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Advances in the application of neuropeptides in insect control

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Abstract

The development of a new approach for the generation of a novel type of putative insect control agents based on backbone cyclic peptidomimetic antagonists of insect-neuropeptides is reported. The approach, termed the backbone cyclic neuropeptide based on antagonist (BBC-NBA) was applied to the insect pyrokinin/pheromone biosynthesis activating neuropeptide (PBAN) family as a model, and led to the discovery of a potent linear lead antagonist and several highly potent, metabolically stable BBC peptidomimetic antagonists, devoid of agonistic activity, which inhibited in vivo PBAN-mediated activities in moths.

Keywords: PBAN; Insect neuropeptides; Neuropeptide antagonists

1. Introduction

The success of modern agriculture in achieving and maintaining high-yield crops strongly depends on controlling insect pests via the intensive utilization of insecticides. To date, organo-synthetic chemical insecticides are the main means of protection against the damage caused by insects to crops. Uncontrolled application of chemical insecticides in the last decades has led to acquired resistance in insects, has contaminated the environment with toxic residues that endanger humans and other life forms, and has disrupted the ecological balance in crop fields. The growing concern regarding the toxic effects of insecticides led to the implementation of strict regulations in the Western World which are being adopted also in Third World countries. These regulations limit the application of the existing organo-chemical insecticides and ban further application of the more toxic ones.

The strategic approach which is directing the worldwide research and development (R&D) efforts is aimed toward the identification and development of novel families of non-toxic, insect-specific compounds, which are safe and compatible with integrated pest management (IPM) programs, which will eventually replace organo-synthetic chemicals as the mainstream pest control compounds.

The initial products that have emerged from this effort during the past decade were based on a variety of chemical and biological technologies. These bio-insecticidal, bio-rational products include: bacterial toxins (BT), micro-organisms, mating disruption pheromones, insect growth regulators (IGRs), genetically engineered pest-resisting crops, natural enemies, and natural products extracted from plants. The main drawback of these technologies and products, which constitute approximately 2–3% of the insecticides market, is that they cannot replace the organo-chemicals as the mainstream insecticides, because of their high production cost and limited applicability.

In the quest for a novel group of non-toxic insecticides which could eventually replace the toxic organo-chemical compounds and overcome the limitations introduced by the existing bio-insecticides, entomological studies concentrate on the search for targets and compounds which will serve as a basis for the development of highly effective, selective and environmentally friendly insect control agents and will emerge as a new mainstream of insecticide technology.

Insect neuropeptides are a prime target in the development of novel insect control agents, since they regulate most of the key functions in insects such as: embryonic and post-embryonic development, homeostasis, osmoregulation, migration, oviposition and mating (for review

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see Gäde, 1997). Their blockers (antagonists) disrupt and interfere with the normal growth, development and behavior of insects, and, therefore, can yield receptor-selective, insect-specific insecticides. Such antagonists are derived from and resemble the natural peptides but are peptidomimetic in nature. A similar approach has recently been applied to human neuropeptides as a novel direction in the drug industry.

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 first 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.

Recently, we have developed a novel approach, termed backbone cyclic neuropeptide- based antagonist (BBC-NBA), which overcomes the above limitations. The approach was applied to the insect pheromone biosynthesis activating neuropeptide (PBAN) and resulted in the discovery of highly potent, stable and selective antagonists.

The basic concepts that make up the BBC-NBA technology (backbone cyclization and cycloscan) are explained, and the advantages that are introduced by these methods, as well as their application to the insect PBAN for the discovery of highly potent antagonists are described.

1.1. Pheromone biosynthesis activating neuropeptide

PBAN is an important neuropeptide that mediates some of the key functions in insects. PBAN was first reported by Raina and Klun (1984), as the neuropeptide that regulates sex pheromone production in female moths, and its amino acid sequence was determined in *Helicoverpa zea* (Hez-PBAN; PBAN 1-33NH_2) (Raina et al., 1989). Since then, six other PBAN molecules have been isolated from five additional moth species, and their entire primary structures have been determined (Kitamura et al., 1989, 1990; Masler et al., 1994; Duportets et al., 1998; Jacquin-Joly et al., 1998; Choi et al., 1998), and the c-DNA and genes of PBAN have been cloned (Davis et al., 1992; Kawano et al., 1992, 1997; Ma et al., 1994; Choi et al., 1998; Duportets et al., 1998; Jacquin-Joly et al., 1998). PBAN molecules were found to be C-terminally amidated neuropeptides consisting of 33–34 amino acid residues, and comparison of their primary structures revealed that they share a high degree of homology and an identical pentapeptide C-terminal sequence (Phe–Ser–Pro–Arg–Leu–NH_2) which also constitutes the active core required for its biological activity (Altstein et al., 1995, 1996a, 1997; Kochansky et al., 1997; Nagasawa et al., 1994; Raina and Kempe, 1990, 1992). Since 1984, the presence of PBAN-like activity has been demonstrated in a variety of moths and in other non-Lepidopteran species, and its mode of action has been studied extensively (for review see Raina, 1993; Gäde, 1997).

