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5-[4-(3,3-Dimethylbutoxycarbonyl)phenyl]-4-pentynoic Acid and Its Derivatives Inhibit Ionotropic γ-Aminobutyric Acid Receptors by Binding to the 4′-Ethynyl-4-*n*-propylbicycloorthobenzoate Site

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Abstract—Acyclic noncompetitive antagonists of ionotropic γ-aminobutyric acid (GABA) receptors, bearing an ester or ether linkage, were designed, synthesized, and assayed for their inhibition of the specific binding of [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB), a radiolabeled noncompetitive antagonist, to rat brain and housefly head membranes. 5-[4-(3,3-Dimethylbutoxycarbonyl)phenyl]-4-pentynoic acid (DBCPP), a butyl benzoate analogue, was found to competitively inhibit the binding of [^{3}H]EBOB in rat brain membranes, with an IC_{50} of 88 nM. The potency conferred by the *p*-substituent decreased in the order $C{\equiv}C(CH_2)_2COOH > C{\equiv}C(CH_2)_2COOCH_3 > C{\equiv}CH >$ Br. Pentyl phenyl ethers were equally potent compared with butyl benzoates, while phenyl pentanoates and benzyl butyl ethers were less potent. These compounds were generally less active in housefly head membranes than in rat brain membranes. The introduction of an isopropyl group into the 1-position of the 3,3-dimethylbutyl group of a butyl benzoate and two benzyl butyl ethers caused an increase in potency in housefly GABA receptors, whereas this modification at the corresponding position of other compounds led to an unchanged or decreased potency. In the case of rat receptors, this modification resulted in a decrease in potency except for a phenyl pentanoate. To confirm that DBCPP interferes with GABA receptor function, we performed whole-cell patch clamp experiments with rat dorsal root ganglion neurons in the primary culture. Repeated co-applications of GABA and DBCPP suppressed GABA-induced whole-cell currents with an IC_{50} of 0.54 μM and a Hill coefficient of 0.7. These findings indicate that DBCPP and its derivatives inhibit ionotropic GABA receptors by binding to the EBOB site and that there might be structural difference in the noncompetitive antagonist-binding site between rat and housefly GABA receptors.

Introduction

The γ-aminobutyric acid $(GABA)_A$ receptor is a hetero-oligomeric ion channel protein that mediates inhibitory neurotransmission in the vertebrate central nervous system.[1] GABA inhibits neuronal firing by increasing the passage of chloride ions into the cell through the channel. The $GABA_A$ receptor has binding sites for several classes of medicinal or toxic compounds and for ions, in addition to the binding site for GABA. These chemicals allosterically modulate the action of GABA on the receptor by binding to sites distinct from the GABA binding site. Similar but pharmacologically different ionotropic GABA receptors are present in the central and peripheral nervous systems of invertebrate species.[2,3]

Noncompetitive antagonists inhibit the function of ionotropic GABA receptors by binding to a site that is labeled by a high-affinity, selective radioligand, [^{3}H]4′-ethynyl-4-*n*-propylbicycloorthobenzoate (EBOB).[4] The noncompetitive antagonists include diverse classes of compounds such as plant terpenoid toxins (picrotoxinin,[5] picrodendrins,[6] etc.), insecticides (lindane,[7,8] dieldrin,[9] etc.), and convulsants (bicyclophosphates,[10] bicycloorthocarboxylates,[11] dithianes,[12] etc.). On the basis of the antagonists' structure–activity relationships, we previously constructed a model of the binding site that can accommodate structurally diverse antagonists. The generated model predicted that four important

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interacting subsites are related to the antagonist activity;[13] however, no antagonists supposed to interact with all four sites have been found, probably because the conformational rigidity of known antagonists precludes the interaction of any one of them with all four subsites.

In the present study, we have designed and synthesized four classes of flexible acyclic antagonists (Fig. 1) as molecular probes to verify the existence of the four important subsites, and we here report that 5-[4-(3,3-dimethylbutoxycarbonyl)phenyl]-4-pentynoic acid (DBCPP) and its derivatives are noncompetitive antagonists interacting with the EBOB site. We also discuss the structure–activity relationships of the acyclic antagonists in relation to our binding-site model.

Results

Effects of acyclic esters and ethers on [^{3}H]EBOB binding

Butyl benzoates. 3,3-Dimethylbutyl benzoates (**1**, **3**, **5** and **7**) inhibited specific [^{3}H]EBOB binding to rat brain membranes, with IC_{50}s of 0.088–3.77 μM (Table 1). 5-[4-(3,3-Dimethylbutylbutoxycarbonyl)phenyl]-4-pentynoic acid (DBCPP, **7**) was the most potent inhibitor (Table 1 and Fig. 2A). The change of the *p*-substituent caused a decrease in potency in the order $C{\equiv}C(CH_2)_2COOH > C{\equiv}C(CH_2)_2COOCH_3 > C{\equiv}CH > Br$. The introduction of an isopropyl group into the 1-position of the 3,3-dimethylbutyl group resulted in a reduction in potency. In housefly head membranes, benzoates with a Br and C≡CH substituent were weakly active or inactive, and those with $C{\equiv}C(CH_2)_2COOCH_3$ and $C{\equiv}C(CH_2)_2COOH$ were moderately active. The introduction of an isopropyl group into the 1-position of the 3,3-dimethylbutyl group led to an increased (**3** versus **4**), unchanged (**7** versus **8**), or decreased (**5** versus **6**) potency.

Figure 2B and C show the effects of 88 nM DBCPP on the saturation isotherm and the Scatchard plot of [^{3}H]EBOB binding to rat brain membranes, respectively. DBCPP increased an apparent dissociation constant (K_d) of EBOB from 2.45 to 4.74 nM without the significant alteration of the maximum number of binding sites (B_{max}) (2.38 and 2.36 pmol/mg protein in the absence and presence of DBCPP, respectively), indicating that DBCPP competes with EBOB for a common binding site in rat brain $GABA_A$ receptors. Scatchard analysis was also performed for the inhibition of [^{3}H]EBOB binding by 3 μM **8** in housefly head membranes, suggesting that this type of benzoate acts as a noncompetitive antagonist in housefly GABA receptors (data not shown).

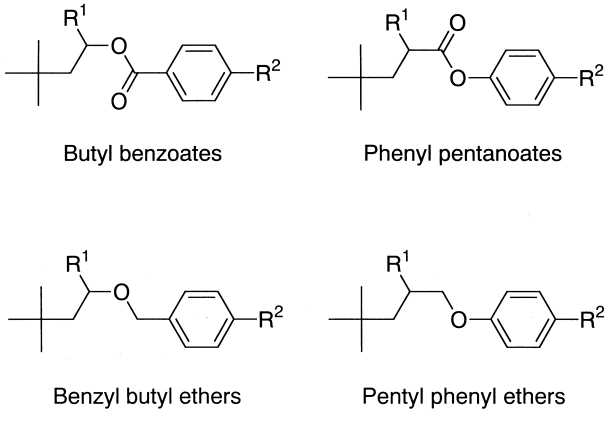


Figure 1. Structures of acyclic esters and ethers synthesized in the present study.

Phenyl pentanoates. Phenyl pentanoate **9**, bearing $C{\equiv}C(CH_2)_2COOCH_3$ at the *p*-position, poorly inhibited [^{3}H]EBOB binding in rat brain membranes (Table 1). The introduction of an isopropyl group into the 2-position of **9** led to a moderately active inhibitor. In housefly head membranes, both compounds were weakly active or inactive. Analogues with a Br and C≡CH substituent were devoid of inhibitory activity in both rat brain and housefly head membranes (data not shown).

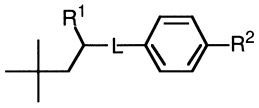
Benzyl butyl ethers. Alkyl benzyl ethers **13**, **15** and **17** were moderately effective in inhibiting [^{3}H]EBOB binding to rat brain membranes. Replacement of C≡CH with $C{\equiv}C(CH_2)_2COOCH_3$ and $C{\equiv}C(CH_2)_2COOH$ resulted in a slight increase in potency. Compounds **15** and **17** were less potent in housefly head membranes than in rat brain membranes, while **13** was equally active in both membranes. The introduction of an isopropyl group into the 1-position of the 3,3-dimethylbutyl group of these compounds caused a decrease in potency in rat brain membranes. In contrast, the introduction of an isopropyl group in **15** and **17** led to inhibitors with increased potencies in housefly head membranes.

