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Synthesis and Insecticidal Activity of Novel Carbamate Derivatives as Potential Dual-Binding Site Acetylcholinesterase Inhibitors

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In biological systems, bivalent ligands often possess increased functional affinity for their receptors compared with monovalent ligands. On the basis of the structure of acetylcholinesterase (AChE), a series of novel carbamate heterodimetic derivatives were designed and synthesized with the aim of increasing the potency toward AChE inhibition. The AChE inhibitory ability of all the novel compounds was tested using AChE obtained from the brain of the housefly. The bioassay results showed that compounds **6j**, **6k**, **6m**, **6n**, **6p**, and **6q** had increased inhibitory activities in comparison with parent phenyl *N*-methylcarbamate (MH) at the concentration of 100 mg/L. Among them, the most potent AChE inhibitor of these compounds was **6q** (IC_{50} = 12 μM), which showed 62-fold greater AChE inhibitory activity than that of MH and 12-fold greater activity than metolcarb (MT), which suggested that the 3-nitrophenoxy moiety of compound **6q** was able to interact with the aromatic amino acid residues lining the gorge and the phenyl *N*-methylcarbamate moiety was able to interact with the catalytic sites of AChE, simultaneously. The insecticidal activities of compounds **6j**, **6k**, **6m**, **6n**, **6p**, and **6q** were further evaluated. Consistent with the result in vitro bioassay, those compounds demonstrated better activities against *Lipaphis erysimi* than parent compound **MH** at the concentration of 300 mg/L, and compound **6q** showed the best insecticidal activity, causing 98% mortality after 24 h of treatment.

KEYWORDS: Acetylcholinesterase; carbamate heterodimetic derivatives; inhibitory activity; phenyl *N*-methylcarbamate

INTRODUCTION

Multivalent ligand−receptor interactions, known as the cluster effect, are defined as specific simultaneous associations of multiple ligands presented on a molecule that bind to multiple receptors presented on a biological entity (*1*−*3*). It is well-known that multivalent binding interactions could enhance functional affinity (*4*−*7*) and receptor selectivity (*8*−*10*) compared to the monomeric ones, thus providing a broad range of benefits and unique roles that are not easily achievable with monovalent interactions.

Conventional carbamate insecticides, such as metolcarb, methomyl, thiodicarb, carbofuran, carbosulfan, and aldicarb have been used to control insect pests throughout the world during the past several decades. However, the intensive use of carbamate insecticides has resulted in the development of resistance to these insecticides (*11*−*14*). As the discovery of new modes of action insecticides has become ever-increasingly challenged, development of new insecticides with known modes of action remains an important strategy. The carbamate insecticides inhibit acetylcholinesterase (AChE: EC 3.1.1.7), a key enzyme in the nervous system of insects, by covalently carbamylating the serine residue within the active site gorge (*15*). AChE terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine. The remarkable features of AChE are its deep and narrow gorge, referred to as the "active site gorge", and a peripheral site existing at the gorge mouth. Fourteen aromatic residues lined a substantial portion of the surface of the gorge (~40%). Those residues and their flanking sequences are highly conserved in AChEs among different species (*16*), providing multiple hydrophobic binding sites (*17*−*19*). On the basis of the unique structure of AChE, many potential dual-site binding inhibitors of AChE have been synthesized and tested as drugs to treat or alleviate Alzheimer's disease (AD) (*20*−*24*). For example, Donepezil, approved in 1996 by the U.S. FDA and marketed as Aricept, features a unique orientation along the active site gorge, extending from the anionic subsite at the bottom of the gorge to the peripheral anionic site at the top of the gorge, via aromatic $\pi-\pi$ stacking interactions with conserved aromatic acid residues (*25*). Recently, several AChE−serotonin transporter (SERT) dual inhibitors were reported; the increased AChE inhibitory potency was attributed to the simultaneous binding of the units to the

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active and the hydrophobic site of the AChE (*19*). It was proposed that the phenoxy moiety interacted efficiently with the conserved aromatic amino acid residues lining the gorge and resulted in the increasing AChE inhibitory ability.

