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# SUMMARY OF DOCTORAL THESIS

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Title: Interaction Mechanism of 1-Phenyl-1*H*-1,2,3-triazole Noncompetitive Antagonists with Ionotropic GABA Receptors
(1-フェニル-1*H*-1,2,3-トリアゾール非競合拮抗体のイオンチャネル型 GABA レセプターとの相互作用機構)

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter, which is widely distributed in the nervous system of vertebrates and invertebrates. The ionotropic GABA receptor is a ligand-gated ion channel that mediates fast inhibitory neurotransmission between neuronal cells. Insect GABA receptors are a promising target for insecticides. The present study was undertaken to study the molecular mechanisms of the interaction of 1-phenyl-1*H*-1,2,3-triazoles with GABA receptors.

First of all, 4- or 5-substituted 1-phenyl-1*H*-1,2,3-triazoles were synthesized and examined for their ability to inhibit the specific binding of [$^3$H]EBOB, a radiolabeled noncompetitive antagonist, to housefly, rat, and human ß3 GABA receptors. The ß3 receptor was used as a model of insect GABA receptors, as the ß3 receptor has high homology with insect receptors in the putative antagonist binding site. The assay results indicated that 4-substituted 1-phenyl-1*H*-1,2,3-triazoles were more potent competitive inhibitors of [$^3$H]EBOB binding than the 5-substituted regioisomers in the case of all receptors, and that most of the synthesized analogues were more active in housefly and human ß3 receptors than in rat receptors. Three-dimensional quantitative structure-activity relationship (3D-QSAR) analysis demonstrated that both the 4-trifluoromethyl-2,6-dichloro substitution on the phenyl ring and a small, bulky, hydrophobic substituent at the 4-position of the triazole ring were necessary for high potency to housefly and human ß3 receptors. As the human ß3 receptor resembled the housefly receptor in terms of their recognition of phenyltriazoles, the human ß3 receptor serves as a useful tool for screening antagonists that have high affinity for insect GABA receptors.

Next, sixteen 4,5-disubstituted 1-phenyl-1*H*-1,2,3-triazoles were synthesized, and a total of 49 analogues, including 4- and 5-monosubstituted

analogues, were used to determine their affinities for human ß3 and α1ß2γ2 GABA receptors. Most of the tested compounds showed selectivity for ß3 over α1ß2γ2 receptors. The assay and 3D-QSAR analysis results indicated that an electronegative substituent at the 4-position of the benzene ring, a compact, hydrophobic substituent at the 4-position of the triazole ring, and a small, electronegative substituent at the 5-position of the triazole ring played significant roles for the high potency in ß3 receptors. Ligand-docking studies using homology models of GABA receptors suggested that the amino acid residues at the 2′ - and 6′ -positions of the second transmembrane domain of GABA-gated channels played an important role in binding phenyltriazoles. The flexibility of the alignment of phenyltriazoles in the channel lumen of human ß3 receptors and a difference in the hydrophobic environment at the 2′ -position of ß3 homopentamers and α1ß2γ2 heteropentamers might be two of the reasons why phenyltriazoles select ß3 over α1ß2γ2 receptors.

The above findings indicate that 1-phenyl-1*H*-1,2,3-triazoles are noncompetitive GABA receptor antagonists, and that substituents on the phenyl and triazole rings are critical for the high potency. Some of the analogues exhibited high affinity for housefly and ß3 receptors and significant insecticidal activity comparable to those of fipronil, a GABA receptor antagonist/commercial insecticide. The information obtained from the 3D-QSAR and ligand-docking studies should prove helpful not only for understanding the mechanisms of the interaction of noncompetitive antagonists with GABA receptors, but also for the discovery of novel and safe insect pest control chemicals.