Pentyl phenyl ethers. The dependence of potency on the *p*-substituent was similar to that of other classes of compounds. Compounds **23** and **25** exhibited a high level of potency, similar to the corresponding butyl benzoates. These compounds were more potent in rat brain membranes than in housefly head membranes. The effects of the isopropyl group were negative in both rat brain and housefly head membranes.

Effects of DBCPP on GABA-induced currents

When the membrane potential was held at −60 mV in the normal external solution, the bath application of GABA produced an inward current in rat dorsal root ganglion neurons. Responses to low concentrations of GABA were maintained at a stable level over a period of up to 60 min after the rupture of the membrane (data not shown). The currents induced by 30 μM GABA were suppressed when 0.1 or 1 μM DBCPP was co-applied (Fig. 3A). During DBCPP suppression, the desensitization of GABA-induced currents was accelerated. Currents were completely recovered after the membranes were washed with a DBCPP-free solution (data not shown).

Table 1. Potency of DBCPP and its derivatives in inhibiting [^{3}H]EBOB binding to rat brain and housefly head membranes



No.	R^1	L	R^2	IC_{50} (μM)[a]	
				Rat	Housefly
Butyl benzoate					
1	H	-O–CO-	Br	3.77 (2.69–5.27[b])	>10 (22.4[c])
2	$CH(CH_3)_2$	-O–CO-	Br	>10 (15.4[c])	>10 (15.9[c])
3	H	-O–CO-	C≡CH	0.67 (0.53–0.84[b])	>10 (27.3[c])
4	$CH(CH_3)_2$	-O–CO-	C≡CH	>10 (39.1[c])	7.84 (5.06–12.16[b])
5	H	-O–CO-	$C{\equiv}C(CH_2)_2COOCH_3$	0.22 (0.18–0.29[b])	4.15 (2.76–6.23[b])
6	$CH(CH_3)_2$	-O–CO-	$C{\equiv}C(CH_2)_2COOCH_3$	4.83 (3.43–6.80[b])	>10 (49.9[c])
7 (DBCPP)	H	-O–CO-	$C{\equiv}C(CH_2)_2COOH$	0.088 (0.068–0.110[b])	3.41 (2.26–5.15[b])
8	$CH(CH_3)_2$	-O–CO-	$C{\equiv}C(CH_2)_2COOH$	1.41 (1.10–1.81[b])	2.90 (1.88–4.47[b])
Phenyl pentanoate					
9	H	-CO–O-	$C{\equiv}C(CH_2)_2COOCH_3$	>10 (48.4[c])	>10 (17.6[c])
10	$CH(CH_3)_2$	-CO–O-	$C{\equiv}C(CH_2)_2COOCH_3$	4.67 (3.31–6.59[b])	>10 (35.1[c])
Benzyl butyl ether					
11	H	-O–CH_2-	Br	>10 (32.5[c])	>10 (17.5[c])
12	$CH(CH_3)_2$	-O–CH_2-	Br	>10 (10.6[c])	>10 (11.2[c])
13	H	-O–CH_2-	C≡CH	3.41 (2.68–4.34[b])	5.45 (3.79–7.84[b])
14	$CH(CH_3)_2$	-O–CH_2-	C≡CH	>10 (13.8[c])	6.02 (4.05–8.95[b])
15	H	-O–CH_2-	$C{\equiv}C(CH_2)_2COOCH_3$	1.99 (1.50–2.64[b])	>10 (29.9[c])
16	$CH(CH_3)_2$	-O–CH_2-	$C{\equiv}C(CH_2)_2COOCH_3$	>10 (49.2[c])	7.96 (5.01–12.67[b])
17	H	-O–CH_2-	$C{\equiv}C(CH_2)_2COOH$	1.68 (1.30–2.17[b])	>10 (37.2[c])
18	$CH(CH_3)_2$	-O–CH_2-	$C{\equiv}C(CH_2)_2COOH$	5.51 (4.09–7.41[b])	2.72 (2.06–3.61[b])
Pentyl phenyl ether					
19	H	-CH_2–O-	Br	>10 (41.0[c])	>10 (16.8[c])
20	$CH(CH_3)_2$	-CH_2–O-	Br	>10 (9.3[c])	>10 (−0.4[c])
21	H	-CH_2–O-	C≡CH	5.16 (3.67–7.27[b])	5.62 (3.74–8.45[b])
22	$CH(CH_3)_2$	-CH_2–O-	C≡CH	>10 (26.6[c])	>10 (37.5[c])
23	H	-CH_2–O-	$C{\equiv}C(CH_2)_2COOCH_3$	0.13 (0.10–0.18[b])	4.94 (3.13–7.79[b])
24	$CH(CH_3)_2$	-CH_2–O-	$C{\equiv}C(CH_2)_2COOCH_3$	9.05 (5.12–16.00[b])	>10 (11.1[c])
25	H	-CH_2–O-	$C{\equiv}C(CH_2)_2COOH$	0.10 (0.080–0.13[b])	5.21 (3.58–7.59[b])
26	$CH(CH_3)_2$	-CH_2–O-	$C{\equiv}C(CH_2)_2COOH$	2.90 (2.21–3.82[b])	3.78 (3.23–4.41[b])

[a]Determined using 0.5 nM [^{3}H]EBOB.
[b]95% Confidence limit.
[c]Inhibition percentage at 10 μM.

Figure 3B shows the concentration–response relationship for the suppression of GABA-induced currents by co-application with DBCPP. The currents induced by 10-s co-applications of 30 μM GABA and several concentrations of DBCPP were measured when they reached a steady-state level after repeated co-applications. DBCPP suppressed GABA-induced currents in a concentration-dependent manner with an IC_{50} of 0.54 μM and a Hill coefficient of 0.7.

Discussion

Structure–activity relationships

The present study demonstrates that four classes of acyclic compounds are inhibitors of the specific binding of [^{3}H]EBOB, a radiolabeled noncompetitive GABA antagonist, to rat brain and housefly head membranes. The compounds synthesized are butyl benzoates, phenyl pentanoates, benzyl butyl ethers, and pentyl phenyl ethers. Butyl benzoates are regarded as ring-opened analogues of bicycloorthocarboxylate-type antagonists such as EBOB. Phenyl pentanoates are the compounds in which the acid and alcohol sides are reversed compared with those of butyl benzoates. Benzyl butyl ethers and pentyl phenyl ethers are the compounds that have a methylene unit in place of the carbonyl group of butyl benzoates and phenyl pentanoates, respectively. These compounds were more potent in rat $GABA_A$ receptors than in housefly GABA receptors, or equipotent in both receptors, except for four compounds (**4**, **14**, **16** and **18**). The structural requirements for high potency are an appropriately *p*-substituted phenyl group and a suitable alkyl group on each side of their structures as well as a central electronegative link (L of the structure in Table 1). In rat brain membranes, butyl benzoates and pentyl phenyl ethers were more potent than were phenyl pentanoates and benzyl butyl ethers. This finding might indicate that the presence of oxygen atom(s) in a symmetrical position with respect to a line passing through the *p*-substituents of the phenyl group is

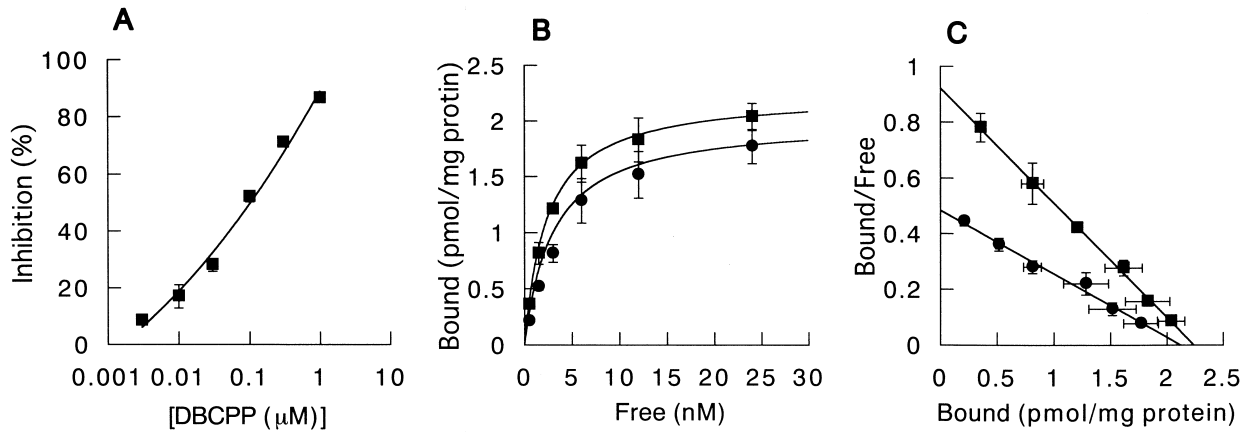


Figure 2. Inhibition of [^{3}H]EBOB binding by DBCPP in rat brain membranes: (A) concentration–inhibition curves; (B) saturation isotherms of [^{3}H]EBOB binding in the absence (■) and presence (●) of 88 nM DBCPP; (C) Scatchard plots in the absence (■) and presence (●) of 88 nM DBCPP.

