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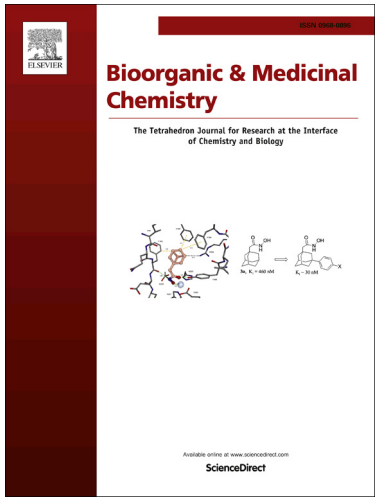

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PII: S0968-0896(14)00916-X
DOI: http://dx.doi.org/10.1016/j.bmc.2014.12.058
Reference: BMC 11990

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 23 October 2014
Revised Date: 8 December 2014
Accepted Date: 24 December 2014

Please cite this article as: Nagaoka, H., Nishiwaki, H., Kubo, T., Akamatsu, M., Yamauchi, S., Shuto, Y., Docking model of the nicotinic acetylcholine receptor and nitromethylene neonicotinoid derivatives with a longer chiral substituent and their biological activities, *Bioorganic & Medicinal Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.bmc.2014.12.058

Docking model of the nicotinic acetylcholine receptor and nitromethylene neonicotinoid derivatives with a longer chiral substituent and their biological activities

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Keywords

Nitromethylene neonicotinoids; QSAR; docking model; *Musca domestica*; nicotinic acetylcholine receptor

Abbreviations

Ac, *Aplysia californica*; CH-IMI, nitromethylene analogue of imidacloprid; IMI, imidacloprid; LBD, ligand-binding domain; Ls, *Lymnaea stagnalis*; nAChR, nicotinic acetylcholine receptor; NIA, propargyl propyl phenylphosphonate (Niagara 16388); QSAR, quantitative structure-activity relationship; SAR, structure-activity relationship

ABSTRACT

In the present study, nitromethylene neonicotinoid derivatives possessing substituents that contain a sulfur atom, oxygen atom or aromatic ring at position 5 on the imidazolidine ring were synthesized to evaluate their affinity for the nicotinic acetylcholine receptor (nAChR) and their insecticidal activity against adult female houseflies. Comparing the receptor affinity of the alkylated derivative with the receptor affinity of compounds possessing either ether or thioether groups revealed that conversion of the carbon atom to a sulfur atom did not influence the receptor affinity, whereas conversion to an oxygen atom was disadvantageous for the receptor affinity. The receptor affinity of compounds possessing a benzyl or phenyl group was lower than that of the unsubstituted compound. Analysis of the three-dimensional quantitative structure–activity relationship using comparative molecular field analysis demonstrated that steric hindrance of the receptor should exist around the C3 of an *n*-butyl group attached at position 5 on the imidazolidine ring. A docking study of the nAChR-ligand model suggested that the ligand-binding region expands as the length of the substituent increases by brushing against the amino acids that form the binding region. The insecticidal activity of the compounds was positively correlated with the receptor affinity by considering log P and the number of heteroatoms, including sulfur and oxygen atoms, in the substituents, suggesting that the insecticidal activity is influenced by the receptor affinity, hydrophobicity, and metabolic stability of the compounds.

1. INTRODUCTION

Neonicotinoids are neuroactive insecticides that act on the nicotinic acetylcholine receptor (nAChR) at the postsynaptic membrane in insects. They are widely used as agricultural insecticides and for residential pest control. The affinity of neonicotinoids for nAChR is lower in mammals than in target pests, which is why these insecticides exert highly selective toxicity against pests over mammals. Imidacloprid (IMI, **1** in Fig. 1) was developed as the first neonicotinoid insecticide that contained pyridine and imidazolidine rings.[1] Many structure–activity relationship analyses have been reported, and some important neonicotinoid pharmacophores have been suggested.[1-3] We focused on the ethylene moiety of the imidazolidine ring, which is considered to be at an important metabolic position in the housefly *Musca domestica*.[4] The ethylene moieties of IMI and its nitromethylene analogue (CH-IMI, **2** in Fig. 1) have not been recognized as important pharmacophores because acyclic neonicotinoids, including nitenpyram, acetamiprid, and dinotefuran, have been developed. We eventually determined that 5*R*-methylated and 5*R*-ethylated imidacloprid derivatives (**3** and **4** in Fig. 1) were equipotent to the unsubstituted compound **2**, suggesting that this region could be considered a pharmacophore.[5,6] In addition, based on quantitative structure–activity relationship (QSAR) analyses of the receptor affinity and on a docking study using the receptor model of houseflies combined with a synthesized alkylated derivative, it was hypothesized that a space that can accept a certain sized substituent (up to *n*-propyl group, **5** in Fig. 1) should exist around position 5 of the imidazolidine ring in the ligand-binding region of the receptor.[6] Because the ligand-binding pocket space in the receptor is constructed of several aromatic amino acid residues, such as tyrosine and tryptophan,[6] it is expected that a substituent that interacts with these amino acid residues and backbone peptide bonds in the ligand-binding region would have a high affinity for the receptor. The oxygen atom can interact with peptide bonds via hydrogen bonding, and the sulfur atom and benzyl and phenyl groups are also expected to interact with aromatic amino acid residues through sulfur-π and π-π interactions.[7]

In this study, we synthesized various CH-IMI derivatives with substituents that possessed an oxygen atom, sulfur atom, or aromatic ring, which are expected to interact with the ligand-binding region of nAChR, to evaluate their receptor affinity and insecticidal activity (**6**–**18** in Fig. 1). To elucidate the physicochemical properties of ligands interacting with the receptor, three-dimensional QSARs were analyzed using the comparative molecular field analysis (CoMFA) method. The docking model was also reconstructed to discuss the mode of binding to the receptor. Furthermore, the relationship between receptor affinity and insecticidal activity against houseflies was quantitatively analyzed to determine the factors other than receptor affinity that influence insecticidal activity.

2. MATERIALS AND METHODS

2.1. Insects

An insecticide-susceptible strain of the housefly (*Musca domestica* L, Takatsuki strain) was reared at 25°C in our laboratory.

2.2. Chemicals

Compounds **6**-**18** were newly synthesized using the synthetic scheme shown in Fig. 2. The reagents used for the syntheses were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), Nacalai Tesque, Inc. (Kyoto, Japan), Tokyo Chemical Industry Co, Ltd. (Tokyo, Japan), and Aldrich Chemical Co. (Milwaukee, WI, USA). The metabolic inhibitor NIA 16388 (NIA; propargyl propyl phenylphosphonate) was our stock sample.[5,6] ^{1}H and ^{13}C NMR analyses were performed using a JEOL ECS-400 NMR spectrometer in deuterochloroform ($CDCl_3$), deuteromethanol (CD_3OD) or deuterium oxide (D_2O) with tetramethylsilane (for $CDCl_3$ and CD_3OD) or sodium 3-(trimethylsilyl)-1-propanesulfonate (for D_2O) as the internal standard. The authenticity of the final compounds was also confirmed by HRMS using a Xevo Q-TOFMS (Waters, UK). The melting points of the compounds were measured using a Yanaco melting point apparatus (Kyoto, Japan) and were uncorrected. Optical rotation values were determined

using a P-2100 polarimeter (Jasco, Tokyo, Japan).

(*S*)-Methyl-3-*tert*-butoxycarbonyl-2,2-dimethyloxazolidine-4-formate (**20**, step a)

One hundred milliliters of a methanol solution containing D-serine (4.5 g, 43 mmol, **19**) was cooled in a salt-ice bath at 0°C, and $SOCl_2$ (18.6 mL, 258 mmol) was added dropwise. The resulting mixture was stirred for 12 h at ambient temperature and then concentrated *in vacuo*. After coevaporating the solvent with diethyl ether multiple times to remove excess $SOCl_2$, the residue was dissolved in 100 mL of CH_2Cl_2, to which Et_3N (15.7 mL, 113 mmol) was added at 0°C. To this solution was added di-*tert*-butyl dicarbonate (11.3 g, 52 mmol) under stirring, and the resulting mixture was refluxed until the starting material was consumed, as determined by TLC (methanol). The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in ethyl acetate (100 mL) and then washed with saturated $NaHCO_3$ followed by washing with brine (×3). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford *N*-*tert*-butoxycarbonyl serine methyl ester (Boc-Ser-OMe) as the intermediate as an oil, which was used in the subsequent reaction without further purification. The crude Boc-NH-Ser-OMe was dissolved in a mixture of acetone (100 mL) and 2,2-dimethoxypropane (81.0 mL, 659 mmol). To the resulting mixture was added BF_3-Et_2O complex (1.1 mL, 9 mmol) at ambient temperature, and the reaction mixture was stirred for 12 h. After determining that the reaction was complete by TLC, 1.1 mL of Et_3N was added to the mixture to quench the reaction, and the solvent was removed *in vacuo*. The brown oil was then partitioned between Et_2O and saturated $NaHCO_3$ (aq.). The aqueous layer was extracted with Et_2O (×5), and the organic layers were combined, dried over Na_2SO_4, and concentrated. The resulting brown oil was purified by column chromatography (hexane:ethyl acetate = 10:1) to afford **20** as a yellow oil (9.6 g, 84%). The ^1H and ^{13}C NMR spectra were consistent with previously reported spectra.[8]

(*R*)-3-*tert*-Butoxycarbonyl-2,2-dimethyl-4-phthalimidomethyloxazolidine (**21**, step b)

After a mixture of $NaBH_4$ (5.2 g, 139 mmol) and LiCl (5.9 g, 139 mmol) in dry EtOH (30

mL) was stirred for 30 min at 0°C, compound **20** (6.0 g, 23 mmol) dissolved in 15 mL of dry THF was added. The resulting mixture was warmed to room temperature and stirred for 12 h. The precipitate was filtered over Celite and washed with EtOH. The filtrate was then evaporated and partitioned between ethyl acetate and brine. After the aqueous layer was extracted with ethyl acetate (×5), the organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to afford the intermediate alcohol as a yellow oil. To a reaction mixture of dry THF (100 mL) containing 11 g (42 mmol) of PPh_3, 8.2 g (56 mmol) of phthalimide, and 6.6 g (28 mmol) of the yellow oil, 22.7 mL (40% in toluene, 50 mmol) of diethyl azodicarboxylate dissolved in dry THF (20 mL) was added dropwise while stirring in an ice bath. After 12 h at ambient temperature, the solvent was evaporated, and the resulting residue was purified by column chromatography (hexane:ethyl acetate = 3:1) to afford **21** as white crystals (5.4 g, 53%).

