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# SUMMARY OF DOCTORAL THESIS

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Title: Synthesis and pharmacological characterization of 3-isothiazolols and 3-isoxazolols as competitive antagonists of insect GABA receptors

（昆虫GABA受容体の競合的アンタゴニストとしての3-イソチアゾロール類および3-イソキサゾロール類の合成と薬理学的特性）

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γ-Aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the nervous system of animals. Insect ionotropic GABA receptors (GABARs) are important targets for insecticides and parasiticides. Noncompetitive antagonists (NCAs) of GABARs such as fipronil have been exploited as commercial insecticides. However, no potent competitive antagonist (CA) of insect GABARs is available at present. CAs might be utilized to develop novel insecticides as they inhibit GABAR activation by acting at the orthosteric site, which differs from the allosteric sites of NCA insecticides. The objective of this study is to identify effective CAs of insect GABARs. Three classes of five-membered heterocyclic compounds, including 4-substituted 5-(4-piperidyl)-3-isothiazolols (thio-4-PIOL), 4,5-substituted 3-isoxazolols, and 4-substituted 5-(4-piperidyl)-3-isoxazolols (4-PIOL), were synthesized and examined for their antagonism of insect GABARs.

Eleven 4-substituted thio-4-PIOL analogues were first synthesized in eight steps. The antagonism of common cutworm (CC), small brown planthopper (SBP), and housefly (HF) GABARs by the thio-4-PIOLs were examined by a fluorometric imaging plate reader membrane potential assay or a two-electrode voltage clamp (TEVC) method. Thio-4-PIOL showed weak antagonism of three insect GABA receptors. The antagonistic activity of thio-4-PIOL was enhanced by introducing bicyclic aromatic substituents into the 4-position of the isothiazole ring. The 2-naphthyl and the 3-biphenylyl analogues displayed antagonist potencies with half maximal inhibitory concentrations ($IC_{50}$s) in the low micromolar range. The 2-naphthyl analogue induced a parallel rightward shift of the GABA concentration–response curve, suggesting competitive antagonism by these analogues. Both compounds exhibited weak insecticidal activities against HFs. Thus, the orthosteric site of insect GABA receptors might be a potential target site of insecticides. Ligand docking studies using a HF GABAR homology model predicted that the orthosteric site

contains two cavities large enough to accommodate bicyclic aromatic 4-substituents of thio-4-PIOL analogues.

A series of 4,5-disubstituted 3-isoxazolols, including muscimol analogues, were next synthesized and examined for their activities against four splice variants (ac, ad, bc, and bd) of HF GABARs expressed in *Xenopus* oocytes using the TEVC assay. Muscimol was a more potent agonist than GABA in all four splice variants, whereas synthesized analogues did not exhibit agonism but rather antagonism in HF GABARs. The introduction of bicyclic aromatic groups at the 4-position of muscimol and the simultaneous replacement of the aminomethyl group with a carbamoyl group at the 5-position to afford six 4-aryl-5-carbamoyl- 3-isoxazolols resulted in compounds that exhibited significantly enhanced antagonism with $IC_{50}$ values in the low micromolar range in the ac variant. The inhibition of GABA-induced currents by 100 μM analogues was approximately 1.5- to 4-fold greater in the ac and bc variants than in the ad and bd variants. 4-(3-Biphenylyl)-5-carbamoyl-3-isoxazolol displayed competitive antagonism, with $IC_{50}$ values of 30, 34, 107, and 96 μM in the ac, bc, ad, and bd variants, respectively, and exhibited moderate insecticidal activity against HFs, with a median lethal dose of 5.6 nmol/HF. These findings suggest that these 3-isoxazolol analogues are novel lead compounds for the development of insecticides that act at the orthosteric site of HF GABARs. Docking studies indicated that a cation-π interaction between the 3-biphenylyl group and an Arg residue in loop C of the orthosteric site might be beneficial for the antagonism of HF GABARs.

To better understand the molecular interactions of ligands with the orthosteric sites of GABARs, three 4-aryl/arylalkyl-4-PIOL and a 5-(3-biphenylyl)-4-(4-piperidyl)-1-hydroxypyrazole were examined for their antagonism with regard to the three insect GABARs. The 3-isoxazolol was preferable to the 3-isothiazolol and 1-hydroxypyrazole in antagonism to CC and HF GABARs. Of the tested analogues, 4-(3-biphenylyl)-4-PIOL displayed the greatest antagonism for CC and HF GABARs, with $IC_{50}$ values of 3.4 and 10.2 μM, respectively. In contrast to the antagonism of the two GABARs, 4-(3-biphenylyl)-4-PIOL showed partial agonism for the case of SBP GABARs, with a half maximal effective concentration of 31.3 μM. Homology models and docking simulations revealed that a cation-π interaction between an analogue and an Arg residue in loop C or E of the orthosteric site is a key component of antagonism. This specific phenomenon was lacking in the interactions between 4-(3-biphenylyl)-4-PIOL and the orthosteric site of SBP GABARs. To our knowledge, 4-(3-biphenylyl)-4-PIOL is the most potent CA of insect GABARs reported to date. These findings in the studies for this dissertation provide important insights into designing and developing novel insecticides that target the orthosteric site of insect GABARs.