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Competitive antagonism of housefly γ-aminobutyric acid receptors by iminopyridazine butanoic acids

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Abstract

The competitive antagonistic activities of a series of gabazine (3) based 3-substituted iminopyridazine butanoic acid analogs [4-(3-aryl/heteroaryl-1,6-dihydro- 6-iminopyridazin-1-yl) butanoic acid hydrochlorides] 4a-4k (Fig. 1) were examined at housefly (*Musca domestica*) GABA receptors expressed in *Xenopus* oocytes using two-electrode voltage-clamp (TEVC) technique. The 4-biphenylyl analog 4e exhibited the highest inhibition (approximately 68%) of GABA-induced currents at 100 μM with an IC_{50} value of 40.81 ± 3.89 μM. The 2-naphthyl analog 4h was the second most active compound with approximately 48% inhibition. The 4-biphenylyl analog 4e demonstrated competitive antagonism of housefly GABA receptors. Ligand docking studies into the binding site of housefly GABA receptor homology model predicted that the aromatic 3-substituents are tolerable in the pyridazine ring. The results presented in this paper about GABA receptor competitive antagonists may helpful for design and development of GABA receptor related insecticides.

Introduction

γ-aminobutyric acid (GABA, 1) (Fig. 1) has been established as the widely spread major inhibitory-type neurotransmitter in vertebrate and invertebrate central nervous system (Watanabe *et al.,* 2002; Bowery and Smart, 2006). Based on their structure and pharmacology, there are two distinct types of receptors have been recognized by which GABA mediates its effects: ionotropic and metabotropic GABA receptors (Hevers and Lüddens, 1998; Bettler *et al.,* 2004). The ionotropic GABA receptor is a branch of Cys-loop receptor family of ligand-gated ion channels (LGICs), whereas metabotropic receptor is the member of G-protein coupled receptors. Both of the GABA receptors are detected in insect nervous system. The ionotropic GABA receptors are promising targets of insecticides (Buckingham *et al.* 2017; Casida, 2018). The ionotropic GABA receptors are classified

into two categories: hetero-pentameric type $GABA_A$ receptors, which included α1-6, β1-3, γ1-3, δ, ε, θ, and π subunits, and homo-pentameric type $GABA_C$ receptors with ρ1-3 subunits (Whiting *et al.* 1999; Zhang *et al.,* 2001). Each type of subunit contains a large GABA-binding extracellular domain, a α-helical transmembrane segment, and an intracellular loop. $GABA_A$ receptors with two α1, two β2/β3 and one γ2 subunit combination are the major subtype in the brain (Olsen and Sieghart, 2008; Sigel and Steinmann, 2012). In $GABA_A$ receptors, the binding of GABA or an agonist into the orthosteric binding site of the extracellular domain rapidly opens the integral channel to enhance chloride ions permeability across the neuronal membrane and thus inhibit the action potential generation. Two kinds of antagonists are found other than agonist for the Cys-loop receptor: i)

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competitive antagonists, which share the same orthosteric binding site with agonists, and ii) noncompetitive antagonists, binding in the allosteric site in the channel domain (Johnston, 1996). Competitive antagonists binding to orthosteric site stabilize the closed conformation of GABA receptor channels to display insecticidal activities (Liu *et al.,* 2015). Ionotropic GABA receptors play a significant role both in the central and peripheral nervous systems of insect (Okada *et al.,* 2009; Ozoe, 2013). There are three distinct classes of ionotropic GABA receptor subunits have been cloned so far from a number of insect species: Rdl, a subunit

