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Synthesis and Structure−Activity Relationships of 1-Phenyl-1*H*-1,2,3-triazoles as Selective Insect GABA Receptor Antagonists

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To study the interaction of phenylheterocycles with γ-aminobutyric acid (GABA) receptors, 4- or 5-alkyl-(or phenyl)-1-phenyl-1*H*-1,2,3-triazoles were synthesized and examined for their ability to inhibit the specific binding of [^{3}H]-4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB), a noncompetitive antagonist, to the housefly and rat GABA receptors, as well as to the β3 subunit homo-oligomer of the human GABA receptor investigated as a model receptor. 4-Substituted 1-phenyl-1*H*-1,2,3-triazoles were found to be more potent competitive inhibitors than the 5-substituted regioisomers in the case of all receptors. The 4-*tert*-butyl or 4-*n*-propyl analogue of 1-(2,6-dichloro-4-trifluoromethylphenyl)-1*H*-1,2,3-triazole exhibited the highest level of inhibition of [^{3}H]EBOB binding to all receptors. Most of the synthesized analogues were more active in terms of the inhibition of EBOB binding to the housefly and human β3 GABA receptors than to the rat receptor. The 4-cyclohexyl analogue showed the highest (185-fold) housefly versus rat receptor selectivity. A 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 played significant roles in conferring high potency in cases involving the housefly and human β3 receptors. The human β3 receptor resembled the housefly receptor in terms of their recognition of phenyltriazoles, whereas 3D-QSAR analysis revealed a slight difference between the two receptors in terms of their mechanisms of recognition of the para-substituent on the phenyl moiety. Some of the triazoles synthesized here exhibited insecticidal activity, which was correlated with their ability to inhibit [^{3}H]EBOB binding to the housefly receptor. Thus, 1-phenyl-1*H*-1,2,3-triazoles with the appropriate substituents exert insecticidal activity by selectively acting at the site for noncompetitive antagonism of insect GABA receptors.

KEYWORDS: 1-Phenyl-1,2,3-triazole; GABA receptor; antagonist; insecticide; fipronil

INTRODUCTION

γ-Aminobutyric acid (GABA) performs a significant role as a major inhibitory neurotransmitter in both vertebrates and invertebrates, including insects. In mammals, GABA binds to two major receptor types, ionotropic and G protein-coupled receptors, which are crucial for the accurate and proper functioning of the central nervous system (*1*). The ionotropic receptors are heteropentameric ligand-gated ion channels that are assembled from five of eight subunit classes (α, β, γ, δ, ϵ, θ, π, and ρ), four of which contain several variants. The major receptor in the brain is composed of α1, β2, and γ2 subunits. GABA influences the opening of the channels to increase the membrane conductance of chloride ions, thereby suppressing the generation of action potentials (*2*). Insects have similar, but pharmacologically different, GABA receptors from those of mammals, and insect receptors reside not only in the central but also in the peripheral nervous system (*3*, *4*); thus, the readily accessible peripheral GABA receptors have been considered as a promising target for insect control chemicals. In insects, the Rdl subunit, encoded by a dieldrin resistance-associated gene, is the only subunit known to form a chloride channel (*3*).

Fipronil (**Figure 1**) is a phenylpyrazole insecticide that acts as a noncompetitive antagonist on insect ionotropic GABA receptors (*5*) while it blocks glutamate-gated chloride channels (*6*). Electrophysiological studies have shown that fipronil

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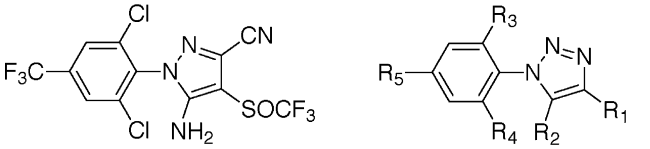


Fipronil 1-Phenyl-1*H*-1,2,3-triazoles

Figure 1. Structures of fipronil and 1-phenyl-1*H*-1,2,3-triazoles.

blocked GABA-induced currents in *Drosophila* homo-oligomeric GABA-gated chloride channels (Rdl) expressed in *Xenopus* oocytes (*7*, *8*) and that an analogue of fipronil antagonized the action of GABA in the ventral ganglia of *Drosophila* larvae (*9*). In addition, two types of related phenyl heterocyclic compounds (PHCs), that is, 1-phenyl- and 2-phenyl-1,2,3-triazoles, have been reported to block GABA-activated Cl^- currents in cell bodies isolated from locust metathoracic ganglia (*10*) and nematode somatic muscle cells (*11*), respectively. Fipronil was found to more potently inhibit the specific binding of [^{3}H]-4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB), a noncompetitive antagonist of ionotropic GABA receptors, to housefly head membranes than to mouse brain membranes (*12*). The low sensitivity of mammalian GABA receptors to fipronil was shown to result from the difference in subunit compositions between mammalian and insect receptors (*13*). 2-Phenylpyrimidines and -1,3-thiazines are also recognized as inhibitors of [^{3}H]EBOB binding to housefly head and mouse brain membranes (*14*). Moreover, 3-phenylpyrimidinones are thought to function in a similar manner (*15*). We previously demonstrated that a variety of five- and six-membered nitrogen-containing heterocyclic compounds bearing a 2,4,6-trisubstituted phenyl group interact with ionotropic GABA receptors (*16*). The quantitative structure−activity relationships (QSARs) of anti-GABAergic PHCs have not yet been completely elucidated, although the dipole moment of PHCs was hypothesized to be responsible for their insecticidal activity (*17*), and favorable and unfavorable moieties of PHCs have been predicted by three-dimensional (3D)-QSAR analysis (*18*).

The present study was undertaken to gain a better understanding of the interaction of PHCs with GABA receptors with the ultimate goal of contributing to the design of safe, novel insecticides. To this end, 1-phenyl-1*H*-1,2,3-triazoles with various substituents at the 4- and 5-positions of the triazole ring and on the phenyl ring (**Figure 1**) were synthesized and examined for their effects on [^{3}H]EBOB binding to housefly, rat, and human β3 GABA receptors. We report here the results of the QSAR analysis of the phenyltriazoles. A preliminary report of some of this work has been published previously in abstract form (*19*).

MATERIALS AND METHODS

General. ^{1}H NMR spectra were recorded on a JEOL JNM-A 400 spectrometer, and chemical shifts are given in parts per million relative to tetramethylsilane. Mass spectra were obtained on a Hitachi M80-B spectrometer. Melting points were determined using a Yanagimoto MP 500D apparatus and remain uncorrected. [*propyl*-2,3-^{3}H]EBOB (30.0 or 47.5 Ci/mmol) was purchased from Perkin-Elmer Life Sciences Japan, Co. Ltd. (Tokyo, Japan). Phenyltriazoles **13**−**15** were available from our earlier study (*16*). General chemicals were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

General Procedure for the Synthesis of 4(or 5)-Alkyl/phenyl-1-phenyl-1*H*-1,2,3-triazoles (Scheme 1). 2,6-Dichloro-4-trifluoromethylaniline (670 mg, 2.91 mmol) was added to a mixture of water (50 mL) and concentrated HCl (50 mL), and the mixture was stirred at room temperature for 30 min. Sodium nitrite (200 mg, 2.91 mmol) dissolved in water (5 mL) was added in a dropwise manner to the mixture, which was kept at 0−5 °C. After the mixture had been stirred for 30 min, sodium azide (190 mg, 2.91 mmol) dissolved in water (5m L) was added in a dropwise manner to the mixture, and the mixture was stirred overnight at room temperature. After the reaction was complete, the mixture was extracted with CH_2Cl_2 (50 mL × 2), and the extract was dried with sodium sulfate overnight. Then the CH_2Cl_2 was evaporated, and the residue was chromatographed on silica gel (*n*-hexane), giving 2,6-dichloro-4-trifluoromethylphenyl azide as a red oil. 2,6-Dichloro-4-trifluoromethylphenyl azide (412 mg, 1.61 mmol), an alkyne (1.61 mmol), and toluene (3 mL) were placed in a sealed tube and heated at 110 °C for 16 h. After the reaction, the mixture was partitioned between water and ether. The ether layer was dried with sodium sulfate overnight. The ether was evaporated, and the residue was subjected to silica gel column chromatography (*n*-hexane/EtOAc = 30:1), thus affording the 4-alkyl/phenyl and 5-alkyl/phenyl isomers of the desired compound. Compounds **1**−**12** and **24**−**33** were synthesized according to this method. The analytical data for the phenyltriazoles are available as Supporting Information.