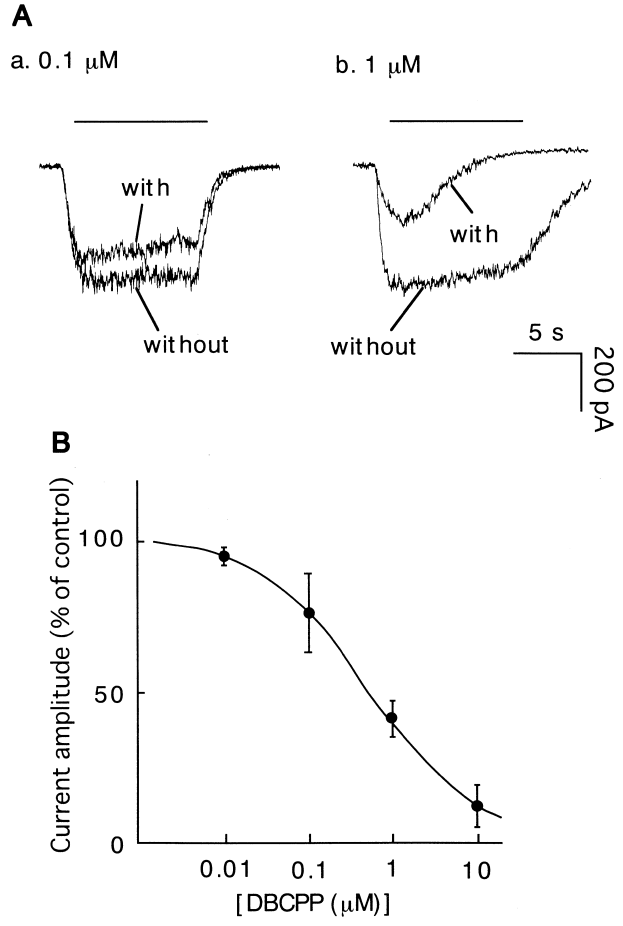


Figure 3. Suppression of GABA-induced currents by co-application of DBCPP and GABA in rat dorsal root ganglion neurons: (A) current records in response to 10-s application of 30 μM GABA with and without 0.1 (a) or 1 μM (b) DBCPP; (B) dose–response relationship for the suppression of GABA-induced peak currents by co-application of DBCPP.

advantageous for high potency (Fig. 4). In housefly head membranes, however, butyl benzoates and pentyl phenyl ethers were not necessarily more potent than were benzyl butyl ethers. As for the aromatic moiety, the introduction of C≡CH, C≡C(CH$_2$)$_2$COOCH$_3$ and C≡C(CH$_2$)$_2$COOH into the *p*-position of the phenyl group markedly increased the potency compared with the Br analogues. This substitution has been reported to confer high potency to dithiane-type antagonists.[14] The significance of the special interaction of these functional groups with the binding site should be pursued further in future work. The isopropyl group (R^1 of the structure in Table 1) in the alkyl moiety was introduced because a similar modification rendered bicyclophosphorothionate-type antagonists capable of exhibiting selectivity for housefly GABA receptors versus rat GABA$_A$ receptors.[15] However, the isopropyl group's effect was not prominent in the flexible, acyclic compounds examined in the present study, although the enhancement of potency for housefly receptors was observed in **4** (versus **3**), **16** (versus **15**) and **18** (versus **17**).

Site of action

Scatchard analysis showed that the inhibition of [^{3}H]EBOB binding by DBCPP and **8** is competitive, suggesting their noncompetitive antagonism toward ionotropic GABA receptors (Fig. 2). To confirm this finding by a functional assay, whole-cell patch clamp experiments were performed with rat dorsal root ganglion neurons in the primary culture. When DBCPP was co-applied with GABA for 10 s, the current was suppressed in a dose-dependent manner (Fig. 3). During DBCPP suppression, the desensitization of GABA-induced currents was accelerated. These findings indicate that the DBCPP suppression of the GABA-induced currents was use-dependent, being accelerated by frequent openings of GABA-gated channels. Both biochemical and electrophysiological data thus lead to the conclusion that DBCPP and its derivatives are noncompetitive antagonists that share a common binding site with EBOB.

Interaction with the binding site

On the basis of the structure–activity relationships of diverse classes of noncompetitive antagonists, we previously postulated that there are four major interacting subsites in the noncompetitive antagonist-binding site

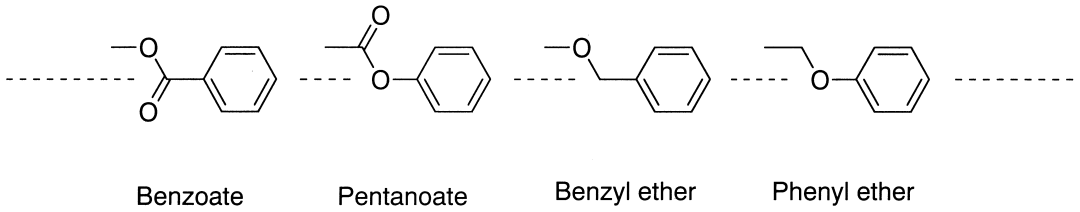
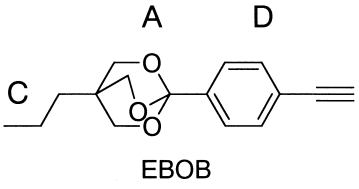
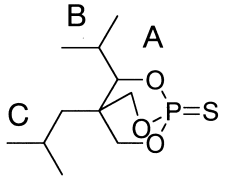


Figure 4. Partial structures of acyclic GABA antagonists and position(s) of their oxygen atom(s).

within ionotropic GABA receptors (Fig. 5).[6] Compounds capable of interacting with two of the four subsites were supposed to act as antagonists, although the molecular mechanisms by which the antagonists exert their effects remain to be elucidated. Subsite A probably accepts an electronegative part of ligands. In both sides of subsite A, there might be spaces (subsites C and D) that accommodate hydrophobic and hydrophobic/electronegative parts of ligands. In addition, we infer the existence of one more subsite (B), which is located near subsite C. With regard to subsite B, we have reported that bicyclophosphorothionate GABA antagonists, bearing an isopropyl group at the 3-position, exhibit selectivity for housefly GABA receptors versus rat $GABA_A$ receptors, while the reversed selectivity is the case for unsubstituted analogues.[15] This finding allows us to speculate that the effect of the isopropyl group on the receptor selectivity is due to a structural difference around subsite B between insect and mammalian receptors. This hypothesis has been substantiated by three-dimensional quantitative structure–activity relationship analyses.[6,15,16]



EBOB



Bicyclophosphorothionate

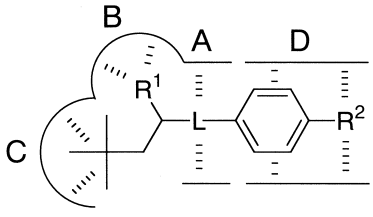


Figure 5. Possible interaction of noncompetitive antagonists with their binding site. A, B, C and D refer to subsites A, B, C and D. L = -O–CO-, -CO–O-, -O–CH_2-, -CH_2–O-; R^1 = H, *i*-C_3H_7; R^2 = Br, C≡CH, C≡$C(CH_2)_2COOCH_3$, C≡$C(CH_2)_2COOH$.