which disturb the GABA receptor functions. Gabazine (3) (Fig. 1) is a recognized competitive antagonist for mammalian GABA$_A$ receptors (Chambon *et al.* 1985, Ueno *et al.* 1997). However, it was weak to moderately active competitive antagonist against insect GABA receptors (Hosie and Sattelle, 1996; Satoh *et al.,* 2005; Narusuye *et al,* 2007). In our previous studies, we synthesized gabazine based iminopyridazine butanoic acid analogs modifying the 3-position of pyridazine ring and studied their antagonistic activities against three insect species. The antagonistic activities of the synthesized compounds were examined in
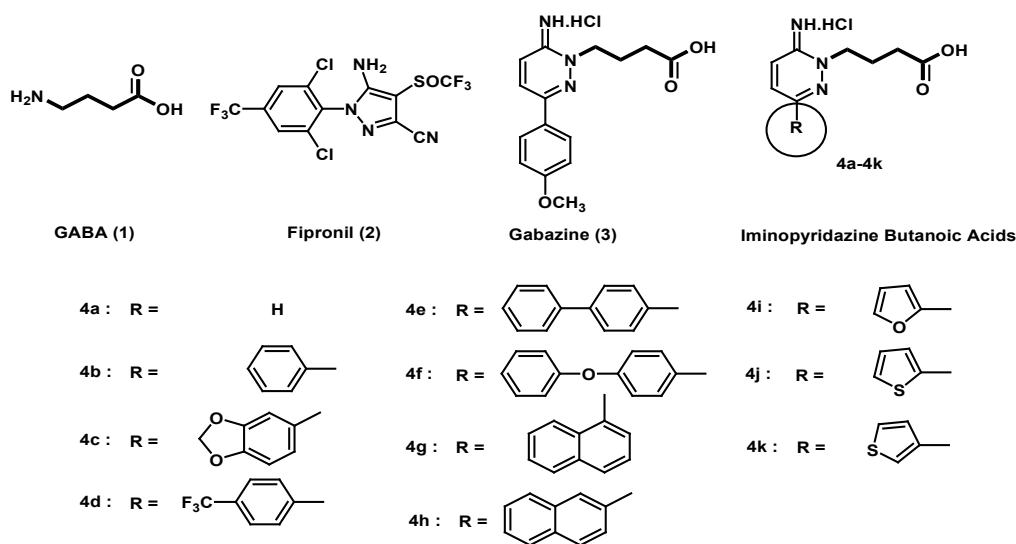
Fig. 1. Chemical structures of GABA receptor agonist GABA (1), the GABA receptor noncompetitive antagonist fipronil (2), the GABA receptor competitive antagonist gabazine (3), and the iminopyridazine butanoic acids (4a-4k). GABA structural units are shown in bold lines

encoded by the gene *Rdl* (resistant to dieldrin), GRD (the GABA$_A$ and glycine receptor-like subunit of *Drosophila*), and LCCH3 (ligand-gated chloride channel 3) (Sattelle *et al.,* 1991; Hosie *et al.,* 1997; Buckingham *et al.,* 2005). Although structurally insect GABA receptors are identical to mammalian GABA receptors, they have distinct pharmacological behavior, which tolerate GABA receptors to act as significant targets for insecticides and parasiticides (Buckingham *et al.,* 2005). As for example, a well known noncompetitive antagonist fipronil (2) (Fig. 1) is extensively used as insecticides (Raymond-Delpech *et al.* 2005). It has been reported that resistance to fipronil were developed in several insects for its extensive use (Nakao *et al.* 2010, Nakao *et al.* 2011, Nakao *et al.* 2012). Although efforts are continuing to develop new drugs, no potent competitive antagonists are developed till date for insect GABA receptors

cloned GABA receptors expressed in *Drosophila* S2 cell lines from small brown planthoppers (*Laodelphax striatella*) and common cutworms (*Spodoptera litura*) by Fluorometric Imaging Plate Reader (FLIPR) Membrane Potential (FMP) assays. Whole-cell patch-clamp technique was also used to study their activities in native GABA receptors of American cockroaches (*Periplaneta americana*) (Rahman *et al.,* 2012). In this study, we would like to examine the antagonistic activities of those iminopyridazine butanoic acid analogs [4-(3-aryl/heteroaryl-1,6-dihydro-6-iminopyridazin-1-yl)but anoic acid hydrochlorides] 4a-4k (Fig. 1) against housefly (*Musca domestica* Linnaeus) GABA receptors expressed in *Xenopus* oocytes using Two-Electrode Voltage Clamp (TEVC) technique, to know the variation of activities in GABA receptors of different insect species. The information described in this paper would be utilized for the development

of insect pest control chemicals in future.

Materials and methods

Synthesis of cRNAs of housefly (*Musca domestica*) RDL and expression in *Xenopus* oocytes

The capped cRNAs of housefly GABA receptor subunit of ac variant [DDBJ accession Nos. AB177547 (complete cds of RDL_{bd}), AB824728 (partial cds of exon 3a version), and AB824729 (partial cds of exon 6c version)] were synthesized using T7 polymerase mMESSAGE mMACHINE T7 Ultra Kit (Ambion, Austin, TX, USA), the primer pcDNA3-cRNAF (5′-CTCTCTGGCTAACTAGAGAACC-3′) according to a previously described report (Eguchi *et al.*, 2006). The cRNAs were precipitated using LiCl, dissolved in sterilie RNase-free water to a concentration of 543 ng/nl, and stored at -20 °C prior to use.

