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Synthesis and Antiviral Activity of Novel Chiral Cyanoacrylate Derivatives

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2-Cyanoacrylate is an important kind of herbicide targeted in photosystem II. Starting from cyano ethyl acetate, the chiral title compounds were synthesized under microwave irradiation, which has the advantages of shorter reaction time, higher yield, and simpler procedure. A half-leaf method was used to determine the inhibition and curative efficacies of the eight chiral products against tobacco mosaic virus in vivo. It was found that chiral compound **IIc-*R*** possesses moderate inhibition and curative effect in vivo with rates of 89.1 and 43.1%, respectively. In the MTT test, these new chiral compounds were found to possess weak antiproliferation activities to PC3 and A431 cells.

KEYWORDS: 2-Cyanoacrylates; TMV inhibitory agent; antiproliferation bioactivity; microwave; chirality

1. INTRODUCTION

2-Cyanoacrylates are of considerable importance because of their versatile biological activity and the possibility for application in agrochemistry, for example, herbicides that disrupt photosynthetic electron transportation at a common binding domain on the 32 kDa polypeptide of the photosystem II (PSII) reaction center (*1*, *2*). Among these cyanoacrylates, (*Z*)-ethoxyethyl 2-cyano-3-(4-chlorophenyl) methylamino-3-isopropyl-acrylate (CPNPE) exhibits the highest Hill inhibitory activity yet reported (*3*−*8*). To the best of our knowledge, there are no studies of the antiviral activity of cyanoacrylate in the literature (*9*). In our previous work, we designed and synthesized cyanoacrylates containing a 4-(trifluoromethyl)phenylamino moiety, which exhibited moderate antiviral bioactivity against tobacco mosaic virus (TMV) (*10*). In view of these facts and in continuation of our interest in the chemistry of cyanoacrylate, we contemplated undertaking the synthesis of, as yet, unreported novel chiral compounds containing cyanoacrylate moieties in order to obtain chiral compounds possessing better biological activity. To explore the bioactivity of the chiral analogues of such compounds, also considering that chiral compounds are the focus of much interest in modern agricultural chemistry because the chiral isomers are always biologically more active than their racemic mixtures and are more environmentally friendly due to their lower usage (*11*), we decided to design and synthesize some chiral cyanoacrylates with antiviral activity through replacement of the methylthio moiety by the (*R*)- or (*S*)-1-phenylethanamine in some 2-cyano-3-(methylthio)-3-substituted-phenylaminoacrylates. At the same time, many effective methods for the preparation of cyanoacrylate have been developed including prolonged heating of amine with 2-cyano-3-(methylthio)-3-aminoacrylate. However, these methods have many problems such as long reaction times and many side reactions (*12*). Recently, organic reactions irradiated by microwaves have been developed as safe and convenient methods for the synthesis of phosphonyl/*S*-methyl ketene thioacetals (*13*). The application of microwave energy to accelerate the organic reaction is of increasing interest and offers several advantages over conventional techniques (*14*). Those synthetic reactions normally requiring lengthy periods can be achieved conveniently and rapidly in a microwave oven. Hence, we report herein a new method for the preparation of chiral cyanoacrylate from (*R*)- or (*S*)-1-phenylethanamine in refluxing *n*-propanol under microwave irradiation producing chiral cyanoacrylate with moderate or high yields (**Scheme 1**; **Table 1**). Several ethyl 3-[(*R* or *S*)-1-phenylethylamino]-3-(4-substitutedphenylamino)-2-cyanoacrylates were synthesized, and the bioassay tests showed that some of these title compounds exhibit good antiviral activity in vivo and anticancer activity in vitro. The crystal structure of **IIa-*R*** was determined by X-ray single-crystal structure analysis from which an (*E*) configuration of the title compounds was confirmed (**Figure 1**, **Figure 2**). To the best of our knowledge, this is the first report on the synthesis, anti-TMV bioactivity, and antitumor bioactivity of chiral cyanoacrylate derivatives.

2. MATERIALS AND METHODS

2.1. Synthetic Procedures. The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., Beijing, China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR22 spectrometer with KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-INOVA 400 MHz spectrometer in $CDCl_3$ at room temperature using tetramethyl-