Mp 124-126. $[\alpha]_D^{25}$ -32.4 (c 1.0, $CHCl_3$). NMR δ_H ($CDCl_3$): 7.85 (2H, m), 7.72 (2H, m), 4.36 (1H, m), 4.00-3.81 (4H, m), 1.64 (3H, s), 1.46 (3H, s), 1.33 (9H, s). NMR δ_C ($CDCl_3$): Major rotamer, 168.5, 152.6, 134.1, 132.3, 123.3, 94.4, 80.2, 65.8, 55.8, 40.7, 28.2, 27.2, 24.3; Minor rotamer, 168.3, 151.7, 133.6, 132.1, 123.1, 94.1, 80.2, 65.5, 55.2, 40.2, 28.0, 26.7, 22.9.

(2'*S*)-*N*-(2'-(6-Chloro-3pyridyl)methylamino)-3'-hydroxypropyl-phthalimide (**22**, step c)

A concentrated HCl solution (20 mL) was added to THF (30 mL) containing **21** (2.0 g, 6 mmol) while stirring at ambient temperature to remove the Boc group. After stirring for 30 min, the solvent was evaporated to afford a deprotected amine hydrochloride salt as a white solid, which was used in the subsequent reaction without further purification. The crystal was dissolved in 30 mL of CH_3CN, to which 5.3 mL (38 mmol) of Et_3N and 0.9 g (6 mmol) of 2-chloro-5-chloromethylpyridine hydrochloride were added. After refluxing overnight, the solvent was evaporated, and the resulting residue was purified by column chromatography (hexane:ethyl acetate = 1:5) to afford **22** as white crystals (0.4 g, 19%).

Mp 103-105. $[\alpha]_D^{25}$ -15.7 (c 0.36, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.33 (1H, d, J = 2.8 Hz),

7.87-7.85 (2H, m), 7.77-7.75 (2H, m), 7.66 (1H, dd, *J* = 8.4, 2.4 Hz), 7.23 (1H, d, *J* = 8 Hz), 3.96-3.79 (4H, m), 3.60 (1H, dd, *J* = 11.6, 5.2 Hz), 3.49 (1H, dd, *J* = 12.0, 4.4 Hz), 2.96 (1H, m), 2.85 (1H, br). NMR δ_C ($CDCl_3$): 169.1, 150.2, 149.4, 138.8, 134.5, 134.4, 131.7, 124.0, 123.5, 61.4, 57.7, 47.8, 38.1.

(*R*)-3-*tert*-Butoxycarbonyl-2,2-dimethyloxazolidine-4-methoxyethane (**24**, step d)

To a dry THF solution containing compound **23** (2.5 g, 11 mmol), which was prepared from L-serine *via* steps a and b, 0.5 g (20 mmol) of NaH was added. After stirring for 5 min, 3.4 g (22 mmol) of iodoethane was added. After stirring for 10 h at ambient temperature, 1 mL of H_2O was added to the reaction mixture, the solvent was removed *in vacuo*, and the resulting residue was purified by column chromatography (hexane:ethyl acetate = 4:1) to afford **24** as an oil (1.3 g, 47%). $[\alpha]_D^{25}$ -18.6 (c 1.27, $CHCl_3$). NMR δ_H ($CDCl_3$): 4.06 (1H, m), 3.95 (2H, m), 3.51 (2H, m), 3.46 (2H, m), 1.55 (6H, m), 1.48 (9H, s), 1.19 (3H, m). NMR δ_C ($CDCl_3$): Major rotamer, 151.6, 93.6, 79.6, 69.8, 66.5, 65.6, 56.3, 28.3, 26.7, 23.0,15.1; Minor rotamer, 152.1, 93.1, 80.1, 69.0, 66.5, 65.3, 56.4, 28.3, 27.4, 24.3, 15.1.

(*S*)-*tert*-Butyl (1-ethoxy-3-hydroxypropane-2-yl)carbamate (**25**, step e)

To 20 mL of a MeOH solution containing compound **24** (1.3 g, 5 mmol), 2.0 g (10 mmol) *p*-toluenesulfonic acid was added, and the reaction mixture was stirred at ambient temperature for 18 h. The solvent was evaporated, and the resulting residue was dissolved in $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ (aq.) (×3). The organic layer was dried over Na_2SO_4 and concentrated to afford **25** as an oil (1.4 g, 71%). $[\alpha]_D^{25}$ -4.2 (c 1.02, $CHCl_3$). NMR δ_H ($CDCl_3$): 3.50 (3H, m), 2.92 (2H, m), 1.26 (9H, s), 0.87 (5H, m). NMR δ_C ($CDCl_3$): 130.8, 95.2, 72.5, 66.9, 31.9, 30.0, 22.7, 14.1.

(*S*)-2-Amino-3-(ethylthio)propanoic acid (**27**, step f)

To a 0.2 M $Ba(OH)_2$ solution (70 mL) of D-cysteine (**26**, 2.0 g, 13 mmol), 2.8 mL (15 mmol) of diethyl sulfate was added while stirring at ambient temperature. After 3 h, 0.6 mL

of conc. H_2SO_4 was added to stop the reaction, and the precipitate was filtered. After evaporation of the resulting filtrate, the pH was adjusted to 5.0 with NH_3 aq., and the solution was recrystallized with EtOH to afford **27** as white crystals (1.6 g, 85%). Mp 174-176. $[\alpha]_D^{25}$ +36.6 (c 0.1, MeOH). NMR δ_H (D_2O): 3.93 (1H, q, *J* = 4.0 Hz), 3.14 (1H, dd, *J* = 15.2, 4.0 Hz), 3.04 (1H, dd, *J* = 14.4, 7.2 Hz), 2.63 (2H, dd, *J* = 14.4, 6.4 Hz), 1.25 (3H, t, *J* = 7.4 Hz). NMR δ_C (D_2O): 176.0, 56.4, 34.4, 28.3, 16.7.

N-*tert*-Butoxycarbonyl-D-norleucinol (**28**, step g)

$LiAlH_4$ (1.8 g, 46 mmol) was suspended in 50 mL of dry THF in an ice bath, and then D-norleucine (3.0 g, 23 mmol) was slowly added. The reaction mixture was warmed to room temperature and refluxed for 5 h. Three milliliters of H_2O and 1.5 mL of 2 M NaOH aq. were added to the reaction mixture to quench the reaction, and the quenched solution was used in the subsequent reaction without further purification. To the mixture solution, 5.5 g (23 mmol) of di-*tert*-butyl dicarbonate was added, and the reaction mixture was refluxed for 5 h. The resulting precipitate was filtered over Celite, and the precipitate was washed with 150 mL of THF. The filtrate was then concentrated *in vacuo* to afford **28** as an oil, which was used in the subsequent reaction without further purification because no by-products were observed in the NMR spectra. (5.8 g, quant) $[\alpha]_D^{25}$ +51.5 (c 1.0, $CHCl_3$). NMR δ_H ($CDCl_3$): 4.85 (1H, br), 3.62-3.54 (2H, m), 3.20 (1H, m), 1.54-1.52 (2H, m), 1.45 (9H, s), 1.39-1.29 (4H, m), 0.90 (3H, t, *J* = 2.6 Hz). NMR δ_C ($CDCl_3$): 156.5, 79.3, 65.5, 52.7, 31.1, 28.3, 27.3, 22.5, 13.9.

(2'*R*)-*N*-(2'-*tert*-Butoxycarbonylamino)hexylphthalimide (**29**, step h)

To a reaction mixture of dry THF (80 mL) containing 9.0 g (34 mmol) of PPh_3, 6.7 g (46 mmol) of phthalimide, and 5.0 g (23 mmol) of compound **28**, 9.7 mL (40% in toluene, 41 mmol) of diethyl azodicarboxylate dissolved in dry THF (20 mL) was added dropwise while stirring in an ice bath. After stirring for 12 h at ambient temperature, the solvent was evaporated, and the resulting residue was purified by column chromatography

(hexane:ethyl acetate = 3:1) to afford **29** as white crystals (5.4 g, 68%). Mp 115-118. $[\alpha]_D^{25}$ -20.7 (c 1.0, $CHCl_3$). NMR δ_H ($CDCl_3$): 7.84-7.83 (2H, m), 7.70-7.68 (2H, m), 4.59 (1H, br), 3.97 (1H, m), 3.74-3.63 (2H, m), 1.56 (1H, m), 1.47-1.30 (5H, m), 1.21 (9H, s), 0.91-0.90 (3H, m). NMR δ_C ($CDCl_3$): 168.4, 155.6, 133.7, 132.0, 123.1, 78.9, 49.6, 42.4, 32.5, 28.0, 27.8, 22.4, 13.9.

