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Identification of selective and non-selective, biostable β-amino acid agonists of recombinant insect kinin receptors from the southern cattle tick *Boophilus microplus* and mosquito *Aedes aegypti*

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ABSTRACT

The multifunctional arthropod 'insect kinins' share the evolutionarily conserved C-terminal pentapeptide motif Phe-X^1-X^2-Trp-Gly-NH_2, where X^1 = His, Asn, Ser, or Tyr and X^2 = Ser, Pro, or Ala. Eight different analogs of the insect kinin C-terminal pentapeptide active core in which the critical residues Phe^1, Pro^3 and Trp^4 are replaced with β^3-amino acid and/or their β^2-amino acid counterparts were evaluated on recombinant insect kinin receptors from the southern cattle tick, *Boophilus microplus* (Canestrini) and the dengue vector, the mosquito *Aedes aegypti* (L.). A number of these analogs previously demonstrated enhanced resistance to degradation by peptidases. Single-replacement analog β^2Trp^4 and double-replacement analog [β^3Phe^2, β^3Pro^3] of the insect kinins proved to be selective agonists for the tick receptor, whereas single-replacement analog β^3Pro^3 and double-replacement analog [β^3Phe, β^3Pro^3] were strong agonists on both mosquito and tick receptors. These biostable analogs represent new tools for arthropod endocrinologists and potential leads in the development of selective, environmentally friendly arthropod pest control agents capable of disrupting insect kinin-regulated processes.

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1. Introduction

The insect kinins are multifunctional neuropeptides found in several arthropod and invertebrate groups [2,5,26,28,36]. They were first isolated from the cockroach, *Leucophaea maderae*, according to their stimulatory actions on hindgut contraction [12–14,24]. Shortly after their discovery, insect kinins were shown to have diuretic activity on isolated Malpighian tubules of the yellow fever mosquito *Aedes aegypti* and the cricket *Acheta domesticus* [3,8]. More recently, insect kinins, and/or analogs, have been reported to inhibit weight gain by larvae of the tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*), both serious agricultural pests [19,20,31].

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The insect kinins share the evolutionarily conserved C-terminal pentapeptide motif Phe-X^1-X^2-Trp-Gly-NH_2, where X^1 = His, Asn, Ser, or Tyr and X^2 = Ser, Pro, or Ala [11,36]. The C-terminal pentapeptide kinin core is the minimum sequence required for full cockroach myotropic and cricket diuretic activity on assays with tissues [19,24] and for bioluminescence response in CHO-K1 cells expressing kinin receptors [9,27,35]. Activity in these assays and the receptor expressing system is completely lost when the C-terminal amide of the insect kinin is replaced with a negatively charged acid moiety [22,35]. Evaluation of an Ala-replacement analog series of the C-terminal pentapeptide confirmed the importance of Phe^1 and Trp^4 side chains, because the replacement of these two positions with Ala also leads to the complete loss of activity on mosquito and tick receptor expressing systems and in myotropic and diuretic assays. These studies also show that the variable position 2 tolerates a wide range of chemical characteristics, from acid to basic residues and from hydrophilic to hydrophobic, although aromatic residues at this position are associated with the highest potencies in Malpighian tubule fluid secretion assays [24,29,35]. The natural achetakinins elicit cricket Malpighian tubule fluid secretion at EC_{50} values ranging from 18 to 325 pM [3]. The active core pentapeptide is equipotent with the parent nonapeptide in this assay. Due to kinin peptide susceptibility to both exo- and endopeptidases in the insect hemolymph and gut, insect kinin peptides cannot be directly used as pest control agents and/or research tools by insect neuroendocrinologists. Members of the insect kinin family are hydrolyzed, and therefore inactivated, by tissue-bound peptidases of insects [4,6,16]. Two susceptible hydrolysis sites in insect kinins have been reported in the active core sequence Phe^1-Tyr^2-Pro^3-Trp^4-Gly^5-NH_2. The primary site is between Pro^3 and the Trp^4 residue, with a secondary site N-terminal to the Phe^1 residue in natural extended insect kinin sequences [20,37]. It has been demonstrated experimentally that the primary hydrolysis site is susceptible to cleavage by angiotensin converting enzyme (ACE) from the housefly and both the primary and secondary hydrolysis sites can be cleaved by neprilysin (NEP) [4,16,20,21,25,29].