After purchasing the mature female African clawed frog (*Xenopus laevis*) from Shimizu Laboratory Supplies Co., Ltd., Kyoto, Japan, the frogs were raised in a 12 h light/dark cycle controlled environment at 16-18 °C. The frogs were anesthetized with 0.1% (w/v) aq. ethyl *m*-aminobenzoate methanesulfonate solution and the ovarian lobes were surgically taken out. The removed ovarian lobes were treated with 2 mg/ml collagenase (Sigma-Aldrich, Tokyo, Japan) in Ca^{2+}-free standard oocyte SOS solution (100 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl, 5 mM HEPES maintaining pH 7.6) at room temperature for 90 to 120 min. Then the oocytes were gently washed with sterile SOS (100 mM NaCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 2 mM KCl, 5 mM HEPES maintaining pH 7.6) containing 2.5 mM of sodium pyruvate (Sigma-Aldrich), 100 U/ml penicillin (Invitrogen), 50 µg/ml gentamycin (Gibco), and 100 µg/ml streptomycin (Invitrogen). After washing, the oocytes were incubated overnight at 16 °C in the buffer solution. 5 ng of cRNAs (dissolved in 9.2 ml of RNase-free water) were injected in every oocyte and incubated in the same condition for 48 h.

Two-electrode voltage clamp (TEVC) experiments

Two-electrode voltage clamp (TEVC) experiments were performed as previous report (Rahman *et al.*, 2014). A Warner Instruments OC-725C Oocyte Clamp amplifier (Hamden, CT) was used to record GABA-induced currents at a -80 mV holding potential. After recordings, the analysis of currents was done by World Precision Instruments Inc. Data-Trax2™ software (Sarasota, FL). The fabrication of glass capillary electrodes were done with a Sutter Instrument P-97 micropipette puller and filled by 2.0 M KCl solution with a variation of resistance from 0.5 to 2.0 MΩ. After placing the *Xenopus* oocytes in a bath to record currents, the oocytes were constantly perfused with SOS solution at 18 to 22 °C. GABA was dissolved in SOS for agonist assays and applied to the oocytes for 3 s with intervals of 60 s. Increasing concentrations of GABA were sequentially applied to produce concentration-response curves. For antagonist assays, DMSO was used to dissolve gabazine (3) and the iminopyridazine butanoic acids 4a-4k and then diluted with SOS to the final concentrations (0.1% DMSO, v/v). Half maximal effective concentration, EC_{50} of GABA (10 µM) was applied to the oocytes several times for 3 s as control applications and was applied successively during the rest of the experiments. Iminopyridazine butanoic acid solutions were applied alone for 60 s prior to the co-application of GABA and the repetition of co-application was continued for 3 s at 60 s breaks until the maximum stable inhibition was obtained. The calculation of the inhibition (%) was done from the average ratio of two least responses during the application of iminopyridazine butanoic acid to the average of several responses induced by GABA (EC_{50}, 10 µM). OriginPro 8J data analysis and graphing software of OriginLab was used to obtain the EC_{50} and the IC_{50} (Half maximal inhibitory concentration) values from concentration-response relationships by nonlinear regression analysis.

Homology modeling and docking studies

The housefly (*Musca domestica*) Rdl_{ac} subunits GABA receptor homology model was constructed based on the X-ray crystal structure of *Caenorhabditis elegans* GluCl (Glutamate-gated chloride channel) as a template (PDB: 3RIF) (Hibbs and Gouaux, 2011). The model was generated using MOE 2011.10 software (Chemical Computing Group). ClustalW software was used to align the α subunit of *Caenorhabditis elegans* GluCl and Rdl_{ac} subunit of housefly sequence. AMBER99 force field was used for the geometry optimization. Molecular Builder of MOE was used to create the zwitterionic form of GABA and protonated imino structure of 4h. ASEDock 2011.01.27 software was used for docking of the generated ligands into the binding site of created model. The ligands with stable conformations were found by the conformational search. The MMFF94x forced field was used to minimize the energy of ligands and the receptor. MOE Site Finder was used to identify the docking site. The docking mode having the highest score was selected for the final presentation.

