequence that defines members of this family of neuropeptides. To overcome the limitations inherent in the physical characteristics of peptides, the development of peptidomimetic analogs has become an important strategy for improving the therapeutic potential of peptides.

**Peptidomimetic approach**

Peptidomimetics is a broader term used to refer to pseudopeptides and nonpeptides designed to perform the functions of a peptide. Generally these peptidomimetics are derived by the structural modification of the lead peptide sequence to overcome a number of metabolic limitations, such as proteolytic degradation that restrict the use of peptides as therapeutic and/or agrochemical control agents.

One such peptidomimetic approach is the incorporation of bulky or sterically-hindered residues adjacent to hydrolysis susceptible peptide bonds. This approach has led to the development of biostable mimetic analogs of several insect neuropeptide families (including the pyrokinins) that have demonstrated aphicidal activity that approaches or exceeds the potency of commercially available aphicides, whereas the natural peptides remain inactive. Another peptidomimetic approach is the incorporation of a biostable, isosteric replacement motif for the hydrolysis susceptible peptide bond.

**Conclusion**

The discovery of additional insecticide targets is extremely important in mankind's continuously ongoing arms' race against pest insects. It may address the existing and increasing problems with product selectivity and with the evolution of resistance in insect populations, respectively. While the currently-applied commercial insecticidal products are only targeting a very limited number of insect proteins, the international research community has already identified a much larger set of novel and potentially

promising insecticide targets. Such targets constitute a solid basis for ongoing and future molecular screenings, designs and development programs. Moreover, and in particular because of the accessibility and the unique relationship between the target's structural and functional properties, several neurohormone receptors may provide the selectivity that is often a big problem with the currently available pesticides. Many of these candidate targets are GPCRs that play crucial roles in the control of insect physiology. However, there is still an urgent need to gather more fundamental knowledge about the in vivo structural and functional properties of most insect neurohormone receptor molecules, which up-to-date have often been (over)expressed in heterologous systems, such as mammalian cell lines or Xenopus oocytes. Several emerging research strategies will most likely further contribute to a reduction of the costs for developing new pest control products and research methods. These involve an improved rational design of synthetic receptor ligands, such as peptidomimetic compounds, that could be further optimised by means of high-throughput screening assays towards highly effective and selective pesticide products. Within the next few years, whole genome sequence will not be limited for model insect species. Genome sequence information for more and more non-model insects, especially for those economically and ecologically important species, will become available, which will greatly speed up pesticide discovery. GPCR is one of the largest multi-gene families that play crucial roles in diverse developmental, physiological and behavior responses, which is why GPCRs have drawn the most attention in the pharmaceutical industry. Thus, about 40% of therapeutic drugs target human GPCRs. Although many insect GPCRs have been deorphanized, the knowledge gained from these studies has not yet been applied for discovery of new insecticides. Large-scale RNAi screen has been successfully used in dissecting signal transduction networks in vitro as well as in vivo. RNAi screens have been used to identify 25 GPCRs that are critical for growth, development and survival of the red flour beetle. In the future, many more studies using information available from insect genomes that are being sequenced will help to explore this class of molecules in the development of pest control agents.

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