Further studies on the regulation of sex pheromone biosynthesis in moths have revealed that sex pheromone biosynthesis can be elicited by additional neuropeptides isolated from various insects, all of which share the common C-terminal pentapeptide of PBAN (Phe–Xxx–Pro–Arg–Leu–NH_2; Xxx = Ser, Gly, Thr, Val), (Abernathy et al., 1995; Fónagy et al., 1992; Kuniyoshi et al., 1992a, b; Teal et al., 1996). Among these peptides are the pyrokinins (Lem-PK, Lom-PK-I and Lom-PK-II) and the locustamyotropins (Lom-MT-I–IV) (myotropic peptides isolated from the Madeira cockroach *Leucophaea maderae* (Fabricius) and the migratory locust, *Locusta migratoria* (Nachman et al., 1993; Schoofs et al., 1993); pheromonotropin (Pss-PT) an 18-amino acid peptide isolated from *Pseudaletia (Mythimna) separata* (Walker) (Matsumoto et al., 1992) and diapause hormone (Bom-DH) isolated from the silkworm, *Bombyx mori* (L.) (Imai et al., 1991). These peptides have recently been designated the pyrokinin/PBAN family. In addition to their ability to stimulate sex pheromone biosynthesis in moths, members of this family have been found to control a variety of other physiological and behavioral functions such as: melanization and reddish coloration in moth larvae (Matsumoto et al., 1990; Altstein et al., 1996b), contraction of the locust oviduct (Schoofs et al., 1991), myotropic activity of the cockroach and locust hindgut (Nachman et al., 1986; Schoofs et al., 1991), egg diapause in the silkworm (Imai et al., 1991) and acceleration of pupariation in fleshfly *Sarcophaga bullata* (Parker) larvae (Nachman et al., 1997).

Three reasons led to the application of the BBC-NBA approach to the generation of receptor-selective agonists and antagonists to the pyrokinin/PBAN family: (i) the major role the pyrokinin/PBAN family plays in the physiology of moths and other insects; (ii) knowledge of the amino acid sequence and the large body of information derived from structure–activity relationship (SAR) studies; and (iii) the availability of biological assays which enable agonistic and antagonistic activities to be determined quantitatively.

1.2. Backbone cyclic neuropeptide-based antagonist (BBC-NBA) approach

The BBC-NBA approach is based on backbone cyclization of peptides and the cycloscan concept (see below) and comprises the following steps:

(1) Identification of the neuropeptide that controls the required function.

(2) Elucidation of the shortest sequence of the neuropeptide that constitutes the active site.
(3) Discovery of a peptidic linear lead antagonist on the basis of the sequence found in (2).
(4) Discovery of a potent BBC antagonist devoid of agonistic activity, based on (3).
(5) Determination of the structural requirements for antagonistic activities, on the basis of (4).
(6) Design and synthesis of an insecticide prototype (a small, metabolically stable, selective bioavailable, cost-effective peptidomimetic compound), based on the information obtained in (5), ready for formulation, toxicology and field trials.

The BBC-NBA approach is dependent on the availability of simple and quantitative in vitro and in vivo bioassays for monitoring bioactivity and on the availability of an advanced chemistry to design, synthesize and determine the structure of compounds of interest. The approach is general and can be applied to any insect neuropeptide.

The rational behind the steps and the basic concepts (backbone cyclization and cycloscan) that make up the BBC-NBA approached are described below.

1.2.1. Dislcosure of the active sequence in the neuropeptide

There are two types of antagonists: a competitive antagonist, that binds to the same site as the agonist but does not elicit signal transduction; and a type that inhibits binding and/or signal transduction by means of allosteric effects. Antagonists of the first type are usually derived from agonists but exhibit different structural and conformational features; those of the second type are usually non-peptides and are identified by screening of natural product libraries or combinatorial libraries.