As mentioned above, the compounds synthesized in the present study are open-ring analogues of bicycloorthobenzoates. It thus seems reasonable to assume that they act at the noncompetitive antagonist site in such a way that their benzene rings lie in the same position, i.e. subsite D (Fig. 5). We expected that esters and ethers with R^1 = *i*-C_3H_7 would interact with all four subsites in insect receptors, and that they would exhibit high potency in inhibiting [^{3}H]EBOB binding to housefly GABA receptors. Actually, although the compounds showed affinity for the EBOB binding site, they are less potent than expected. Stable conformers of the conformationally flexible compounds might be incapable of fitting to the binding site in terms of free energy,[17] whereas compact, rigid compounds, such as 3-isopropyl substituted bicyclophosphorothionates, are suitable for binding. However, the isopropyl group's effect, i.e. decreasing affinity for rat receptors and increasing affinity for housefly receptors, was observed, at least in **4**, **16** and **18**, being suggestive of the presence of subsite B in the housefly antagonist site and structural difference between the rat and housefly antagonist sites.

In conclusion, we have reported here, for the first time, a series of acyclic noncompetitive antagonists of ionotropic GABA receptors. In particular, **5**, DBCPP (**7**), **23** and **25** were found to be potent antagonists, probably interacting with subsites A, C, and D in our rat binding-site model. While the data presented here also suggests the existence of four subsites in the noncompetitive antagonist site, further modification will be necessary to prepare highly active noncompetitive antagonists that interact with all four subsites.

Experimental

Chemistry

General. Compounds were checked for their purity by thin layer chromatography on silica gel G, mass spectrometry, and ^{1}H NMR spectrometry. Mass spectra were obtained on a Hitachi M-80B mass spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz on a JEOL JNM-A-400 spectrometer, unless otherwise noted. Spin multiplicities are described as: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), sp (septet), dsp (doublet of septets), or m (multiplet). Melting points were determined on a Yanako MP-500D apparatus and are uncorrected. Distillation was performed using a Shibata GTO-350D glass tube oven.

3,3-Dimethylbutyl 4-bromobenzoate (1). A solution of 3,3-dimethyl-1-butanol (2.04 g, 20 mmol) and Et_3N (2.02 g, 20 mmol) in dry Et_2O (20 mL) was added dropwise to an ice-cold, stirred solution of 4-bromobenzoyl chloride (4.37 g, 20 mmol) in dry Et_2O (30 mL). After 6-h stirring at room temperature, the solution was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried, and concentrated. The residue was purified by column chromatography on silica gel with benzene to give **1** as a colorless liquid (3.51 g, 61.8% yield): ^{1}H NMR δ 0.99 (9H, s, $(CH_3)_3C$), 1.70 (2H, t, $J=7.3$ Hz, $(CH_3)_3CCH_2$), 4.37 (2H, t, $J=7.3$ Hz, CH_2O), 7.57, 7.89 (4H, AA′XX′, $J=8.8$, 2.1 Hz, Ar); CIMS m/z 285 (M + 1, 99.5%), 286 (M + 2, 66.7%), 287 (M + 3, 100%), 288 (M + 4, 61.3%).

1-Isopropyl-3,3-dimethylbutyl 4-bromobenzoate (2). A solution of 2,5,5-trimethyl-3-hexanol (1.44 g, 10 mmol), 4-bromobenzoic acid (2.01 g, 10 mmol), dicyclohexylcarbodiimide (3.50 g, 17 mmol), and 4-(dimethylamino)-pyridine (0.37 g, 3 mmol) in toluene (200 mL) was stirred at room temperature for 4 days. The solution was extracted with $CHCl_3$, and the $CHCl_3$ extract was washed with water, dried, and concentrated. The residue was purified by column chromatography on silica gel with benzene to give **2** as a colorless liquid (1.34 g, 41.2% yield): ^{1}H NMR δ 0.90 (9H, s, $(CH_3)_3C$), 0.92, 0.94 (6H, 2d, $J=6.8$ Hz, $CH(CH_3)_2$), 1.46 (1H, dd, $J=14.9$, 1.5 Hz, CH_2), 1.65 (1H, dd, $J=14.9$, 9.1 Hz, CH_2), 1.92 (1H, dsp, $J=6.8$, 4.5 Hz, $CH(CH_3)_2$), 5.17 (1H, ddd, $J=9.1$, 4.5, 1.5 Hz, CHO), 7.58, 7.91 (4H, AA′XX′, $J=8.5$, 2.3 Hz, Ar); CIMS m/z 327 (M + 1, 30.0%), 329 (M + 3, 24.3%).

3,3-Dimethylbutyl 4-ethynylbenzoate (3). A solution of **1** (0.57 g, 2 mmol), (trimethylsilyl)acetylene (0.39 g, 4 mmol), triphenylphosphine (24 mg, 0.09 mmol), and palladium(II) acetate (12 mg, 0.05 mmol) in Et_3N (7 mL) was refluxed under a nitrogen atmosphere for 24 h. After the solution was concentrated, a mixture of dry tetrahydrofuran (THF) (12 mL) and a THF solution of 1 M tetrabutylammonium fluoride (2.4 mL) were added to the residue at 0 °C. After 2-h stirring at room temperature, the solution was concentrated and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with water, dried, and concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane: CH_2Cl_2 (9:1) to give **3** as a yellow solid (264 mg, 57.3% yield): mp 50.7 °C; ^{1}H NMR δ 1.00 (9H, s, $(CH_3)_3C$), 1.71 (2H, t, $J=7.3$ Hz, $(CH_3)_3CCH_2$), 3.22 (1H, s, C≡CH), 4.38 (2H, t, $J=7.3$ Hz, CH_2O), 7.55, 7.89 (4H, 2d, $J=8.3$ Hz, Ar); CIMS m/z 231 (M + 1, 100%).

1-Isopropyl-3,3-dimethylbutyl 4-ethynylbenzoate (4). This compound was obtained as a yellow liquid (8.8% yield) from **2**, using the procedure described for **3**: ^{1}H NMR δ 0.91 (9H, s, $(CH_3)_3C$), 0.92, 0.94 (6H, 2d, $J=6.8$ Hz, $CH(CH_3)_2$), 1.46 (1H, dd, $J=14.9$, 1.5 Hz, CH_2), 1.66 (1H, dd, $J=14.9$, 9.0 Hz, CH_2), 1.93 (1H, dsp, $J=6.8$, 4.4 Hz, $CH(CH_3)_2$), 3.22 (1H, s, C≡CH), 5.18 (1H, ddd, $J=9.0$, 4.4, 1.5 Hz, CHO), 7.55, 8.00 (4H, AA′XX′, $J=8.5$, 1.6 Hz, Ar); CIMS m/z 273 (M + 1, 3.3%).

Methyl 5-[4-(3,3-dimethylbutoxycarbonyl)phenyl]-4-pentynoate (5). A solution of 4-pentynoic acid (2.5 g, 25.5 mmol) and H_2SO_4 (1.5 g) in dry MeOH (50 mL, 1.2 mol) was heated under reflux for 12 h. The solution was extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with a saturated $NaHCO_3$ solution and concentrated to give crude methyl 4-pentynoate (2.3 g, 79.2% yield). A solution of **1** (1.4 g, 5 mmol), methyl 4-pentynoate (1.3 g, 12 mmol), bis(triphenylphosphine)palladium(II) chloride (87.5 mg, 0.13 mmol), and copper iodide(I) (25 mg, 0.13 mmol) in Et_3N (50 mL) was refluxed under a nitrogen atmosphere for 24 h. The solution was extracted with Et_2O, and the Et_2O extract was dried and concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane: CH_2Cl_2 (9:1) to give **5** as a white solid (1.25 g, 79.3% yield): mp 32.2 °C; ^{1}H NMR δ 0.99 (9H, s $(CH_3)_3C$), 1.70 (2H, t, $J=7.3$ Hz, $(CH_3)_3CCH_2$), 2.65, 2.76 (4H, 2t, $C{\equiv}C(CH_2)_2$), 3.73 (3H, s, $COOCH_3$), 4.37 (2H, t, $J=7.3$ Hz, CH_2O), 7.43, 7.94 (4H, 2d, $J=8.1$ Hz, Ar); CIMS m/z 317 (M + 1, 62.0%).

Methyl 5-[4-(1-isopropyl-3,3-dimethylbutoxycarbonyl)-phenyl]-4-pentynoate (6). This compound was obtained as a yellow liquid (53.4% yield), using the procedure described for **5**: ^{1}H NMR δ 0.90 (9H, s, $(CH_3)_3C$), 0.92, 0.94 (6H, 2d, $J=6.8$ Hz, $CH(CH_3)_2$), 1.45 (1H, dd, $J=14.9$, 1.5 Hz, CH_2), 1.65 (1H, dd, $J=14.9$, 9.0 Hz, CH_2), 1.92 (1H, dsp, $J=6.8$, 4.4 Hz, $CH(CH_3)_2$), 2.65, 2.76 (4H, ddd, $C{\equiv}C(CH_2)_2$), 3.72 (3H, s, $COOCH_3$), 5.18 (1H, ddd, $J=9.0$, 4.4, 1.5 Hz, CHO), 7.43, 7.96 (4H, AA′XX′, $J=8.7$, 1.8 Hz, Ar); CIMS m/z 359 (M + 1, 20.0%).

