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# Synthesis and antiviral bioactivities of novel chiral bis-thiourea-type derivatives containing α-aminophosphonate moiety

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Starting from 1-((1*R*,2*R*)-2-aminocyclohexyl)-3-substituted thioureas (**3a–c**) and substituted isothiocyanates (**9a–d**), chiral bis-thiourea derivatives containing α-aminophosphonate moiety **10a–l** were prepared and completely characterized by elemental analysis, physical and spectral (IR, $^{1}$H NMR, $^{13}$C NMR, $^{31}$P NMR) data. The results of bioassay revealed that compounds **10a** and **10e** possessed appreciable curative bioactivities on cucumber mosaic virus (CMV) at 0.5 mg/mL *in vivo* (inhibitory rate = 60.3%, 64.8% respectively) and tobacco mosaic virus (TMV) at 0.5 mg/mL *in vivo* (inhibitory rate = 50.3%, 50.8% respectively), which were comparable to the values shown by standard reference (58.7%) and commercial product Ningnanmycin (56.3%), respectively. Chiral compound **10e** displayed more potent antiviral activity ($EC_{50}$ = 0.149 mg/mL) than Ningnanmycin ($EC_{50}$ = 0.201mg/mL) against CMV.

**chiral bis-thiourea, α-aminophosphonate moiety, antiviral activity, synthesis**

## 1 Introduction

The plant virus disease, often considered as plant cancer, has long been a challenging problem to control in the agricultural sector. The cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) pose serious threats to the production of crop throughout the world. The application of traditional pesticides with high residue level and negative impact on the environment has not proved effective to enhance productivity. Ningnanmycin is a commercial antiviral agent first isolated from *Strepcomces noursei var·xichangensisn*. Var. by the Chengdu Institute of Biology, the Chinese Academy of Sciences. It is a type of microbial pesticide, which can enhance resistance in host plants by destruction of coat protein of TMV. Despite being useful in the treatment of plants affected by TMV, the use of Ningnanmycin for the field trial is largely limited due to its sensitivity towards moisture and light. Therefore, the search for new antiplant viral agents still remains as one of the most important tasks in pesticide science [1].

The application of thiourea derivatives as potential fungicides, herbicides, rodenticides, as well as anti-bacterial, anti-plant viral and anti-microbial therapeutic agents has been reported since early 1950 [2–5]. Selected chiral derivatives of these compounds are also applied in typical organocatalytic asymmetric transformations leading to new carbon-carbon bond formation [6–9]. Numerous synthetic studies of aromatic and heterocyclic chiral thioureas were undertaken in recent years to evaluate their suitability as non-nucleoside inhibitors (NNRTI) of the reverse transcriptase (RT) enzyme of the human immunodeficiency virus (HIV-1) [10–14]. Another interesting class of compounds called α-aminophosphonates which are essentially structural analogues to natural amino acids, exhibit diverse biological activities such as plant virucidal, plant growth regulatory,

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herbicidal, fungicidal and antitumor effects [15–17]. In view of different biological and chemical applications of these compounds, development of suitable synthetic methodologies for their preparation in pure form has been a topic of great interest in the past few decades [18–22]. In order to incorporate the two aforementioned active pharmacophores in a single molecule, we prepared a series of chiral monothiourea derivatives containing an α-aminophosphonate moiety by the addition of *O*,*O*′-dialkylisothiocyanato(phenyl) methylphosphonate to a chiral amine. The compounds exhibited a curative activity level close to commercial Ningnanmycin against TMV [23]. Since multiple thiourea functionalities can present opportunities for creating more active compounds through diverse hydrogen-bonded networks, we studied herein the effect of some novel bis-thiourea derivatives bearing an α-aminophosphonate moiety in the design of new anti-plant viral compounds. The basis for the design of target compounds and the synthetic route from isothiocyanate and diaminocyclohexane are shown in Figure 1 and Scheme 1 respectively. Preliminary bioassay results showed that some of these compounds possess a certain degree of antiviral activity against CMV and TMV at 0.5 mg/mL *in vivo* as shown in Table 4. According to the bioassay, compounds **10a** and **10e** had significant curative bioactivities on CMV (inhibitory rate = 60.3%, 64.8% respectively) and TMV (inhibitory rate = 50.3%, 50.8% respectively), which were comparable to the values shown by standard reference (58.7%) and commercial product-Ningnanmycin (56.3%). The chiral compound **10e** displayed more potent antiviral activity ($EC_{50}$ = 0.149 mg/mL) than Ningnanmycin ($EC_{50}$ = 0.201 mg/mL) against CMV.