Results and discussion

In this study, gabazine (3) based 3-substituted iminopyridazine butanoic acid derivatives [4-(3-aryl/heteroaryl-1,6-dihydro-6-iminopyridazin-1-yl)butanoic acid hydrochlorides] 4a-4k (Fig. 1) were
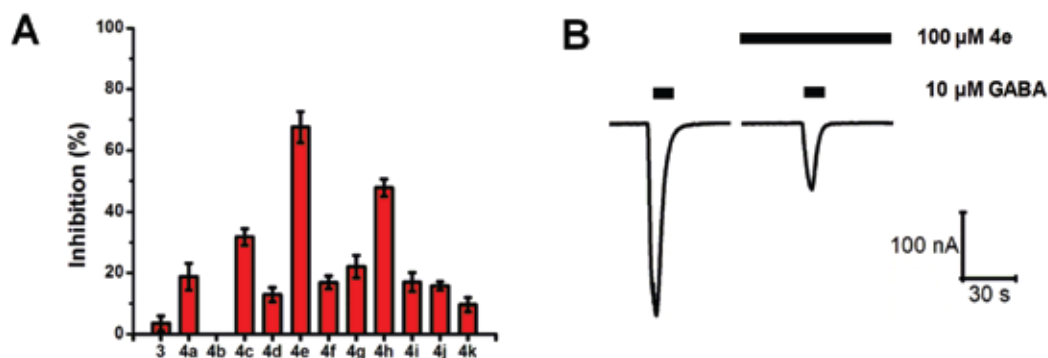
Fig. 2. Inhibition of GABA-induced currents in housefly (Musca domestica) GABA receptors expressed in Xenopus oocytes. (A) Inhibition of GABA-induced currents by 100 μM gabazine (3) and iminopyridazine butanoic acids (4a-4k). Data are as means ± SEM of three or four assays obtained from at least two frogs. (B) Representative current traces that shows the inhibition of GABA-induced current by 100 μM 4e. GABA EC50 (10 μM) were applied to activate the receptors
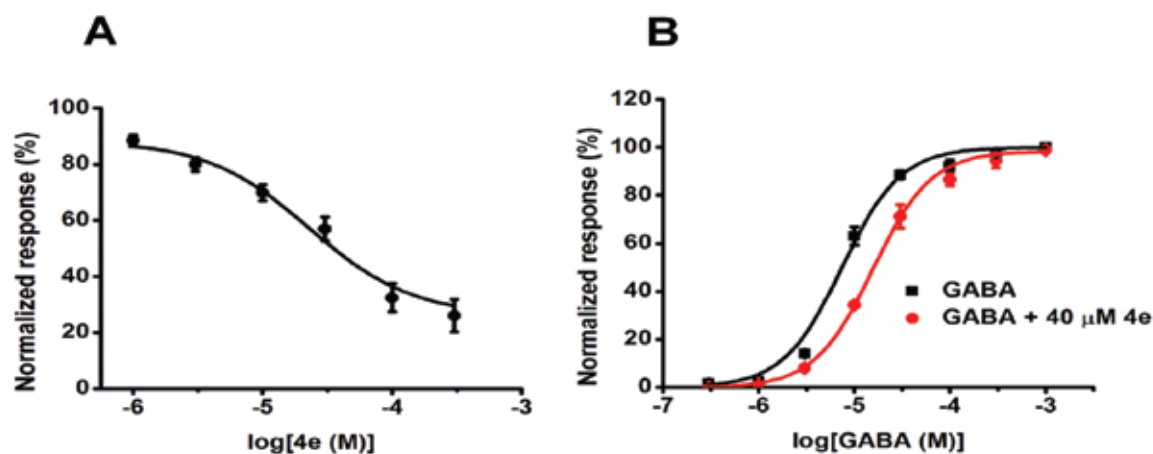
Fig. 3. Concentration-response curves. (A) Inhibitory concentration-response curves for 4e in housefly GABA receptors expressed in Xenopus oocytes. The data are the means ± SEM of three assays obtained from at least two frogs. (B) Concentration-response curves of GABA alone (black square) and GABA in the presence of 40 μM 4e (red circles) in housefly GABA receptors expressed in Xenopus oocytes. The data are the means ± SEM of three or four assays obtained from at least two frogs. Currents were normalized relative to 1 mM GABA-induced currents

evaluated at housefly (*Musca domestica*) GABA receptors expressed in *Xenopus* oocytes using two-electrode voltage-clamp technique. cRNAs encoding the housefly Rdl$_{ac}$ subunit were injected and transiently expressed in *Xenopus* oocytes as a previous report (Ozoe *et al.,* 2013). The synthesis and structure determination of gabazine and its analogs were done according to our previous study (Rahman *et al.,* 2012). At first, gabazine (3) and its

analogs 4a-4k were examined in the absence and in the presence of GABA EC$_{50}$ value to determine whether they behave as agonists or antagonists. No compounds showed agonism but all compounds exhibited antagonism at 100 μM in housefly GABA receptors. The inhibition percentages of gabazine (3) and compounds 4a-4k at 100 μM are presented in Fig. 2A. Partial data (only the inhibition %) of compounds 4e and 4h were reported in