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Scheme 1

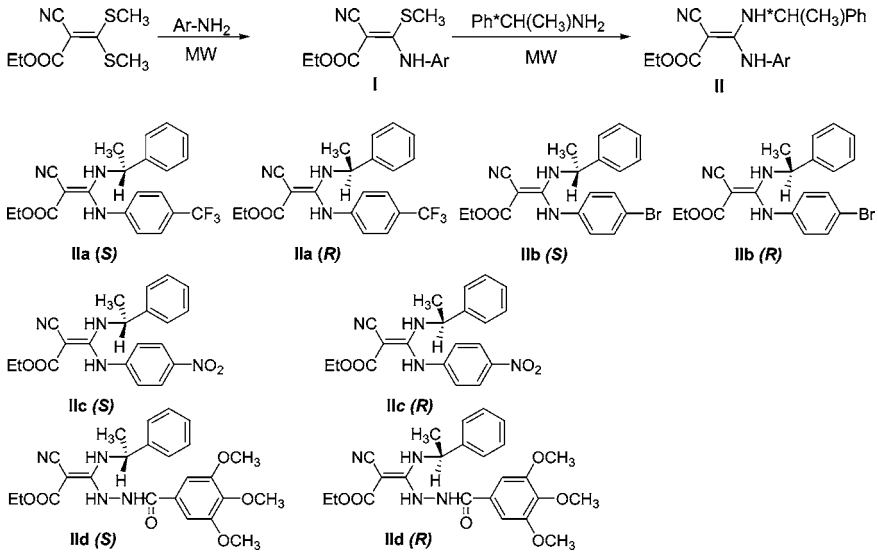

Table 1. Results at Different Reaction Conditions for Synthesis of **IIa-*R***

entry	solvent	time (min)	temp (°C)	yield[a] (%)
1[b]	*n*-propanol	5	97	24.1
2[b]	*n*-propanol	10	97	30.3
3[b]	*n*-propanol	20	97	53.1
4[b]	*n*-propanol	30	97	54.1
5[b]	*n*-propanol	15	40	no product
6[b]	*n*-propanol	30	40	9.8
7[b]	*n*-propanol	20	50	21.5
8[b]	*n*-propanol	20	60	33.1
9[b]	*n*-propanol	20	70	41.2
10[b]	*n*-propanol	20	80	44.2
11[c]	*n*-propanol	20	97	no product
12[c]	*n*-propanol	60	97	no product
13[c]	*n*-propanol	120	97	no product
14[c]	*n*-propanol	180	97	no product
15[c]	*n*-propanol	240	97	no product
16[c]	*n*-propanol	360	97	22.1
17[c]	*n*-propanol	600	97	37.8
18[b]	ethanol	45	78	47.6
19[b]	methanol	45	65	31.1
20[b]	isopropanol	45	105	46.9

[a] Isolated yields. [b] Reactions were carried out in 20 mL of solvent under microwave irradiation, power 600 W. [c] Reactions were carried out in 20 mL of solvent under stirring without microwave irradiation.

silane (TMS) as internal reference. D_2O exchange was applied to confirm the assignment of the signals of NH protons. The mass spectra were taken on an HP5988A spectrometer operated at 70 eV. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Microwave irradiations were carried out in an XH-100A CNC microwave-catalysis apparatus at 600 W (Beijing Xianghu Technical Development Co., Beijing, China). The instrument was equipped with a refluxing apparatus, together with an internal magnetic stirring motor. The reagents were all analytically or chemically pure. 2-Cyano-3,3-(dimethylthio)acrylate was prepared according to a literature method (*15*).

2.2. General Procedure for the Preparation of Intermediates Ia−d. To an oven-dried three-neck 50 mL round-bottom flask fitted with a magnetic stirring bar was added 2-cyano-3,3-(dimethylthio)acrylate (2.17 g, 0.01 mol), substituted aniline (0.01 mol), 60% NaH (0.80 g, 0.02 mol), DMF (6 mL), and toluene (6 mL). The resulting mixture was irradiated in the microwave oven at 80 °C and 600 W for 15 min. The mixture was then poured into ice water (40 mL) and separated. The aqueous phase was acidified with 10% HCl to pH 6−7 and filtered. The residue was dried and recrystallized from ethanol−water to give the title compounds.

*2.2.1. (E)-Ethyl 3-[4-(trifluoromethyl)phenylamino]-2-cyano-3-(methylthio)acrylate (**Ia**):* white crystal; mp, 80−82 °C; yield, 62.1%; IR (KBr) ν_{max} 3216, 2980, 2208, 1655, 1591, 1563, 1396, 1381, 1324, 1298, 1265, 1167, 1150, 1124, 1113, 1065, 1022, 779 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.27 (t, J = 6.4 Hz, 3H, CH_3−C), 2.05 (s, 3H, S−CH_3), 4.54 (q, 2H, O−CH_2), 7.82−7.69 (m, 4H, Ar−H), 11.40 (s, 1H, NH); *m/z* (EI-MS) 318 (M^+). Anal. Calcd for $C_{14}H_{13}F_3N_2O_2S$: C, 50.91; H, 3.97; N, 8.48. Found: C, 50.94; H, 3.96; N, 8.34.