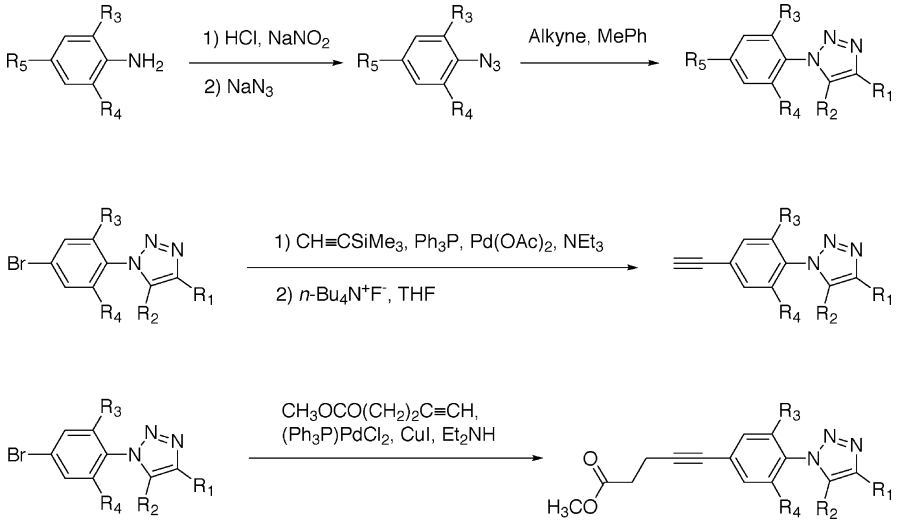
Procedure for the Synthesis of 4(or 5)-*n*-Butyl-1-[4-(4-methoxycarbonyl-1-butynyl)phenyl]-1*H*-1,2,3-triazoles (Scheme 1). A mixture of **28** (120 mg, 0.43 mmol), methyl 4-pentynoate (48 mg, 0.43 mmol), *trans*-dichlorobis(triphenylphosphine)palladium(II) (3.1 mg, 4.3 μmol), copper(I) iodide (0.41 mg, 2.2 μmol), and diethylamine (2.6 mL) was refluxed under a nitrogen atmosphere for 24 h. The diethylamine was evaporated, and the residue was partitioned between water and EtOAc. After the EtOAc layer had been dried with sodium sulfate, the solution was concentrated. The residue was subjected to silica gel column chromatography (*n*-hexane/EtOAc = 30:1) to afford **18** (117 mg, 87.5%) as brown crystals. Compounds **16**, **17**, and **19** were synthesized in a similar manner. The analytical data for **16**−**19** are available as Supporting Information.

Procedure for the Synthesis of 4(or 5)-*n*-Butyl-1-(4-ethynylphenyl)-1*H*-1,2,3-triazoles (Scheme 1). A mixture of **26** (114 mg, 0.33 mmol), triphenyl phosphine (4.5 mg, 0.017 mmol), palladium(II) acetate (1.8 mg, 8.2 μmol), (trimethylsilyl)acetylene (30 mg, 0.33 mmol), and triethylamine (5 mL) was heated at 90 °C under a nitrogen atmosphere for 14 h. The triethylamine was evaporated, and dry THF (5 mL) was added to the residue under a nitrogen atmosphere. After the solution had cooled to 10 °C, 1.0 M tetrabutylammonium fluoride in THF (0.5 mL) was added in a dropwise manner to the solution, with stirring under a nitrogen atmosphere. The solution was stirred at room temperature for 2 h. Then, the THF was evaporated, and the residue was partitioned between water and EtOAc. After the EtOAc layer had been dried with sodium sulfate, the solution was concentrated. The residue was subjected to silica gel column chromatography (*n*-hexane/EtOAc = 30:1) to give **20** (34.0 mg, 35.2%) as brown crystals. Compounds **21**−**23** were synthesized in a similar manner. The analytical data for **20**−**23** are available as Supporting Information.

Regioselective Synthesis of 1-(2,6-Dichloro-4-trifluoromethylphenyl)-4-phenyl-1*H*-1,2,3-triazole. This reaction was carried out according to the method of Rostovtsev et al. (*20*). Ethynylbenzene (240 mg, 2.3 mmol) and 2,6-dichloro-4-trifluoromethylphenyl azide (600 mg, 2.3 mmol) were suspended in a 1:1 mixture of water and *tert*-butyl alcohol (12 mL). Sodium ascorbate (230 μL of freshly prepared 1 M solution in water, 0.23 mmol) was added to the mixture, followed by copper(II) sulfate pentahydrate (5.8 mg, 0.023 mmol) in 77 μL of water. The resulting heterogeneous mixture was stirred vigorously overnight. The reaction mixture was diluted with water and cooled in an ice bath, and a light yellow precipitate was collected by filtration. The yellow precipitate was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:1) to afford 595 mg (99%) of a pure product as white crystals, which gave a ^{1}H NMR spectrum identical to that of **1** synthesized according to the method described above.

Generation of *Drosophila* Schneider 2 (S2) Cells Stably Expressing the Human GABA Receptor β3 Subunit Homo-oligomer. The cDNA encoding the β3 subunit of the human GABA receptor (accession number NM_000814) was amplified by PCR from first-strand cDNA derived from normal adult human brain tissue (BioChain). A *Kpn*I/*Apa*I fragment containing the 1.4 kb open reading frame of the β3 subunit gene (hsβ3) was inserted into the *Drosophila* expression vector pMT (Invitrogen), in which cDNA expression is driven by the

Scheme 1



metallothionein promoter and induced by copper sulfate. S2 cells were co-transfected with pMThsβ3 and the selection vector pCoHygro by a calcium phosphate transfection method, according to the manufacturer's instructions (Invitrogen, the DES kit). The transfected cells were maintained for ~3 weeks in selection medium containing hygromycin B. Antibiotic-resistant polyclonal cells were used for the binding assays described below.

Membrane Preparation and [^{3}H]EBOB Binding Assays. Housefly head membranes were prepared from adult houseflies (*Musca domestica* L.) of the WHO/SRS strain according to a modification of the method of Deng et al. (*21*). Housefly heads were homogenized in 10 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose (buffer A) with a glass−Teflon homogenizer; then, the homogenate was filtered through four layers of 64-μm mesh nylon screen and centrifuged at 500*g* for 5 min. The supernatant was filtered through four layers of 64-μm mesh nylon screen and centrifuged at 25000*g* for 30 min. The pellets were suspended in buffer A and were kept on an ice bath for 30 min. The suspension was centrifuged at 25000*g* for 30 min. The pellets were resuspended in 10 mM sodium phosphate buffer, pH 7.5, containing 0.3 M NaCl (buffer B) and were used immediately for the binding assays.

Rat brain membranes were prepared from 5-week-old male Wistar rats according to a modification of the method of Squires et al. (*22*). Forebrains stored at −80 °C were thawed and homogenized in ice-cold 1 mM EDTA using a glass−Teflon homogenizer. The homogenate was centrifuged at 1000*g* for 10 min. The supernatant was then centrifuged at 25000*g* for 30 min. The resulting pellets were suspended in 1 mM EDTA and dialyzed three times (2 h each) in cellophane tubing against 2 L of distilled, deionized water at 4 °C. After dialysis, the inner solution was centrifuged at 25000*g* for 30 min, and the pellets were stored at −80 °C until use.

S2 cells expressing human homo-oligomeric β3 receptors were grown at 28 °C in Schneider's *Drosophila* medium (Gibco) containing 10% fetal bovine serum (Gibco), using a shaking incubator at 50 rpm. Schneider's *Drosophila* medium was supplemented with hygromycin B at a concentration of 300 μg/mL. S2 cells were grown at a viability of >96% and a density of 6−20 × 10^6 cells/mL of culture medium for ~3−4 days. Copper sulfate was added to the medium at a concentration of 500 μM/mL 24 h before the preparation of protein. S2 cell-containing Schneider's *Drosophila* medium was centrifuged at 1000*g* for 5 min, and after the supernatant had been discarded, the cells were washed with buffer A. The pellets were resuspended in buffer A and centrifuged at 1000*g* for 5 min, and after the supernatant had been discarded, the pellets were washed with buffer A. The membranes were prepared by homogenizing the pelleted cells in buffer A with a glass−Teflon homogenizer. The supernatant was centrifuged at 25000*g* for 20 min at 4 °C. The resulting pellets were superficially washed with buffer B, suspended in buffer B, and used immediately for the binding assays.