our previous study for comparison (Rahman *et al.,* 2014). The inhibition percentage of gabazine (with 4-methoxy group at 3-position of pyridazine ring) is presented here as standard which exhibited very weak activity with only 3.53% inhibition of GABA-induced currents in housefly GABA receptors at 100 μM. However, it showed significant inhibition of GABA-activated currents at the same concentration in small brown planthopper and common cutworm GABA receptors expressed in *Drosophila* S2 cells. But, it displayed very low activity in American cockroach (*Periplaneta americana*) GABA receptors when tested at 500 μM in an abdominal ganglion (Rahman *et al.* 2012). An analog lacking any 3-substituent in pyridazine ring (4a) showed little, but enhanced antagonism than gabazine (3). Compound 4b, which has a phenyl group at 3-position of pyridazine ring, displayed no activity. The substitution of 4-methoxy group of gabazine with 3,4-methylenedioxy group, led to compound 4c, exhibited approximately 9-fold increased activity than gabazine with 31.8% inhibition. This analog 4c showed complete and more than 85% inhibition in small brown planthopper and common cutworm GABA receptors, respectively (Rahman *et al.* 2012). The lone pairs of methylenedioxy group might have some interactions with the binding site of the receptors. The 4-trifluoromethylphenyl analog 4d demonstrated comparable antagonistic activity to that of 4a. Replacement of 4-methoxyphenyl group of gabazine (3) with 4-biphenylyl group, yielding 4e, led to *ca*. 19-fold higher inhibition than gabazine which exhibited the highest antagonistic activity in this receptor. This compound also exhibited highest inhibition in American cockroach GABA receptors with 92.0% inhibition; although it had moderate activity in small brown planthopper and common cutworm GABA receptors in our previous study (Rahman *et al.* 2012). The inhibition of GABA-activated currents by 100 μM of 4-biphenylyl analog 4e is presented in Fig. 2B. Introduction of 4-phenoxyphenyl and 1-naphthyl groups in the 3-position of pyridazine ring of 4a to give compounds 4f and 4g, respectively showed similar inhibition pattern compared to 4a. In contrast, the 2-naphthyl analog 4h showed ~ 14-fold enhanced inhibition than gabazine (3) which was the second greatest inhibition in the receptor. This analog also displayed higher inhibition in small brown planthopper, common cutworm, and American cockroach GABA receptors (Rahman *et al.,* 2012). The higher activity of 4e and 4h indicate that bulky long aromatic substituents are tolerable at the 3-position of pyridazine ring. However, the introduction of cyclobutyl group at the 4-position of pyridazine ring of compounds 4e and 4h were proved detrimental to iminpyridazine activity in

housefly GABA receptors (Rahman *et al.,* 2014). High similarity (71.1%) between housefly GABA receptor amino acid sequence (GenBank accession AB177547) and American cockroach GABA receptor partial sequence (GenBank accession FJ612451) were found (Rahman *et al.,* 2012) which might led the analogs 4a-4k comparable antagonistic activities in housefly and American cockroach GABA receptors. Replacement of the 4-methoxy group of gabazine with 5-membered heteroaromatic substituents affording 4i, 4j, and 4k, resulted in approximately 3- to 5-fold increased inhibition percentage than that of gabazine. The IC_{50} value of 4e was calculated to be 40.81 ± 3.89 (SEM) μM (n = 3) from concentration-response curve (Fig. 3A). Different potencies were shown by gabazine (3) and its analogs 4a-4k against different insect GABA receptors. The variations of results in different receptors indicate that there are structural or functional differences might exist in orthosteric binding sites of GABA receptors among insect species. Although the potencies of the analogs were low, the results presented in this study signify that the iminopyridazine butanoic acid scaffold might be useful for developing housefly GABA receptors competitive antagonists.

Mode of antagonism

To determine the mode of antagonism of iminopyridazine butanoic acid analogs 4a-4k, GABA concentration-response relationships in the presence and in the absence of 4e were examined in housefly GABA receptors expressed in *Xenopus* oocytes. A parallel rightward shift of GABA concentration-response curves in the presence of 4e (40 μM) indicating a competitive mechanism of these compounds (Fig. 3B). The calculated EC_{50} of GABA in the absence and in the presence of 4e were 7.42 ± 0.56 μM and 22.77 ± 1.59 μM (SEM), respectively. These gabazine based analogs were previously acted as competitive antagonists in American cockroach GABA receptors as well (Rahman *et al.,* 2012).