*2.2.2. (E)-Ethyl 3-(4-bromophenylamino)-2-cyano-3-(methylthio)-acrylate (**Ib**):* white crystal; mp, 130−132 °C; yield, 68.1%; IR (KBr) ν_{max} 3150, 2993, 2205, 1659, 1556, 1487, 1412, 1377, 1254, 1165, 1028, 843, 779, 660, 588, 519, 411 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.26 (t, J = 6.8 Hz, 3H, CH_3−C), 2.85 (s, 3H, S−CH_3), 4.32 (d, J = 8.8 Hz, 2H, O−CH_2), 7.63−7.65 (m, 4H, Ar−H), 11.22 (s, 1H, NH−Ar); *m/z* (EI-MS) 328 (M^+). Anal. Calcd for $C_{13}H_{13}BrN_2O_2S$: C, 45.76; H, 3.84; N, 8.21. Found: C, 45.80; H, 3.78; N, 8.18

*2.2.3. (E)-Ethyl 3-(4-nitrophenylamino)-2-cyano-3-(methylthio)acrylate (**Ic**):* white crystal; mp, 118−120 °C; yield, 60.1%; IR (KBr) ν_{max} 3305, 2208, 1653, 1547, 1537, 1269, 1173, 1124, 1119, 1024 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.25 (t, 3H, J = 7.2 Hz, CH_3−C), 2.31 (s, 3H, S−CH_3), 4.26 (q, 2H, J = 7.2 Hz, O−CH_2), 7.70 (d, 2H, J = 8.4 Hz, Ar−H), 7.81 (d, 2H, J = 8.4 Hz, Ar−H), 11.49 (s, 1H, NH); *m/z* (EI-MS) 307 (M^+). Anal. Calcd for $C_{13}H_{13}N_3O_4S$: C, 50.81; H, 4.23; N, 13.68. Found: C, 50.94; H, 4.19; N,13.44.

*2.2.4. (E)-Ethyl 3-(3,4,5-trimethoxybenzoamido)-2-cyano-3-(methylthio)acrylate (**Id**):* white crystal; mp, 162−163 °C; yield, 92.1%; IR (KBr) ν_{max} 3449, 3333, 2194, 1689, 1610, 1580, 1533, 1504, 1356, 1344, 1327, 1315, 1281, 1232, 1130, 997, 743 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.38 (t, J = 6.4 Hz, 3H, C−CH_3), 2.65 (s, 3H, S−CH_3), 3.82−3.85 (m, 10H, 3 × OCH_3 + ArCONH), 4.32 (q, 2H, O−CH_2), 7.24−7.60 (m, 2H, Ar−H), 12.51 (s, 1H, ArCONNH); *m/z* (EI-MS) 395 (M^+). Anal. Calcd for $C_{17}H_{21}N_3O_6S$: C, 51.64; H, 5.35; N, 10.63. Found: C, 51.63; H, 5.41; N, 10.65.

2.3. General Procedure for the Preparation of Title Compounds II. A solution of intermediate **I** (0.4 mmol) in *n*-PrOH (20 mL) was stirred, followed by the addition of (*R*)-1-phenylethanamine or (*S*)-1-phenylethanamine (0.4 mmol). The mixture was irradiated in the microwave oven at 97 °C at 600 W for 20 min. The solvent was removed under reduced pressure. The crude product was purified by column chromatography on a silica gel (eluent, ethyl acetate/petroleum ether, 2:8, v/v) to give the title compounds.

*2.3.1. (E)-Ethyl 3-[(R)-1-phenylethylamino]-3-(4-(trifluoromethyl)-phenylamino)-2-cyanoacrylate (**IIa-R**) (**Figure 1**):* white crystal; mp, 145−147 °C; yield, 53.1%; $[\alpha]_{25}^{D}$ −210.2 (*c* 0.4828, acetone); IR (KBr) ν_{max} 3258, 3125, 2980, 2195, 1666, 1620, 1595, 1327, 1284, 1115, 1067, 702 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.26 (t, J = 6.4 Hz, 3H, CH_3−C), 1.45 (d, J = 6.4 Hz, 3H, C−CH_3), 4.18−4.28 (br, 3H, OCH_2 + NCH), 7.15−7.31 (m, 9H, Ar−H), 9.61 (d, J = 6.4 Hz, 1H, C−NH),

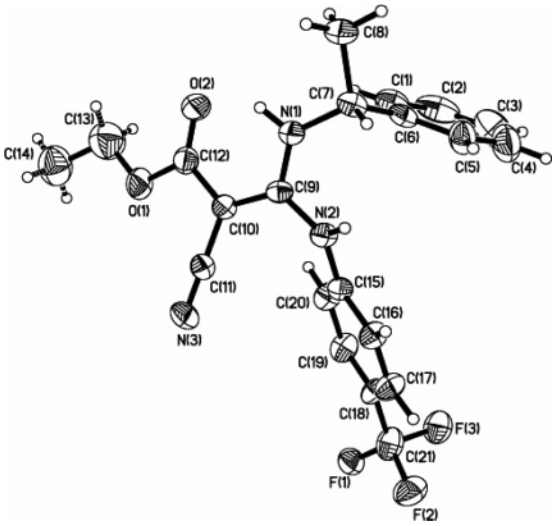

Figure 1. Molecular structure of compound **IIa-*R***.