From this perspective it is clear that an initial requirement for the development of a competitive antagonist is the identification of the smallest active sequence in the neuropeptides that binds and activates the receptor; this is the basis on which modifications can be made, leading to the discovery of a lead antagonist.

Elucidation of the active site of a neuropeptide is achieved by means of SAR studies. In the case of large neuropeptides (having more than 15 amino acid residues) peptide mapping is performed. This involves determination of the activities of peptide fragments containing up to 10 amino acid residues, that span the entire sequence of the neuropeptide. In case of smaller neuropeptides or when active peptides are discovered by peptide mapping, des-amino acid scan is performed, namely, SAR studies of a library of peptides that lack 1–5 amino acids from either the C- or the N-terminus. It should be mentioned that in a neuropeptide family in which there is sequence homology among the various members of the family, the homologous sequence is usually the active region.

1.2.2. Discovery of a competitive lead antagonist

Lead antagonists are usually partial antagonists that bind to the receptor site but can only partially activate the transduction system. Improvement of antagonistic activity (high potency, selectivity and lack of agonistic activity) can be achieved by SAR or by imposing conformational constraints (see below).

Most of the lead competitive antagonists discovered hitherto were based on vertebrate neuropeptide agonists. The following empirical practices for the conversion of agonists to antagonists have emerged from these studies: (i) systematic replacement of the naturally occurring L-amino acids by their non-natural D isomers or replacement of amino acid residues with D hydrophobic amino acid residues, such as D–Phe or D–Trp (Hruby et al., 1990; Hruby, 1992; Rees et al., 1974; Vale et al., 1972; Piercey et al., 1981; Sawyer et al., 1981; Rosell et al., 1983; Folkers et al., 1984; Vevrek and Stewart, 1985; Heinz-Erian et al., 1987; Rhaleb et al., 1991; Cody et al., 1995; Collins et al., 1996; Maretto et al., 1998); (ii) omission of amino acid residues from agonistic sequences, or omission or replacement of functional side chains (e.g. [Sar^1]-Angiotensin II (1–7)amide in which Asp^1 in angiotensin was replaced with Sar and Phe^8 was replaced with an amide group, or [D-Phe^6,Des-Met^{14}]-Bombesin (6–14)ethyl amide in which the six N-terminal amino acids and Met^{14} were omitted) (Coy et al., 1989); (iii) replacement of a C-terminal amide with a free acid (as in bombesin and gastrin) (Llinares et al., 1999; Rodriguez et al., 1987); (iv) reduction of peptide bonds, as in the case of bombesin (Coy et al., 1988); (v) conformational and/or topographical alteration (for reviews see Hruby, 1981a, b, 1992; Goodman and Ro, 1995; Becker et al., 1999; Collins et al., 1996). Implementation of the above empirical practices for given agonists necessitates detailed knowledge of the SAR, and any available information regarding the bioactive conformation.

To the best of our knowledge these approaches have been applied to a very limited number of insect neuropeptides for the discovery of antagonists. The D–Phe approach was applied to the insect neuropeptide proctolin and resulted in the discovery of a few peptides with antagonistic activity (Kuczer et al., 1999) and recently, we discovered a lead antagonist for the insect PBAN by applying these empirical rules (Gilon et al., 1997; Zeltser et al., 2000).

1.2.3. Improvement of the antagonistic activity by conformational constraint

As mentioned above, one major problem that hampers the use of neuropeptides (e.g., the linear lead antagonists discussed above) as insect control agents is related to the facts that they are susceptible to proteolytic degradation, have low bioavailability, are non-selective because of conformational flexibility, and are not cost effective. An approach to overcoming the first three limitation is the

introduction of conformational constraint into peptides, which leads to slower equilibrium rate, thus reducing the flexibility of the peptide.