5-[4-(3,3-Dimethylbutoxycarbonyl)phenyl]-4-pentynoic acid (7). A mixture of **5** (80 mg, 0.25 mmol), LiOH (42 mg, 1 mmol), THF (5 mL), and H_2O (3 mL) was stirred in an ice bath for 30 min. The solution was washed with *n*-hexane, and the aqueous layer was acidified with dilute HCl and extracted with EtOAc. The EtOAc extract was dried and concentrated. The residue was recrystallized from Et_2O to give **7** as a white solid (18 mg, 23.8%): mp 116.0 °C; ^{1}H NMR δ (DMSO-d_6) 0.96 (9H, s, $(CH_3)_3C$), 1.65 (2H, t, $J=7.1$ Hz, $(CH_3)_3CCH_2$), 2.54, 2.67 (4H, 2t, $J=6.8$ Hz, $C{\equiv}C(CH_2)_2$), 4.33 (2H, t, $J=7.1$ Hz, CH_2O), 7.51, 7.91 (4H, 2d, $J=8.2$ Hz, Ar), 12.36 (1H, br s, COOH); CIMS m/z 303 (M + 1, 15.0%).

5-[4-(1-Isopropyl-3,3-dimethylbutoxycarbonyl)phenyl]-4-pentynoic acid (8). This compound was obtained as a colorless viscous liquid (1.2% yield), using the procedure described for **7**: ^{1}H NMR δ (DMSO-d_6) 0.86 (9H, s, $(CH_3)_3C$), 0.87, 0.89 (6H, 2d, $J=7.3$ Hz, $CH(CH_3)_2$), 1.50 (1H, d, $J=14.8$ Hz, $(CH_3)_3CCH_2$), 1.63 (1H, dd, $J=14.8$, 9.0 Hz, $(CH_3)_3CCH_2$), 1.87 (1H, m, $CH(CH_3)_2$), 2.55, 2.68 (4H, 2t, $J=7.1$ Hz, $C{\equiv}C(CH_2)_2$), 5.07 (1H, dd, $J=9.0$, 4.4 Hz, CHO), 7.52, 7.93 (4H, 2d, $J=8.3$ Hz, Ar), 12.37 (1H, br s, COOH); CIMS m/z 345 (M + 1, 30.0%).

Methyl 5-[4-(4,4-dimethylpentanoyloxy)phenyl]-4-pentynoate (9). A solution of 3,3-dimethyl-1-butanol (20.7 g, 202.9 mmol) in pyridine (2 g, 25 mmol) was added

dropwise to an ice-cold, stirred solution of PBr_3 (23 g, 85.8 mmol) containing a few drops of pyridine. After 12-h reflux, the solution was distilled to give 1-bromo-3,3-dimethylbutane (33.0 g, 99.2% yield): bp 136–138 °C. A solution of 1-bromo-3,3-dimethylbutane (5.6 g, 34 mmol) in dry Et_2O (50 mL) was added dropwise to an ice-cold, stirred mixture of magnesium turnings (0.82 g, 34 mg-atom) in dry Et_2O (50 mL). After the solution was stirred for 2 h at room temperature, dry CO_2 gas was passed into the solution for 1 h. The reaction mixture was poured dropwise into a 25% NH_4Cl solution and extracted with EtOAc. The EtOAc extract was washed with water and concentrated. The residue was washed with an $NaHCO_3$ solution and washed with *n*-hexane. The aqueous layer was acidified with 1 N HCl and extracted with EtOAc. The EtOAc extract was dried and concentrated to give crude 4,4-dimethylpentanoic acid (1.95 g, 43.9% yield). The reaction of 4,4-dimethylpentanoic acid with 4-bromophenol in a manner similar to that described for **2** gave 4-bromophenyl 4,4-dimethylpentanoate as a white solid (27.5% yield): mp 54.8 °C. **9** was obtained as a white solid (77.3% yield) from 4-bromophenyl 4,4-dimethylpentanoate and methyl 4-pentynoate, using the procedure described for **5**: mp 41.2 °C; ^{1}H NMR δ 0.95 (9H, s, $(CH_3)_3C$), 1.67, 2.52 (4H, 2m, $(CH_3)_3C(CH_2)_2$), 2.63, 2.72 (4H, 2ddd, $C{\equiv}C(CH_2)_2$), 3.72 (3H, s, $COOCH_3$), 7.00, 7.38 (4H, AA′XX′, J = 8.8, 2.3 Hz, Ar); CIMS m/z 317 (M + 1, 45.3%).

Methyl 5-[4-(2-isopropyl-4,4-dimethylpentanoyloxy)-phenyl]-4-pentynoate (10). A solution of diisopropylamine (2.3 g, 23 mmol) in dry THF (15 mL) was stirred at −20 °C under a nitrogen atmosphere. To this solution were successively added an *n*-hexane solution of 1.6 M *n*-BuLi (15.5 mL) and 4,4-dimethylpentanoic acid (1.5 g, 12 mmol) at such a rate that the temperature does not exceed 0 °C. After hexamethylphosphoramide (2.5 g, 13 mmol) was added to the solution, the mixture was stirred at room temperature for 30 min. To the mixture maintained at 0 °C was added isopropyl iodide (3.0 g, 17 mmol), and the mixture was stirred at room temperature for 3 h. After the reaction mixture was acidified with 3 N HCl, it was extracted with petroleum ether. The petroleum ether extract was washed with H_2O, concentrated, and extracted with a saturated $NaHCO_3$ solution. The aqueous solution was washed with *n*-hexane, acidified with 3 N HCl, and extracted with EtOAc. The EtOAc extract was dried, concentrated, and purified by column chromatography on silica gel with petroleum ether:EtOAc (3:1) to give 2-isopropyl-4,4-dimethylpentanoic acid (1.2 g, 58.4%) as a colorless liquid. The reaction of 2-isopropyl-4,4-dimethylpentanoic acid and 4-bromophenol in a manner similar to that described for **2** gave 4-bromophenyl 2-isopropyl-4,4-dimethylpentanoate as a colorless liquid (45.3% yield). Compound **10** was obtained as a colorless liquid (25.4% yield) from 4-bromophenyl 2-isopropyl-4,4-dimethylpentanoate and methyl 4-pentynoate, using the procedure described for **5**: ^{1}H NMR δ 0.95 (9H, s, $(CH_3)_3C$), 1.02, 1.03 (6H, 2d, J = 6.8 Hz, $CH(CH_3)_2$), 1.36 (1H, dd, J = 14.2, 1.2 Hz, $(CH_3)_3CCH_2$), 1.90 (1H, dd, J = 14.5, 10.4 Hz, $(CH_3)_3$ CCH_2), 1.93 (1H, sp, J = 6.8 Hz, $CH(CH_3)_2$), 2.42, 2.44 (1H, ddd, J = 10.4, 6.8, 1.2 Hz, CHCO), 2.63, 2.72 (4H, 2ddd, $C{\equiv}C(CH_2)_2$), 3.72 (3H, s, $COOCH_3$), 6.98, 7.38 (4H, AA′XX′, J = 8.5, 2.3 Hz, Ar); CIMS m/z 359 (M + 1, 100%).

4-Bromobenzyl 3,3-dimethylbutyl ether (11). 4-Bromobenzyl bromide (4.96 g, 20 mmol) was added to sodium 3,3-dimethyl butoxide prepared from 3,3-dimethyl-1-butanol (3.06 g, 30 mmol) and sodium (0.46 g, 20 mg-atom) in dry *n*-butyl ether (20 mL). The mixture was refluxed for 2 h and partitioned between Et_2O and H_2O. The Et_2O layer was concentrated, purified by column chromatography on silica gel with benzene, and distilled to give **11** as a colorless liquid (2.29 g, 42.5% yield): bp 115–118 °C/4 mmHg; ^{1}H NMR δ 0.92 (9H, s, $(CH_3)_3C$), 1.56 (2H, t, J = 7.5 Hz, $(CH_3)_3CCH_2$), 3.52 (2H, t, J = 7.5 Hz, CH_2CH_2O), 4.44 (2H, s, OCH_2Ph), 7.21, 7.46 (4H, AA′XX′, J = 8.5, 2.0 Hz, Ar); CIMS m/z 269 (M-1, 100%), 270 (M, 99.3%), 271 (M + 1, 99.5%), 272 (M + 2, 99.3%), 273 (M + 3, 59.3%).