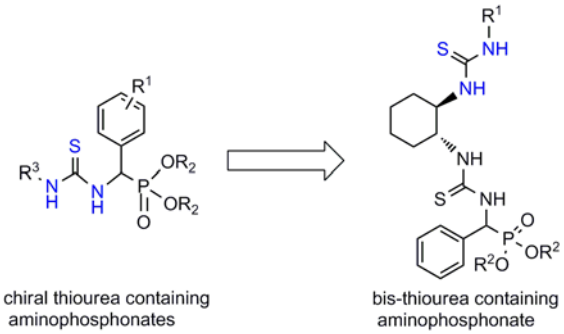
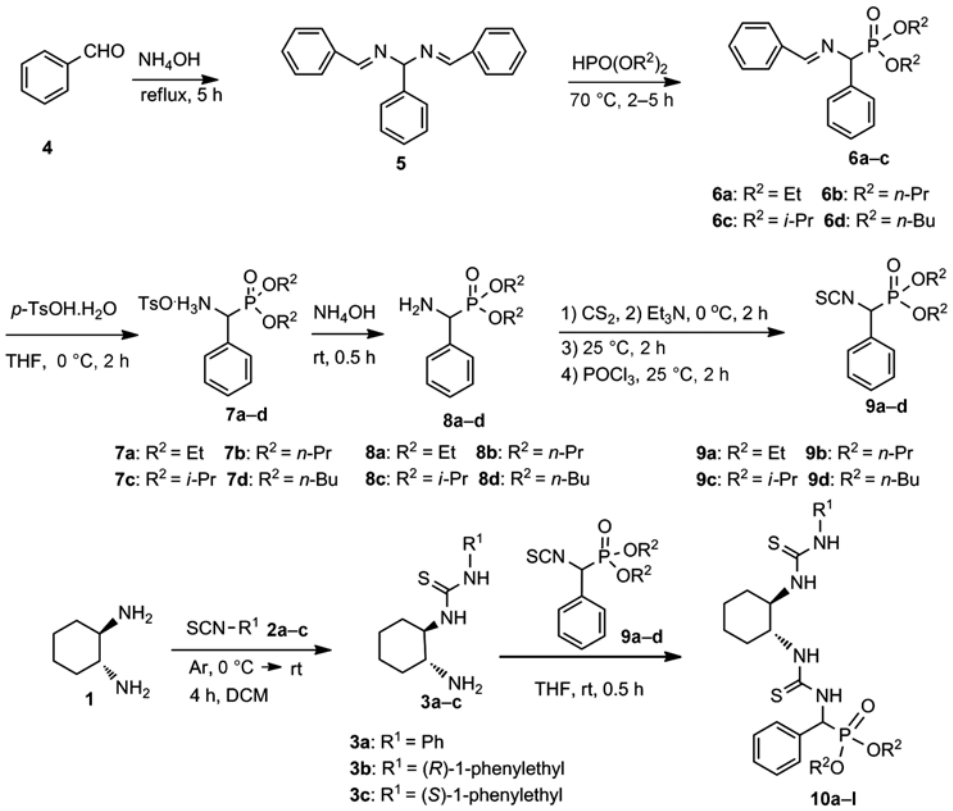



**Figure 1** Design of the target compounds.




**Scheme 1** Synthetic route to chiral bis-thiourea analogues **10a–l** containing α-aminophosphonate moiety.

| Compd | $R_1$ | $R_2$ | Compd | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| **10a** | Ph | Et | **10b** | Ph | *n*-Pr |
| **10c** | Ph | *i*-Pr | **10d** | Ph | *n*-Bu |
| **10e** | (*R*)-1-phenylethyl | Et | **10f** | (*S*)-1-phenylethyl | Et |
| **10g** | (*R*)-1-phenylethyl | *n*-Pr | **10h** | (*S*)-1-phenylethyl | *n*-Pr |
| **10i** | (*R*)-1-phenylethyl | *i*-Pr | **10j** | (*S*)-1-phenylethyl | *i*-Pr |
| **10k** | (*R*)-1-phenylethyl | *n*-Bu | **10l** | (*S*)-1-phenylethyl | *n*-Bu |