[^{3}H]EBOB binding assays were carried out according to the methods of Deng et al. (*21*) and Cole and Casida (*23*). Housefly head membranes (200 μg of protein), cell membranes expressing human β3 receptors (50 μg of protein), or rat brain membranes (125 μg of protein) were incubated with various concentrations of phenyltriazoles and 0.5 nM [^{3}H]EBOB in 1 mL of buffer B at 22 °C for 70 min (housefly and human β3 receptors) or at 37 °C for 90 min (rat receptor). The protein content was measured according to the method of Bradford (*24*). After incubation, the mixture was filtered through Whatman GF/B filters and rapidly rinsed twice with 5 mL of cold (10 °C) buffer B using a Brandel M-24 cell harvester. The radioactivity of [^{3}H]EBOB that had specifically bound to the membranes on the filters was measured with a liquid scintillation counter. Nonspecific binding was determined in the presence of 5 μM unlabeled EBOB. Experiments were repeated at least three times. The IC_{50} values were calculated from the mean values using the standard probit method. Scatchard analysis was performed using 1.0 nM [^{3}H]EBOB and various concentrations of cold EBOB. Experiments were repeated five times.

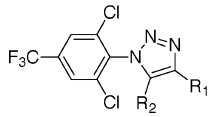
Molecular Modeling and 3D-QSAR Studies. All computations were carried out using the molecular modeling software SYBYL, ver. 6.9 (Tripos). The structures of all phenyltriazoles were constructed on the basis of the X-ray crystal structure of **9** and were fully optimized by following the semiempirical molecular orbital method AM1. Comparative molecular field analysis (CoMFA) was used to study the 3D-QSAR of phenyltriazoles in the human β3 and housefly GABA receptors. The molecules were superimposed at the 1-, 2-, 3-, 4-, and 5-positions of the triazole ring by the Fit Atoms menu of SYBYL for CoMFA.

Insecticidal Assays. Insecticidal activity was determined by the topical application of acetone (1 μL) containing various concentrations of phenyltriazoles on the dorsal surface on the thorax of adult female houseflies (*M. domestica* L., WHO/SRS strain, 3−5 days old after emergence). Thirty flies were used for each dosage. An acetone solution (1 μL) of piperonyl butoxide (10 μg), a cytochrome P450 inhibitor, was applied topically to each fly 1 h before the application of the phenyltriazoles. The flies were supplied with sugar and water and were kept at 25 °C. The rate of mortality was determined after 24 h. Experiments were repeated three times. The LD_{50} values were calculated from the mean values using the standard probit method.

RESULTS

Synthesis of 1-Phenyl-1*H*-1,2,3-triazoles. A total of 30 1-phenyl-1*H*-1,2,3-triazoles (**1**−**12** and **16**−**33** in **Tables 1** and **2**) were synthesized in the present study. The 1,3-dipolar cycloaddition between an alkyne and a phenyl azide gave a mixture of *anti*- and *syn*-triazole regioisomers, that is, 4-substituted- and 5-substituted-1-phenyl-1*H*-1,2,3-triazoles. The *anti*-

Table 1. Potencies of 1-(2,6-Dichloro-4-trifluoromethylphenyl)-4(or 5)-(alkyl or phenyl)-1*H*-1,2,3-triazoles in Terms of the Inhibition of [^{3}H]EBOB Binding to Human β3, Housefly, and Rat GABA Receptors



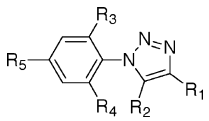
			[^{3}H]EBOB binding, IC_{50} (nM)				
compd	R_1	R_2	human β3	housefly	rat	RS[a]	insecticidal activity, LD_{50} (pmol/fly)
1	Ph	H	154 (101–242[b])	183 (110–309[b])	>10000 (22.7[c])	>55	7110 (5490–9290[b])
2	H	Ph	570 (362–925[b])	1270 (734–2420[b])	>10000 (32.1[c])	>8	
3	*n*-Hex	H	470 (340–661[b])	1060 (616–1980[b])	8500 (7020–10400[b])	8	
4	H	*n*-Hex	1040 (780–1410[b])	>10000 (43.8[c])	>10000 (29.5[c])		
5	*n*-Pen	H	33.4 (22.4–47.2[b])	192 (115–328[b])	4310 (3550–5250[b])	22	3990 (3280–4840[b])
6	H	*n*-Pen	1670 (1200–2380[b])	5070 (3220–9020[b])	>10000 (19.1[c])	>2	
7	*n*-Bu	H	3.35 (2.56–4.37[b])	40.0 (25.0–66.0[b])	1660 (1230–2220[b])	42	1930 (1280–3050[b])
8	H	*n*-Bu	>10000 (49.9[c])	1630 (1180–2240[b])	>10000 (12.0[c])	>6	
9	*t*-Bu	H	0.997 (0.777–1.297[b])	9.90 (5.59–16.40[b])	643 (503–816[b])	65	1.86 (0.69–3.95[b])
10	H	*t*-Bu	>10000 (49.6[c])	>10000 (46.0[c])	>10000 (9.0[c])		
11	*n*-Pr	H	2.57 (2.03–3.24[b])	9.04 (5.49–14.01[b])	730 (498–1198[b])	81	8.66 (1.29–11.67[b])
12	H	*n*-Pr	909 (528–1440[b])	1060 (640–1910[b])	>10000 (12.1[c])	>9	
13	c-Hex	H	14.1 (10.7–18.3[b])	35.4[d] (20.9–60.0[b])	6560[d] (4490–9580[b])	185	76.9 (57.7–101.7[b])
14	$(CH_2)_3Cl$	H	1.96 (1.43–2.69[b])	27.6[d] (17.0–44.9[b])	1770[d] (1360–2320[b])	64	
15	Ph	NH_2	24.4 (16.0–41.9[b])	34.9[d] (25.9–47.0[b])	1090[d] (840–1410[b])	31	

[a] Selectivity for housefly versus rat receptor ($IC_{50}^{rat}/IC_{50}^{fly}$). [b] Ninety-five percent confidence limits. [c] Inhibition (percent) at 10 μM. [d] Reference *16*.

isomers were obtained in yields that were 1.1−3.2-fold higher than those of the *syn*-isomers. The regioisomers were separated by silica gel column chromatography. The regiochemistry of 1-(2,6-dichlorophenyl)-1*H*-1,2,3-triazoles was established on the basis of an X-ray crystallographic analysis of **9** (**Figure 2** and Supporting Information). The regiochemistry of the 1-(2,6-unsubstituted phenyl)-1*H*-1,2,3-triazoles was determined by NOE observation between the ortho-protons of the phenyl group and the 1-protons of the *n*-butyl group of the *syn*-isomers. The ^{1}H NMR spectra showed that the triazole proton of the 4-substituted 1-(2,6-unsubstituted phenyl)triazoles was shifted downfield compared to that of the corresponding 1,5-substituted triazoles, as has been reported for other triazoles (*25*−*28*). In contrast, the opposite relationship was observed in the case of 1-(2,6-dichlorophenyl)-1*H*-1,2,3-triazoles; that is, the 1,4-substituted triazoles had a triazole signal that was upfield of that of the corresponding 1,5-substituted triazoles. The only exceptions were observed with **1** and **2**, which produced the triazole proton signals at almost the same chemical shift. The regiochemistry of **1** (*anti*) and **2** (*syn*) was determined on the basis of the fact that copper(I)-catalyzed regioselective cycloaddition (*20*) gave **1** and that the ortho-protons of the 4-phenyl group of **1** were shifted downfield compared to those of the 5-phenyl group of **2**, as are the protons of the alkyl groups of other 4- or 5-substituted triazoles. Phenyltriazoles with an ethynyl or a 4-methoxycarbonyl-1-butynyl group at the 4-position of the phenyl group were prepared by palladium-catalyzed cross-coupling between the corresponding bromo analogues and terminal alkynes.