(2'*R*)-*N*-(2'-(6-Chloro-3-pyridyl)methylamino)hexylphthalimide (**30**, step i)

To 50 mL of a THF solution containing 5.2 g (15 mmol) of compound **29, a** conc. HCl solution (20 mL) was added dropwise while stirring at ambient temperature. After stirring overnight, the solvent was distilled to afford the deprotected amine as a white solid, which was used in the subsequent reaction without further purification. The product was dissolved in 30 mL of CH_3CN, to which 10.7 mL (77 mmol) of Et_3N and 2.5 g (15 mmol) of 2-chloro-5-chloromethylpyridine hydrochloride were added. After the reaction mixture was refluxed overnight, the solvent was evaporated, and the resulting residue was purified by column chromatography (hexane:ethyl acetate = 2:1) to afford **30** as a yellow oil (1.5 g, 27%). $[\alpha]_D^{25}$ -23.3 (c 1.0, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.25 (1H, d, *J* = 2.4 Hz), 7.84-7.82 (2H, m), 7.75-7.72 (2H, m), 7.57 (1H, dd, *J* = 8.4, 2.4 Hz), 7.12 (1H, d, *J* = 8.0 Hz), 3.86 (1H, d, *J* = 13.6 Hz), 3.78 (1H, d, *J* = 13.6 Hz), 3.72-3.69 (2H, m), 2.90 (1H, m), 1.34 (6H, m), 0.91 (3H, t, *J* = 6.7 Hz). NMR δc ($CDCl_3$): 168.6, 149.9, 149.3, 138.8, 135.0, 134.0, 131.8, 123.7, 123.2, 56.1, 47.1, 41.3, 32.4, 27.8, 22.7, 13.9.

(2'*S*)-*N*-(2'-(6-Chloro-3pyridyl)methylamino)-3'-(methoxymethoxy)propyl-phthalimide (**31**, step j)

To a reaction mixture of dry CH_2Cl_2 (2 mL) containing 0.2 g (0.6 mmol) of compound **22** and 0.4 mL (2 mmol) of *N,N*-diisopropylethylamine, 0.09 mL (1 mmol) of methoxymethyl chloride was added while stirring at ambient temperature. After 1 h, 0.5 mL of MeOH was added to the reaction mixture, which was then stirred for an additional 30 min. The reaction mixture was washed with saturated $NaHCO_3$ (×3). The organic layer was dried over

Na_2SO_4, filtered, and concentrated to afford **31** as a red oil (0.28 g, quant.). $[\alpha]_D^{25}$ -62.1 (c 2.0, $CHCl_3$). NMR δ_H ($CDCl_3$): 7.99 (1H, d, *J* = 2.0 Hz), 7.73-7.64 (4H, m), 7.30 (1H, dd, *J* = 8.2, 2.6 Hz), 6.67 (1H, d, *J* = 8.4 Hz), 5.23 (2H, s), 4.54 (2H, d, *J* = 2.4 Hz), 4.42 (1H, d, *J* = 6.4 Hz), 4.35 (1H, d, *J* = 6.4 Hz), 4.01 (1H, m), 3.56 (2H, d, *J* = 2.4 Hz), 2.90 (3H, s). NMR δ_C ($CDCl_3$): 167.9, 149.8, 149.6, 139.0, 133.9, 133.1, 131.4, 123.4, 122.9, 96.5, 66.1, 60.8, 56.1, 54.3, 39.2.

(*R*)-5-Butyl-1-(6-chloro-3-pyridylmethyl)-2-(nitromethylene)imidazolidine (**6**, step k).

To an EtOH solution containing 1.5 g (4 mmol) of compound **30**, 1.0 mL (21 mmol) of hydrazine monohydrate was added, and the mixture was refluxed for 3 h while stirring. After the insoluble residue was removed by filtration, the resulting filtrate was evaporated to afford the crude deprotected amine as an oil, which was used for the subsequent reaction without further purification. After the oil was dissolved in 25 mL of ethanol, 0.6 g (3 mmol) of 1,1-bis(methylthio)-2-nitroethylene and 0.5 g (3 mmol) of K_2CO_3 were added, and the reaction mixture was refluxed overnight. After removing K_2CO_3 by filtration, the filtrate was evaporated *in vacuo*, and the resulting residue was purified by column chromatography (ethyl acetate) to afford **6** as white crystals (0.8 g, 76%). Mp 102-103. $[\alpha]_D^{25}$ +96.1 (c 1.15, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.73 (1H, br), 8.29 (1H, d, *J* = 2.4 Hz), 7.60 (1H, dd, *J* = 8.0, 2.0 Hz), 7.35 (1H, d, *J* = 8.0 Hz), 6.57 (1H, s), 4.38 (2H, q, *J* = 16.8 Hz), 3.92 (1H, q, *J* = 10.0 Hz), 3.85 (1H, q, *J* = 8.7 Hz), 3.50 (1H, t, *J* = 9.0 Hz), 1.74 (1H, m), 1.54 (1H, m), 1.31-1.24 (4H, m), 0.88 (3H, t, *J* =7.0 Hz). NMR δ_C ($CDCl_3$): 159.1, 151.0, 148.1, 137.5, 129.9, 124.5, 96.4, 60.2, 47.7, 44.0, 31.4, 26.5, 22.3, 13.7. ESIMS *m/z* $[M+H]^+$: calcd for $C_{14}H_{20}N_4O_2Cl$, 311.1262; found, 311.1275.

S-1-(6-Chloro-3-pyridylmethyl)-5-hydroxymethyl-2-(nitromethylene)imidazolidine (**7**)

Mp 189-192. $[\alpha]_D^{25}$ +10.1 (c 1.09, MeOH). NMR δ_H (CD_3OD): 8.36 (1H, d, *J* = 2.4Hz), 7.80 (1H, dd, *J* = 8.6, 2.6 Hz), 7.48 (1H, d, *J* = 8.4 Hz), 6.70 (1H, s), 4.62 (2H, d, *J* = 12.8 Hz), 4.02 (1H, m), 3.90 (1H, t, *J* = 10.8 Hz), 3.78 (1H, dd, *J* = 12.2, 3.4 Hz), 3.69

(1H, dd, *J* = 10.6, 7.4 Hz), 3.63 (1H, dd, *J* = 12.0, 4.0 Hz). NMR δ_C (CD_3OD): 151.8, 149.7, 139.9, 133.0, 125.8, 97.6, 62.7, 61.8, 46.2, 45.4, 30.7. ESIMS *m/z* $[M+H]^+$: calcd for $C_{11}H_{14}N_4O_3Cl$, 285.0749; found, 285.0754.

S-1-(6-Chloro-3-pyridylmethyl)-5-methoxymethyl-2-(nitromethylene)imidazolidine (**8**)
Mp 148-151. $[\alpha]_D^{25}$ +28.5 (c 0.12, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.70 (1H, br), 8.31 (1H, d, *J* = 2.4Hz), 7.60 (1H, dd, *J* = 8.4, 2.4 Hz), 7.35 (1H, d, *J* = 8.0 Hz), 6.54 (1H, s), 4.46 (2H, q, *J* = 16.8 Hz), 4.04 (1H, m), 3.91 (1H, t, *J* = 10.2 Hz), 3.57 (1H, m), 3.52-3.45 (2H, m), 3.27 (3H, s). NMR δ_C ($CDCl_3$): 159.4, 151.3, 148.4, 137.6, 130.2, 124.5, 96.6, 72.6, 59.8, 59.1, 45.4, 45.1. ESIMS *m/z* $[M+H]^+$: calcd for $C_{12}H_{16}N_4O_3Cl$, 299.0902; found, 299.0911.

S-1-(6-Chloro-3-pyridylmethyl)-5-ethoxymethyl-2-(nitromethylene)imidazolidine (**9**)
Mp 123-125. $[\alpha]_D^{25}$ +105.6 (c 0.51, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.70 (1H, br), 8.32 (1H, d, *J* = 2.4Hz), 7.60 (1H, dd, *J* = 8.2, 2.6 Hz), 7.35 (1H, d, *J* = 8.0 Hz), 6.54 (1H, s), 4.47 (2H, q, *J* = 18.5 Hz), 4.04 (1H, m), 3.89 (1H, t, *J* = 10.4 Hz), 3.57 (1H, m), 3.55-3.50 (2H, m), 3.41 (2H, q, *J* = 7.0 Hz), 1.14 (3H, t, *J* = 7.0 Hz). NMR δ_C ($CDCl_3$): 159.4, 151.4, 148.5, 137.6, 130.1, 124.5, 96.6, 70.8, 67.1, 59.8, 45.5, 45.2, 14.9. ESIMS *m/z* $[M+H]^+$: calcd for $C_{13}H_{18}N_4O_3Cl$, 313.1056; found, 313.1067.

S-1-(6-Chloro-3-pyridylmethyl)-2-(nitromethylene)-5-propoxymethylimidazolidine (**10**)
Mp 111-114. $[\alpha]_D^{25}$ +101.7 (c 1.05, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.69 (1H, br), 8.31 (1H, d, *J* = 2.4Hz), 7.60 (1H, dd, *J* = 8.2, 2.0 Hz), 7.34 (1H, d, *J* = 8.0 Hz), 6.53 (1H, s), 4.47 (2H, q, *J* = 17.5 Hz), 4.05 (1H, m), 3.90 (1H, t, *J* = 10.0 Hz), 3.59-3.50 (3H, m), 3.32 (2H, t, *J* = 6.6 Hz), 1.57-1.48 (2H, m), 0.87 (3H, t, *J* = 7.4 Hz). NMR δ_C ($CDCl_3$): 159.4, 151.3, 148.5, 137.6, 130.2, 124.5, 96.6, 73.4, 71.0, 59.9, 45.5, 45.2, 22.6, 10.5. ESIMS *m/z* $[M+H]^+$: calcd for $C_{14}H_{20}N_4O_3Cl$, 327.1229; found, 327.1224.