## 2 Materials and methods

### 2.1 Instruments

Melting points were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. IR spectra were recorded on a Bruker VECTOR 22 spectrometer in KBr disk. $^{1}$H, $^{13}$C and $^{31}$P NMR (solvent $CDCl_3$ or $D_3CCOCD_3$ or DMSO-$d_6$) spectral analyses were performed on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Elemental analyses were performed on an Elementar Vario-III CHN analyzer. Analytical TLC was performed on Silica Gel $GF_{254}$. Column chromatographic purification was carried out using Silica Gel. $GF_{254}$. Commercial reagents were used as received, unless otherwise indicated. Reactions were performed under a positive pressure of dry argon in oven-dried or flame-dried glassware equipped with a magnetic stir bar. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. Dichloromethane (DCM) was freshly distilled from calcium hydride ($CaH_2$). All reagents were of analytical reagent grade or chemically pure. All solvents were dried, deoxygenated and redistilled before use.

### 2.2 Synthetic procedures

Intermediates **1**, **2**, **4** were purchased from Alfa Aesar. Intermediates **3** were synthesized by slightly modifying a literature procedure [24]. Intermediates **5**, **6**, **7** and **8** were prepared following standard synthetic protocols [25]. Intermediates **9** were prepared according to a reported method [23, 26] with minor modifications of reaction temperature and reaction time.

### 2.3 General procedure for the preparation of the intermediates 3a–c

A 100 mL oven dried round bottom flask containing (*R,R*)-1,2-diaminocyclohexane (3.0 mmol) in 15 mL dry DCM was placed under an atmosphere of dry argon and cooled to 0 °C. Subsequently, a solution of intermediates **2a–c** (3.0 mmol) in dry DCM (15 mL) was added dropwise through a syringe over a period of 4 h. The resulting yellow solution was left to stir overnight. The reaction mixture was concentrated *in vacuo* and the yellow residue was purified by column chromatography using a mixture of methanol, $CH_2Cl_2$ and ethyl acetate (1:1:1) as the eluent. The data for intermediates **3a–c** can be found in the Supporting Information.

### 2.4 General procedure for the preparation of chiral compounds 10a–l

To a well stirred mixture of intermediates **9a–d** (1.0 mmol) in tetrahydrofuran (10 mL) at room temperature was added dropwise the corresponding intermediates **3a–c** (1.0 mmol). The reaction mixture was stirred further for an additional 0.5 h at room temperature, the solvent was removed by evaporation and the crude product was purified by chromatography on silica using a mixture of petroleum ether and ethyl acetate (2:1) as the eluent to give compounds **10a–l** in 75%–86% yields. The data for **10a** is shown below, while data for **10b–l** can be found in the Supporting Information section.