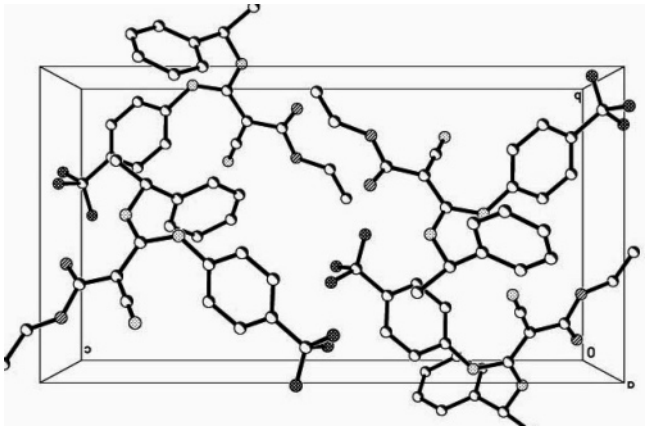

Figure 2. Packing diagram of the unit cell of compound **IIa-*R***.

10.54 (s, 1H, Ar−NH); *m*/*z* (EI-MS) 389 (M^+); Anal. Calcd for $C_{21}H_{20}F_3N_3O_2$: C, 62.52; H, 5.00; N, 10.42. Found: C, 62.70; H, 4.96; N, 10.36.

*2.3.2. (E)-Ethyl 3-[(S)-1-phenylethylamino]-3-(4-(trifluoromethyl)-phenylamino)-2-cyanoacrylate (**IIa-S**):* white crystal; mp, 135−137 °C; yield, 54.1%; $[\alpha]_{25}^{D}$ +253.2 (*c* 0.7972, acetone); IR (KBr) ν_{max} 3258, 3125, 2980, 2195, 1666, 1620, 1595, 1448, 1327, 1284, 1256, 1115, 1067, 845, 702, 550 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.32 (t, J = 6.4 Hz, 3H, CH_3−C), 1.44 (d, J = 6.4 Hz, 3H, C−CH_3), 4.24−4.35 (br, 3H, OCH_2 + NCH), 7.15−7.27 (m, 9H, Ar−H), 9.60 (d, J = 6.0 Hz, 1H, C−NH), 10.53 (s, 1H, Ar−NH); *m*/*z* (EI-MS) 403 (M^+). Anal. Calcd for $C_{21}H_{20}F_3N_3O_2$: C, 62.52; H, 5.00; N, 10.42. Found: C, 62.27; H, 4.99; N, 10.36.

*2.3.3. (E)-Ethyl 3-[(R)-1-phenylethylamino]-3-(4-bromophenylamino)-2-cyanoacrylate (**IIb-R**):* white crystal; mp, 152−153 °C; yield, 50.9%; $[\alpha]_{25}^{D}$ −207.8 (*c* 0.4620, acetone); IR (KBr) ν_{max} 3258, 3125, 2980, 2195, 1666, 1620, 1595, 1448, 1327, 1284, 1256, 1115, 1067, 845, 702, 550 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.23 (t, J = 7.2 Hz, 3H, CH_3−C), 1.47 (d, J = 6.8 Hz, 3H, C−CH_3), 4.14−4.4.20 (m, J = 3.6 Hz, 2H, O−CH_2), 4.65−4.4.69 (m, 1H, N−CH), 6.99−7.46 (m, 9H, Ar−H), 9.51 (d, J = 6.2 Hz, 1H, C−NH), 10.45 (s, 1H, Ar−NH); *m*/*z* (EI-MS) 414 (M^+). Anal. Calcd for $C_{20}H_{20}BrN_3O_2$: C, 58.00; H, 4.87; N, 10.13. Found: C, 57.98; H, 4.87; N, 10.14.

*2.3.4. (E)-Ethyl 3-[(S)-1-phenylethylamino]-3-(4-bromophenylamino)-2-cyanoacrylate (**IIb-S**):* white crystal; mp, 153−155 °C; yield, 51.1%; $[\alpha]_{25}^{D}$ +221.7 (*c* 0.4556, acetone); IR (KBr) ν_{max} 3273, 3115, 2984, 2193, 1663, 1607, 1582, 1541, 1487, 1445, 1292, 1271, 1092, 812, 768, 700, 512 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.19 (s, 3H, CH_3−C), 1.40 (d, J = 6.8 Hz, 3H, C−CH_3), 4.15−4.4.21 (m, 2H, OCH_2), 4.49−4.69 (m, 1H, N−CH), 7.21−6.81 (m, 9H, Ar−H), 9.51 (d, J = 6.0 Hz, 1H, C−NH), 10.45 (s, 1H, Ar−NH); *m*/*z* (EI-MS) 414 (M^+). Anal. Calcd for $C_{20}H_{20}BrN_3O_2$: C, 58.00; H, 4.87; N, 10.13. Found: C, 58.07; H, 4.64; N, 10.07.

