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SUMMARY OF DOCTORAL THESIS

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Title: Synthesis and Structure-Activity Relationships of Iminopyridazine Competitive Antagonists in Insect GABA Receptors
（昆虫GABA受容体におけるイミノピリダジン競合アンタゴニストの合成および構造活性相関）

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter widely distributed in the central nervous system of animals. Insect ionotropic GABARs are important targets for insecticides and parasiticides. Commercially available insecticides such as fipronil act as noncompetitive antagonist. However, a potent competitive antagonist for insect GABARs is not available. The present study aimed to synthesize iminopyridazine (IP) antagonists for insect GABARs by modifying substituents on the pyridazine ring of gabazine.

Twelve 4-(6-imino-3-aryl/heteroarylpyridazin-1-yl)butanoic acids were first synthesized and examined for their antagonism of small brown planthopper (SBP) and common cutworm (CC) GABARs using fluorometric imaging plate reader (FLIPR) membrane potential (FMP) assays. Antagonism of GABARs was also examined in native American cockroach (AC) using a whole-cell patch clamp technique. Gabazine (aryl/heteroaryl = 4-methoxyphenyl) was moderately active in SBP and CC GABARs at 100 μM. However, it was not active in AC GABARs at 500 μM. IPs with ary/heteroaryl = 3,4-methylenedioxyphenyl and 2-naphthyl exhibited complete inhibition of GABA responses in SBP GABARs and inhibited GABA responses by 85.8% and completely in CC GABARs, respectively, at 100 μM. The 2-naphthyl analog displayed 85.4% inhibition of GABA-induced currents in AC GABARs at 500 μM. The 4-biphenyl analog showed 47.6% and 57.3% inhibition of GABA responses in SBP and CC GABARs, respectively, at 10 μM. This analog showed the greatest activity with 92.0% inhibition

of GABA-induced currents in AC GABARs at 500 μM. The high activity of 4-biphenyl and 2-naphthyl analogs suggests that long aromatic substituents at the 3-position of the pyridazine ring are tolerated. The 3-thienyl analog demonstrated a competitive mode of inhibition in AC GABARs. Hydrophobic interactions were predicted to predominate in the area of the orthosteric site that accommodates the 3-substituents when docked into the homology model of HF GABAR containing Rdl_{bd} subunits.

Thirteen 1,3-di- and 1,3,4-trisubstituted IPs with various substituents on the pyridazine ring of gabazine were next synthesized and examined for their antagonism of SBP and CC GABARs using FMP assays. Antagonism of HF GABARs expressed in *Xenopus* oocytes was also examined using a two-electrode voltage clamp technique. 4-[4-Cyclobutyl-6-imino-3-(2-naphthyl)pyridazin-1-yl]butyronitrile showed 79.7% and complete inhibition of GABA-activated responses in SBP and CC GABARs, respectively at 100 μM. However, this analog showed <30% inhibition in HF GABARs at the same concentration. Removal of the cyclobutyl group from this analog resulted in 93.0% and complete inhibition of GABA responses in SBP and CC GABARs, respectively, and it increased the inhibition percentage to 58.6% in HF GABARs, with an IC_{50} value of 75.5 μM. 4-[3-(4-Biphenyl)-6-iminopyridazin-1-yl]butyronitrile and the 2-fluoro-4-biphenylyl congener exhibited 83.4% and 86.7% inhibition of GABA responses in SBP GABARs, respectively, and complete inhibition in CC GABARs. These two analogs showed 79.6% and 83.6% inhibition in HF GABARs, with IC_{50}s of 37.9 μM and 42.3 μM, respectively. Ethyl 3-[3-(4-biphenyl)-6-iminopyridazin-1-yl]propylphosphonate analog showed the highest activity with 87.3% inhibition, with an IC_{50} value of 18.8 μM in HF GABARs. 4-[3-(4-Biphenyl)-6-iminopyridazin-1-yl]butyronitrile and ethyl 3-[3-(4-biphenyl)-6-iminopyridazin-1-yl]propylphosphonate exhibited a competitive mode of antagonism of GABA responses in HF GABARs. Docking simulation of an IP into a HF Rdl_{ac} GABAR homology model predicted that the orthosteric GABA-binding site accommodates an IP with a cyano functionality and an aromatic substituent at the 3-position of the pyridazine ring. The results obtained from this study might provide useful information for the designing of new type of insecticide.