Continuing with our research on dual-site inhibitors of AChE in the pesticide field (*26*, *27*) and hypothesizing that the phenoxy moiety would be able to interact with aromatic amino acid residues through $\pi-\pi$ interactions, we have designed and synthesized a series of novel carbamate derivatives incorporated with a phenoxy moiety as potential AChE inhibitors to enhance the insecticidal activity and to overcome the resistance via additional interaction in the gorge of the AChE.

MATERIALS AND METHODS

Synthesis. ^{1}H NMR spectra were recorded in $CDCl_3$ with a Bruker DPX300 spectrometer, using tetramethylsilane as internal standard (performed at China Agricultural University). Elemental analyses (C, H, N) were performed at the Institute of Chemistry, Chinese Academy of Sciences. Melting points were measured on a WRS-2A melting point apparatus and are uncorrected. The solvents and reagents were used as received or were dried prior to use as needed.

1-(3-Bromopropoxy)-4-(trifluoromethyl)benzene (2b). To a solution of 1,3-dibromopropane (46.5 g, 0.23 mol) in acetone (200 mL) were added 4-trifluoromethylphenol (15 g, 0.092 mol) and potassium carbonate (19 g, 0.138 mol). After 16 h of refluxing, the reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated, and the resulting mixture was purified by column chromatography on silica gel (ethyl acetate/hexane = 1:70) to give 1-(3-bromopropoxy)-4-(trifluoromethyl)benzene (22.88 g, 88% yield) as a yellow liquid. Intermediates **2a**−**2k** were synthesized via the same procedures accordingly.

4-(3-(4-(Trifluoromethyl)phenoxy)propoxy)phenol (3b). A mixture of *p*-hydroquinone (4.8 g, 0.044 mol), compound **2b** (5 g, 0.018 mol), and potassium carbonate (7.3 g, 0.053 mol) in acetone (100 mL) was refluxed for 24 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated, and the resulting mixture was purified by column chromatography on silica gel (ethyl acetate/hexane = 1:10) to give 4-(3-(4-(trifluoromethyl)phenoxy)propoxy)phenol (3.9 g, 71% yield) as a yellow liquid. Intermediates **3a**−**3d**, **4a**−**4d**, and **5a**−**5k** were synthesized via the same procedures accordingly.

4-(3-(4-(Trifluoromethyl)phenoxy)propoxy)phenyl Methylcarbamate (6b). Compound **3b** (1.05 g, 3.36 mmol) and methylcarbamic chloride (0.63 g, 6.72 mmol, 90%) were dissolved in dichloromethane (50 mL) in an ice bath, and then 20% sodium hydroxide aqueous solution (2 mL) was added to the solution for 5 min at 0−5 °C. After the mixture had been stirred for 20 min in an ice bath, 5% brine (50 mL) was added, and the mixture was stirred for another 20 min at room temperature. The organic layer was isolated and washed with water twice and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 1:3) to give 4-(3-(4-(trifluoromethyl)phenoxy)propoxy)-phenyl methylcarbamate (0.8 g, 65% yield) as a white solid. Target compounds **6a**−**6s** were synthesized via the same procedures accordingly.

4-(3-(4-(Trifluoromethyl)phenoxy)propoxy)phenyl Dimethylcarbamate (7b). Compound **3b** (1.25 g, 4 mmol) and *N*,*N*-dimethylcarbamic chloride (0.64 g, 6 mmol) were dissolved in *N*,*N*-dimethylformamide (10 mL), and then potassium carbonate (0.83 g, 6 mmol) was added. After the mixture had been stirred at room temperature for 8 h, the reaction mixture was poured into water (10 mL) and then extracted with ethyl acetate (2 × 20 mL). The combined organic extracts were washed with brine and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 1:3) to give 4-(3-(4-(trifluoromethyl)phenoxy)propoxy)phenyl dimethylcarbamate (**7b**) (1.13 g, 73.8% yield). Target compounds **7a**−**7l** were synthesized via the same procedures accordingly.

The structures, appearance, melting points, and yields of target compounds **6a**−**6s** and **7a**−**7l** are listed in **Table 1**, and their ^{1}H NMR and elemental analysis are given in the Supporting Information.