*Data for the title chiral compound **10a***

White crystal; mp 199–201 °C; yield, 85%; IR (KBr, $cm^{-1}$): $\nu$ 3321, 3289, 3273, 3250, 1552, 1531, 1211, 1020 $cm^{-1}$; $^{1}$H NMR (DMSO-$d_6$, ppm) $\delta$ 9.46 (1H, br, NH), 8.46 (1H, br, NH), 7.84 (1H, br, NH), 7.48 (1H, br, NH), 7.35 (2H, d, $J$ = 7.5 Hz, ArH), 7.28 (2H, t, $J$ = 7.2 Hz, ArH), 7.22 (1H, t, $J$ = 7.8 Hz, ArH), 7.16 (2H, d, $J$ = 6.9 Hz, ArH), 7.00 (2H, t, $J$ = 7.3 Hz, ArH), 6.93 (1H, t, $J$ = 7.1 Hz, ArH), 6.11 (1H, dd, $J$ = 9.7, 9.8 Hz, CHP), 4.15 (2H, br, $OCH_2$), 4.03–3.99 (2H, m, $OCH_2$), 3.92–3.87 (1H, m, CH cycl), 3.82–3.78 (1H, m, CH cycl), 2.19–2.11 (2H, m, $CH_2$ cycl), 1.64 (2H, br, $CH_2$ cycl), 1.27–1.17 (7H, m, $2\times CH_2$ cycl + $CH_3$), 1.06 (3H, t, $J$ = 5.0 Hz, $CH_3$); $^{13}$C NMR (125 MHz, DMSO-$d_6$, ppm) $\delta$ 183.0, 180.0, 139.0, 137.4, 129.2, 128.7, 128.4, 128.0, 124.4, 123.3, 63.2, 62.9, 57.6, 56.6, 55.3, 54.1, 32.7, 31.8, 24.7, 16.8, 16.6; $^{31}$P NMR (200 MHz, DMSO-$d_6$, ppm) $\delta$ 22.4. Anal. calcd for $C_{25}H_{35}N_4O_3PS_2$: C 56.16, H 6.60, N 10.48; found: C 56.31, H 6.50, N 10.66.

### 2.5 Antiviral biological assay

*Purification of tobacco mosaic virus (TMV)*

Using Gooding's method [27], the upper leaves of *Nicotiana tabacum* L inoculated with TMV were selected and ground in a phosphate buffer, then filtered through double layer pledget. The filtrate was centrifuged at 10000 *g*, treated twice with PEG and centrifuged again. The whole experiment was carried out at 4 °C. Absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer.

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

*Curative effect of compounds against TMV in vivo*

Growing leaves of *Nicotiana tabacum* L of the same ages were selected. TMV (concentration of $6\times10^{-3}$ mg/mL) was dipped to inoculate 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 counted and recorded 3–4 d after inoculation [28]. For each compound, three repetitions were measured. The inhibition rate of the compound was then calculated according to the following formula ('av' means average).

$$\text{Inhibition rate}\,(\%) = \frac{\begin{array}{c}\text{av local lesion numbers of control}\\ \text{(not treated with compound)}\end{array}}{\begin{array}{c}\text{av local lesion numbers of control}\\ \text{(not treated with compound)}\end{array}} - \frac{\text{av local lesion numbers smeared with drugs}}{\begin{array}{c}\text{av local lesion numbers of control}\\ \text{(not treated with compound)}\end{array}} \times 100\%$$

*Extraction of cucumber mosaic virus (CMV)*

The leaves of *Nicotiana tabacum* L. inoculated with CMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The supernatant liquid obtained after centrifugation of the filtrate at 8000 *g* was employed as the virus extract. The entire experiment was carried out at 4 °C [29].

*Preparation of medicaments*

Tested compounds and 2% Ningnanmycin aqua used as a reference antiviral agent were first diluted with a little amount of *N,N*-dimethylformamide (DMF) and then the resulting mixture was dissolved in distilled water containing 1% Tween 20 at 500 μg/mL [29].

*Curative effect of compounds against CMV in vivo*

Growing leaves of *Chenopodium amaranticolor* of the same age were selected. Crude extracts of CMV were dipped and inoculated with a brush on the whole leaves, which were previously scattered with silicon carbide. After inoculation, the leaves were washed with water for 0.5 h and then dried. The compound solution was smeared on the left side of leaves, and the solvent was smeared on the right side for control. All plants were cultivated in an incubator at 28 ± 1 °C and 10000 Lux illumination. The local lesion numbers appearing 6–7 d after inoculation were counted. Three repetitions were conducted for each compound [30]. The inhibition rate of the compound was calculated according to the following formula (av denotes average).