S-1-(6-Chloro-3-pyridylmethyl)-5-methylthiomethyl-2-(nitromethylene)imidazolidine (**11**)

Mp 115-117. $[\alpha]_D^{25}$ +102.9 (c 0.13, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.71 (1H, br), 8.31 (1H, d, *J* = 2.4Hz), 7.60 (1H, dd, *J* = 8.4, 2.4 Hz), 7.37 (1H, d, *J* = 8.4 Hz), 6.55 (1H, s), 4.42 (2H, d, *J* = 4.0 Hz), 4.02 (1H, m), 3.98 (1H, q, *J* = 9.4 Hz), 3.72 (1H, dd, *J* = 9.6, 6.0 Hz), 2.79 (1H, dd, *J* = 13.4, 3.8 Hz), 2.68 (1H, dd, *J* = 13.2, 7.6 Hz), 2.10 (3H, s). NMR δ_C ($CDCl_3$): 159.2, 151.6, 148.3, 137.6, 129.6, 124.8, 96.7, 59.2, 47.7, 44.8, 36.3, 16.1. ESIMS *m/z* $[M+H]^+$: calcd for $C_{12}H_{16}N_4O_2SCl$, 315.0680; found, 315.0683.

S-1-(6-Chloro-3-pyridylmethyl)-5-ethylthiomethyl-2-(nitromethylene)imidazolidine (**12**)

Mp 114-117. $[\alpha]_D^{25}$ +57.1 (c 0.92, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.71 (1H, br), 8.31 (1H, d, *J* = 2.8Hz), 7.59 (1H, dd, *J* = 8.2, 2.6 Hz), 7.37 (1H, d, *J* = 8.0 Hz), 6.55 (1H, s), 4.41 (2H, d, *J* = 4.8 Hz), 3.97 (2H, m), 3.72 (1H, m), 2.81 (1H, dd, *J* = 13.4, 3.8 Hz), 2.69 (1H, dd, *J* = 13.4, 7.4 Hz), 2.52 (2H, q, *J* = 7.3 Hz), 1.23 (3H, t, *J* = 7.4 Hz). NMR δ_C ($CDCl_3$): 159.2, 151.7, 148.4, 137.6, 129.5, 124.8, 96.7, 59.5, 47.7, 44.8, 33.8, 26.8, 14.7. ESIMS *m/z* $[M+H]^+$: calcd for $C_{13}H_{18}N_4O_2SCl$, 329.0825; found, 329.0839.

S-1-(6-Chloro-3-pyridilmethyl)-2-(nitromethylene)-5-propylthiomethylimidazolidine (**13**)

Mp 90-93. $[\alpha]_D^{25}$ +101.7 (c 1.05, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.70 (1H, br), 8.31 (1H, d, *J* = 2.4Hz), 7.59 (1H, dd, *J* = 8.0, 2.4 Hz), 7.37 (1H, d, *J* = 8.4 Hz), 6.54 (1H, s), 4.41 (2H, d, *J* = 5.2 Hz), 3.97 (2H, m), 3.72 (1H, m), 2.80 (1H, dd, *J* = 13.0, 3.8 Hz), 2.68 (1H, dd, *J* = 13.4, 7.4 Hz), 2.46 (2H, t, *J* = 7.2 Hz), 1.56 (2H, m), 0.97 (3H, t, *J* = 7.2 Hz). NMR δ_C ($CDCl_3$): 159.2, 151.7, 148.4, 137.6, 129.6, 124.8, 96.6, 59.5, 47.7, 44.8, 34.9, 34.2, 22.8, 13.3. ESIMS *m/z* $[M+H]^+$: calcd for $C_{14}H_{20}N_4O_2SCl$, 343.0989; found, 343.0996.

R-1-(6-Chloro-3-pyridylmethyl)-5-methoxyethyl-2-(nitromethylene)imidazolidine (**14**)

Mp 69-72. $[\alpha]_D^{25}$ +8.32 (c 0.75, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.71 (1H, br), 8.29 (1H, d, *J* = 2.4Hz), 7.57 (1H, dd, *J* = 8.2, 2.2 Hz), 7.35 (1H, d, *J* = 8.0 Hz), 6.56 (1H, s), 4.36 (2H, d, *J* = 7.2 Hz), 3.95 (2H, m), 3.58 (1H, m), 3.42 (2H, t, *J* = 5.8 Hz), 3.29 (3H, s), 2.01 (1H, m), 1.81 (1H, m). NMR δ_C ($CDCl_3$): 159.3, 151.5, 148.3, 137.5, 129.8, 124.7, 96.7, 68.3, 58.9,

58.7, 48.3, 44.4, 32.2. ESIMS *m/z* $[M+H]^+$: calcd for $C_{13}H_{18}N_4O_3Cl$, 313.1055; found, 313.1067.

S-1-(6-Chloro-3-pyridylmethyl)-5-(methoxymethoxy)methyl-2-(nitromethylene)imidazolidine (**15**)

Mp 129-130. $[\alpha]_D^{25}$ +142.8 (c 0.16, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.71 (1H, br), 8.32 (1H, d, *J* = 2.8Hz), 7.59 (1H, dd, *J* = 8.4, 2.8 Hz), 7.35 (1H, d, *J* = 8.4 Hz), 6.55 (1H, s), 4.56 (2H, q, *J* = 6.7 Hz), 4.46 (2H, d, *J* = 2.8 Hz), 4.06 (1H, m), 3.93 (1H, t, *J* = 10.2 Hz), 3.66 (2H, d, *J* = 5.2 Hz), 3.62 (1H, m), 3.32 (3H, s). NMR δ_C ($CDCl_3$): 159.4, 151.5, 148.5, 137.6, 129.9, 124.6, 96.7, 96.6, 67.4, 59.5, 55.8, 45.3, 45.2. ESIMS *m/z* $[M+H]^+$: calcd for $C_{13}H_{18}N_4O_4Cl$, 329.1004; found, 329.1017.

S-1-(6-Chloro-3-pyridilmethyl)-5-methylthioethyl-2-(nitromethylene)imidazolidine (**16**)

Mp 138-140. $[\alpha]_D^{25}$ +43.7 (c 0.2, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.72 (1H, br), 8.30 (1H, d, *J* = 2.8 Hz), 7.59 (1H, dd, *J* = 8.2, 2.8 Hz), 7.37 (1H, d, *J* = 8.4 Hz), 6.56 (1H, s), 4.38 (2H, q, *J* = 17.7 Hz), 4.00 (1H, m), 3.96 (1H, m), 3.55 (1H, m), 2.48 (2H, m), 2.08 (3H, s), 2.02 (1H, m),1.87(1H, m). NMR δ_C ($CDCl_3$): 159.2, 151.5, 148.3, 137.6, 129.7, 124.7, 96.7, 59.2, 47.8, 44.5, 31.2, 29.2, 15.7. ESIMS *m/z* $[M+H]^+$: calcd for $C_{13}H_{18}N_4O_2SCl$, 329.0833; found, 329.0839.

R-1-(6-Chloro-3-pyridylmethyl)-2-(nitromethylene)-5-phenylimidazolidine (**17**)

Mp 237-239. $[\alpha]_D^{25}$ +88.1 (c 0.5, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.81 (1H, br), 8.05 (1H, d, *J* = 2.4 Hz), 7.45 (4H, m), 7.32 (1H, d, *J* = 8.4 Hz), 7.27 (1H, d, *J* = 2.8 Hz), 7.26 (1H, d, *J* = 2.4 Hz), 6.73 (1H, s), 4.71 (1H, t, *J* = 9.0 Hz), 4.32 (1H, d, *J* = 16.4 Hz), 4.17 (1H, t, *J* = 10.2 Hz), 3.94 (1H, d, *J* = 16.0 Hz), 3.74 (1H, t, *J* = 9.4 Hz). NMR δ_C ($CDCl_3$): 159.0, 151.7, 149.0, 138.1, 136.4, 129.8, 129.7, 128.9, 127.5, 124.8, 96.8, 63.7, 51.1, 44.0. ESIMS *m/z* $[M+H]^+$: calcd for $C_{16}H_{16}N_4O_2Cl$, 331.0953; found, 331.0962.

R-5-Benzyl-1-(6-chloro-3-pyridylmethyl)-2-(nitromethylene)imidazolidine (**18**)
Mp 137-139. $[\alpha]_D^{25}$ +57.4 (c 1.4, $CHCl_3$). NMR δ_H ($CDCl_3$): 8.68 (1H, br), 8.18 (1H, d, J = 2.0 Hz), 7.51 (1H, dd, J = 8.0, 2.8 Hz), 7.31 (4H, m), 7.10 (1H, d, J = 8.4 Hz), 7.09 (1H, d, J = 7.6 Hz), 6.58 (1H, s), 4.30 (2H, d, 16.6 Hz), 4.05 (1H, m), 3.73 (1H, t, J = 9.8 Hz), 3.54 (1H, dd, J = 9.6, 6.8 Hz), 3.10 (1H, dd, J = 14.0, 5.6 Hz), 2.79 (1H, dd, J = 13.4, 8.6 Hz). NMR δ_C ($CDCl_3$): 159.2, 151.8, 148.4, 137.6, 135.1, 129.4, 129.2, 128.9, 127.6, 124.8, 96.7, 60.9, 47.7, 44.7, 38.7. ESIMS *m/z* $[M+H]^+$: calcd for $C_{17}H_{18}N_4O_2Cl$, 345.1114; found, 345.1118.