*2.3.5. (E)-Ethyl 3-[(R)-1-phenylethylamino]-3-(4-nitrophenylamino)-2-cyanoacrylate (**IIc-R**):* white crystal; mp, 201−203 °C; yield, 65.6%; $[\alpha]_{25}^{D}$ −297.4 (*c* 0.7616, acetone); IR (KBr) ν_{max} 3233, 2980, 2193, 1670, 1601, 1518, 1340, 1302, 1283, 1258, 1090, 853, 764, 700, 554 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.20 (t, J = 6.8 Hz, 3H, CH_3−C), 1.49 (d, J = 6.4 Hz, 3H, C−CH_3), 4.09−4.22 (m, 2H, O−CH_2), 4.87 (t, J = 7.2 Hz, 1H, N−CH), 7.20−8.14 (m, 9H, Ar−H), 9.44 (d, J = 6.8 Hz,1H, C−NH), 9.75 (s, 1H, Ar−NH); *m*/*z* (EI-MS) 380 (M^+). Anal. Calcd for $C_{20}H_{20}N_4O_4$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.05; H, 5.25; N, 14.66.

*2.3.6. (E)-Ethyl 3-[(S)-1-phenylethylamino]-3-(4-nitrophenylamino)-2-cyanoacrylate (**IIc-S**):* white crystal; mp, 203−205 °C; yield, 71.2%; $[\alpha]_{25}^{D}$ +283.3 (*c* 1.0140, acetone); IR (KBr) ν_{max} 3235, 3219, 2980, 2194, 1670, 1601, 1551, 1518, 1497, 1443, 1387, 1340, 1302, 1281, 1257, 1113, 1090, 853, 764, 748, 700, 553 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.20 (t, J = 6.8 Hz, 3H, CH_3−C), 1.49−1.50 (d, J = 6.8 Hz, 3H, C−CH_3), 4.09−4.22 (m, 2H, O−CH_2), 4.89 (t, J = 7.2 Hz, 1H, N−CH), 7.20−8.14 (m, 9H, Ar−H), 9.44 (d, J = 6.8 Hz, 1H, C−NH), 9.75 (s, 1H, Ar−NH); *m*/*z* (EI-MS) 380 (M^+). Anal. Calcd for $C_{20}H_{20}N_4O_4$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.15; H, 5.00; N, 14.70.

*2.3.7. (E)-Ethyl 3-[(R)-1-phenylethylamino]-3-(3,4,5-trimethoxybenzoamido)-2-cyanoacrylate (**IId-R**):* white crystal; mp, 168−169 °C; yield, 98.1%; $[\alpha]_{25}^{D}$ −53.5 (*c* 0.5468, acetone); IR (KBr) ν_{max} 3302, 2974, 1628, 1582, 1531, 1504, 1412, 1352, 1236, 1128, 997, 839, 698 cm^{-1}; δ_H (400 MHz, $CDCl_3$) 1.56−1.62 (m, 6H, 2C−CH_3), 3.83−3.88 (m, 12H, OCH_2 + CONH + 3 × OCH_3), 5.30 (s, 1H, N−CH), 7.00−7.38 (m, 7H, Ar−H), 9.44 (d, J = 6.4 Hz, 1H, C−NH), 9.75 (s, 1H, ArCON−NH); *m*/*z* (EI-MS) 468 (M^+). Anal. Calcd for $C_{24}H_{28}N_4O_6$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.35; H, 6.00; N, 11.70.

*2.3.8. (E)-Ethyl 3-[(S)-1-phenylethylamino]-3-(3,4,5-trimethoxybenzoamido)-2-cyanoacrylate (**IId-S**):* white crystal; mp, 158−160 °C; yield, 97.8%; $[\alpha]_{25}^{D}$ +57.3 (*c* 0.4844, acetone); IR (KBr) ν_{max} 3302, 2974, 1628, 1582, 1531, 1504, 1412, 1352, 1236, 1128, 997, 698; δ_H (400 MHz, $CDCl_3$) 1.55 (d, J = 6.8 Hz, 6H, 2CH_3−C), 3.82−3.85 (m, 12H, OCH_2 + ArCONH + 3 × OCH_3), 5.27−5.31 (m, 1H, N−CH), 6.70−7.38 (m, 7H, Ar−H), 9.44 (d, J = 6.4 Hz, 1H, C−NH), 9.75 (s, 1H, ArCON−NH); *m*/*z* (EI-MS) 468 (M^+). Anal. Calcd for $C_{24}H_{28}N_4O_6$: C, 61.53; H, 6.02; N, 11.96. Found: C, 61.45; H, 5.80; N, 11.80.