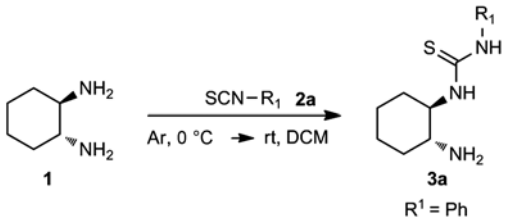
$$\text{inhibition rate}(\%) = \frac{\begin{array}{c}\text{av local lesion no. of right leave}\\ (\text{not treated with compound})\end{array}}{\begin{array}{c}\text{av local lesion no. of right leave}\\ (\text{not treated with compound})\end{array}} - \frac{\begin{array}{c}av\ \text{local lesion no. of left leave}\\ (\text{smearedwithcompound})\end{array}}{\begin{array}{c}\text{av local lesion no. of right leave}\\ (\text{not treated with compound})\end{array}} \times 100\%$$

## 3 Results and discussion

### 3.1 Synthesis

To optimize the conditions for the preparation of intermediate **3a**, the reaction was first carried out at 0 °C by making dropwise addition of intermediate **2a** (3.0 mmol in dry DCM) over different periods of time to a well-stirred solution of (*R,R*)-1,2-diaminocyclohexane **1** in dry DCM. It may be observed from Table 1 that the desired product **3a** could be obtained in 56% yield (Table 1, entry 3) when the isothiocyanate was added into the mixture over a period of 4 h. Addition of intermediate **2a** in one shot and over 2 h lowered the yield of intermediate **3a** to 31% and 44%, respectively (Table 1, entries 1 and 2), whereas increase of

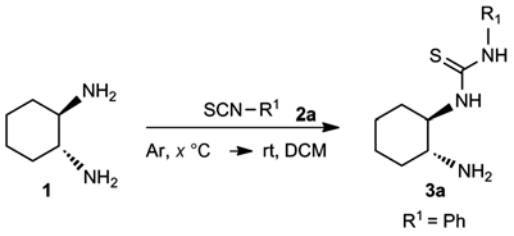
**Table 1** Effect of different rates of addition of isothiocynate **2a** on the synthesis of **3a** [a]




| Entry | Addition period (h) | Yield [b] (%) |
|---|---|---|
| 1 | one shot | 31 |
| 2 | 2 | 44 |
| 3 | 4 | 56 |
| 4 | 6 | 54 |
| 5 | 8 | 56 |
| 6 | 10 | 51 |

a) Reaction conditions: A mixture of 1 equiv of (*R,R*)-1,2-diaminocyclohexane **1** in dry DCM was placed under an atmosphere of argon and cooled to 0 °C. Then, a solution of intermediates **2a** (1 equiv) in dry DCM was added drop wise via syringe over different periods of time, as indicated. The resulting yellow solution was left to stir overnight. b) Isolated yield after purification via column chromatography on silica gel.

**Table 2** Effect of temperature on the synthesis of **3a** from diamine **1** and isothiocynate **2a** [a]




| Entry | Reaction temperature *x* (°C) | Yield [b] (%) |
|---|---|---|
| 1 | 20 | 38 |
| 2 | 0 | 56 |
| 3 | −10 | 40 |
| 4 | −20 | 32 |

a) Reaction conditions: A mixture of 1 equiv of (*R,R*)-1,2-diaminocyclohexane **1** in dry DCM was placed under an atmosphere of argon at *x* °C. Then, a solution of intermediate **2a** (1 equiv) in dry DCM was added drowse via syringe over 4 h. The resulting yellow solution was left to stir overnight. b) Isolated yield after purification via column chromatography on silica gel.

addition time beyond 4 h did not affect the overall yield of the product significantly (Table 1, entries 4, 5, 6). Keeping other parameters intact, the reaction temperature was then varied from −20 °C to +20 °C (Table 2). The optimal yield of 56% (Table 2, entry 2) was observed at 0 °C. Having established the most suitable reaction conditions for intermediate **3a** in DCM, the role of different solvents e.g. THF, DCM, acetonitrile, DMF and toluene was then studied for the synthesis of title chiral compound **10a** from the reaction of intermediate **3a** with intermediate **9a**. As can be seen from the data presented in Table 3, under optimal conditions when intermediate **3a** was reacted with 1 molar equiv of intermediate **9a** in THF at room temperature for 0.5 h, the target chiral compound **10a** was obtained in 85% yield (Table 3, entry 1).