4-Bromobenzyl 1-isopropyl-3,3-dimethylbutyl ether (12). This compound was obtained as a colorless liquid (10.8% yield) by the reaction of 2,5,5-trimethyl-3-hexanol and 4-benzyl bromide in 1,4-dioxane as described for **11**: bp 120–124 °C/4 mmHg; ^{1}H NMR δ 0.87, 0.89 (6H, 2d, J = 6.8 Hz, $CH(CH_3)_2$), 0.92 (9H, s, $(CH_3)_3C$), 1.28 (1H, dd, J = 14.6, 2.4 Hz, $(CH_3)_3CCH_2$), 1.36 (1H, dd, J = 14.6, 7.3 Hz, $(CH_3)_3CCH_2$), 2.05 (1H, dsp, J = 6.8, 3.4 Hz, $CH(CH_3)_2$), 3.31 (1H, ddd, J = 7.3, 3.4, 2.4 Hz, CHO), 4.34 , 4.50 (2H, 2d, J = 11.5 Hz, OCH_2Ph), 7.21, 7.44 (1H, AA′XX′, J = 8.5, 2.0 Hz, Ar); CIMS m/z 311 (M-1, 39.3%), 313 (M + 1, 57.3%), 314 (M + 2, 10.7%), 315 (M + 3, 22.0%).

4-Ethynylbenzyl 3,3-dimethylbutyl ether (13). This compound was prepared as a yellow liquid (6.3% yield) from **11** and trimethylsilylacetylene, using the procedure described for **3**: ^{1}H NMR δ 0.95 (9H, s, $(CH_3)_3C$), 1.57 (2H, t, J = 7.4 Hz, $(CH_3)_3CCH_2$), 3.06 (1H, s, C≡CH), 3.53 (2H, t, J = 7.4 Hz, CH_2CH_2O), 4.49 (2H, s, OCH_2Ph), 7.29, 7.47 (4H, 2d, J = 8.3 Hz, Ar); CIMS m/z 217 (M + 1, 100%).

4-Ethynylbenzyl 1-isopropyl-3,3-dimethylbutyl ether (14). This compound was prepared as a black liquid (17.9% yield) from **12** and trimethylsilylacetylene, using the procedure described for **3**: ^{1}H NMR δ 0.88, 0.89 (6H, 2d, J = 6.8 Hz, $CH(CH_3)_2$), 0.93 (9H, s, $(CH_3)_3C$), 1.27 (1H, dd, J = 14.6, 2.3 Hz, $(CH_3)_3CCH_2$), 1.37 (1H, dd, J = 14.6, 7.5 Hz, $(CH_3)_3CCH_2$), 2.06 (1H, dsp, J = 6.8, 3.6 Hz, $CH(CH_3)_2$), 3.05 (1H, s, C≡CH), 3.31 (1H, ddd, J = 7.5, 3.6, 2.3 Hz, CHO), 4.40, 4.55 (2H, 2d, J = 11.6 Hz, OCH_2Ph), 7.30, 7.46 (4H, 2d, J = 8.2 Hz, Ar); CIMS m/z 259 (M + 1, 29.8%).

Methyl 5-[4-(3,3-dimethylbutoxymethyl)phenyl]-4-pentynoate (15). This compound was obtained as a yellow liquid (37.9% yield) from **11** and methyl 4-pentynoate, using the procedure described for **5**: ^{1}H NMR δ 0.91 (9H, s, $(CH_3)_3C$), 1.56 (2H, t, J = 7.4 Hz, $(CH_3)_3CCH_2$), 2.64, 2.73 (4H, 2ddd, $C{\equiv}C(CH_2)_2$), 3.51 (2H, t, J = 7.4 Hz, CH_2CH_2O), 3.72 (3H, s, $COOCH_3$), 4.47 (2H, s,

OCH_2Ph), 7.25, 7.37 (4H, 2d, J=8.2 Hz, Ar); CIMS m/z 303 (M+1, 83.0%).

Methyl 5-[4-(1-isopropyl-3,3-dimethylbutoxymethyl)-phenyl]-4-pentynoate (16). This compound was obtained as a black liquid (52.4% yield) from **12** and methyl 4-pentynoate, using the procedure described for **5**: ^{1}H NMR δ 0.87, 0.88 (6H, 2d, J=6.8 Hz, CH(CH_3)$_2$), 0.92 (9H, s, (CH_3)$_3$C), 1.28 (1H, dd, J=14.6, 2.2 Hz, (CH$_3$)$_3$CCH_2), 1.36 (1H, dd, J=14.6, 7.6 Hz, (CH$_3$)$_3$CCH_2), 2.05 (1H, dsp, J=6.8, 3.4 Hz, CH(CH$_3$)$_2$), 2.63, 2.73 (4H, 2ddd, C≡C(CH_2)$_2$), 3.30 (1H, ddd, J=7.6, 3.5, 2.2 Hz, CHO), 3.72 (3H, s, COOCH_3), 4.37, 4.53 (2H, 2d, J=11.6 Hz, OCH_2Ph), 7.26, 7.34 (4H, 2d, J=8.3 Hz, Ar); CIMS m/z 345 (M+1, 62.3%).

5-[4-(3,3-Dimethylbutoxymethyl)phenyl]-4-pentynoic acid (17). A mixture of **15** (100 mg, 0.33 mmol), KOH (20 mg, 0.36 mmol), H_2O (10 mg, 0.56 mmol), MeOH (0.5 mL) and CH_2Cl_2 (20 mL) was stirred at room temperature for 3 h. The aqueous layer was acidified with 1 N HCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was concentrated and extracted with an $NaHCO_3$ solution. The aqueous layer was washed with *n*-hexane, acidified with 1 N HCl, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried and concentrated. The residue was recrystallized to yield **17** as a white solid (15.3 mg, 16.3% yield): mp 125.1 °C; ^{1}H NMR δ 0.91 (9H, s, (CH_3)$_3$C), 1.56 (2H, t, J=7.4 Hz, (CH$_3$)$_3$CCH_2), 2.71 (4H, m, C≡C(CH_2)$_2$), 3.51 (2H, t, J=7.4 Hz, CH$_2$CH_2O), 4.47 (2H, s, OCH_2Ph), 7.25, 7.36 (4H, 2d, J=8.3 Hz, Ar); CIMS m/z 289 (M+1, 21.1%).

5-[4-(1-Isopropyl-3,3-dimethylbutoxymethyl)phenyl]-4-pentynoic acid (18). This compound was obtained as a yellow solid (35.5% yield) from **16**, using the procedure described for **17**: mp 65.2 °C; ^{1}H NMR δ 0.87, 0.88 (6H, 2d, J=6.8 Hz, CH(CH_3)$_2$), 0.92 (9H, s, (CH_3)$_3$C), 1.28 (1H, dd, J=14.6, 2.2 Hz, (CH$_3$)$_3$CCH_2), 1.36 (1H, dd, J=14.6, 7.4 Hz, (CH$_3$)$_3$CCH_2), 2.05 (1H, dsp, J=6.8, 3.7 Hz, CH(CH$_3$)$_2$), 2.73 (4H, m, C≡C(CH_2)$_2$), 3.30 (1H, ddd, J=7.4, 3.7, 2.2 Hz, CHO), 4.38, 4.53 (2H, 2d, J=11.7 Hz, OCH_2Ph), 7.26, 7.35 (4H, 2d, J=8.3 Hz, Ar); CIMS m/z 331 (M+1, 3.3%).