Effects of the 4- and 5-Substituents of 1-Phenyl-1*H*-1,2,3-triazoles on Interaction with GABA Receptors. Using [^{3}H]-EBOB, a specific radioligand that binds to the noncompetitive antagonist binding site of GABA receptors (*21*, *23*), a series of phenyltriazoles were assayed to examine the effects of a phenyl or an alkyl group at the 4- or 5-position of the triazole moiety upon interaction with GABA-gated chloride channels. **Table 1** lists the respective potencies of 1-(2,6-dichloro-4-trifluoromethylphenyl)-1*H*-1,2,3-triazoles in terms of their ability to inhibit specific [^{3}H]EBOB binding to human β3, housefly, and rat GABA receptors. Comparison of the 4- and 5-substituted isomers of the phenyltriazoles revealed that the potencies of the former isomers were generally higher than those of the latter isomers. Among this series of compounds (**1**−**15**), **11** and **9** were found to be the most potent in the nanomolar range in the housefly and human β3 receptors, followed by **14**, **13**, **15**, and **7** and then by **5** and **1**. On the basis of the IC_{50}^{fly} values, the rank order of potency with respect to the 4-substituent was as follows: *t*-Bu, *n*-Pr > $(CH_2)_3Cl$, c-Hex, *n*-Bu > *n*-Pen, Ph > *n*-Hex. Replacement of the terminal methyl group of the 4-*n*-butyl group of **7** with a chlorine atom to produce **14** did not result in a marked change in potency. The introduction of an amino group into the 5-position of **1** led to a >5-fold increase

Table 2. Potencies of 1-(Substituted phenyl)-4(or 5)-*n*-butyl-1*H*-1,2,3-triazoles in Terms of the Inhibition of [^{3}H]EBOB Binding to Human β3, Housefly, and Rat GABA Receptors



					[^{3}H]EBOB binding, IC_{50} (nM)				
compd	R_1	R_2	R_3/R_4	R_5	human β3	housefly	rat	RS[b]	insecticidal activity, LD_{50} (nmol/fly)
16	*n*-Bu	H	Cl	MCB[a]	>10000 (19.3[d])	>10000 (8.9[d])	>10000 (20.1[d])		
17	H	*n*-Bu	Cl	MCB	>10000 (43.8[d])	>10000 (16.2[d])	>10000 (3.2[d])		
18	*n*-Bu	H	H	MCB	>10000 (34.7[d])	>10000 (9.4[d])	>10000 (47.0[d])		
19	H	*n*-Bu	H	MCB	>10000 (22.0[d])	>10000 (–0.6[d])	>10000 (–1.3[d])		
20	*n*-Bu	H	Cl	C≡CH	41.6 (28.6–58.2[c])	670 (500–920[c])	>10000 (42.3[d])	15	
21	H	*n*-Bu	Cl	C≡CH	>10000 (12.0[d])	>10000 (28.5[d])	>10000 (10.6[d])		
22	*n*-Bu	H	H	C≡CH	>10000 (47.4[d])	>10000 (29.4[d])	4060 (2900–6020[c])		
23	H	*n*-Bu	H	C≡CH	9100 (7130–13540[c])	>10000 (8.5[d])	≈10000 (52.5[d])		
24	*n*-Bu	H	Cl	Cl	98.7 (65.0–138.8[c])	480 (340–660[c])	>10000 (48.2[d])	>20	65.1 (41.1–120.7[c])
25	H	*n*-Bu	Cl	Cl	4900 (4020–6160[c])	>10000 (37.9[d])	>10000 (24.5[d])		
26	*n*-Bu	H	Cl	Br	37.4 (25.7–52.3[c])	290 (200–420[c])	>10000 (48.1[d])	>34	
27	H	*n*-Bu	Cl	Br	940 (607–1521[c])	>10000 (49.5[d])	>10000 (18.6[d])		
28	*n*-Bu	H	H	Br	3830 (2690–5770[c])	>10000 (29.2[d])	>10000 (32.5[d])		
29	H	*n*-Bu	H	Br	3280 (2720–3970[c])	>10000 (22.1[d])	>10000 (26.5[d])		
30	*n*-Bu	H	H	CF_3	≈10000 (51.1[d])	>10000 (21.1[d])	>10000 (32.2[d])		
31	H	*n*-Bu	H	CF_3	6360 (5230–7950[c])	>10000 (12.2[d])	>10000 (11.3[d])		
32	*n*-Bu	H	Cl	H	4220 (2540–8030[c])	>10000 (17.1[d])	>10000 (18.1[d])		
33	H	*n*-Bu	Cl	H	2460 (1680–3790[c])	>10000 (3.4[d])	>10000 (11.9[d])		

[a] MCB, $C{\equiv}C(CH_2)_2CO_2CH_3$. [b] Selectivity for housefly versus rat receptor ($IC_{50}^{rat}/IC_{50}^{fly}$). [c] Ninety-five percent confidence limits. [d] Inhibition (percent) at 10 μM.

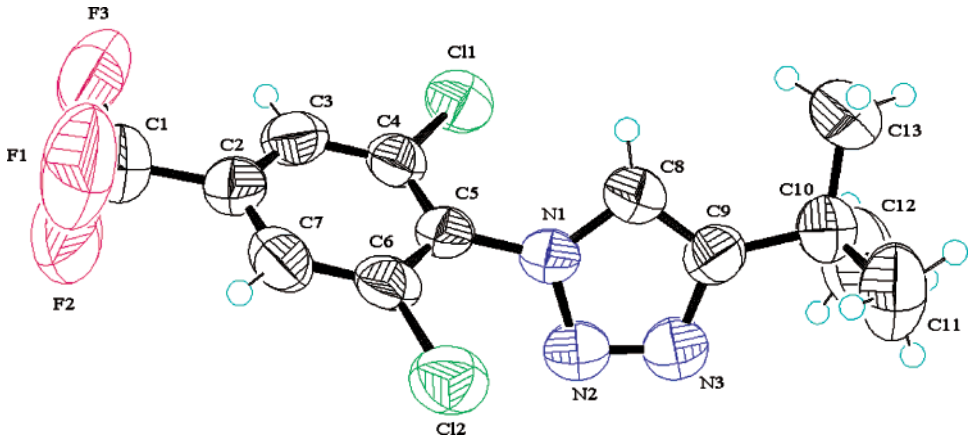


Figure 2. X-ray crystal structure of **9**.

in potency in the case of all three receptors, as seen with **15**. Most compounds remained basically inactive (IC_{50} > 10 μM) in the rat receptor or were much less active than in the case of other receptors. Even the most potent inhibitors (**11** and **9**) had IC_{50} values of several hundred nanomolar, and therefore it was concluded that these compounds exhibited high selectivity ($IC_{50}^{rat}/IC_{50}^{fly}$) for the housefly versus the rat GABA receptor (**Table 1**). **Figure 3** shows the concentration−inhibition curves of these selectively active phenyltriazoles. Compound **13** was found to be the most selective (185-fold) analogue in this regard,

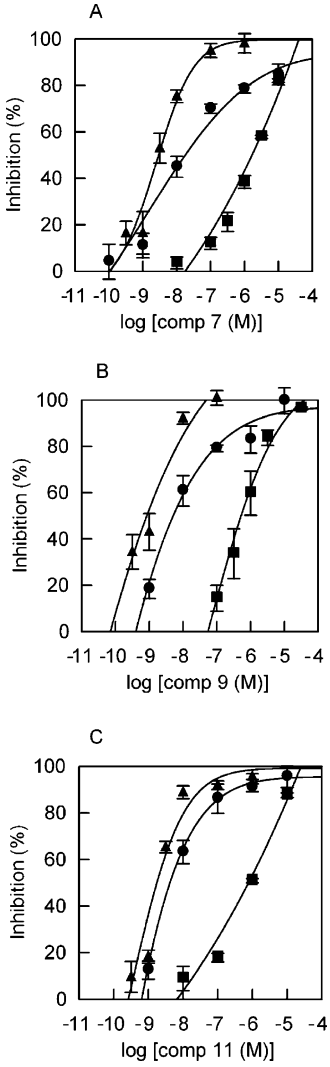


Figure 3. Dose–response curves of **7** (**A**), **9** (**B**), and **11** (**C**) in inhibiting [^{3}H]EBOB binding to rat (■), housefly (●), and human β3 (▲) GABA receptors. The data are the mean ± standard deviation for at least three experiments, each performed in duplicate.

as previously reported (*16*). To determine whether phenyltriazoles bind to the same site as that for EBOB binding, a Scatchard analysis of [^{3}H]EBOB binding to the cloned human β3 receptor was carried out in the presence of **9**. The two lines obtained in the presence and absence of **9** intersected near the abscissa axis, thus indicating that the two compounds do indeed bind to the same site (**Figure 4**).