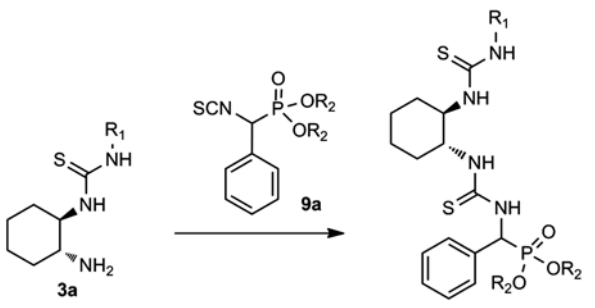
### 3.2 Antiviral activity and structure-activity relationship

To make a judgment of the antiviral potency of the synthesized chiral compounds **10a–l**, the commercially available plant virucide Ningnanmycin, perhaps the most successful registered anti-plant viral agent available in China, was used as the control. The bioassay against CMV and TMV is assayed by the reported method [27–30], and antiviral results of all the compounds against CMV and TMV are listed in Table 4. The results showed that some of our designed compounds had moderate to good antiviral activities at 0.5 mg/mL against CMV and TMV *in vivo*.

All the synthesized compounds **10a–l** exhibited curative activities ranging from 29.9%–64.8% against CMV at 0.5 mg/mL. Amongst them, **10e** [$R_1$ = (*R*)-1-phenylethyl, $R^2$=Et], **10a** [$R^1$ = Ph, $R^2$ = Et] and **10g** [$R^1$ = (*R*)-1-phenylethyl, $R^2$ = *i*-Pr] showed much higher curative activities (64.8%, 60.3% and 49.7%, respectively) which were comparable to that of the standard reference (58.7%). As for curative bioactivities against TMV, it is evident from Table 4 that compounds **10a**, **10e** and **10f** [$R^1$ = (*S*)-1-phenylethyl, $R^2$ = Et] afforded the best results withinhibitory rates of 50.3%, 50.8% and 44.1%, respectively. In fact, **10a** and **10e** proved almost as effective as the commercial product Ningnanmycin (56.3%). The nature of substituents present in the title compounds seems to affect the overall result to a great extent. Comparison of the values as displayed by **10a–l**, clearly reveals that the title compounds derived from diethyl phosphite ($R^2$ = Et) were far more active than those derived from other dialkyl phosphites ($R^2$ = *n*-Pr, *i*-Pr and *n*-Bu. Again, as expected of these synthesized chiral compounds, bioactivity is dependent on the nature of absolute configurations and hence on the type of isothiocyanates **2** involved in their preparation. The chiral thioureas **10e**, **10g**, **10i** and **10k** derived from (*R*)-enantiomer [$R^1$ = (*R*)-1-phenylethyl] displayed superior activity against CMV (inhibitory rate = 64.8%, 49.0%, 47.0% and 37.9% respectively) than **10f**, **10h**, **10j** and **10l** (inhibitory rate = 41.9%, 32.7%, 31.0% and 31.1% respectively) which were prepared from (*S*)-enantiomer [$R^1$ = (*S*)-1-phenylethyl] of isothiocyanate **2**. Similarly, the thioureas **10e**, **10g**, **10i** and **10k** derived from (*R*)-enantiomers [$R^1$ = (*R*)-1-phenylethyl] were more active against CMV (inhibitory rate = 64.8%, 49.0%, 47.0% and 37.9% respectively) compared to their respective (*S*) enantiomers **10f**, **10h**, **10j** and **10l** (inhibitory rate = 41.9%, 32.7%, 31.0% and 31.1% respectively).

Selected compounds **10a**, **10e**, **10f** and **10g** displaying

**Table 3** Compatibility of different solvents for the synthesis of **10a** [a]




| Entry | Solvent | Yield [b] (%) |
|---|---|---|
| 1 | THF | 85 |
| 2 | DCM | 80 |
| 3 | $CH_3CN$ | 78 |
| 4 | DMF | 72 |
| 5 | Toluene | 75 |

a) Reaction conditions: The reaction was conducted with 1 equiv of intermediate **3a** and 1 equiv of intermediate **9a** in various solvents at room temperature. The solution was stirred for 0.5 h. b) Isolated yield after purification via column chromatography on silica gel.