Homology modeling and molecular interactions

To understand the mechanism of the interactions of iminopyridazine butanoic acid analogs 4a-4k with the orthosteric binding site of housefly GABA receptor, docking studies of GABA (1) and the 2-naphthyl analog 4h were performed into the orthosteric binding site of housefly Rdl$_{ac}$ GABA receptor homology model generated based on the X-ray crystal structure of *Caenorhabditis elegans* GluCl
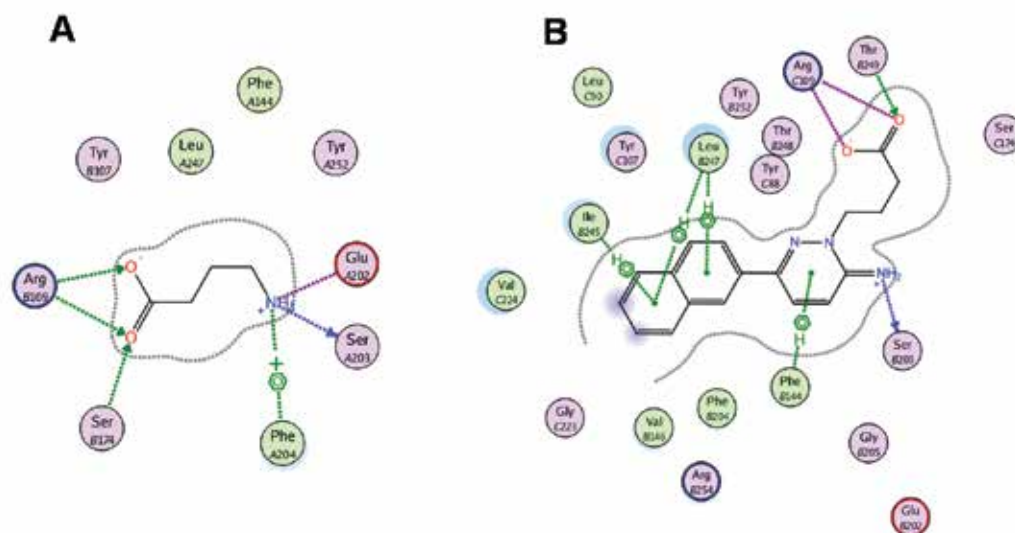
Fig. 4. Interaction profiles of (A) GABA and (B) 4h with the orthosteric binding site of housefly GABA receptors homology model constructed based on the X-ray crystal structure of *Caenorhabditis elegans* GluCl (glutamate-gated chloride channel)

(Glutamate-gated chloride channel) as a template. The docking and interaction studies of GABA demonstrated that the backbone carbonyl oxygen of Ser203 and the side chain of Glu202 act as hydrogen acceptors for the protonated amino group. On the other side, Arg109 and Ser174 act as hydrogen donors for the carboxylate anion of GABA (Fig. 4A). Simulation of molecular dynamics and experimental data of *Drosophila* Rdl GABA receptors showed that Arg109 plays a vital role in the interaction with GABA (Ashby *et al.,* 2012). Aromatic amino acids Phe204 and Tyr252 exist adjacent to the protonated amino group of GABA might produce cation/π interactions as recommended for Rdl *Drosophila* GABA receptors (Lummis *et al.,* 2011). The docking and interaction studies of 2-naphthyl analog 4h into the orthosteric binding site showed that hydrogen bonding of the protonated imino and dissociated carboxyl groups play important role with the surrounding amino acid residues such as Ser203, Arg109, Thr249, Phe144, Leu247, and Ile245 (Fig. 4B). The docking and interaction studies revealed that the large 3-substituent were tolerable in the receptor cavity. 3-Substituted aromatic group may create CH/π type interactions with Leu247 and Ile245. Phe144 also produce CH/π type interaction with pyridazine moiety. The 4-biphenylyl analog 4e also demonstrated similar type of interactions as involved by GABA when docked into the orthosteric binding site of housefly GABA receptors (Rahman *et al.* 2012).

Conclusion

The evaluation of competitive antagonistic activities of iminopyridazine butanoic acids [4-(3-aryl/heteroaryl-1,6-dihydro-6-iminopyridazin-1-yl)butanoic acid hydrochlorides] 4a-4k demonstrated that the bulky long aromatic groups are tolerable at the 3-position of pyridazine ring of gabazine in the orthosteric binding site of housefly (*Musca domestica*) GABA receptors. The data presented in this study could be proved useful for the development of insect pest control chemicals, albeit the potencies of the compounds were low.