In Vitro AChE Inhibitory Bioassay. To evaluate the biological profiles of these novel compounds, AChE inhibiton was assayed in comparison with phenyl *N*-methylcarbamate (MH) and metolcarb (MT). AChE was prepared from heads of adult houseflies (female/ male = 1:1). Inhibitory activities of these compounds were measured against AChE according to a previously reported method (*26*, *27*).

In Vivo Insecticidal Activities Assay. To evaluate the insecticidal activity of synthesized compounds **6j**, **6k**, **6m**, **6n**, **6p**, and **6q**, insecticidal activities were carried out with *Lipaphis erysimi*. These insects were reared in a room maintained at 25 (±1) °C, 60 (±5)% relative humidity, and 14 h light photoperiod. Stock solutions of test compound were prepared in acetone at a concentration of 10 g/L and then diluted to the required test concentrations with water (containing 0.1% Triton X-100).

Disks of Chinese cabbage leaves with 35 apterous adult *L. erysimi* were prepared. Leaf disks with insects were separately dipped in a test solution for 10 s. After drying, the leaves were placed in a plastic dish (6 cm diameter). The dishes were placed into an incubator at 25 °C, and mortality was determined after 24 h by the number in the treated dishes relative to that in the untreated controls. The untreated control experiments were carried out under the same conditions without test compounds. This insecticidal activity of MT was also evaluated against *L. erysimi* and utilized as positive control. All experiments and the respective controls were carried out in three replicates, and the corrected mortality was calculated (**Table 2**).

RESULTS AND DISCUSSION

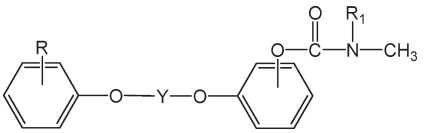
On the basis of the dual-site binding strategy in rational design of AChE inhibitors, phenyl carbamate was selected as a parent compound to bind to the active site gorge, and the substituted phenoxy moiety was chosen as a pharmacophoric element corresponding to the hydrophobic sites. Therefore, we designed a series of compounds containing phenyl carbamate and substituted phenoxy linked by different carbon numbers of alkylene to simultaneously bind to the catalytic site and the hydrophobic site as potential dual-site binding inhibitors of AChE. The synthesis, AChE inhibitory assays, and insecticidal activities against *L. erysimi* are reported here.

Synthesis. During the preparation of the intermediate (3-bromoalkoxy)benzene (**Scheme 1**), both bromine atoms of the α,ω-dibromoalkanes could possibly be replaced by the substituted phenoxy to generate the bis-substituted byproduct. However, the reactions provided the monosubstituted products (**2**) as the major products in excellent isolated yields by using an excess of α,ω-dibromoalkanes. The reaction of intermediates **2** with different dihydroxybenzenes (**Scheme 2**) also gave the desired monosubstituted products by using an excess of dihydroxybenzene. Target compounds **6** and **7** were synthesized by reacting *N*-methylcarbamoyl chloride and *N*,*N*-dimethylcarbamoyl chloride with intermediates **3**, **4**, and **5**, respectively (**Scheme 3**).

In Vitro Inhibition of AChE Activities. The AChE inhibitory ability of all the new compounds was tested using AChE obtained from the brain of the housefly. As shown in **Table 1**, compounds **6j**, **6k**, **6m**, **6n**, **6p**, and **6q** exhibited increased inhibitory activities in comparison with parent phenyl *N*-methylcarbamate (MH) at the concentration of 100 mg/L. These six compounds had three common structural features. First, these compounds possessed the structure of *N*-methylcarbamate; second, the *N*-methylcarbamate group was in the meta-position of the aromatic ring; third, the linker between phenyl carbamate and the substituted phenoxy moiety was a chain of three or four carbon atom alkoxy units. The IC_{50} values of compounds **6j**, **6k**, **6m**, **6n**, **6p**, and **6q** (**Table 2**) demonstrated higher inhibitory activity for AChE than MH. The most potent AChE inhibitor of these compounds was **6q** (IC_{50} = 12 μM), which showed 62-fold greater AChE inhibitory activity than MH and 12-fold greater activity than MT.