2.4. Crystal Structure Determination. In the determination of the structure of the single crystal, X-ray intensity data were recorded on a Rigaku Raxis-IV diffraction meter using graphite monochromated Mo Kα radiation (λ = 0.71073 Å). In the range of 2.06° ≤ θ ≤ 25.01°, 3741 independent reflections were obtained. Intensities were corrected for Lorentz and polarization effects and empirical absorption, and all data were corrected using the SADABS (*16*) program. The structure was solved by direct methods SHELXS-97 program (*17*). All of the non-hydrogen atoms were refined on *F*2 anisotropically by a full-matrix least-squares method. The hydrogen atoms were located from the difference Fourier map, but their positions were not refined. The contributions of these hydrogen atoms were included in structure−factor calculations. The final least-squares cycle gave wR = 0.1786, R = 0.0707, for the 11111 reflection with $I > 2\sigma(I)$; the weighting scheme was $w = 1/[\sigma^2(F_o^2) + (0.1046P)^2 + 0.000P]$, where $P = [(F_o^2) + 2F_c^2]/3$. The maximum and minimum difference peaks and holes are 0.338 and −0.179 e•A^{-3}, respsctively. s = 1.203, and $(\Delta/\sigma)_{max}$ = 0.026(4). Atomic scattering factors and anomalous dispersion corrections were taken from the *International Table for X-ray Crystallography* (*18*). Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC-271061 (available free of charge at http://www.ccdc.cam.ac.uk/conts/retrieving/html or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.).

2.5. Antiviral Biological Assay.

2.5.1. Purification of Tobacco Mosaic Virus. Using Gooding's method (*19*), the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000*g*,

treated with PEG twice, and centrifuged again. The whole experiment was processed at 4 °C. Absorbance value was estimated at 260 nm by ultraviolet spectrophotometer.

$$\text{virus concn} = (A_{260} \times \text{dilution ratio})/E_{1\text{cm}}^{0.1\%,260\text{nm}}$$

2.5.2. Inhibition Effect of Compound on TMV in Vivo. The virus was inhibited by mingling with the compound solution at the same volume for 30 min. The mixture was then inoculated on the left side of the leaves of *N. tabacum* L., whereas the right side of the leaves was inoculated with the mixture of solvent and the virus for control. The local lesion numbers were recorded 3−4 days after inoculation (*20*). Three repetitions were conducted for each compound.

2.5.3. Cure Effect of Compound on TMV in Vivo. The leaves of *N. tabacum* L. growing at the same ages were selected. TMV at a concentration of 6×10^{-3} mg/mL was dipped and inoculated on the whole leaves. Then the leaves were washed with water and dried. The compound solution was smeared on the left side, and the solvent was smeared on the right side for control. The local lesion numbers were then recorded 3−4 days after inoculation (*20*). For each compound, three repetitions were conducted to ensure the reliability of the results.

$$\text{inhibition rate (\%)} = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{av local lesion numbers smeared with drugs}}{\text{av local lesion numbers without drugs}}$$

2.6. Herbicidal Activity Bioassay. Several plants including *Brassica campestris*, *Amaranthus retroflexus* L., and *Echinochloa crus-galli* were used to test the herbicidal activity of compounds. All of the seeds were bought from the Institute of Crop, Tianjin Agriculture Science Academy, People's Republic of China. The seeds were planted in 6-cm-diameter plastic boxes containing artificial mixed soil. Before plant emergence, the boxes were covered with plastic film to retain moisture. Plants were grown in the greenhouse. The dosage (activity ingredient) was 1500 g/ha. Purified compounds were dissolved in 100 μL of dimethylformamide (DMF), with the addition of a little Tween 20, and then were sprayed using a laboratory belt sprayer delivering a spray volume of 750 L ha^{-1}. The same amount of water was sprayed as control. Compounds were sprayed to treat the soil immediately after seed planting and were sprayed to the stem and leaves after seed germination. The fresh weight of aerial tissues was measured 10 days after treatment. The inhibition percent of aerial tissue fresh weight is used to describe the control efficiency of compounds.