Effects of Substituents of the Phenyl Moiety of 1-Phenyl-1*H*-1,2,3-triazoles on Interaction with GABA Receptors. The effects of the substituents of the 1-phenyl group on specific [^{3}H]-EBOB binding were examined. The substituents on the phenyl ring exerted marked effects on the potency of triazoles in the inhibition of EBOB binding to the human β3, housefly, and rat receptors (**Table 2**). Among the 4(or 5)-*n*-butyl-1-phenyl-1*H*-1,2,3-triazoles (**16**−**33**) with substitution patterns other than the 2,6-dichloro-4-trifluoromethyl substitution on the phenyl group, **20**, **24**, and **26** were the only compounds found to be active in the case of both human β3 and housefly GABA receptors. Compounds **23**, **25**, **27**−**29**, and **31**−**33** showed marginal activity in the case of the human β3 receptor, whereas they were almost inactive in the case of the housefly and rat receptors. 1-(4-Monosubstituted phenyl)-4(or 5)-*n*-butyl-1*H*-1,2,3-triazoles **22**, **18**, and **23** exhibited higher activity in the case of the rat GABA receptor than in case of the housefly receptor, although this activity was very low. Comparison of **20**, **22**, **24**, **26**, **28**, **30**, and **32** with **7** demonstrated the importance of both the *p*-CF_3 group and the two *o*-chlorine atoms; the rank order of potency regarding the phenyl substitution was 2,6-Cl_2-4-CF_3 > 2,6-Cl_2-4-Br > 2,4,6-Cl_3 ≥ 2,6-Cl_2-4-C≡CH. Compounds with a 4-(4-methoxycarbonyl-1-butynyl) group on the phenyl group exhibited no activity.

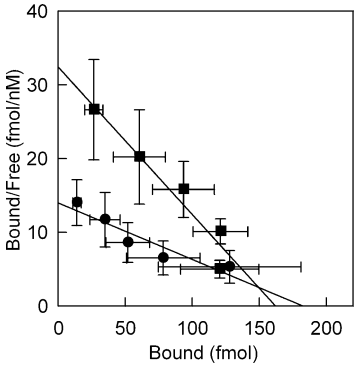


Figure 4. Scatchard plots of [^{3}H]EBOB binding to the cloned human β3 receptor in the presence (●) and absence (■) of 1 nM **9**. The amount of specifically bound [^{3}H]EBOB is expressed as fmol/0.05 mg of protein. B_{max} = 3.26 pmol/mg of protein, K_d = 5.00 nM in the absence of **9**. B_{max} = 3.64 pmol/mg of protein, K_d = 13.1 nM in the presence of 1 nM **9**. The data are the mean ± standard deviation for five experiments each performed in duplicate.

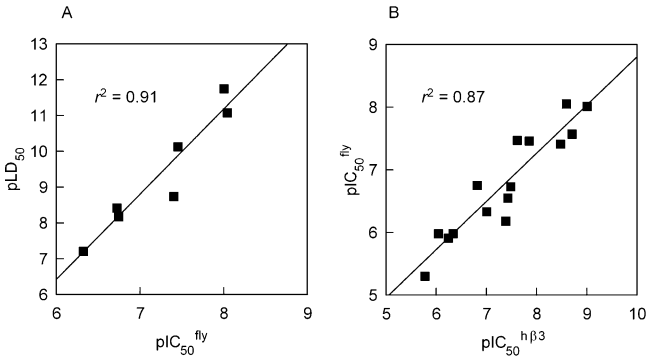


Figure 5. Correlation between the inhibitory activity of selected 1-phenyl-1*H*-1,2,3-triazoles in terms of the inhibition of [^{3}H]EBOB binding to the housefly GABA receptor and their insecticidal activity (**A**) and correlation between the inhibitory potencies of selected 1-phenyl-1*H*-1,2,3-triazoles in the inhibition of [^{3}H]EBOB binding to human β3 and housefly GABA receptors (**B**).

Insecticidal Activity. To test the validity of [^{3}H]EBOB binding as an in vitro assay, 1-phenyl-1*H*-1,2,3-triazoles with a wide range of potency were selected and examined for their in vivo potency, that is, insecticidal activity against houseflies pretreated with piperonyl butoxide. Compound **9** showed the highest insecticidal activity among the tested compounds, with an LD_{50} value of 1.86 pmol/fly. The plot of pLD_{50} values (log-[1/LD_{50} (mol)]) versus pIC_{50}^{fly} values (log[1/IC_{50}^{fly} (M)]) of seven compounds (**1**, **5**, **7**, **9**, **11**, **13**, and **24**) gave a high correlation (r^2 = 0.91) (**Figure 5A**). This finding indicated that the interaction of phenyltriazoles with the housefly GABA receptor leads to significant insecticidal activity.

Relationship between the Ability of 1-Phenyl-1*H*-1,2,3-triazoles To Inhibit the Binding of [^{3}H]EBOB to Human β3 and Housefly GABA Receptors. To validate the relevance of the human β3 receptor as a model of the housefly receptor, we plotted the potencies (pIC_{50} or log[1/IC_{50} (M)]) of 15 triazoles (**1**−**3**, **4**, **6**, **7**, **9**, **11**−**15**, **20**, **24**, and **26**) in inhibiting [^{3}H]EBOB

Table 3. CoMFA Results of 1-Phenyl-1*H*-1,2,3-triazoles: $\log(1/IC_{50}) = a +$ [CoMFA Field Terms]

							cross-validated		relative contribution	
model	*a*	*C*[a]	*n*	*s*	r^2	$F_{c,n-c-1}$	s_{press}	q^2	steric	electrostatic
model for housefly receptor	6.608	4	16	0.192	0.962	70.546	0.558	0.684	0.679	0.321
model for β3 receptor	5.199	5	24	0.282	0.960	85.850	0.845	0.638	0.551	0.449

[a] Optimum component.

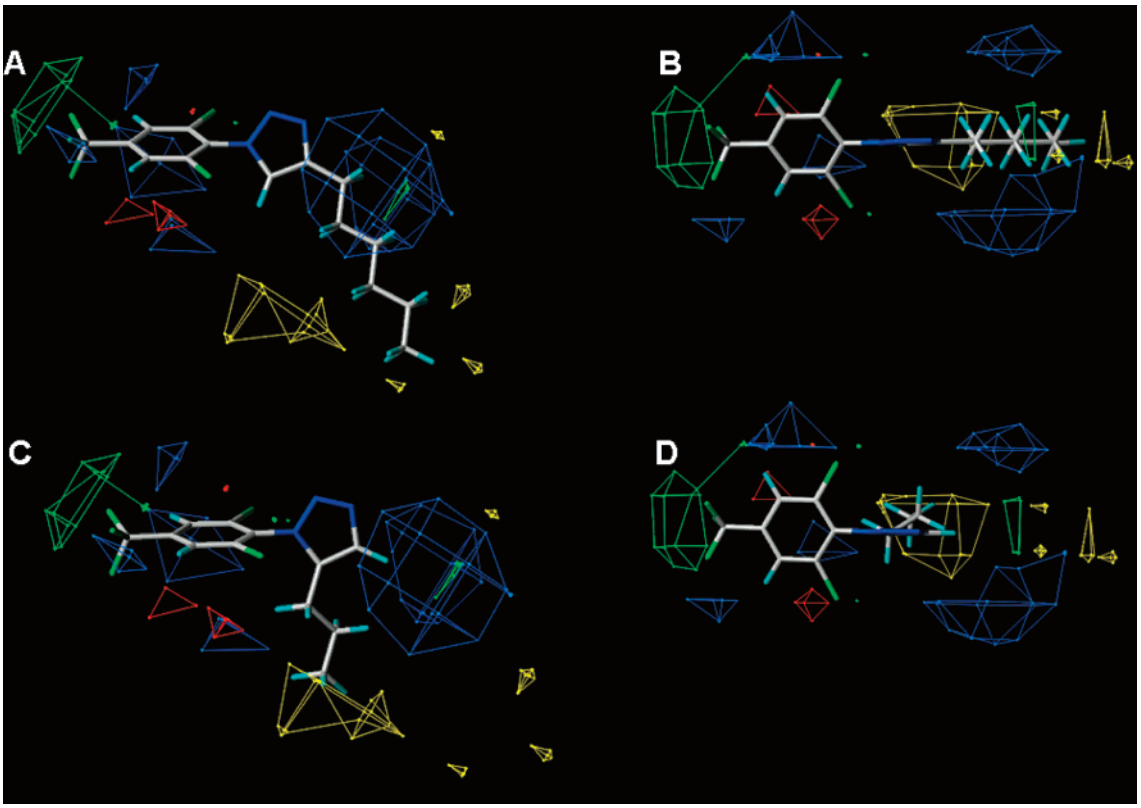


Figure 6. CoMFA contour maps showing the results of analyses of the human β3 GABA receptor: (**A**, **B**) orthogonal views of maps with **3**; (**C**, **D**) orthogonal views of maps with **12**.

binding obtained using membranes from human β3 receptor-expressing S2 cells against those obtained using housefly head membranes (**Figure 5B**). This plot yielded a good correlation ($r^2 = 0.87$), indicating a similarity in ligand recognition between the two receptors.