The data in **Table 1** show that all *N*,*N*-dimethylcarbamate heterodimetic derivatives exhibited the same low inhibition of

Table 1. Melting Point, Yield, and in Vitro Inhibition of AChE for Target Compounds at 100 mg/L



compd	R	Y	$-OOCNR_1CH_3$	R_1	appearance	mp (°C)	yield (%)	inhibition rate of AChE (%)
MH								46
6a	4-CF_3	CH_2CH_2	*p*-	H	white solid	163−164	55.5	2
6b	4-CF_3	$CH_2CH_2CH_2$	*p*-	H	white solid	152−154	40.6	2
6c	4-CF_3	$CH_2CH_2CH_2CH_2$	*p*-	H	white solid	99−101	52.5	4
6d	4-CF_3	$CH_2CHCHCH_2$	*p*-	H	white solid	151−152	48.4	3
6e	4-CF_3	CH_2CH_2	*o*-	H	white solid	142−143	49.3	13
6f	4-CF_3	$CH_2CH_2CH_2$	*o*-	H	white solid	91−93	48.7	25
6g	4-CF_3	$CH_2CH_2CH_2CH_2$	*o*-	H	white solid	94−95	46.6	19
6h	4-CF_3	$CH_2CHCHCH_2$	*o*-	H	white solid	103−106	48.6	17
6i	4-CF_3	CH_2CH_2	*m*-	H	white solid	86−88	52.5	20
6j	4-CF_3	$CH_2CH_2CH_2$	*m*-	H	white solid	94−96	52.0	61
6k	4-CF_3	$CH_2CH_2CH_2CH_2$	*m*-	H	white solid	92−95	45.5	62
6l	4-CF_3	$CH_2CHCHCH_2$	*m*-	H	white solid	105−107	46.5	12
6m	4-NO_2	$CH_2CH_2CH_2$	*m*-	H	white solid	103−105	50.2	69
6n	4-NO_2	$CH_2CH_2CH_2CH_2$	*m*-	H	white solid	123−126	52.5	80
6o	4-NO_2	$CH_2CHCHCH_2$	*m*-	H	white solid	153−154	47.8	45
6p	2-NO_2	$CH_2CH_2CH_2CH_2$	*m*-	H	pale yellow solid	66−67	49.2	80
6q	3-NO_2	$CH_2CH_2CH_2CH_2$	*m*-	H	pale yellow solid	66−67	49.2	92
6r	2-OCH_3	$CH_2CH_2CH_2CH_2$	*m*-	H	pale yellow solid	70−74	45.2	46
6s	4-Cl	$CH_2CH_2CH_2CH_2$	*m*-	H	pale yellow solid	78−81	49.5	30
7a	4-CF_3	CH_2CH_2	*p*-	CH_3	white solid	109−112	52.4	6
7b	4-CF_3	$CH_2CH_2CH_2$	*p*-	CH_3	pale yellow solid	119−121	46.1	1
7c	4-CF_3	$CH_2CH_2CH_2CH_2$	*p*-	CH_3	white solid	113−114	47.5	6
7d	4-CF_3	$CH_2CHCHCH_2$	*p*-	CH_3	white solid	99−101	45.8	0
7e	4-CF_3	CH_2CH_2	*o*-	CH_3	pale yellow solid	64−67	50.4	15
7f	4-CF_3	$CH_2CH_2CH_2$	*o*-	CH_3	pale yellow viscous oil	_	46.5	25
7g	4-CF_3	$CH_2CH_2CH_2CH_2$	*o*-	CH_3	yellow viscous oil	_	50.8	7
7h	4-CF_3	$CH_2CHCHCH_2$	*o*-	CH_3	white solid	_	54.1	7
7i	4-CF_3	CH_2CH_2	*m*-	CH_3	white solid	_	47.4	8
7j	4-CF_3	$CH_2CH_2CH_2$	*m*-	CH_3	pale yellow viscous oil	_	48.8	27
7k	4-CF_3	$CH_2CH_2CH_2CH_2$	*m*-	CH_3	pale yellow viscous oil	_	48.5	20
7l	4-CF_3	$CH_2CHCHCH_2$	*m*-	CH_3	white solid	69−72	49.8	13