4-Bromophenyl 4,4-dimethylpentyl ether (19). A solution of 4,4-dimethylpentanoic acid (5.5 g, 42.3 mmol) in dry Et_2O (50 mL) was added dropwise to a suspension of $LiAlH_4$ (1.6 g, 42.3 mmol) in dry Et_2O (100 mL). After 5-h reflux, the suspension was slowly poured into an ice-cold 15% H_2SO_4 solution (100 mL). The solution was partitioned between EtOAc and H_2O, and the EtOAc layer was dried and concentrated to give 4,4-dimethyl-1-pentanol as a colorless liquid (2.1 g, 44.8% yield). A solution of 4,4-dimethyl-1-pentanol (2.2 g, 18.9 mmol) in pyridine (0.5 g, 6.3 mmol) was added to an ice-cold, stirred solution of PBr_3 (2.2 g, 8.2 mmol) containing a few drops of pyridine. After 12-h reflux, the solution was distilled to give 1-bromo-4,4-dimethylpentane as a colorless liquid (1.8 g, 54.4% yield): bp 148–149 °C. A solution of 1-bromo-4,4-dimethylpentane (1.8 g, 10.3 mmol) in dry THF (6 mL) was added to an ice-cold, stirred suspension of NaH (0.3 g, 12.5 mmol) in dry THF (10 mL) and dry *N*,*N*-dimethylformamide (DMF) (4 mL) under a nitrogen atmosphere. The suspension was stirred in an ice bath for 1 h and at room temperature for 1 h. To the suspension cooled in an ice-bath was added 4-bromophenol (1.77 g, 10.3 mmol). After stirring in an ice bath for 1 h and at room temperature for 24 h, a small amount of MeOH was added, and the solution was extracted with Et_2O. The Et_2O extract was washed with water, dried, and concentrated. The residue was purified by column chromatography on silica gel with benzene: *n*-hexane (1:1) to give **19** as a colorless liquid (650 mg, 23.4% yield): ^{1}H NMR δ 0.92 (9H, s, (CH_3)$_3$C), 1.31 (2H, m, (CH$_3$)$_3$CCH_2), 1.71–1.78 (2H, m, CH$_2$CH_2CH$_2$), 3.89 (2H, t, J=6.7 Hz, CH$_2$O), 6.77, 7.36 (4H, AA′XX′, J=9.0, 2.8 Hz, Ar); CIMS m/z 270 (M, 45.0%), 271 (M+1, 18.3%), 272 (M+2, 44.3%), 273 (M+3, 16.7%).

4-Bromophenyl 2-isopropyl-4,4-dimethylpentyl ether (20). A mixture of 2-isopropyl-4,4-dimethylpentanoic acid (370 mg, 2.2 mmol), dry methanol (50 mL), and H_2SO_4 (3 g) was refluxed for 12 h. The solution was extracted with CH_2Cl_2, and the CH_2Cl_2 extract was washed with an $NaHCO_2$ solution, dried, and concentrated to give methyl 2-isopropyl-4,4-dimethylpentanoate as a yellow liquid (216 mg, 54.0% yield). Methyl 2-isopropyl-4,4-dimethylpentanoate (3.2 g, 17 mmol) in dry THF (15 mL) was added dropwise to an ice-cold, stirred suspension of $LiAlH_4$ (3.2 g, 85 mmol) in dry THF (100 mL), and the suspension was refluxed for 10 h. The suspension was poured into a 3 N H_2SO_4 solution and extracted with EtOAc. The EtOAc extract was dried and concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane:EtOAc (9:1) to give 4,4-dimethyl-2-isopropyl-1-pentanol as a colorless liquid (1.9 g, 72.1% yield). A solution of PBr_3 (1.5 g, 5.5 mmol) containing a few drops of pyridine was slowly added to a stirred solution of 2-isopropyl-4,4-dimethyl-1-pentanol (1.7 g, 11 mmol) and pyridine (0.2 g, 2.5 mmol) at −20 °C. The solution was stirred at −20 °C for 1 h and was heated at 80–90 °C for 12 h. After cooling in an ice bath, H_2O was added to destroy excess PBr_3, and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was dried, concentrated, and distilled to give 1-bromo-2-isopropy-4,4-dimethylpentane as a colorless liquid (1.0 g, 42.9% yield): bp 60–65 °C/20 mmHg. To an ice-cold, stirred suspension of NaH (260 mg, 11 mmol), dry THF (18 mL), and dry DMF (7 mL) was slowly added a solution of 4-bromophenol (470 mg, 2.7 mmol) in dry THF (5 mL) under a nitrogen atmosphere. After the mixture was stirred in an ice bath for 1 h and at room temperature for 1 h, a solution of 1-bromo-2-isopropyl-4,4-dimethylpentane (600 mg, 2.7 mmol) in dry THF (5 mL) was slowly added to the ice-cold, stirred mixture. The mixture was stirred in an ice bath for 1 h and at room temperature for 24 h. A small amount of MeOH was added to the mixture to destroy excess NaH, and the solution was extracted with Et_2O. The Et_2O extract was washed with H_2O, dried, and concentrated. The residue was purified by column chromatography on silica gel with *n*-hexane to give **20** as a colorless liquid (435 mg, 51.1% yield): ^{1}H NMR δ 0.88, 0.91 (6H, 2d, J=6.8 Hz, CH(CH_3)$_2$),

0.92 (9H, s, $(CH_3)_3C$), 1.05 (1H, dd, $J=14.3$, 6.3 Hz, $(CH_3)_3CH_2$), 1.26 (1H, dd, $J=14.3$, 2.7 Hz, $(CH_3)_3CH_2$), 1.74 (1H, m, $CHCH_2O$), 1.96 (1H, dsp, $J=6.8$, 3.8 Hz, $CH(CH_3)_2$), 3.76 (1H, dd, $J=9.2$, 7.6 Hz, CH_2O), 3.80 (1H, dd, $J=9.2$, 5.1 Hz, CH_2O), 6.77, 7.35 (4H, AA′XX′, $J=9.0$, 2.8 Hz, Ar); EIMS m/z 312 (M, 20.0%), 314 (M + 2, 19.0%).

4-Ethynylphenyl 4,4-dimethylpentyl ether (21). This compound was obtained as a colorless liquid (15.0% yield) from **19** and trimethylsilylacetylene, using the procedure described for **3**: 115-120 °C/2 mmHg; ^{1}H NMR δ 0.92 (9H, s, $(CH_3)_3C$), 1.32 (2H, m, $(CH_3)_3CCH_2$), 1.72–1.79 (2H, m, $CH_2CH_2CH_2$), 2.99 (1H, s, C≡CH), 3.93 (2H, t, $J=6.7$ Hz, CH_2O), 6.83, 7.41 (4H, AA′XX′, $J=8.8$, 2.4 Hz, Ar); CIMS m/z 217 (M + 1, 83.0%).

4-Ethynylphenyl 2-isopropyl-4,4-dimethylpentyl ether (22). This compound was obtained as a yellow liquid (18.8% yield) from **20** and trimethylsilylacetylene, using the procedure described for **3**: ^{1}H NMR δ 0.88, 0.91 (6H, 2d, $J=6.8$ Hz, $CH(CH_3)_2$), 0.92 (9H, s, $(CH_3)_3C$), 1.06 (1H, dd, $J=14.4$, 6.6 Hz, $(CH_3)_3CH_2$), 1.26 (1H, dd, $J=14.4$, 2.7 Hz, $(CH_3)_3CH_2$), 1.75 (1H, m, $CHCH_2O$), 1.96 (1H, dsp, $J=6.8$, 3.8 Hz, $CH(CH_3)_2$), 2.98 (1H, s, C≡CH), 3.80 (1H, dd, $J=9.3$, 7.6 Hz, CH_2O), 3.84 (1H, dd, $J=9.3$, 5.4 Hz, CH_2O), 6.83, 7.41 (4H, AA′XX′, $J=8.9$, 2.4 Hz, Ar); CIMS m/z 259 (M + 1, 8.2%).

Methyl 5-[4-(4,4-dimethylpentoxy)phenyl]-4-pentynoate (23). This compound was obtained as a yellow liquid (72.2% yield) from **19** and methyl 4-pentynoate, using the procedure described for **5**: ^{1}H NMR δ (DMSO-d_6) 0.91 (9H, s, $(CH_3)_3C$), 1.31 (2H, m, $(CH_3)_3CCH_2$), 1.71–1.78 (2H, m, $CH_2CH_2CH_2$), 2.60–2.66, 2.69–2.74 (4H, 2m, $C\equiv C(CH_2)_2$), 3.72 (3H, s, $COOCH_3$), 3.91 (2H, t, $J=6.7$ Hz, CH_2O), 6.79, 7.30 (4H, AA′XX′, $J=9.0$, 2.4 Hz, Ar); CIMS m/z 303 (M + 1, 100%).