2.3. Evaluation of Receptor Affinity

The assay method was essentially the same as that in our previous reports.[5,6] From the concentration–response curve, the molar concentration for 50% inhibition (IC_{50}) of [^{3}H]imidacloprid binding to the receptor was calculated. The K_i value was calculated according to the following equation using PRISM ver 5.0:

$K_i = IC_{50}/1 + ([L]/K_d)$

where [L] is the final concentration of the radioligand (10 nM) and K_d (3.66 nM) is the dissociation constant of [^{3}H]imidacloprid for the receptor fraction. The K_i values of the test compounds were obtained from three separate assays performed in duplicate and are listed in Table 1.

2.4. 3D-QSAR Analysis Using CoMFA

For CoMFA, compounds **1**–**18**, which were synthesized in this study, and compounds **19**–**50**, whose pK_i values have previously been reported,[6] were included in the data set. The calculations for the optimization and superposition of the test chemicals were performed using the molecular modeling software package SYBYL ver. 7.1 (Tripos Associates, Inc., St Louis, MO). Following the previous report, the initial conformations of the compounds were constructed and their structures were optimized.[6] For the superposition of these compounds, four atoms were selected on the basis of our previous

report,[6,9,10] *i.e.*, the nitrogen atoms of the pyridine ring and at the 1 position of the imidazolidine ring, the carbon atom at the 2 position of the imidazolidine ring, and the bridging carbon atom between the aromatic and imidazolidine rings. The potential energy fields of each superposed conformer were calculated at the lattice intersections. The electrostatic (Coulomb potential) and steric (Lennard-Jones potential) field energies were calculated at each lattice point using a sp^3-carbon atom with a charge of +1.0 as a probe. The data for the receptor affinities of compounds **1**–**50** were correlated with these parameters using the partial least-squares method. The steric and electrostatic potential contour maps with *n*-propylthiomethyl CH-IMI **13** determined using Eq. 1 presented in section **3.2** of the Results are shown in Fig. 3. The pK_i values calculated using Eq. 1 are shown in Table 2.

2.5. Construction of Ligand-binding Domain Model of Housefly nAChR Combined with a CH-IMI Derivative Using PDFAMS Software

Based on the crystallographic data of Ac-AChBP bound with IMI (PDB, 3C79), the homology model of the ligand-binding domain (LBD) in the housefly nAChR was constructed using the homology modeling software PDFAMS Pro 2.0 (In-Silico Sciences, Inc., Tokyo, Japan) according to previous reports.[6,12] The amino acid sequence of subunit 6 (isoform II) of *M. domestica* AChR (GenBank ID ABJ09669), which was the most common among the six splicing variants in subunit 6 of the housefly receptor,[13] was aligned with the sequence of Ac-AChBP using PDFAMS, as shown in Fig. 4. In our previous study, we confirmed that employing the sequence of α2 (GenBank ID ABD37617) or α5 (GenBank ID ABY40460) subunit rather than the sequence of subunit 6 to construct the ligand-binding domain model did not affect the results.[6] Thus, only subunit 6 was considered in the present study. The LBD was constructed using two of the same subunits because the Ac-AChBP template was constructed as a homopentamer, and it is currently unknown whether the nAChR of houseflies is constructed as a homopentamer or heteropentamer. Using the simulated annealing method,[14] the three-dimensional structure of the LBD was constructed.

The coordinate of IMI was fixed during the simulated annealing. The constructed model was then energy-minimized using the force field and partial charges of the molecular mechanics MMFF94.[15,16] A graphical image of the surface of the ligand-binding pocket was created using the multichannel surface tool of the MOLCAD module in the SYBYL software (Fig. 5).

2.6. Docking Study Using FRED Software

For the docking studies, MAKE RECEPTOR, OMEGA, FRED, and VIDA of OpenEye Scientific Software Inc. (Santa Fe, NM) were employed. The CH-IMI-binding region of the housefly nAChR model constructed in section **2.5.**, which was adopted as a template receptor, was calculated using the "MAKE RECEPTOR" tool (ver. 3.0.0). The mol2 file of the three-dimensional structure of the compound possessing an *n*-propylthiomethyl group (**13**), which was constructed using the Sybyl software for CoMFA analysis, was submitted to OMEGA (ver. 2.5.1.4) to generate possible conformers. Docking of compound **13** was performed using FRED (Fast Rigid Exhaustive Docking; ver. 3.0.0) against up to 200 energy-stable conformers generated by OMEGA. VIDA (ver. 4.2.1) was used to view the models, as shown in Fig. 6.

2.7. Evaluation of Insecticidal Activity

The assay method was essentially the same as that used in our previous report.[5,6] To evaluate the insecticidal activity, female houseflies anesthetized using carbon dioxide were topically treated with methanol containing synergists piperonyl butoxide (PBO) and NIA16388 (NIA) [0.2% (w/v)]. After 1 h, 0.22 μL of a 50% ethanol solution containing a test chemical at various concentrations was injected into the dorsal side of the thorax of reanesthetized flies. Insecticidal activity was evaluated 1 h after injection. The ED_{50} values (effective dose for inducing paralysis or death in 50% of the houseflies) were calculated using a probit transformation and are listed in Table 1.

2.8. Analysis of the Relationship between Receptor Affinity and Insecticidal Activity

Linear regression analysis between the receptor affinity and insecticidal activity was performed using the QREG 2.05 software.[17] The hydrophobicities, log P, of compounds **3** – **18**, **28** and **32** were measured using the shake flask method in a water/*n*-octanol system.[18] The log P values of compounds **19** - **21**, **22** - **24**, **25** - **27**, **31**, and **33** – **35** were referred to as the values of compounds **3**, **4**, **5**, **28** and **32**, respectively, because the corresponding compound has the same substituent. The numbers of sulfur and oxygen atoms were defined as the indicator valuables, such as $I_{thioether}$ and I_{ether}, respectively. Compounds **29** and **30** were omitted from the analysis because their insecticidal activity could not be measured even when applied at the high dose. The physicochemical parameters employed are listed in Table 3.

3. RESULTS

3.1. Receptor Affinity

The inhibition constant K_i (nM) was employed as an indicator of receptor affinity (Table 1). The receptor affinity decreased as the number of carbon atoms in the side chain increased from a methyl group to a propyl group (compound **3** vs. **5**), whereas the affinity of compound **6,** which possessed a butyl group, was 4.6-fold higher than the affinity of compound **5**, suggesting that the increase in the number of carbon atoms in the linear direction is not always disadvantageous in terms of affinity. Comparing the affinity of compound **7** with the affinity of compound **3**, the introduction of a hydroxyl group was found to be disadvantageous for affinity. The receptor affinities of compounds **8**, **9**, and **10**, in which the hydroxyl group of compound **7** was replaced with a methoxy, ethoxy, and propoxy group, respectively, were 169-, 37-, and 2.4-fold lower than the receptor affinity of compound **7**, respectively. The introduction of oxygen atoms was disadvantageous for affinity (for example, propyl **5** vs. methoxymethyl **8**), but elongation of the carbon chains (from **8** to **10**) increased the binding affinity. The affinities of compounds **11**, **12**, and **13**, in which the oxygen atoms of compounds **8**, **9**, and **10** were changed to sulfur atoms, were 46-,

58-, and 4.6-fold higher than the affinities of the corresponding ether compounds, respectively, suggesting that the introduction of sulfur atoms was more advantageous for affinity than oxygen atoms. In addition, the affinities of compounds **11** and **12** were 2-fold lower than the affinities of compounds **5** and **6**, demonstrating that conversion of carbon atoms to sulfur atoms did not remarkably influence the receptor affinity. The conversion of the carbon atom to a sulfur atom did not influence the receptor affinity, whereas the conversion to an oxygen atom decreased the affinity, suggesting that the atom at this position of the substituent would interact with the receptor. Compounds **9** and **14**, in which the carbon atoms at the 2- and 3-positions of the *n*-butyl group were converted to oxygen atoms, respectively, exhibited similar receptor affinities, demonstrating that the position of the oxygen atom introduced into the *n*-butyl group did not affect affinity. The affinity of compound **15,** which possessed two oxygen atoms on the side chain, was 38-fold lower than the affinity of compound **10**, demonstrating that an increase in the number of oxygen atoms was disadvantageous for receptor affinity. The affinity of the methylthioethylated compound **16** was 5.4-fold lower than the affinity of the ethylthiomethylated compound **12**, demonstrating that the position of the sulfur atom influenced receptor affinity. The affinities of compounds **17** and **18,** which possessed a benzene ring, were 2684- and 5.4-fold lower than the affinity of compound **2**, respectively, demonstrating that the introduction of a benzene ring was disadvantageous for receptor affinity.

3.2. CoMFA Analysis

Statistical analysis of the receptor affinities of 50 compounds using CoMFA was performed using Eq. 1, as follows:

$$pK_i = 5.79 + [\text{CoMFA field terms}] \quad \text{(Eq. 1)}$$

$$n = 50,\ s = 0.47,\ r^2 = 0.93,$$

$$CN = 5,\ \text{Cross-validated}\ [s_{cv} = 1.03,\ q^2 = 0.68],\ RC\ [Steric = 0.64,\ Electrostatic = 0.36]$$

In this and the following equations, *n* is the number of compounds, *s* is the standard deviation, and *r* is the correlation coefficient. *CN* indicates the number of latent variables, and s_{cv} and *q* are the standard deviation and correlation coefficient obtained from the leave-one-out cross-validation, respectively. *RC* refers to the relative contribution of steric and electrostatic effects to variations in receptor affinity. The pK_i values calculated using Eq. 1 are shown in Table 2. The major steric and electrostatic potential contour maps with the *n*-propylthiomethyl CH-IMI analogue **13** were drawn according to Eq. 1 (Fig. 3). The blue areas in Fig. 3A indicate regions in which the more positive electrostatic features of the compounds increased activity, whereas the red area shows the region in which negative electrostatic features were favorable. A positive electrostatic potential region appeared around the chlorine atom of the pyridine ring and the hydrogen atom attached at N3 of the imidazolidine ring, consistent with earlier reports.[6,9,10] In addition, a negative electrostatic region appeared around the nitrogen atom of the pyridine ring, also consistent with previous research.[6] In this study, a blue region newly appeared around the C3 and C4 positions of the *n*-propylthiomethyl group attached at position 5 of the imidazolidine ring. Oxygen atoms are more electronegative than carbon and sulfur atoms, and the receptor affinities of substituents with an oxygen atom at this position were lower than the receptor affinities of the other compounds, which explains why the blue region appeared (relatively positive charges would be favorable for receptor affinity). In Fig. 3B, the green and yellow regions denote sterically favorable and unfavorable moieties for receptor affinity, respectively. Both green and yellow regions appeared around the C2–C4 positions of the *n*-propylthiomethyl group attached at position 5 of the imidazolidine ring, suggesting that steric hindrance should exist around these regions.