Molecular Modeling and 3D-QSAR. To obtain quantitative information regarding the steric and electrostatic requirements for the effects of 1-phenyl-1*H*-1,2,3-phenyltriazoles on the human β3 and housefly receptors, 3D-QSAR analyses were performed using the CoMFA module in the SYBYL program. The results are summarized in **Table 3**. For the human β3 and housefly receptors, respectively, 24 and 16 active phenyltriazoles were successfully included in the CoMFA analysis. The squared cross-validated correlation coefficient (q^2) values were 0.638 and 0.684 for the human β3 and housefly receptor models, respectively, and these q^2 values indicate the validity of the suggested mode of interaction [i.e., a q^2 value of $\geq$0.25 corresponds to the conventional 95% confidence interval for the probability of a chance correlation (*29*)].

Figures 6 and **7** show the contour maps of the CoMFA analysis of the human β3 and housefly receptors, respectively. The green contours indicate sterically favorable regions that enhance the affinity for receptors, whereas the yellow contours are sterically unfavorable regions. The blue contours indicate regions in which positively charged moieties of a ligand increase receptor affinity, and the red contours represent regions in which negatively charged moieties of a ligand increase receptor affinity. Both the human β3 and housefly receptor models provided analogous contour maps, except that the para-substituent of the phenyl group was surrounded by a red contour in the housefly receptor model, whereas in the case of the human β3 receptor model, the red contour was replaced with a green contour. That is, the housefly receptor probably recognizes the electronegativity of the para-substituent, whereas the human β3 receptor might recognize it as a sterically fitting substituent. The presence of the green and blue contours around the 4-position of the triazole ring in both human β3 and housefly receptor models displays a binding site area in which a substituent of a high-affinity ligand is accommodated. The blue contours indicate that lipophilic interactions between the binding site and ligands enhance the antagonist activity. These findings are consistent with the results showing that the optimal substituent of the triazole ring is a *tert*-butyl or *n*-propyl group at the 4-position. The longer alkyl chains at the 4- and 5-positions of the triazole ring sterically hinder binding as demonstrated by the yellow contours.

DISCUSSION

In the present study, we synthesized 30 1-phenyl-1*H*-1,2,3-triazoles and performed [^{3}H]EBOB binding assays to evaluate

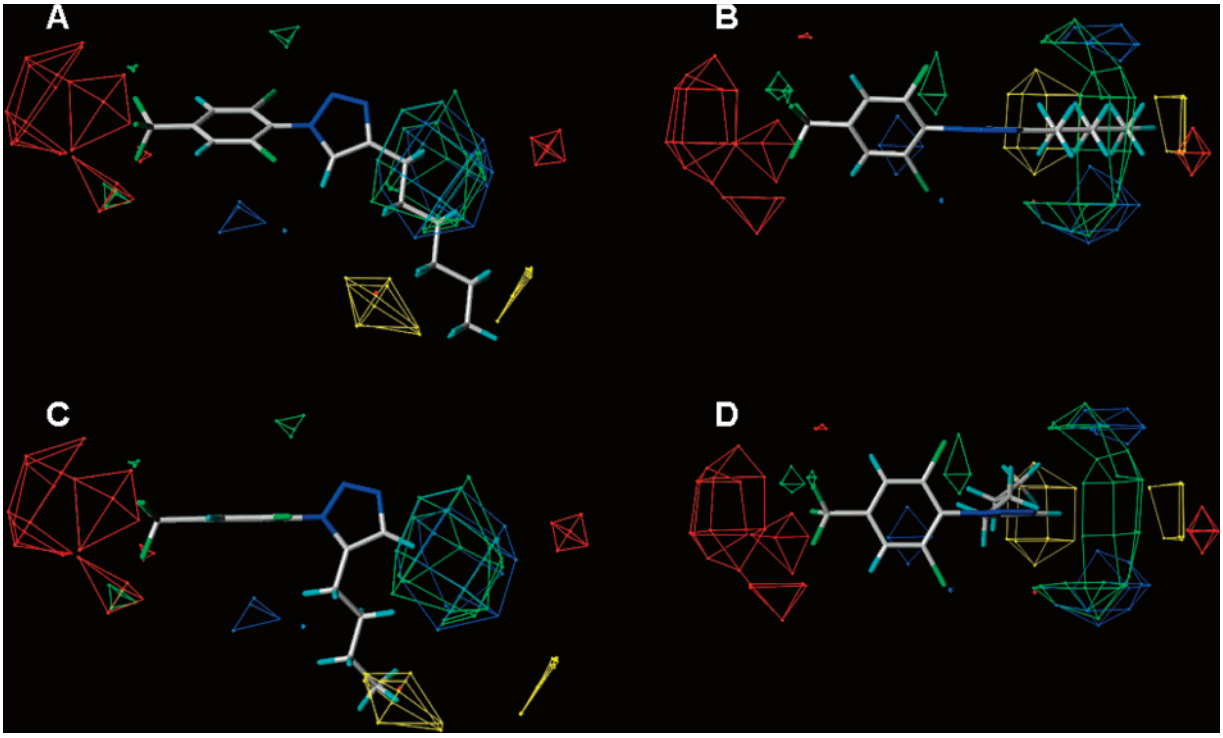


Figure 7. CoMFA contour maps showing the results of analyses of the housefly GABA receptor: (**A**, **B**) orthogonal views of maps with **3**; (**C**, **D**) orthogonal views of maps with **8**.