2.7. MTT Assay against Cell Viability and Proliferation (*21*). All compounds tested were dissolved in dimethyl sulfoxide (DMSO) (1−100 μM solution) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density of 3×10^3 cells/well in 100 μL of the proper culture medium and treated with the compounds at concentrations of 1−100 μM for 72 h. In parallel, the cells were treated with 0.1% DMSO as control. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Roche Molecular Biochemicals, 1 465 007) was performed 30 h later according to the instructions provided by Roche. This assay is based on the cellular cleavage of the tetrazolium salt, MTT, into a formazan that is soluble in cell culture medium and is measured at 550 nm directly in 96-well assay plates. Absorbance is directly proportional to the number of living cells in culture. Two types of cells were used in these studies, PC3 (prostate cancer) and A431 (uterus cancer) cell lines (provided by the Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Science) were cultivated in F-12 (for PC3) or RPMI 1640 (for A431) supplemented with 10% fetal bovine serum (provided by TBD & HyBio. Co.) and 2 mM L-glutamine. Tissue culture reagents were obtained from Gibco BRL. Also, three repetitions were conducted for each compound.

3. RESULTS AND DISCUSSION

3.1. Synthesis. To optimize the reaction conditions, the synthesis of **IIa-*R*** was carried out under several conditions. The effects of different solvents, reaction time, and the use or not of microwave irradiation on the reaction were investigated, and the results are shown in **Table 1**. The results indicated that microwave irradiation can accelerate the reaction (entries 1−10). When there is no microwave irradiation, the reaction was relatively slow and no product was detected within 4 h (entries 11−15). When the reaction time was prolonged to 6−10 h without microwave irradiation, **IIa-*R*** could be obtained in 22.1 and 37.8% yield, respectively (entries 16 and 17). While under microwave irradiation, the yield of **IIa-*R*** increased from 24.1 to 53.1% when the reaction time was prolonged from 5 to 20 min in refluxing *n*-propanol at 97 °C (entries 1−3). When the reaction time was prolonged further to 30 min under microwave irradiation, a tiny improvement of yield (54.1%, entry 4) was obtained compared to that at 20 min (53.1%, entry 3). As for the reaction temperature, it could be seen that the yield was relatively low when the reaction was carried out at low temperature (entries 5−10, 18, and 19) compared with that at 97 °C (entry 3). No substantial improvement was observed when the reaction system was heated to 105 °C (entry 20). Hence, it is better for the reaction to be performed at 97 °C than in lower or higher temperature.

Table 2. Comparison of Yields between the Microwave-Assisted and Classical Synthesis of **II**

			yield[a] (%)	
entry	compound	solvent	microwave assisted[b]	classical method[c]
1	**IIa-*R***	*n*-propanol	53.1	38.9
2	**IIa-*S***	*n*-propanol	54.1	38.9
3	**IIb-*R***	*n*-propanol	50.9	31.1
4	**IIb-*S***	*n*-propanol	51.1	33.3
5	**IIc-*R***	*n*-propanol	65.6	48.9
6	**IIc-*S***	*n*-propanol	71.2	56.4
7	**IId-*R***	*n*-propanol	98.1	88.1
8	**IId-*S***	*n*-propanol	97.8	88.8

[a] Yields of isolated products. [b] Reaction conditions: reaction under microwave irradiation in *n*-propanol, power 600 W, at 97 °C for 20 min. [c] Reaction conditions: using *n*-propanol as solvent, at 97 °C under stirring without microwave irradiation for 10 h.

The present new method for the synthesis of chiral cyanoacrylate **II** under microwave irradiation offers several advantages including faster reaction rates, fewer byproducts, and higher yields, as compared with the drawbacks of the classical method, which involves a long tedious process (∼10 h) and gives low yields. In **Table 2** it is easy to observe that the average yields of products obtained with the microwave method are ∼10% higher than those obtained with the classical method. The short reaction time of the microwave method is the advantage of this method in relation to the other method; the average time ratio between the two methods is 1:30.

3.2. Antiviral Activity. The antiviral activity of compound **II** against TMV is assayed by the reported method (*18*). It could be seen from **Tables 3** and **4** that these newly synthesized chiral compounds exhibit promising antiviral activities against TMV in vivo. Apparently and interestingly, the antiviral data indicate that the (*R*) or (*S*) configuration substantially affects bioactivity and that the (*R*) configuration is vital. For example, the bioactivity to TMV of the (*R*) isomers of **IIa**, **IIb**, **IIc**, and **IId** are all better than those of the (*S*) isomers. It could be also seen that the nature of the substituent on the phenyl group affects the antiviral activity substantially. The electron-withdrawing property of the substituent is favorable to their antiviral bioactivity, as shown by the (*R*) isomer of **IIc** having a more potent antiviral activity than the other (*R*) or (*S*) isomers and

Table 3. Inhibition Effect of the New Chiral Compounds to TMV in Vivo

	agent										
	ningnanmycin	virus A	antofine	**IIa-*R***	**IIa-*S***	**IIb-*R***	**IIb-S**	**IIc-*R***	**IIc-*S***	**IId-*R***	**IId-*S***
concn (mg/L)	500	500	50	500	500	500	500	500	500	500	500
inhibition rate (%)	88.9	78.4	14.0	28.9	6.1	53.4	0	89.1	5.3	46.4	7.5