Conformational constraint can be imposed by various methods (for reviews see Hruby, 1981a, b, 1992; Goodman and Ro, 1995). Cyclization of peptides is one of the most common and attractive methods to introduce conformational constraint into peptides and thus restrict their conformational space (Kessler, 1982). The conformational constraint confers the following attributes on the peptides: (i) selectivity: the cyclic structure may restrict the conformational space to a conformation which mediates one function of the peptide and excludes those which mediate other functions; (ii) enhanced metabolic stability: the cyclic structure may exclude the conformation which is recognized by degrading enzymes from the conformational space, thus preventing enzymatic degradation; (iii) increased biological activity: the rigidified structure will be more potent than the linear one, since it spends more time in the bioactive conformation because of the much slower equilibrium between the conformations. However, this is only true when the conformational space of the cyclic peptide overlaps with the bioactive conformation. In most cases cyclization will yield an inactive peptide because of mismatching (Altstein et al., 1999a); (iv) improved bioavailability because of reduction of polarity.

Nature has also chosen the cyclization route for restricting the conformation of peptides, and many natural cyclic peptides are known today, some of which are in therapeutic use (e.g., insulin, oxytocin and cyclosporin).

There are four modes of cyclization in peptides: (i) forming a covalent bond between two side chains (e.g., an amide bond between aspartic acid and lysine, or a disulphide bond between two cysteine residues); (ii) covalently linking the amino and carboxy termini of the peptide; (iii) forming a bond between an Asp or Glu side chain and the amino terminus; and (iv) forming a bond between a lysine or ornithine side chain and the carboxy terminus (for review see Gilon et al., 1991).

The four natural modes of cyclization cannot easily be applied to most of the peptides, for two reasons: (i) not every peptide contains functional amino acid residues which can be covalently interconnected and more importantly (ii) even when there are such residues, in most cases they are crucial for the biological activity of the peptide and using them for ring closure causes a loss of or marked reduction in the bioactivity. The same holds true for the amino and carboxy termini, which might be important for the activity of naturally occurring peptides. In addition, the natural modes of cyclization result in only a small number of conformational combinations which are too few to effectively screen the conformational space available for a linear peptide with a given sequence.

1.2.4. Backbone cyclization: a tool for imposing conformational constraint on peptides

In order to overcome the above-mentioned problems, the concept of backbone cyclization was developed (Gilon et al., 1991). According to this concept, the cyclization takes place by means of a covalent interconnection of the peptide backbone atoms (N^{α}(and/or C^{α}) to each other, to side chains, or to amino or carboxyl termini.

By using backbone cyclization, the functionality and the activity of the side chains can be retained during performance of the cyclization. BBC peptides have the following advantages over linear peptides: enhanced stability against proteolytic digestion (Byk et al., 1996; Gilon et al., 1998a), high selectivity (Byk et al., 1996; Gilon et al., 1998a), more potent biological activity (Altstein et al., 1999a), and improved bioavailability. The advantage introduced by the cyclic peptides make them excellent leads for development of insecticide prototypes. In addition, BBC peptides have a constraint conformation which facilitates easy determination of their bioactive conformation by nuclear magnetic resonance (NMR) (Golic-Gradadolnik et al., 1994; Gilon et al., 1998a; Saulitis et al., 1992) and X-ray (Kasher et al., 1999) — provided they are active as the endogenous (parent) peptide. This information is most important for further design of non-peptide small molecules.

1.2.5. Cycloscan: conformationally constrainted BBC peptide libraries

In order to obtain the optimal BBC peptides based on a given sequence, namely the one which best matches the bioactive conformation (and thus exhibits the highest antagonistic activity), there is a need to synthesize a large number of BBC peptides in order to screen the conformational space of the peptide in a systematic way. Cycloscan (Gilon et al., 1998b) is the methodology developed for that purpose. Cycloscan is defined as a selection method based on conformationally constrained BBC peptide libraries intended for efficient screening of the conformational space and thus for fast identification of a BBC peptide lead compound that overlaps the bioactive conformation.

Cycloscan is performed by designing and synthesizing libraries of BBC peptides and screening them with the appropriate bioassay. All the peptides in each library bear the same sequence, and differ from each other in distinct parameters which affect their conformation and hence their bioactivity. This is achieved by the gradual introduction of discrete modifications which ensure efficient screening of the conformational space of the parent peptide. The majority of the peptides in such a library should be inactive, because they do not overlap the bioactive conformation. However, the peptide(s) which do fit into the bioactive conformation should be very potent and should have all the advantages mentioned

above. Such compounds are excellent candidates for neuropeptide–antagonist-based insecticide prototypes.

The main difference between a cycloscan library and the normal combinatorial peptide libraries (either chemical or phage display) is that in the latter every peptide in the library has a different sequence whereas in the former all the peptides in the library have the same sequence and they differ from each other only in their conformation. It is therefore possible to generate a large BBC library for each biologically active lead peptide discovered by peptide mapping or combinatorial libraries.