References

Ashby JA, McGonigle IV, Price KL, Cohen N, Comitani F, Dougherty DA, Molteni C and Lummis SCR (2012), GABA Binding to an insect GABA receptor: a molecular dynamics and mutagenesis study, *Biophys. J.* **103:** 2071-2081. DOI: 10.1016/j.bpj.2012.10.016

Bettler B, Kaupmann K, Mosbacher J and Gassmann M (2004), Molecular structure and physiological functions of GABA$_B$ receptors, *Physiol. Rev.* **84:** 835-867. DOI: 10.1152/physrev.00036.2003

Bowery NG and Smart TG (2006), GABA and glycine as neurotransmitters: a brief history, *Br. J. Pharmacol.* **147:** S109-S119. DOI: 10.1038/sj.bjp.0706443

Buckingham SD, Biggin PC, Sattelle BM, Brown LA and Sattelle DB (2005), Insect GABA receptors: splicing, editing, and targeting by antiparasitics and insecticides, *Mol. Pharmacol.* **68:** 942-951. DOI: 10.1124/mol.105.015313

Buckingham SD, Ihara M, Sattelle DB and Matsuda K (2017), Mechanisms of action, resistance and toxicity of insecticides targeting GABA receptors, *Curr. Med. Chem.* **24:** 2935-2945. DOI: 10.2174/0929867324666 170613075736

Casida JE (2018), Radioligand recognition of insecticide targets, *J. Agric. Food. Chem.* 66: 3277-3290. DOI: 10.1021/acs.jafc.7b05984

Chambon JP, Feltz P, Heaulme M, Restle S, Schlichter R, Biziere K and Wermuth CG (1985), An arylaminopyridazine derivative of γ-aminobutyric acid (GABA) is a selective and competitive antagonist at the GABA$_A$ receptor site, *Proc. Natl. Acad. Sci. USA* **82:** 1832-1836. DOI: 10.1073/pnas.82.6.1832

Eguchi Y, Ihara M, Ochi E, Shibata Y, Matsuda K, Fushiki S, Sugama H, Hamasaki Y, Niwa H, Wada M, Ozoe F and Ozoe Y (2006), Functional characterization of *Musca* glutamate- and GABA-gated chloride channels expressed independently and coexpressed in *Xenopus* oocytes, *Insect Mol. Biol.* **15:** 773-783. DOI: 10.1111/j.1365-2583.2006.00680.x

Hevers W and Lüddens H (1998), The diversity of GABA$_A$ receptors pharmacological and electrophysiological properties of GABA$_A$ channel subtypes, *Mol. Neurobiol.* **18:** 35-86. DOI: 10.1007/BF02741459

Hibbs RE and Gouaux E (2011), Principles of activation and permeation in an anion-selective Cys-loop receptor, *Nature* **474:** 54-60. DOI: 10.1038/nature10139

Hosie AM, Aronstein K, Sattelle DB and ffrench-Constant RH (1997), Molecular biology of insect neuronal GABA receptors, *Trends Neurosci.* **20:** 578-583. DOI: 10.1016/s0166- 2236(97)01127-2

Hosie AM and Sattelle DB (1996), Agonist pharmacology of two *Drosophila* GABA receptor splice variants, *Br. J. Pharmacol.* 119: 1577-1585. DOI: 10.1111/j.1476-5381.1996.tb16075.x

Johnston GAR (1996), GABA$_A$ receptor pharmacology, *Pharmacol. Ther.* **69:** 173-198. DOI: 10.1016/0163-7258(95)02043-8

Liu G, Ozoe F, Furuta K and Ozoe Y (2015), 4,5-substituted 3-isoxazolols with insecticidal activity act as competitive antagonists of housefly GABA receptors, *J. Agric. Food Chem.* **63:** 6304-6312. DOI: 10.1021/acs.jafc.5b01843

Lummis SCR, McGonigle I, Ashby JA, Dougherty DAJ (2011), Two amino acid residues contribute to a cation-π binding interaction in the binding site of an insect GABA receptor, *J. Neurosci.* **31:** 12371-12376. DOI: 10.1523/JNEUROSCI.1610-11.2011

Nakao T, Hama M, Kawahara N and Hirase K (2012), Fipronil resistance in *Sogatella furcifera*: molecular cloning and functional expression of wild-type and mutant RDL GABA receptor subunits, *J. Pestic. Sci.* **37:** 37-44. DOI:10.1584/JPESTICS.D11-018

Nakao T, Kawase A, Kinoshita A, Abe R, Hama M, Kawahara N and Hirase K (2011), The A2'N mutation of the RDL γ-aminobutyric acid receptor conferring fipronil resistance in *Laodelphax striatellus* (Hemiptera: Delphacidae), *J. Econ. Entomol.* **104:** 646–652. . DOI: 10.1603/EC10391

Nakao T, Naoi A, Kawahara N and Hirase K (2010), Mutation of the GABA receptor associated with fipronil resistance in the whitebacked planthopper, *Sogatella furcifera*, *Pestic. Biochem. Physiol.* **97:** 262-266. DOI: 10.1016/j.pestbp.2010.03.006