Table 2. In Vitro Inhibition of AChE and in Vivo Insecticidal Activity of Some Compounds

	in vitro inhibition of AChE activities				in vivo insecticidal activity (mortality, %)
compd	IC_{50} (95% confidence intervals) (μM)	slope (±SE)	ratio to MH	ratio to MT	*Lipaphis erysimi* (300 mg/L)
MH	766 (711−826)	1.3 (±0.2)	1		76 e[a]
MT	150 (68−330)	0.8 (±0.3)			97 ab
6j	105 (73−149)	1.1 (±0.2)	7	1	85 d
6k	107 (99−115)	1.2 (±0.2)	7	1	92 c
6m	70 (40−118)	0.9 (±0.2)	11	2	89 cd
6n	46 (25−81)	0.6 (±0.2)	17	3	92 c
6p	61 (50−72)	1.5 (±0.3)	13	2	93 bc
6q	12 (11−14)	1.3 (±0.2)	62	12	98 a

[a] Treatments were grouped into statistically distinct classes by applying analysis of variance (ANOVA) at $P = 0.05$ based on Fisher's LSD.

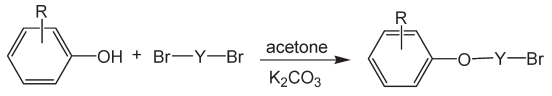
AChE as parent phenyl *N*,*N*-dimethylcarbamate (IC_{50} = 2961 μg mL^{-1}) (*26*), whereas *N*-methylcarbamate heterodimeric molecules showed higher inhibition against AChE than *N*,*N*-dimethylcarbamate heterodimetic molecules. The AChE inhibition potency of the carbamate heterodimetic derivatives varied evidently with the length of the tether chain. Heterodimetic derivatives with a two-carbon-atom chain showed the lowest inhibition of AChE, which may result from too short of a tether length to bind well the dual site of the anzyme. The introduction of butylene with a less flexible linker led to reduced activity, indicating that a flexible chain was favorable for AChE inhibitory activities. Consequently, the variation of the tether chain among the heterodimetic derivatives affected the interaction on binding the dual site in AChE. The optimal tether length among our heterodimetic derivatives was a chain of four-carbon-atom alkoxy moiety. We reasoned that a four-carbon-atom linking chain bearing a phenyl ring might favorably interact with the aromatic residues lining the wall of the AChE gorge. As shown in **Table 1**, the effect of electron-donating (—OCH_3) and electron-withdrawing (—CF_3, —NO_2, —Cl) substituents R at the phenyl ether did not show good regularity to the inhibition of AChE, and nitro-substituted derivatives demonstrated high potency against AChE, which is consistent with the previous study (*19*). This might be due to hydrogen-bond formation between the NO_2

group and hydrogen-bond donor from residues lining the gorge of the AChE.

The mode of action of conventional phenyl carbamate insecticides is that the carbamate part of the molecule is attached to the esteratic site and the substituted aromatic part to the anionic sites of the AChE during the inhibitory process. As the distance between the esteratic and anionic sites was 0.5 nm in the AChE molecule, carbamate insecticides would be the most efficient if the distance between the two groups to be bound to the two sites of the anzyme was also 0.5 nm (*28*−*30*). Compound **6q** showed higher activity than MT, in which the substituent on the phenyl carbamate was longer than 0.5 nm to extend halfway into the gorge. It suggested that the 3-nitrobenzene group of this compound interacted well with the hydrophobic site lining the gorge to promote the affinity of the molecules.

Insecticidal Activities. The insecticidal activities of compounds **6j**, **6k**, **6m**, **6n**, **6p**, and **6q** against *L. erysimi* were investigated, and the results are shown in **Table 2**. As shown in **Table 2**, all compounds showed better activities against *L. erysimi* than parent compound MH at the concentration of 300 mg/L.