3.3. Construction of a Ligand-binding Domain Model of the Housefly nAChR Bound by a CH-IMI Derivative and Docking

The housefly nAChR model constructed based on the crystallographic data of the AChBP of *Lymnaea stagnalis* (PDB code, 2ZJU)[19] suggested the presence of a sterically

permissible region that could accept a substituent up to the length of an *n*-propyl group (Fig. 5A). However, the docking model of the housefly nAChR bound to a CH-IMI derivative, which was reconstructed on the basis of Ac-AChBP (PDB code, 3C79) using PDFAMS, demonstrated the presence of a sterically permissible region that could accept a substituent longer than an *n*-propyl group (Fig. 5B). Four amino acid residues, namely, isoleucine, tyrosine and two tryptophans, were found to construct part of the ligand-binding region (yellow amino acid residues in Fig. 5A). Comparing these residues in Fig. 5A (housefly receptor model constructed based on *Ls*-AChBP) to the ones in Fig. 5B (housefly receptor model constructed based on *Ac*-AChBP) indicated that two tryptophan residues move outside the ligand-binding region and that the orientation of the isoleucine and tyrosine residues is reversed, thus explaining why the ligand-binding region expanded (Fig. 5C).

In the docking model constructed on the basis of *Ac*-AChBP bound by the *n*-propylthiomethyl CH-IMI **13** (with comparatively higher receptor affinity), sulfur-π and van der Waals interactions between the ligand and receptor were observed (Fig. 6). The hydrophobic amino acid residues, aligned in the expanded pocket, should interact with the hydrophobic side chain of the compound.

3.4. Insecticidal Activity and Correlation of Structural Changes with Biological Activities

The insecticidal activity of the synthesized derivatives is presented in terms of their ED_{50} values in Table 1. Although the receptor affinity of compound **6** was 4.6-fold higher than that of compound **5**, the insecticidal activity of compound **6** was 15-fold lower than that of compound **5**, demonstrating that a linear increase in the number of carbon atoms was disadvantageous regarding insecticidal activity. The insecticidal activity of compound **7** was 120-fold lower than that of the methylated compound **3**, suggesting that the introduction of a hydroxyl group would be disadvantageous. Among compounds **8–10**, ethoxymethyl CH-IMI **9** had the lowest insecticidal activity. The insecticidal activities of compounds **8** and **9** were 62- and 25-fold lower than those of the corresponding alkylated

compounds **5** and **6**, demonstrating that the introduction of oxygen atoms was disadvantageous for insecticidal activity, as observed for receptor affinity. The ED_{50} values of compounds **11**–**13**, which possessed sulfur atoms, were not high and ranged from 11–74 pmol/fly, although their K_i values were lower than 1 nM, indicating that these compounds possessed higher receptor affinity. The insecticidal activity of compounds **11** and **12** were 234- and 20-fold lower than those of compounds **5** and **6**, respectively, suggesting that converting the carbon atom to a sulfur atom was disadvantageous for insecticidal activity. Comparing the insecticidal activities of compounds **14** and **15** with the activity of compound **9** revealed that converting carbon atoms to oxygen atoms and the number of oxygen atoms did not influence insecticidal activity. In the case of a sulfur atom rather than an oxygen atom, the insecticidal activity of compound **16** was 2.3-fold lower than that of compound **12**, suggesting that the substituted position does not influence insecticidal activity, as observed for oxygen atoms. The insecticidal activities of compounds **17** and **18** were 1615- and 91-fold lower than that of compound **2**, demonstrating that the introduction of a bulky aromatic ring reduced insecticidal activity. Thus, a compound with higher insecticidal activity was not observed among the synthesized compounds, although compounds **6** (*n*-butyl CH-IMI) and **13** (propylthiomethyl CH-IMI) exhibited nearly identical receptor affinities to the unsubstituted, methylated, and ethylated compounds.

The relationship between insecticidal activity and receptor affinity was quantitatively analyzed considering the other factors of the test compounds using the Hansch-Fujita method, which is one of the conventional QSAR methods, as follows:

$$pED_{50} = 0.62\ (\pm 0.08)\ pK_i - 0.73\ (\pm 0.22)\ \log P - 1.43\ (\pm 0.33)\ I_{thioether} - 0.77\ (\pm 0.23)\ I_{ether} + 6.36\ (\pm 0.77) \quad \text{Eq. 2}$$

$$n = 32,\ s = 0.56,\ r = 0.88,\ F(4\ 27) = 24.312 > 0.999$$

where F is the ratio between the regression and residual variances and the number in parentheses is the 95% confidence interval. This equation suggested that higher receptor

affinity should be advantageous for insecticidal activity, whereas higher hydrophobicity and the introduction of heteroatoms, particularly sulfur atoms, are disadvantageous for insecticidal activity.

4. DISCUSSION

In our previous study,[6] we constructed a docking model of the housefly nAChR bound by a CH-IMI derivative based on the crystallographic data of the AChBP of *L. stagnalis* bound by IMI (PDB code, 2ZJU),[19] which suggested the presence of a sterically permissible region that could accept a substituent up to the length of an *n*-propyl group attached to the imidazolidine ring (Fig. 5A). In this study, however, the receptor affinity of compound **6,** which possessed an *n*-butyl group, was higher than that of compound **5**, and we could not explain this result based on the previous docking model. In fact, a FRED docking simulation indicated that compound **6** was not able to settle in the ligand-binding region of the previous model in the same manner reported earlier (data not shown). In this case, we observed that 2-propanol and IMI bind to one of five agonist-binding regions in Ac-AChBP (PDB, 3C79; Fig. S1A in Supplementary data). In the electron density map of 3C79, there was a meshed region over the ethylene moiety of imidacloprid that was assigned as 2-propanol (data not shown). Both IMI and 2-propanol were registered as ligands in the database (PDB, 3C79), and we hypothesized that the ligand-permissible region should expand if these compounds are recognized as ligands interacting with the receptor for the calculation. The “MAKE RECEPTOR” software supported this hypothesis (Fig. S1B in Supplementary data), and a docking study employing FRED suggested that this region could accept *n*-propylthiomethyl CH-IMI **13** (Fig. S1C). The binding model of the housefly nAChR, which was reconstructed on the basis of Ac-AChBP using PDFAMS, demonstrated the presence of a sterically permissible region that could accept a substituent longer than an *n*-propyl group (Fig. 5B). Comparing the model in this study with the model in the previous study, Tyr73 was notably moved (Fig. 5C). A previous QSAR analysis of trypsin inhibitors such as benzamidines suggested that larger substituents appear to have

pushed an amino acid aside and interact positively with the enzyme surface,[20] which was potentially consistent with our findings. In the X-ray crystallographic data of Ac-AChBP, the relative positions of Tyr73 and IMI were different among the ligand-binding regions (Fig. S1D), suggesting that this tyrosine residue might be flexible. This study suggests the expansion of the ligand-binding region according to the size of a substituent attached to the compound, but it should be considered that the binding mode would change. The surface of the ligand-binding region is not rigid, and the introduction of a bulky group might influence the conformation of the receptor or the binding modes of the compounds. In addition, we constructed the receptor model employing a homodimer. If the subunit compositions of the housefly receptors are resolved, another binding mode might be suggested.

Compound **13,** which has an *n*-propylthiomethyl group, exhibited high receptor affinity, and the docking study suggested that a longer substituent could be acceptable in the expanded ligand-binding region. In the CoMFA study, however, the sterically favorable region (as green in Fig. 3B) did not appear around the tip of the substituents, likely due to the lack of variation of the substituents. More substituents should be employed to clarify the contribution of this region to the receptor affinity.

The analysis of the relationship between insecticidal activity and receptor affinity suggested that higher receptor affinity should be advantageous for insecticidal activity, whereas higher hydrophobicity and the introduction of heteroatoms, particularly sulfur atoms, are disadvantageous for insecticidal activity. Compounds that possess heteroatoms, such as sulfur and oxygen atoms, might be metabolized (for example, cleavage of ether bonds and oxidation of sulfur atoms) before they reach the target site, although synergists were applied before the insecticidal test. Any metabolic pathway that cannot be suppressed by synergists such as NIA and PBO might be implicated.

In this study, we identified some CH-IMI derivatives possessing larger substituents that exhibited high receptor affinity, although their insecticidal activity was not high. In addition, a docking model of the housefly nAChR bound to CH-IMI derivatives suggested that the ligand-binding region expands as the size of the substituent increases. It is clear

that receptor affinity primarily influences insecticidal activity. Based on the receptor model constructed in this study, compounds that exhibit higher receptor affinity should be designed, and the metabolic pathway targeted in insects should be considered, leading to the development of novel neonicotinoid insecticides.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of compounds **6**-**18**. Supplementary Figure S1. The atomic coordinate file of the housefly nAChR model constructed in the present study. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to Profs. Hisashi Miyagawa and Yoshiaki Nakagawa of Kyoto University for gifting radiolabeled imidacloprid. A part of this study was performed at the INCS (Johoku and Tarumi stations) of Ehime University.