Cycloscan can be performed in two general ways: (i) sequence biased cycloscan in which all the peptides have the same primary sequence but differ in the bridge size, chemistry and location; and (ii) combinatorial cycloscan in which cycloscan may be further diversified by replacement of sequential amino acids. In cases where sequence biased cycloscan is applied, the amino acid sequence of the linear lead antagonist is used as a basis for library construction.

The diversity of sequence biased cycloscan is not sequential but conformational, and includes the following modes: (i) the modes of backbone cyclization; (ii) the position of the backbone bridge along the peptide sequence; (iii) the size of the bridge; (iv) the chemistry of the bridge. Each of these diversity parameters have been shown to affect the conformation and hence the biological activity (Altstein et al., 1999a; Bitan et al., 1996, 1997; Byk et al., 1996).

The concepts of backbone cyclization and cycloscan were used initially in our laboratory for obtaining conformationally constrained analogs of naturally occurring vertebrate neuropeptides. The model peptide chosen for the development of these techniques was Substance P (SP) (Gilon et al., 1991). A variety of building units were prepared and incorporated into sequence-biased libraries of BBC SP analogs. The cycloscan parameters such as ring size and ring chemistry, had large effects on the activity of the various analogs (Bitan et al., 1996, 1997; Byk et al., 1996). Overall, the extensive research on BBC SP analogs has clearly proved the feasibility and effectiveness of the concepts of backbone cyclization and cycloscan, and enabled these techniques to be employed on somatostatin (Gilon et al, 1998a) and on the insect family of pyrokinin/PBAN neuropeptides (Gilon et al., 1997; Altstein et al., 1999a; see below).

1.3. Implementation of the BBC-NBA strategy for the discovery of receptor-selective antagonists for the insect pyrokinin/PBAN family

Recently, we have applied the BBC-NBA approach for the discovery of antagonists for the insect pyrokinin/PBAN family. The first stage of the study involved optimization of two in vivo biological assays for evaluation of agonistic and/or antagonistic activities of linear and BBC peptides (Gazit et al., 1990; Altstein et al., 1993, 1996b). With the aid of these assays and using the BBC-NBA approach the following was achieved.

(a) Identification (by SAR studies) of the minimal active sequence of PBAN (MINI-PBAN) that constitutes the active core of the pyrokinin/PBAN molecule (Altstein et al., 1995, 1996a, b, 1997).
(b) Design and synthesis of a biased library of linear peptides (based on the information obtained in step (a) for the identification of a linear lead antagonist (Table 1) (Gilon et al., 1997; Zeltser et al., 2000).
(c) Discovery (by SAR studies) of a peptidic linear lead antagonists from the biased linear library obtained in step (b) (Table 1) (Gilon et al., 1997; Zeltser et al., 2000).
(d) Design and synthesis of BBC peptide libraries (based on the sequence of the lead antagonist found in step (c) — D–Phe sub-library, and the active core found in step (a) — Ser sub-library; Fig. 1) for the discovery of a cyclic antagonist (Gilon et al., 1997; Altstein, 1999a).
(e) Discovery (by SAR studies from the cyclic libraries in step (d)) of several selective and highly potent BBC

Table 1
Amino acid sequence of the biased library of linear peptides and their pheromonotropic antagonistic activity[a]

Peptide	Amino acid sequence	Antagonistic activity
LA-1	H–D–Phe–Tyr–Phe–Ser–Pro–Arg–Leu–NH_2	15
LA-2	H–Arg-D–Phe–Phe–Ser–Pro–Arg–Leu–NH_2	0
LA-3	H–Arg–Tyr–D–Phe–Ser –Pro–Arg–Leu–NH_2	18
LA-4	H–Arg–Tyr–Phe–D–Phe–Pro–Arg–Leu–NH_2	64
LA-5	H–Arg–Tyr–Phe–Ser–D–Phe–Arg–Leu–NH_2	53
LA-6	H–Arg–Tyr–Phe–Ser–Pro–D–Phe–Leu–NH_2	65
LA-7	H–Arg–Tyr–Phe–Ser–Pro–Arg–D–Phe–NH_2	45

[a]Antagonistic activity was determined by injection of each peptide (at 100 pmol) together with 0.1 pmol PBAN1–33NH_2 for 2 h. The antagonistic activity is expressed as 100 minus the ratio (as a percentage) between the pheromone content elicited in the gland by the injection of PBAN1–33NH_2 in the presence and absence of each of the peptides. The amount of sex pheromone elicited by 0.1 pmol PBAN1-33NH_2 was 86 ± 24 ($n = 10$) ng/female and was defined as 100%. Pheromone content was monitored in 9–10 females for each peptide.