Scheme 1. Synthesis of Intermediates **2**

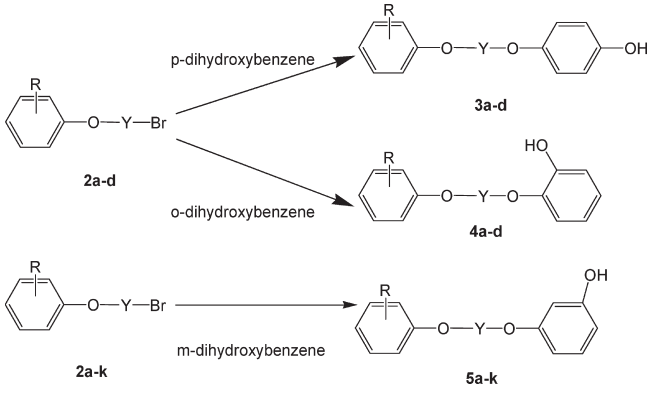


2a-d: R=4-CF_3; Y=CH_2CH_2, $CH_2CH_2CH_2$, $CH_2CH_2CH_2CH_2$, $CH_2CHCHCH_2$

2e-g: R=4-NO_2; Y=$CH_2CH_2CH_2$, $CH_2CH_2CH_2CH_2$, $CH_2CHCHCH_2$

2h-k: R=3-NO_2, 2-NO_2, 2-OCH_3, 4-Cl; Y=$CH_2CH_2CH_2CH_2$

Scheme 2. Synthesis of Intermediates **3**, **4**, and **5**



Compounds **6k**, **6n**, **6p**, and **6q** with four-carbon-atom chains demonstrated increased inhibitory activities, with >90% mortality in comparison with compounds **6j** and **6m** containing three-carbon-atom chains. This suggested the variation of the length of the tether chain had the same effect on insecticidal activities as shown in the in vitro inhibition. Consistent with the in vitro bioassay, compound **6q** showed the best insecticidal activity against *L. erysimi*, exhibiting an activity similar to that of the commercial insecticide MT. This result indicated that those carbamate heterodimetic derivatives not only showed excellent activities in vitro AChE inhibition but also had whole insect activity for *L. erysimi*. Further structural optimization and detailed structure−insecticidal activity relationships will be reported in the future.

In summary, we describe the design, synthesis, and structure−activity relationships (SAR) of a series of carbamate heterodimetic derivatives prepared with the aim of increasing the potency toward AChE inhibition. Heterodimetic derivative **6q** showed 62-fold higher inhibitory activity than that of parental compound and 12-fold higher activity than MT. In addition, compound **6q** exhibited the best insecticidal activity against *L. erysimi*. The results obtained from these studies were further confirmed on the dual-site binding design strategy in the pesticide field.

ABBREVIATIONS USED

AChE, acetylcholinesterase; AD, Alzheimer's disease; SERT, serotonin transporter; MH, *N*-methylcarbamate; MT, metolcarb.

Supporting Information Available: Experiment data are provided for compounds **6a**−**s** and **7a**−**l**. This material is available free of charge via the Internet at http://pubs.acs.org.

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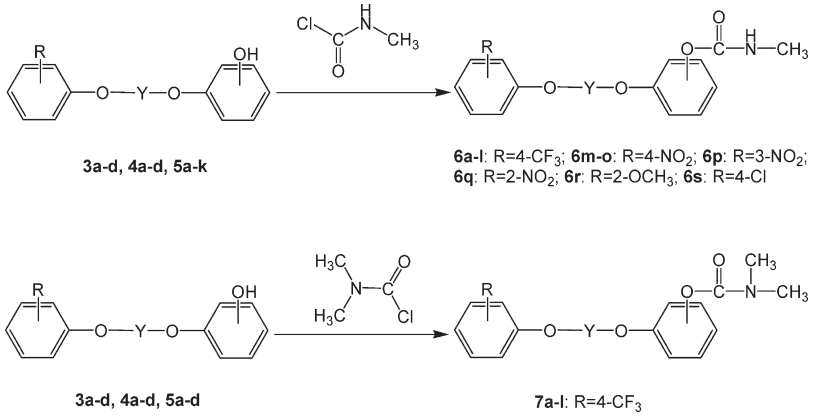
Scheme 3. Synthesis of Target Compounds **6** and **7**



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