Table 4. Curative Effect of the New Chiral Compounds against TMV in Vivo

	agent										
	ningnanmycin	virus A	antofine	**IIa-*R***	**IIa-*S***	**IIb-*R***	**IIb-*S***	**IIc-*R***	**IIc-*S***	**IId-*R***	**IId-*S***
concn (mg/L)	500	500	50	500	500	500	500	500	500	500	500
inhibition rate (%)	28.9	14.6	29.2	39.1	15.2	12.3	3.4	43.1	13.1	26.7	12.1

Table 5. Herbicidal Activities of Chiral Compounds at a Dose of 1.5 kg of Active Ingredient per Hectare

	B. campestris		*A. retroflexus* L.		*E. crus-galli*	
compd	soil treat	stem treat	soil treat	stem control	soil control	stem control
IIa-*R*	16.07	0	0	0	11.43	2.86
IIa-*S*	21.43	30.47	10.53	0	10.00	5.71
IIb-*R*	5.38	10.94	0	0	4.29	0
IIb-*S*	1.29	35.26	0	0	10.00	34.29
IIc-*R*	21.43	17.19	0	0	18.57	0
IIc-*S*	25.00	32.13	0	0	14.29	13.33
IId-*R*	0	3.13	26.32	0	5.71	7.62
IId-*S*	0	18.75	10.53	0	7.14	0

Table 6. Inhibition Rate[a] ($\bar{x} \pm s$) of Compounds **II** to PC3 and A431 Cells at 5 μM ($P < 0.01$)

compd	PC3 cells	A431 cells
IIa-*R*	59.4 ± 4.7	43.1 ± 9.6
IIa-*S*	49.1 ± 6.0	39.9 ± 8.1
IIb-*R*	1.5 ± 0.1	16.1 ± 8.5
IIb-*S*	24.7 ± 11.3	19.3 ± 9.1
IIc-*R*	2.9 ± 0.3	23.6 ± 11.2
IIc-*S*	0.7 ± 0.1	18.1 ± 4.5
IId-*R*	32.1 ± 14.2	25.1 ± 7.8
IId-*S*	3.2 ± 0.2	29.4 ± 9.2
fluorouracil (5-Fu)	41.1 ± 8.2	30.7 ± 7.4

[a] Inhibition rate (%) = $(A_1 - A_2)/A_1 \times 100$, where A_1 is the mean optical density of untreated cells and A_2 is the mean optical density of drug-treated cells.

the standard drugs [virus A (moroxydine hydroxychloride·copper acetate) and antofine]. The inhibition effect of **IIc-*R*** to TMV in vivo is approximately equivalent to that of ningnanmycin.

3.3. Herbicidal Activity. The herbicidal bioactivity of compound **II** was also investigated, and weak bioactivity of the compound was found, as can be seen in **Table 5**.

3.4. Antiproliferation Activity. The anti-cell viability and proliferation activity was assayed according to the MTT method (*19*). The results are listed in **Table 6**. It was found that these chiral compounds exhibit weak activities against the two cancer cells in vitro. The compounds **IIa-*R*** and **IIa-*S*** have relatively higher activity than other chiral compounds. The data given in **Table 6** indicate that the nature of the substituent on the phenyl ring affects the antitumor activity. For example, the antiproliferation activities of compound **IIa-*R*** to PC3 and A431 cells at 5 μM are 59.4 and 43.1%, respectively. No substantial difference was found between the (*R*) and (*S*) configurations in the antitumor bioassay.

3.5. Crystal Structure Analysis. It could be seen from the X-ray single-crystal analysis that chiral compound **IIa-*R*** maintains a planar structure. The configuration of target compound **IIa-*R*** was determined to be (*E*) by X-ray diffraction. The bond length of C(9)—C(10) (1.406 Å) is longer than a normal C═C (1.34 Å), C(12)—O(1) (1.327 Å) is shorter than a normal single C—O (1.44 Å), C(10)—C(12) (1.454 Å) and C(10)—C(11) (1.400 Å) are shorter than a normal C—C (1.54 Å), and C(9)—N(1) (1.315 Å) and C(9)—N(2) (1.365 Å) bonds are shorter than the normal C—N single bond (1.49 Å), which suggest the existence of an electron density localization among N(2)—C(9)—C(10)—C(12)—O(2), C(11), and N(1).

3.6. Conclusion. In summary, we described a practical and efficient procedure for the preparation of chiral cyanoacrylate compounds through the reaction of intermediate **I** with (*R*)-1-phenylethanamine or (*S*)-1-phenylethanamine under microwave irradiation in *n*-PrOH for 20 min at 97 °C. The reactions were, in general, very fast and efficient and gave moderate yields. In the half-leaf method test, the chiral compound **IIc-*R*** was found to possess high antiviral activity against TMV in vivo.

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