the potency of synthesized compounds at GABA receptors. We used three receptor sources, that is, housefly head and rat brain membranes, and S2 cell membranes stably expressing the β3 subunit homo-oligomer of the human GABA receptor, to study the structure−activity relationships of phenyltriazoles and to probe the structure of the binding site. The human β3 receptor was used as a model receptor, and it should be noted that no such homo-oligomeric channel has been identified in the brain (*30*). The rat β3 subunit expressed in HEK293 cells has been reported to form a high-affinity site for the noncompetitive GABA antagonist *tert*-butylbicyclophosphorothionate (*31*). The murine β3 subunit was shown to form a functional but spontaneously opening homo-oligomeric channel when expressed in *Xenopus laevis* oocytes (*32*). Moreover, it has been reported that the human β3 subunit homo-oligomer contained a binding site for [^{3}H]EBOB (*13*), and the human β3 subunit homomer resembled the housefly GABA receptor in terms of its sensitivity to and selectivity for insecticides (*33*). The present study included the human β3 receptor to determine whether this interpretation of the data is just as applicable in the case of phenyltriazoles. The plot of $IC_{50}^{h\beta3}$ versus IC_{50}^{fly} values of phenyltriazoles gave a good correlation, although the human β3 receptor was more sensitive to phenyltriazoles than the housefly receptor, thus supporting the structural similarity between the binding sites of these two receptors. To further confirm the finding, we performed a 3D-QSAR analysis based on $IC_{50}^{h\beta3}$ and IC_{50}^{fly} values. The CoMFA analysis revealed not only a similarity but also a difference between the two receptors in terms of the mode of recognition of ligands. The electronegativity of the para-substituent of the 1-phenyl group is more important in the housefly receptor than in the human β3 receptor; the para-substituent is preferably recognized as a sterically favorable substituent in the case of the human β3 receptor. GABA receptors are known to be formed by five 4-transmembrane subunits. The 2′ amino acid residue in the channel pore-lining α-helix domain (i.e., the second transmembrane domain, TM2) of GABA receptor subunits is thought to

```
                    2′  6′
Housefly Rdl        PARVALGVTTVLTMTTLMSST
Human ß3            AARVALGITTVLTMTTINTHL
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Figure 8. Amino acid residues of the second transmembrane segment of housefly Rdl and human β3 subunits. Pore-facing amino acids are underlined. Accession numbers: housefly Rdl subunit, AB177547; human β3 subunit, NM_000814.

be involved in the formation of the noncompetitive antagonist binding site (*34*−*36*). The only difference between the housefly Rdl and human β3 subunits in terms of the sequence of this region is the 5′ amino acid (Val versus Ile) adjacent to the pore-facing 6′ amino acid (**Figure 8**). The difference in ligand recognition is very unlikely to be explained solely as a result of this amino acid difference. Nevertheless, the good correlation observed here between $IC_{50}^{h\beta3}$ and IC_{50}^{fly} values suggested that the β3 receptor would be a useful tool for screening compounds acting at the housefly GABA receptor.

Some 4-substituted phenyltriazoles were found to be potent inhibitors of [^{3}H]EBOB binding to GABA receptors. Scatchard analysis using **9** showed that the phenyltriazoles and EBOB bind to the same site, at least in the case of the β3 receptor. The compounds that most potently inhibited [^{3}H]EBOB binding to the housefly receptor were the 4-*n*-propyl (**11**) and 4-*tert*-butyl (**9**) analogues of 1-(2,6-dichloro-4-trifluoromethylphenyl)-1*H*-1,2,3-triazole. In addition to these compounds, **7** and **14** strongly inhibited EBOB binding to the human β3 receptor. The requirement of a small hydrophobic substituent at the 4-position of the triazole moiety for high activity was clearly observed, as shown by the green and blue contours in the CoMFA maps (**Figures 6** and **7**). The requirement of a similar hydrophobic moiety has been reported in several studies of different GABA antagonists: 4-alkyl-1-phenylpyrazoles (*37*), 5-alkyl-2-(4-ethynylphenyl)-1,3-dithianes (*38*), 4-alkyl-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octanes (*39*), and 4-alkyl-1-phospha-2,6,7-trioxabicyclo[2.2.2]octane 1-sulfides (*40*). The bulky alkyl group of C2−C3 in length gave high activity. The putative common

hydrophobic moiety has been observed, even in naturally occurring terpenoid GABA antagonists (*41*). The IC_{50} values of **11** and **9** in housefly (9.04 and 9.90 nM, respectively) and human β3 (2.57 and 0.997 nM, respectively) receptors were comparable to the reported IC_{50} values of fipronil in the case of the housefly (2.3 or 6.3 nM) and human β3 (2.4 or 3.1 nM) receptors (*12*, *13*, *42*, *43*) as well as those of the 4-*tert*-butyl and 4-isopropyl analogues of fipronil in the housefly (2.4 and 5.3 nM, respectively) and human β3 (1.8 and 1.9 nM, respectively) receptors (*37*). On the other hand, the IC_{50} values of **11** and **9** in the case of the rat receptor (732 and 643 nM, respectively) were smaller than the reported IC_{50} values of fipronil for the mouse (4300 or 1010 nM) (*12*, *42*) or native human brain receptors (2470 nM) (*13*). In our similar [^{3}H]EBOB assay, the IC_{50} values of fipronil for the housefly and rat receptors were 2.43 and 728 nM, respectively, and thus the selectivity ratio was 299 (Ozoe, unpublished data). Consequently, **11** and **9** resulted in lower housefly versus rat GABA receptor selectivity (81- and 65-fold, respectively) than fipronil. Compound **13** showed the highest selectivity (185-fold) among the phenyltriazoles tested.

The activity of the 4-*n*-butyl-1-phenyl-1*H*-1,2,3-triazoles with a variety of one, two, or three substituents on the benzene ring was examined to gain a better understanding of the significance of the 2,6-dichloro-4-trifluoromethylphenyl group in achieving high levels of activity (**Table 2**). Most of the 1-(4-monosubstituted phenyl)triazoles, including the 4-trifluoromethylphenyl analogue (*30*) (or the didechloro analogue of **7**), exhibited almost no activity in the case of the housefly and rat receptors, whereas four of these triazoles exhibited low activity in the case of the human β3 receptor. It was of note that the 4-ethynyl, 4-bromo, and 4-(4-methoxycarbonyl-1-butynyl) substituents on the benzene ring conferred high potency in the case of other types of GABA antagonists such as 5-phenyl-1,3-dithianes (*38*) and 1-phenyl-2,6,7-trioxabicyclo[2.2.2]octanes (*39*, *44*), but not in the case of the 1-phenyl-1*H*-1,2,3-triazoles. It is also worth noting that removal of the 4-trifluoromethyl group from **7** led to an inactive analogue (**32**) in the case of the housefly and rat receptors, although the resulting analogue retained low activity in the case of the human β3 receptor. The combination of the 4-trifluoromethyl and 2,6-dichloro substitutions was found to be essential for attaining high levels of activity. Likewise, 2,6-dichlorophenyl analogues with the 4-bromo and 4-chloro substituents were potent inhibitors in the housefly and human β3 receptors; the potency of these inhibitors decreased in the order CF_3 > Br > Cl. From analyses based on a wide variety of 4-alkyl-1-phenylpyrazoles (*37*), phenyltriazoles, and related compounds seen in patents, it can be speculated that the 2,4,6-trisubstituted phenyl pattern plays an important role in both the in vivo and in vitro potency