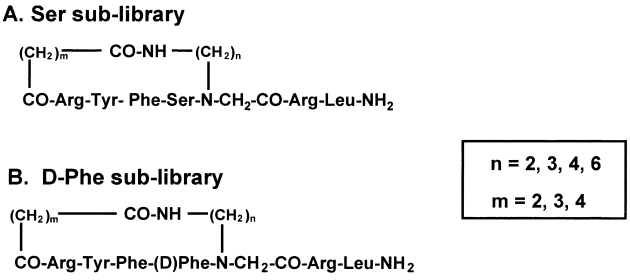

Fig. 1. General structure of the BBC Ser (A) and the D–Phe (B) sub-libraries of peptides.

Table 2
Summary of the pheromonotropic antagonistic activity of the D-Phe BBC peptides

Peptide no.	n[a]	m[a]	$m+n$	Antagonistic activity
19	2	2	4	25
20	2	3	5	96
21	2	4	6	81
22	3	2	5	85
23	3	3	6	24
24	3	4	7	19
25	4	2	6	55
26	4	3	7	1
27	4	4	8	19
28	6	2	8	77
29	6	3	9	34
30	6	4	10	36

[a]See Fig. 1 for details and general scheme of tested peptides.

Note: Antagonistic activity was considered positive only if the tested peptide (at 1 nmol) exhibited over 50% inhibitory activity (50% decrease in pheromone biosynthesis evoked by the injection of 0.5 pmol PBAN1–33NH_2 for 2 h). Antagonistic activity of peptides was determined as described in the legend to Table 1. The amount of sex pheromone evoked by 0.5 pmol PBAN1–33NH_2 ranged from 93 to 113 ng pheromone/female. Pheromone content was monitored with at least 10 females for each of the tested peptides.

anti-PBAN antagonists, devoid of agonistic activity, which inhibit (at 1 nmol) PBAN mediated functions (sex pheromone biosynthesis, Table 2, Gilon et al., 1997, Alstein et al., 1999a and melanization (data not shown).

The results obtained provide a solid proof that neuropeptide antagonists can inhibit biological activities elicited by endogenous neuroendocrine mechanisms and provide valuable information on the structural requirements of pyrokinin/PBAN antagonists (Altstein et al., 1999a).

The design of neuropeptide antagonists for insecticidal applications requires, in addition to the antagonistic properties, the development of novel strategies that will be in line with the common practice of the insecticide industry, namely, non-peptide compounds of low molecular weight, high penetrability through the insect cuticle and gut, environmentally stable and cost-effective in production. Structural data derived from our active and non-active conformationally constrained antagonists as well as a radio receptor assay (RRA) that was developed in our laboratory (Altstein et al., 1999b) for the pyrokimim/PBAN receptor enable us now to proceed toward the development of a high-throughput screening (HTS) assay and the development of novel technologies based on a chemical combinatorial approach for the discovery of potential insecticides/insect control agents based on non-peptide molecules.

2. Concluding remarks

In summary, in this article we present a novel general approach that combines rational design and a selection method for the generation of agonistic and/or antagonistic cyclic peptides based on the sequence of an insect neuropeptide. This approach, applied to PBAN has led to the discovery of several antagonists and agonists which exhibited pheromonotropic activity and effectively inhibited sex pheromone biosynthesis in female *H. peltigera* moths. To the best of our knowledge, this is the first report on the use of backbone cyclization for the design of insect neuropeptide antagonists. Beyond the immediate benefits introduced by the cyclic peptides as selective antagonists, the information on the bioactive conformations of the antagonists that was gained in the course of this study may serve as a basis for the design of improved (small, cost-effective and having enhanced metabolic stability and bioavailability), non-peptide, mimetic agonists and antagonists. Such compounds are potential candidates for agrochemical applications, which could serve, after formulation and preliminary field experiments, as prototypes for the development of a novel group of highly effective, insect-specific and environment-friendly insecticides.

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