Narusuye K, Nakao T, Abe R, Nagatomi Y, Hirase K and Ozoe Y (2007), Molecular cloning of a GABA receptor subunit from *Laodelphax striatella* (Fallén) and patch clamp analysis of the homo-oligomeric receptors expressed in a *Drosophila* cell line, *Insect Mol. Biol.* **16:** 723-733. DOI: 10.1111/j.1365-2583. 2007.00766.x

Okada R, Awasaki T and Ito I (2009), Gamma-aminobutyric acid (GABA)-mediated neural connections in the *Drosophila* antennal lobe, *J. Comp. Neurol.* **514:** 74-91. DOI: 10.1002/cne.21971

Olsen RW and Sieghart W (2008), Subtypes of γ-aminobutyric acid$_A$ receptors: classification on the basis of subunit composition, pharmacology, and function, *Pharmacol. Rev.* **60:** 243-260. DOI: 10.1124/pr.108.00505

Ozoe Y (2013), γ-aminobutyrate- and glutamate-gated chloride channels as targets of insecticides, *Adv. Insect Physiol.* **44:** 211-286. DOI: 10.1016/B978-0-12-394389-7.00004-1

Ozoe Y, Kita T, Ozoe F, Nakao T, Sato K and Hirase K (2013), Insecticidal 3-benzamido-*N*-phenylbenzamides specifically bind with high affinity to a novel allosteric site in housefly GABA receptors, *Pestic. Biochem. Physiol.* **107:** 285-292. DOI: 10.1016/j.pestbp.2013.09.005

Rahman MM, Akiyoshi Y, Furutani S, Matsuda K, Furuta K, Ikeda I and Ozoe Y (2012), Competitive antagonism of insect GABA receptors by the iminopyridazine derivatives of GABA, *Bioorg. Med. Chem.* **20:** 5967-5964. DOI: 10.1016/j.bmc.2012.07.049

Rahman MM, Liu G, Furuta K, Ozoe F and Ozoe Y (2014), Synthesis of 1,3-di- and 1,3,4-trisubstituted 1,6-dihydro-6-iminopyridazines as competitive antagonists of insect GABA receptors, *J. Pestic. Sci.* **39:** 133-143. DOI: 10.1584/jpestics.D14-052

Raymond-Delpech V, Matsuda K, Sattelle BM, Rauh JJ and Sattelle DB (2005), Ion channels: molecular targets of neuroactive insecticides, *Invert. Neurosci.* **5:** 119-133. DOI: 10.1007/s10158-005-0004-9

Satoh H, Daido H and Nakamura T (2005), Preliminary analysis of the GABA-induced current in cultured CNS neurons of the cutworm moth, *Spodoptera litura*, *Neurosci. Lett.* **381:** 125-130. DOI: 10.1016/j.neulet.2005.02.007

Sattelle DB, Lummis SCR, Wong JFH and Rauh JJ (1991), Pharmacology of insect GABA receptors, *Neurochem. Res.* **16:** 363-374. DOI: 10.1007/BF00966100

Sigel E and Steinmann ME (2012), Structure, function, and modulation of GABA$_A$ receptors, *J. Biol. Chem.* **287:** 40224-40231. DOI: 10.1074/jbc.R112.386664

Ueno S, Bracamontes J, Zorumski C, Weiss DS and Steinbach JH (1997), Bicuculline and gabazine are allosteric inhibitors of channel opening of the GABA$_A$ receptor, *J. Neurosci.* **17:** 625-634. DOI: 10.1523/JNEUROSCI.17-02-00625.1997

Watanabe M, Maemura K, Kanbara K, Tamayama T and Hayasaki H (2002), GABA and GABA receptors in the central nervous system and other organs, *Int. Rev. Cytol.* **213:** 1-47. DOI: 10.1016/S0074-7696(02)13011-7

Whiting PJ, Bonnert TP, Mckernan RM, Farrar S, Le Bourdellés B, Heavens RP, Smith DW, Hewson L, Rigby MR, Sirinathsinghji DJ, Thompson SA and Wafford KA (1999), Molecular and functional diversity of the expanding GABA-A receptor gene family, *Ann. N. Y. Acad. Sci.* **868:** 645-653. . DOI: 10.1111/j.1749-6632.1999.tb11341.x

Zhang D, Pan ZH, Awobuluyi M and Lipton SA (2001), Structure and function of GABA$_C$ receptors: a comparison of native versus recombinant receptors, *Trends Pharmacol. Sci.* **22:** 121-132. DOI: 10.1016/s0165-6147(00)01625-4