of this type of GABA antagonists and insecticides. The 5-amino group of fipronil-type pyrazoles interacts with the 2- or 6-substituent on the phenyl group to force the rings twist out of plane, consequently rendering the energetically favorable, orthogonal arrangement (*37*). According to our calculation, fipronil had a dihedral angle of 100.6° formed by the two rings. Moreover, in the case of the 1-phenyl-1*H*-1,2,3-triazoles, the *o*-chlorine atoms on the phenyl group and the 5-substituent on the triazole ring greatly affected the dihedral angle between the planes of the aromatic rings. Molecular orbital calculations showed that the analogues with a 1-(2,6-dichlorophenyl) group and a 5-substituent have dihedral angles of 82.9 $\pm$ 7.8° (n = 11); those with a 1-(2,6-dichlorophenyl) group and a 4-substituent have dihedral angles of 59.4 $\pm$ 1.3° (n = 13); those with the 1-(unsubstituted phenyl) and a 5-substituent have dihedral angles of 45.4 $\pm$ 4.1° (n = 4); and those with the 1-(unsubstituted phenyl) and a 4-substituent have dihedral angles of 23.1 $\pm$ 1.4° (n = 4). Compound **15**, bearing 4-amino and 4-phenyl groups, had a dihedral angle of 66.1°. In phenyltriazoles lacking the *o*-chlorine atoms on the phenyl group and the 5-substituent, the two rings were almost coplanar, whereas in phenyltriazoles containing those substituents, the rings were almost perpendicular. A group of 4-substituted 1-(2,6-dichlorophenyl)-1*H*-1,2,3-triazoles, to which most active triazoles belong, adopted intermediate dihedral angles. In addition to an appropriate 4-substituent of the triazole ring, the angle created by the two rings may also play an important role in the potency and selectivity of 4-substituted 1-phenyl-1*H*-1,2,3-triazoles.

The 4-ethynyl-2,6-dichloro substitution on the benzene ring was not effective at increasing the activity of the 4-alkyl-1-phenyl-1*H*-1,2,3-triazoles (i.e., a 17-fold reduction was observed, in comparison with the IC_{50}^{fly} of the 2,6-dichloro-4-trifluoromethylphenyl analogue); however, the *p*-ethynyl substitution conferred high activity in the case of the 4-alkyl-1-phenylpyrazoles (*37*). The activity of 4-alkyl-1-phenyl-2,6,7-trioxabicyclo-[2.2.2]octane-type GABA antagonists is also known to be greatly enhanced by the introduction of an ethynyl group into the para-position of the phenyl group (*44*). The 4-(4-methoxycarbonyl-1-butynyl) substitution on the benzene ring did not increase the activity of 4(or 5-)-*n*-butyl-1-phenyl-1*H*-1,2,3-triazoles, although the introduction of this substituent into the 4-position of the phenyl group resulted in achieving high potency in the 5-alkyl-2-phenyl-1,3-dithiane-type (*45*, *46*) and straight-chain GABA antagonists (*47*). Taken together, the present findings are expected to provide insights into the orientation of noncompetitive antagonists at the binding site of GABA receptors. Akamatsu et al. (*48*) postulated that the housefly and rat GABA receptors have four major binding subsites for noncompetitive antagonists, as based on the 3D-QSAR analysis of picrotoxinin-type antagonists including dioxatricyclododecenes, hexachlorocyclohexanes, endosulfan, bicyclophosphates, and other related compounds. After reviewing large amounts of data focusing on three classes of antagonists (i.e., plant-derived terpene lactone picrodendrins, fipronil-related heterocyclic compounds, and bicyclophosphorothionates), Ozoe and Akamatsu (*18*) presented a model of the noncompetitive antagonist binding site. According to this model, it was proposed that various types of antagonists, all of which share common structural features, bind to an identical site in the GABA receptor in different or overlapping orientations. Recently, Sammelson et al. (*37*) also reached the same conclusion in a study of 4-alkyl-1-phenylpyrazoles and 4-alkyl-1-phenyl-2,6,7-trioxabicyclo[2.2.2]octanes. A sterically bulky alkyl group at the 4-position, as well as electronegative atoms in both heterocyclic rings, significantly affected binding to the receptor. The *p*-ethynyl group conferred high activity in the case of 1-phenylpyrazoles and 1-phenyl-2,6,7-trioxabicyclo[2.2.2]octanes. **Figure 9** shows the superimposition of three representative types of heterocyclic GABA antagonists overlaid with the emphasis on the heterocyclic rings. As can be seen in this figure, the para-substituent of the dithiane points to a different direction from those of the other two molecules. The introduction of a 4-methoxycarbonyl-1-butynyl group, which is known to be an effective substituent in dithiane-type antagonists, into the 4-position of the phenyl group of 1-phenyl-1*H*-1,2,3-triazoles probably forces deflection of the molecules away from the binding site, thereby rendering the molecules inactive.

In conclusion, the issue regarding whether phenyltriazoles and fipronil bind to an identical site in the GABA receptor still

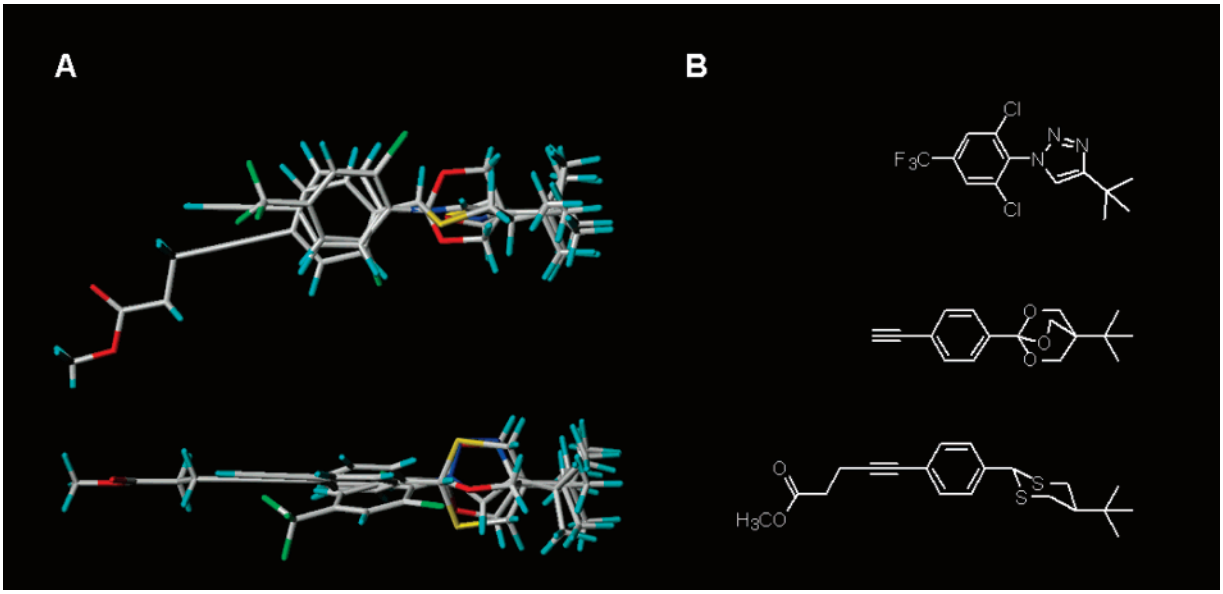

Figure 9. Superimposition of three representative heterocyclic GABA antagonists: (**A**) orthogonal views of the superimposed molecules; (**B**) structures of the superimposed molecules.

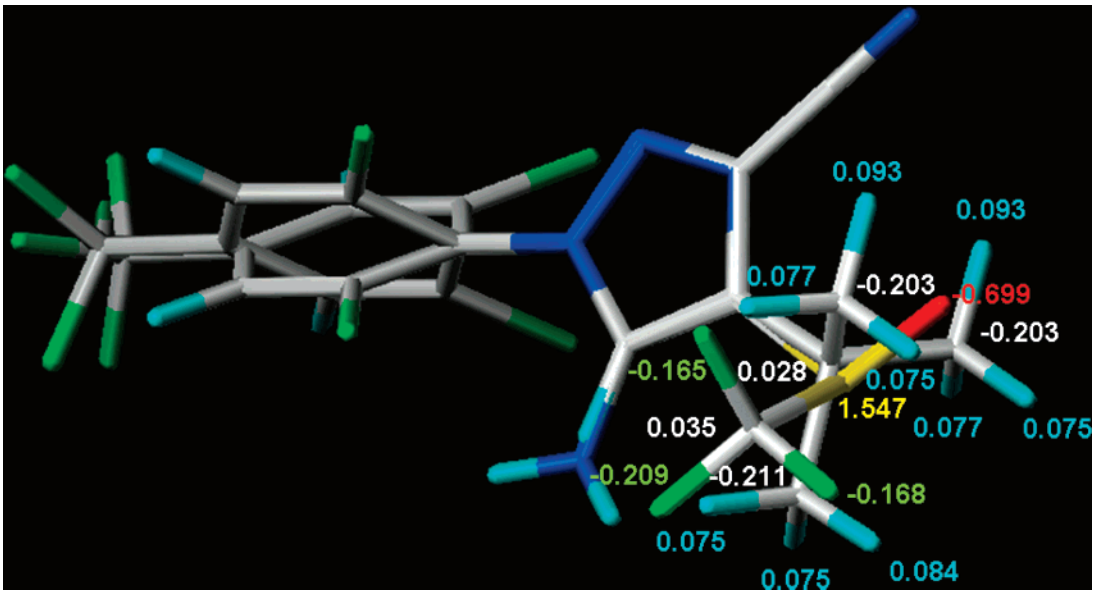

Figure 10. Superimposition of fipronil on **9**. Numbers indicate the electric charges on the atoms.

needs to be addressed. The 4-*tert*-butyl group of phenyltriazole **9** and the trifluoromethylsulfinyl group of fipronil are sterically similar, as pointed out by Sammelson et al. (*37*), but they differ greatly in terms of the electron density of the substituents; the trifluoromethylsulfinyl group has highly negative charges on its fluorine atoms and oxygen atom, whereas the 4-*tert*-butyl group has positive charges on its hydrogen atoms (**Figure 10**). Furthermore, fipronil and its closely related analogues exhibited an almost equal capacity for binding to the GABA receptor of dieldrin-resistant houseflies bearing the *Rdl* mutation (an Ala to Ser mutation at the 2′-position in TM2) to that of susceptible houseflies, whereas **9** showed less of an ability to bind to the former receptor than to the latter receptor (*49*). Recently, a fipronil-resistant *Drosophila* strain was shown to have a second point mutation in the third transmembrane domain in addition to the *Rdl* mutation in TM2 (*50*). These observations led us to speculate that fipronil binds to a site or sites that differ from the site for phenyltriazoles and other antagonists, or else it binds in a quite different orientation. More studies will be needed to clarify this point.

ABBREVIATIONS USED

CoMFA, comparative molecular field analysis; 3D-QSAR, three-dimensional quantitative structure−activity relationship; EBOB, 1-(4-ethynylphenyl)-4-*n*-propyl-2,6,7-trioxabicyclo-[2.2.2]octane or 4′-ethynyl-4-*n*-propylbicycloorthobenzoate; GABA, γ-aminobutyric acid; IC_{50}^{fly}, median inhibition concentration in the housefly GABA receptor; IC_{50}^{rat}, median inhibition concentration in the rat GABA receptor; $IC_{50}^{h\beta3}$, median inhibition concentration in the human β3 GABA receptor; PCR, Polymerase Chain Reaction; PHC, phenyl heterocyclic compound; QSAR, quantitative structure−activity relationship; *Rdl* (resistant to dieldrin), a gene encoding an insect GABA receptor subunit; Rdl, an insect GABA receptor subunit encoded by *Rdl*; TM2, the second transmembrane domain.

ACKNOWLEDGMENT

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Supporting Information Available: Spectroscopic data for phenyltriazoles and X-ray crystallographic data for **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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