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 agents [34]. One such peptidomimetic approach is the incorporation of a β-amino acid. The β-amino acid is similar to an α-amino acid in that they both contain an amino terminus and a carboxyl terminus. However, in a β-amino acid an additional methylene group (–CH_2–) is placed between the α-carbon and either the acid group (designated β^3) or the amino group (designated β^2) (Fig. 1). By incorporating a β-amino acid, many peptides not only retain their biological activity but also demonstrate enhanced resistance to degradation by peptidases. Eight different single and double β-analogs of the insect kinin C-terminal pentapeptide core in which the critical residues Phe^1, Pro^3 and Trp^4 are replaced with β^3-amino acid and/or their β^2-amino acid counterparts were previously synthesized [37]. Several of these analogs display potent diuretic activity in the cricket *A. domesticus* and enhanced resistance to the endopeptidases ACE and NEP, enzymes that deactivate the natural insect kinins. All analogs are also blocked at the N-terminus with an acetyl (Ac) group, which confers resistance to hydrolytic degradation by an additional class of peptidases, the aminopeptidases [7].

Single β group	Double β group
1457, Ac-R[β^3Phe]FPWGa	1552, Ac-R[β^3Phe]F[β^3Pro]WGa
1458, Ac-R[β^2homoPhe]FPWGa	1577, Ac-R[β^3Phe]FF[β^3Pro]WGa
1459, Ac-RFFP[β^3Trp]Ga	1578, Ac-RF[β^3Phe-β^3Pro]WGa
1460, Ac-RFF[β^3Pro]WGa	
1656, Ac-RFFP[β^2Trp]Ga	

In this article we study the comparative interaction of these analogs on the kinin receptors from the dengue vector, the mosquito *A. aegypti* [27] and the southern cattle tick, *Boophilus microplus* [9,10] by a previously described calcium bioluminescence plate assay [27].

2. Materials and methods

2.1. *Peptide synthesis*

Insect kinin analogs were synthesized via Fmoc methodology on Rink Amide resin (Novabiochem, San Diego, CA) using

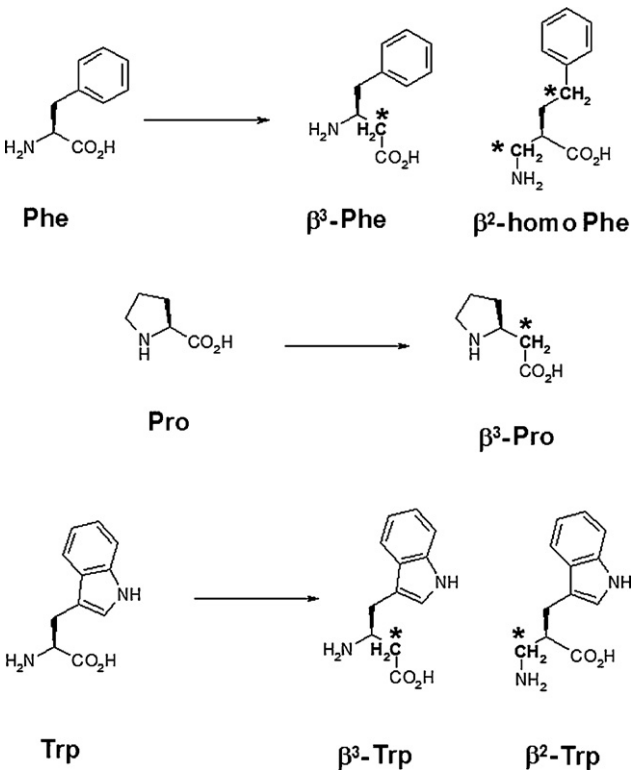

Fig. 1 – β-Amino acid variants [1,15] of natural α-amino acids found in insect kinin neuropeptides. Asterisks identify positions where methylene groups are incorporated.