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# Peptidomics applied: A new strategy for development of selective antagonists/agonists of insect pyrokinin (FXPRLamide) family using a novel conformational-mimetic motif

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ABSTRACT

Applied peptidomics: A PK active core analog incorporating a novel *trans*Pro conformational-mimetic motif, the dihydroimidazole moiety, was found to demonstrate pure, selective agonism in pyrokinin (PK) family bioassays. A second PK core analog incorporating the dihydroimidazole moiety proved to be an antagonist of the diapause-termination activity of another PK assay. The dihydroimidazole analogs feature a modification adjacent to the primary tissue-bound peptidase hydrolysis site that is expected to enhance biostability over natural PK peptides identified by peptidomics. The research identifies a novel scaffold to design mimetic PK analogs as potential environmentally favorable pest management agents capable of disrupting PK-regulated systems.



## 1. Introduction

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* [1] 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 [2]; and a single *Schistocerca* species can process up to 10 pyrokinin sequences [3]. 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) [1] and in stink bugs (T for P; and M for R) [2] and include subfamilies such as PKs, myotropins (MTs), PBAN, diapause hormone (DH), melanization and reddish coloration hormone (MRCH), pheromonotropin (PT), as well as pheromonotropic β and γ peptides derived from the cDNA of moths [1]. The PK family has been shown to stimulate sex pheromone biosynthesis in moths [1,4–6], and mediate critical functions associated with feeding (gut contractions) [1,7,5,8], development (egg diapause, pupal diapause and pupariation) [1,9–11] and defense

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(melanin biosynthesis) [1,7,8] in a variety of insects. The peptides do not exhibit species specificity and experiments have shown that all of the functions listed above can be stimulated by more than one peptide [1,7,10], and that the C-terminal pentapeptide common to the PK neuropeptide class retains activity in each of the disparate functions. 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 [1,12]. 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 [12].

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. Peptidomimetics is a broader term used to refer to pseudopeptides and non-peptides 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 [13]. 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 [14–16]. Another peptidomimetic approach is the incorporation of a biostable, isosteric replacement motif for the hydrolysis susceptible peptide bond; and is the subject of this mini-review.

In this manuscript, we review recent research undertaken to provide definitive evidence of the importance of a *trans* oriented Pro for a wide spectrum of PK bioactivities and to identify a novel *trans*Pro mimetic motif that can serve as a novel scaffold in the development of mimetic PK agonists or antagonists with greater selectivity and biostability than the plethora of natural neuropeptide sequences revealed by peptidomics.

## 2. Results and discussion

The C-terminal pentapeptide FXPRLa is highly conserved and thus, shared by the PK family of neuropeptides. This pentapeptide has further been identified as the active core in pheromonotropic bioassays (X = S) [1,5,7,8] and in an expressed PBAN receptor assay from the moth *Heliothis virescens* [4] and *Spodoptera littoralis* [7,10], although the C-terminal hexapeptide YFXPRLa (X = S) exhibits much greater potency. In the pupal diapause termination assay of the

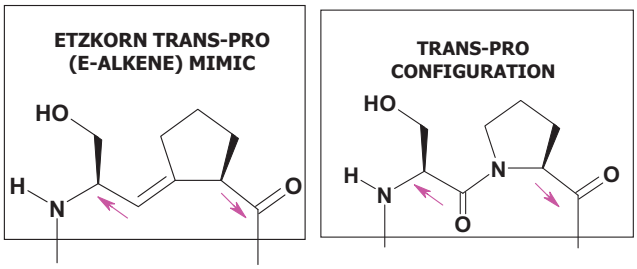


**Fig. 1 – Comparison of a trans-Pro configuration (right) and the (E)-alkene trans-Pro mimetic 'Etzkorn' (SerΨ[(E)—CH=C]Pro) motif (left). In this motif, the peptide bond that binds the amino group of the Pro is locked into a trans orientation by replacement with a double bond, which lacks the ability to rotate between trans and cis orientations as does a normal peptide bond [4,7,9].**

heliothine insect *Helicoverpa zea* the active core for full activity has been identified as the larger C-terminal heptapeptide sequence LWFGPRLa [9], although the C-terminal pentapeptide does elicit a less potent response. The C-terminal pentapeptide common to the PK class has also been found to retain significant activity in other bioassays, such as melanotropic, pupariation, diapause-break and hindgut myotropic preparations.

Nachman et al. conducted a conformational study of the rigid, cyclic PK/PBAN analog *cyclo*[NTSFTPRL] (*cyclo*[Asn$^1$]LPK) in aqueous solution containing no organic solvents using a combination of NMR spectroscopic and molecular dynamics calculations [5,8]. The specific conformation of this constrained, cyclic analog in aqueous solution was shown to be extremely rigid, featuring a *trans*-oriented Pro in the second position of a type-I β-turn over residues Thr-Pro-Arg-Leu within the core region. A *trans*Pro is a defining characteristic of a type I β-turn [5,8].

Despite the conformational constraint imposed upon the cyclic PK analog *cyclo*[Asn$^1$]LPK, it was found to retain a highly significant portion of the pheromonotropic activity of the 33-residue Bom-PBAN-I in a pheromonotropic bioassay in the silkworm *Bombyx mori*. The analog *cyclo*[Asn$^1$]LPK was also found to retain significant bioactivity in several other PK bioassays, including hindgut contractile (cockroach *L. maderae*), oviduct contractile (cockroach *L. maderae*), egg diapause induction (silk worm *B. mori*), diapause termination in the moth *H. zea*, pupariation (flesh fly *Neobelieria bullata*) assay systems, and termination of pupal diapause in heliothines [5,8,9].

### 2.1. *(E)-alkene, trans-Pro isostere*

In order to provide more definitive evidence that a *trans*Pro, and a type I β-turn, represented the active conformation for the PK neuropeptide class, the PK analog **PK-Etz** (Ac-Tyr-Phe-SerΨ[(*E*)—**CH=C**]Pro-Arg-Leu-$NH_2$), incorporating a *trans*Pro isostere ('Etzkorn': SerΨ[(*E*)—**CH=C**]Pro), was evaluated in five diverse PK bioassay systems and on a recombinant PK GPCR receptor cell line. In **PK-Etz**, the peptide bond of the Pro is replaced with a rigid double bond that locks in the *trans* orientation [4,7] (Fig. 1).