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Figure legends

FIGURE 1 Chemical structures of imidacloprid (**1**) and its nitromethylene derivatives **2** - **18**.

FIGURE 2 Synthetic route for imidacloprid derivatives **6** - **18**. (a) $SOCl_2$/MeOH; $(Boc)_2O$/THF; 2,2-dimethoxypropane, BF_3-Et_2O complex/acetone; $NaBH_4$, LiCl/dry THF; (b) phthalimide, DEAD, PPh_3/dry THF; (c) conc. HCl/THF; 2-chloro-5-chloromethylpyridine, Et_3N/acetone; (d) EtI/dry THF; (e) p-TsOH/MeOH; (f) Me_2SO_4, 0.2 M $Ba(OH)_2$/dry CH_2Cl_2; (g) $LiAlH_4$/dry THF; $(Boc)_2O$/THF; (h) phthalimide, DEAD, PPh_3/dry THF; (i) conc. HCl/THF; 2-chloro-5-chloromethylpyridine, Et_3N/acetone; (j) DIEA, MOMCl/dry CH_2Cl_2; (k) N_2H_4-H_2O/EtOH; 1,1-bis(methylthio)-2-nitroethylene, K_2CO_3/EtOH.

FIGURE 3 Contour diagrams of electrostatic (A) and steric (B) fields with *n*-propylthiomethyl CH-IMI **13** according to Eq. 1. (A) Red and blue areas indicate the regions in which negative and positive electrostatic interactions with the receptor-binding site increase receptor affinity, respectively. (B) Green and yellow areas indicate the sterically favorable and unfavorable regions for receptor affinity, respectively.

FIGURE 4 Pairwise sequence alignment of subunit 6 (subtype II) of the nicotinic acetylcholine receptor of *Musca domestica* (MdS6II, GenBank ID ABJ09669) with the acetylcholine binding protein of *Aplysia californica* (Ac-AChBP, PDB, 3C79). An asterisk indicates perfect identity, a colon indicates similar amino acids with conservation, and a period indicates weakly similar amino acids with conservation. Trp35, Tyr73, Trp129, and Ile169 are presented in red.

FIGURE 5 The molecular surface of the ligand-binding site of the housefly receptor model. (A) Ligand-binding domain (LBD) constructed on the basis of the X-ray crystallographic

data of *Ls*-AChBP. Four amino acids (isoleucine, tyrosine and two tryptophans) surrounding the ligand-binding pocket are presented in yellow. (B) LBD constructed on the basis of the X-ray crystallographic data of the acetylcholine binding protein of *Aplysia californica*. The corresponding four amino acids in Fig. 5A are presented in green. (C) Merged image of Figs. 5A-B. In Figs. 5A-B, the color of the contour map indicates the hydrophobicity (brown, hydrophobic; blue, hydrophilic).

FIGURE 6 The amino acid residues for constructing the expanded ligand-binding region of the housefly receptor model and the *n*-propylthiomethyl CH-IMI **13** (drawn using yellow-green bonds).

Table 1 Receptor affinity K_i (nM), insecticidal activity ED_{50} (pmol/fly) and hydrophobicity log P of CH-IMI **2** and its synthesized derivatives **3-18**.[a]

No.	R	Receptor affinity	Insecticidal activity	Log P
		K_i (nM)	ED_{50} (pmol/fly)	
2	H	0.0367[b]	0.117[b]	-0.20
3	Me	0.0428[b]	0.0626[b]	0.09
4	Et	0.0597[c]	0.914[c]	0.47
5	*n*-Propyl	0.258[c]	0.253[c]	0.96
6	*n*-Butyl	0.0564 ± 0.00466	3.77 ± 0.441	1.34
7	Hydroxymethyl	0.157 ± 0.00602	7.49 ± 0.615	-0.55
8	Methoxymethyl	26.6 ± 5.31	15.7 ± 3.80	-0.03
9	Ethoxymethyl	5.76 ± 0.868	94.3 ± 19.4	0.45
10	Propoxymethyl	0.373 ± 0.196	11.0 ± 1.71	1.06
11	Methylthiomethyl	0.581 ± 0.0393	59.2 ± 16.3	0.48
12	Ethylthiomethyl	0.100 ± 0.0170	73.7 ± 1.92	0.95
13	Propylthiomethyl	0.0807 ± 0.0153	11.3 ± 3.50	1.35
14	Methoxyethyl	5.26 ± 0.178	68.9 ± 7.77	0.16
15	Methoxymethoxymethyl	14.0 ± 3.65	63.1 ± 17.9	-0.11
16	Methylthioethyl	0.542 ± 0.233	171 ± 28.5	0.75
17	Phenyl	98.5 ± 25.8	189 ± 13.0	1.23
18	Benzyl	0.198 ± 0.0483	10.7 ± 3.07	1.56

[a] The biological activities of compounds **6**-**18** are presented as the mean ± standard error of the mean (n=3).

[b] The values of the biological activities of compounds are cited from Ref. 5.

[c] The values of the biological activities of compounds are cited from Ref. 6.

Table 2 The reciprocal of the Ki values observed and calculated using the equation derived from the CoMFA analysis.

No.	Compound	pKi Obsd.	CoMFA pKi Calcd.	CoMFA Δ	No.	Compound	pKi Obsd.	CoMFA pKi Calcd.	CoMFA Δ
1	Imidacloprid	8.43	8.18	0.25	**26**	*R*-4-*n*-Propyl	6.68	6.68	0
2	H	10.44	10.17	0.27	**27**	*S*-4-*n*-Propyl	7.13	7.25	-0.12
3	Me	10.37	9.94	0.43	**28**	*R*-5-Isopropyl	8.95	9.45	-0.50
4	Et	10.22	10.11	0.11	**29**	*S*-5-Isopropyl	5.63	5.87	-0.24
5	*n*-Propyl	9.59	8.99	0.60	**30**	*R*-4-Isopropyl	6.23	6.35	-0.12
6	*n*-Butyl	10.25	9.11	1.14	**31**	*S*-4-Isopropyl	6.37	6.76	-0.39
7	Hydroxymethyl	9.80	10.08	-0.28	**32**	*R*-5-Isobutyl	8.34	8.12	0.22
8	Methoxymethyl	7.58	8.43	-0.85	**33**	*S*-5-Isobutyl	7.28	7.49	-0.21
9	Ethoxymethyl	8.24	8.83	-0.59	**34**	*R*-4-Isobutyl	5.56	5.53	0.03
10	Propoxymethyl	9.43	9.25	0.18	**35**	*S*-4-Isobutyl	6.40	6.58	-0.18
11	Methylthiomethyl	9.24	9.02	0.22	**36**	4,4-$(CH_3)_2$	5.81	6.92	-1.11
12	Ethylthiomethyl	10.00	9.44	0.56	**37**	5,5-$(CH_3)_2$	8.98	9.81	-0.83
13	Propylthiomethyl	10.09	9.60	0.49	**38**	phenyl	5.24	5.39	-0.15
14	Methoxyethyl	8.28	8.94	-0.66	**39**	*o*-F	5.43	5.13	0.30
15	Methoxymethoxymethyl	7.85	7.93	-0.08	**40**	*o*-Cl	5.26	4.90	0.36
16	Methylthioethyl	9.27	9.30	-0.03	**41**	*o*-CH_3	5.05	4.77	0.28
17	Phenyl	6.98	6.69	0.29	**42**	*m*-F	6.52	6.28	0.24
18	Benzyl	9.69	9.75	-0.06	**43**	*m*-Cl	6.30	5.47	0.83
19	*S*-5-Me	9.50	9.29	0.21	**44**	*m*-CH_3	4.54	5.42	-0.88
20	*R*-4-Me	8.68	8.11	0.57	**45**	*m*-CH_3O	5.48	5.56	-0.08
21	*S*-4-Me	8.25	7.97	0.28	**46**	*m*-CN	6.75	6.55	0.20
22	*S*-5-Et	7.73	8.14	-0.41	**47**	*p*-F	4.30	4.40	-0.10
23	*R*-4-Et	7.51	7.23	0.28	**48**	*p*-Cl	6.76	6.63	0.13
24	*S*-4-Et	7.61	7.60	0.01	**49**	*p*-CH_3	6.38	6.23	0.15
25	*S*-5-*n*-Propyl	7.60	7.72	-0.12	**50**	Olefin	8.57	9.20	-0.63

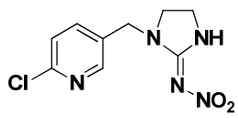
Table 3 The reciprocal of the ED_{50} values observed and calculated using Eq. 2.