Methyl 5-[4-(2-isopropyl-4,4-dimethylpentoxy)phenyl]-4-pentynoate (24). This compound was obtained as a colorless viscous liquid (12.3% yield) from **20** and methyl 4-pentynoate, using the procedure described for **5**: ^{1}H NMR δ 0.88, 0.91 (6H, 2d, $J=6.8$ Hz, $CH(CH_3)_2$), 0.91 (9H, s, $(CH_3)_3C$), 1.05 (1H, dd, $J=14.4$, 6.3 Hz, $(CH_3)_3CH_2$), 1.25 (1H, dd, $J=14.4$, 2.7 Hz, $(CH_3)_3CH_2$), 1.74 (1H, m, $CHCH_2O$), 1.96 (1H, dsp, $J=6.8$, 3.7 Hz, $CH(CH_3)_2$), 2.60–2.66, 2.69–2.76 (4H, 2m $C\equiv C(CH_2)_2$), 3.71 (3H, s, $COOCH_3$), 3.78 (1H, dd, $J=9.3$, 7.6 Hz, CH_2O), 3.82 (1H, dd, $J=9.3$, 5.1 Hz, CH_2O), 6.79, 7.30 (4H, AA′XX′, $J=9.0$, 2.4 Hz, Ar); CIMS m/z 245 (M + 1, 28.4%).

5-[4-(4,4-Dimethylpentoxy)phenyl]-4-pentynoic acid (25). This compound was obtained as a colorless solid (10.2% yield) from **23**, using the procedure described for **7**: mp 135.5 °C; ^{1}H NMR δ (DMSO-d_6) 0.89 (9H, s, $(CH_3)_3C$), 1.28 (2H, m, $(CH_3)_3CCH_2$), 1.63–1.70 (2H, m, $CH_2CH_2CH_2$),2.50, 2.59 (4H, 2m, $C\equiv C(CH_2)_2$), 3.94 (2H, t, $J=6.5$ Hz, CH_2O), 6.88, 7.27 (4H, AA′XX′, $J=8.9$, 2.4 Hz, Ar), 12.30 (1H, s, COOH); CIMS m/z 289 (M + 1, 5.3%).

5-[4-(2-Isopropyl-4,4-dimethylpentoxy)phenyl]-4-pentynoic acid (26). This compound was obtained as white needles (36.4% yield) from **24**, using the procedure described for **7**: mp 96.4–97.6 °C; ^{1}H NMR δ 0.88, 0.90 (6H, 2d, $J=6.8$ Hz, $CH(CH_3)_2$), 0.91 (9H, s, $(CH_3)_3C$), 1.05 (1H, dd, $J=14.4$, 6.6 Hz, $(CH_3)_3CH_2$), 1.25 (1H, dd, $J=14.4$, 2.7 Hz, $(CH_3)_3CH_2$), 1.74 (1H, m, $CHCH_2O$), 1.96 (1H, dsp, $J=6.8$, 3.7 Hz, $CH(CH_3)_2$), 2.66–2.75 (4H, m, $C\equiv C(CH_2)_2$), 3.78 (1H, dd, $J=9.3$, 7.6 Hz, CH_2O), 3.82 (1H, dd, $J=9.3$, 5.4 Hz, CH_2O), 6.79, 7.30 (4H, AA′XX′, $J=8.9$, 2.5 Hz, Ar); CIMS m/z 331 (M + 1, 65.3%).

Equilibrium binding studies

Rat brain P_2 membranes were prepared from 5-week-old male Wistar rats by a modification of the method of Squires et al.[18] Forebrains stored at −80 °C were thawed and homogenized in ice-cold 1 mM EDTA using a Teflon-glass homogenizer. The homogenate was centrifuged at 1000×g for 10 min. The supernatant was then centrifuged at 25,000×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 recentrifuged at 25,000×g for 30 min, and the pellets were stored at −80 °C until use. The frozen pellet was thawed and suspended in 10 mM sodium phosphate buffer containing 300 mM NaCl (pH 7.5) (buffer A) for the binding assays.

Housefly head P_2 membranes were prepared from 5- to 10-day-old adult houseflies (*Musca domestica* L.) of WHO strain by a modification of the method of Deng et al.[19] The heads were homogenized in 10 mM Tris–HCl buffer containing 0.25 M sucrose (pH 7.5) with a glass–Teflon homogenizer, 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 nylon screen and centrifuged at 25,000×g for 30 min. The pellets were suspended in the buffer and allowed to stand in an ice bath for 30 min. The suspension was centrifuged at 25,000×g for 30 min. The pellets were resuspended in buffer A and used immediately for binding assays without freezing.

[^{3}H]EBOB binding assays were carried out according to Cole and Casida[4] and Deng et al.[19] Briefly, DBCPP and its derivatives were incubated with rat brain membranes (125 μg protein) or housefly head membranes (200 μg protein) and 0.5 nM [^{3}H]EBOB (38 Ci/mmol, NEN Life Science Products, Inc.) in 1.0 mL of buffer A at 37 °C for 90 min (rat) or at 22 °C for 70 min (housefly). Protein was measured by the method of Bradford.[20] Test compounds and unlabeled EBOB were added as DMSO solutions (4 μL) to reaction mixtures so as to give the desired final concentrations. After incubation, the mixtures were filtered through GF/B filters (Whatman International Ltd.) and were rapidly rinsed twice with 5 mL of ice-cold buffer A using a Brandel M-24 cell harvester (Biomedical Research and Development Laboratories, Inc.). The radioactivity of [^{3}H]EBOB that specifically bound to membranes on the filters was

measured with a Beckman LS 6000SE liquid scintillation spectrometer. Nonspecific binding was determined in the presence of 5 μM unlabeled EBOB. Each experiment was performed in triplicate and repeated twice. IC_{50} values were obtained by the Probit method.

Whole-cell current recording

The dorsal root ganglia were dissected from the lumbodorsal region of newborn rats (1–5 days postnatal) and were immediately placed into a Ca^{2+} and Mg^{2+}-free phosphate-buffered saline solution supplemented with 6 g/L of glucose. The ganglia were digested in a phosphate-buffered saline solution containing 2.5 mg/mL of trypsin (Sigma Chemical Co.) for 20 min at 37 °C. The ganglia were then dissociated by repeated triturations using a fire-polished Pasteur pipette in Dulbecco's Modified Eagle Medium containing 0.1 mg/mL of fetal bovine serum and 0.08 mg/mL of gentamicin. The dissociated cells were placed on coverslips coated with poly-L-lysine. Neurons were maintained in Dulbecco's Modified Eagle Medium containing serum and gentamicin in a 90% air–10% CO_2 atmosphere controlled at 37 °C. Neurons cultured for 2 to 5 days were used for experiments.

Membrane currents were recorded using the whole-cell patch clamp technique at room temperature (22 °C). Pipette electrodes were made from 0.8-mm (I.D.) borosilicate glass capillary tubes and fire-polished. The electrode had a resistance of about 2 to 3 MΩ when filled with a standard internal solution. The membrane was clamped at −60 mV, and a 5-min period was allowed following the rupture of the membrane to equilibrate the cell interior with a pipette solution. Currents through the electrode were recorded by an Axopatch 200B amplifier (Axon instruments), filtered at 2 kHz, and stored in a microcomputer. Currents were continuously monitored by a chart recorder. Internal and external solutions were designed to eliminate sodium and potassium currents. The standard internal solution contained (in mM): CsCl 140, $MgCl_2$ 1, EGTA 5, and HEPES 10. The pH was adjusted to 7.3 with Tris base, and the osmolarity was adjusted to 290 mOsm. The standard external solution contained (in mM): choline chloride 136, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, and the pH was adjusted to 7.3 with Tris base. GABA was first dissolved in distilled water to make stock solutions. DBCPP was dissolved in DMSO. Stock solutions were then diluted with the standard external solution. The final concentration of DMSO in test solutions was 0.1% (v/v) or less, which had no adverse effect on GABA-induced currents. Test solutions were applied to the cell using a locally developed application system.[9] The application was controlled by a computer-operated magnetic valve. Using this application system, the external solution surrounding the cell could be completely changed within 100 ms. The IC_{50} value and the slope factor (Hill coefficient) of DBCPP were calculated from the equation:

$$I = I_{max} C^n / (C^n + IC_{50}^n)$$

where I is the amplitude of GABA-induced current, I_{max} the maximum current, C the DBCPP concentration, and n the Hill coefficient. The nonlinear regression analysis was carried out using the least squares fitting method (Sigmaplot, Version 4.0) by a microcomputer. Whole-cell current records were analyzed by the pClamp version 6.0 software (Axon instruments).

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