No.	Name	pED_{50} (Obsd.)	pED_{50} (Calcd.)	Δ	LogP	I_{ether}	$I_{thioether}$
2	H	12.93	12.97	-0.04	-0.20	0	0
3	Me	13.20	12.72	0.48	0.09	0	0
4	Et	12.04	12.35	-0.31	0.47	0	0
5	*n*-Propyl	12.60	11.60	1.00	0.96	0	0
6	*n*-Butyl	11.42	11.73	-0.31	1.34	0	0
7	Hydroxymethyl	11.13	12.06	-0.93	-0.55	1	0
8	Methoxymethyl	10.80	10.31	0.50	-0.03	1	0
9	Ethoxymethyl	10.03	10.37	-0.34	0.45	1	0
10	Propoxymethyl	10.96	10.66	0.30	1.06	1	0
11	Methylthiomethyl	10.23	10.31	-0.08	0.48	0	1
12	Ethylthiomethyl	10.13	10.44	-0.31	0.95	0	1
13	Propylthiomethyl	10.95	10.20	0.75	1.35	0	1
14	Methoxyethyl	10.16	10.58	-0.42	0.17	1	0
15	Methoxymethoxymethyl	10.20	9.76	0.44	-0.11	2	0
16	Methylthioethyl	9.77	10.13	-0.36	0.75	0	1
17	Phenyl	9.72	9.84	-0.12	1.23	0	0
18	Benzyl	10.97	11.28	-0.31	1.56	0	0
19	*S*-5-Me	12.47	12.18	0.29	0.09	0	0
20	*R*-4-Me	12.37	11.68	0.69	0.09	0	0
21	*S*-4-Me	12.25	11.40	0.85	0.09	0	0
22	*S*-5-Et	10.03	10.81	-0.78	0.47	0	0
23	*R*-4-Et	10.16	10.67	-0.51	0.47	0	0
24	*S*-4-Et	10.62	10.73	-0.11	0.47	0	0
25	*S*-5-n-Propyl	10.34	10.37	-0.03	0.96	0	0
26	*R*-4-n-Propyl	9.36	9.80	-0.44	0.96	0	0
27	*S*-4-n-Propyl	10.10	10.08	0.02	0.96	0	0
28	*R*-5-Isopropyl	10.48	11.33	-0.85	0.79	0	0
29	*S*-5-Isopropyl	< 9.13 (22%)[a]	-	-	-	-	-
30	*R*-4-Isopropyl	< 9.05 (31%)[a]	-	-	-	-	-
31	*S*-4-Isopropyl	10.47	9.73	0.74	0.79	0	0
32	*R*-5-Isobutyl	11.22	10.56	0.66	1.33	0	0
33	*S*-5-Isobutyl	9.22	9.90	-0.68	1.33	0	0
34	*R*-4-Isobutyl	8.95	8.84	0.11	1.33	0	0
35	*S*-4-Isobutyl	9.45	9.36	0.09	1.33	0	0

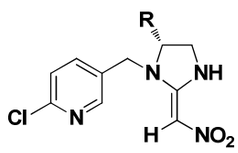
[a] The value in the parentheses was mortality at the concentration presented.

1

Figure 1



1; Imidacloprid



R = H (**2**), Methyl (**3**), Ethyl (**4**), *n*-Propyl (**5**), *n*-Butyl (**6**), Hydroxymethyl (**7**), Methoxymethyl (**8**), Ethoxymethyl (**9**), Propoxymethyl (**10**), Methylthiomethyl (**11**), Ethylthiomethyl (**12**), Propylthiomethyl (**13**), Methoxyethyl (**14**), Methoxymethoxymethyl (**15**), Methylthioethyl (**16**), Phenyl (**17**), Benzyl (**18**).

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Figure 2

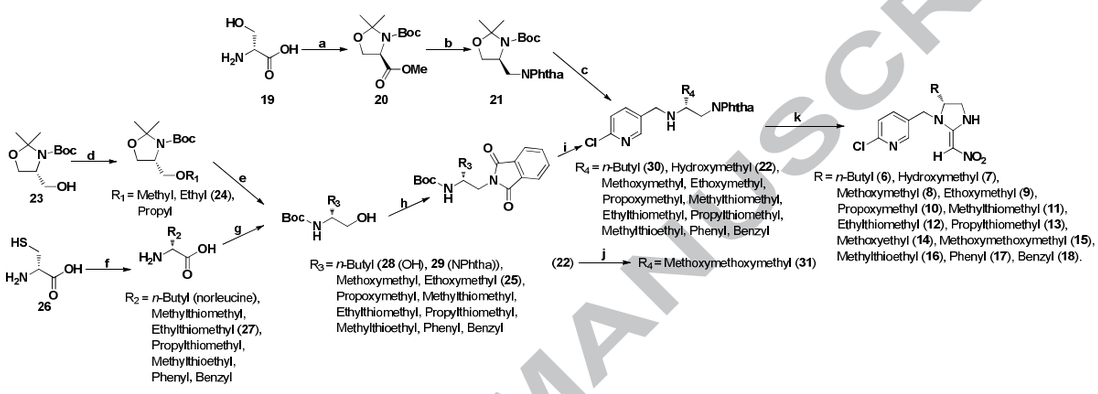


Figure 3

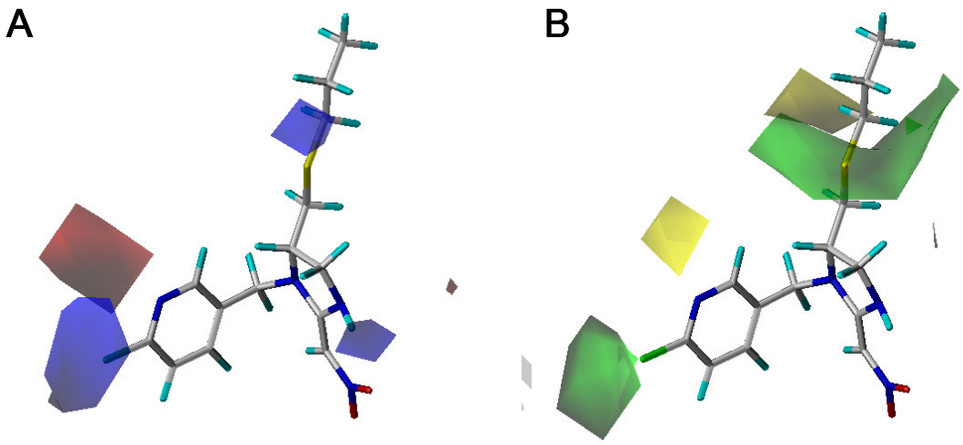


Figure 4

Figure 5

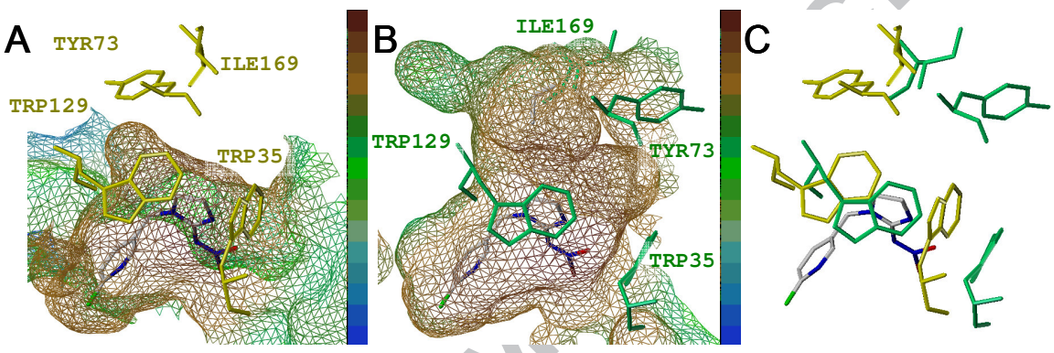
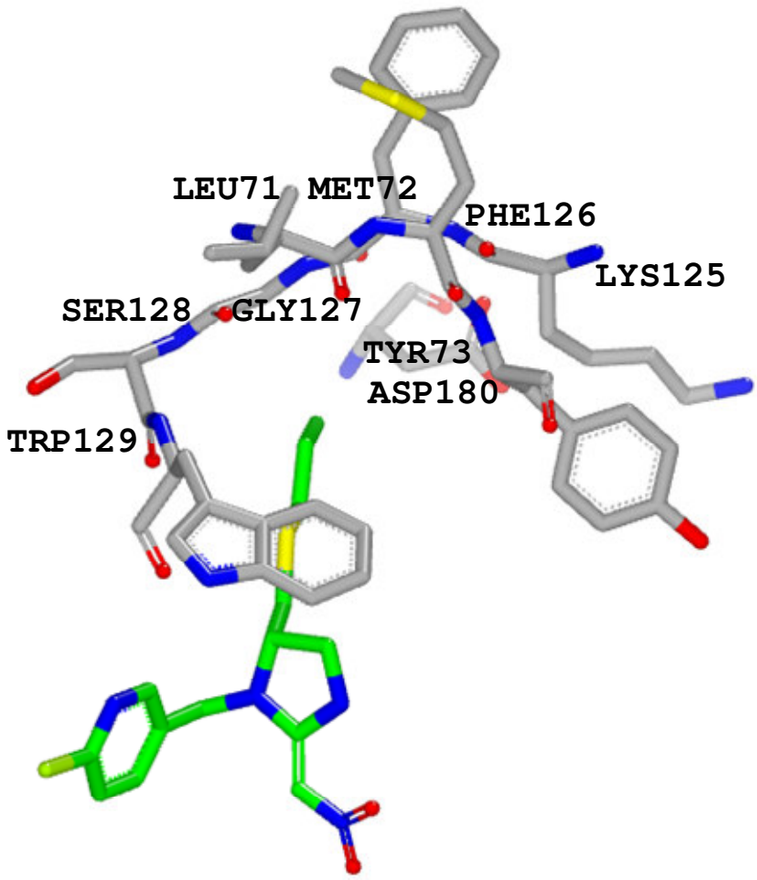


Figure 6



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3 **Graphical abstract**

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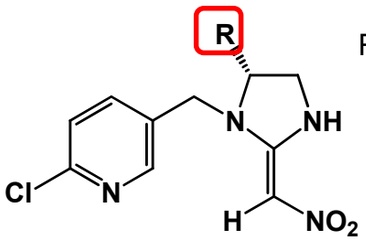

R = *n*-Butyl, Hydroxymethyl, Metoxymethyl, Etoxymethyl, Propoxymethyl, Methylthiomethyl, Ethylthiomethyl, Propylthiomethyl, Methoxyethyl, Methoxymethoxymethyl, Methylthioethyl, Phenyl, Benzyl.

5
6