**Table 4** The curative effect of the new compounds against TMV and CMV *in vivo* [a]

| Agents | Concentration (mg/mL) | Curative effect against CMV (%) | Curative effect against TMV (%) |
|---|---|---|---|
| **10a** | 0.5 | 60.3** | 50.3* |
| **10b** | 0.5 | 37.1* | 31.2* |
| **10c** | 0.5 | 29.9* | 39.3* |
| **10d** | 0.5 | 37.0* | 35.2* |
| **10e** | 0.5 | 64.8** | 50.8** |
| **10f** | 0.5 | 41.9* | 44.1* |
| **10g** | 0.5 | 49.0** | 22.1* |
| **10h** | 0.5 | 32.7* | 28.9* |
| **10i** | 0.5 | 47.0* | 39.4* |
| **10j** | 0.5 | 31.0* | 10.2 |
| **10k** | 0.5 | 37.9* | 29.3* |
| **10l** | 0.5 | 31.1* | 33.2* |
| Ningnamycin | 0.5 | 58.7** | 56.3** |

a) $n$ = 3 for all groups; * $P$ < 0.05, ** $P$ < 0.01.

remarkable curative effects against TMV and CMV (Table 4) were identified for further antiviral-bioassay and the results were compared with commercial plant antiviral agent Ningnanmycin as shown in Table 5. The compound **10a** which was not derived from chiral isothiocyanate ($R^1$ = Ph) and compounds **10f** [$R^1$ = (*S*)-1-phenylethyl] and **10g** [$R^1$ = (*R*)-1-phenylethyl] were fairly effective in curating TMV. Their $EC_{50}$ values were recorded as 0.320, 0.525 and 0.378 mg/mL, respectively. It should also be noted that the curative effects of **10a**, **10e** and **10g** against CMV were significant, being 0.187, 0.149 and 0.359 mg/mL, respectively. Amongst all the studied compounds, the thiourea **10e** prepared from diethyl phosphite and chiral isothiocyanate **2b** [$R^1$ = (*R*)-1-phenylethyl] displayed more potent antiviral activity comparable to Ningnanmycin (0.201 mg/mL) against CMV. It must be noted that the synthesized compounds were stable under ordinary conditions of use and storage and were generally not affected by the trace amount of moisture or light.

In summary, a series of novel chiral bis-thiourea-type derivatives containing an α-aminophosphonate moiety was synthesized from 1-((1*R*,2*R*)-2-aminocyclohexyl)-3-substituted thiourea (**3a–c**) and substituted isothiocyanate (**9a–d**) derived from dialkyl phosphites. The reaction conditions for the multi-step synthesis were carefully optimized to prepare the title compounds. The *in vivo* tests indicated that the bis-thioureas **10a** and **10e**, both derived from diethyl phosphite, possessed good curative bioactivities (inhibitory rate = 60.3%, 64.8% respectively) on CMV, comparable to that of the standard reference (58.7%). They also displayed similar curative bioactivities on TMV (inhibitory rate = 50.3%, 50.8% respectively) as the commercial product Ningnanmycin (56.3%). The nature of dialkyl phosphites and chiral isothiocyanates **2** involved in the preparation of title compound is absolutely vital in the design of new compounds with desired activity. Thus, the antiviral activity of chiral bis-thiourea derivatives was significantly improved by appropriate selection of dialkyl phosphites and suitable control on the substituents and absolute configuration (*R* or *S*) of isothiocyanate **2**. It was found that the chiral thioureas **10e**, **10g**, **10i** and **10k** derived from (*R*)-enantiomer of isothiocyanate displayed better anti-CMV activities than compounds **10f**, **10h**, **10j** and **10l** [$R^1$ = (*S*)-1-phenylethyl] which were derived from (*S*)-enantiomer of isothiocyanate. In addition, the compound **10e** showed more potent antiviral activity ($EC_{50}$ = 0.149 mg/mL) than Ningnanmycin ($EC_{50}$ = 0.201 mg/mL) against CMV. Influence of subtle structural modification and steric parameters on structure activity relationships for identifying lead bioactive compound would be taken up in our future course of investigation.

**Table 5** Antiviral activities *in vivo* (%) of chiral compounds **10a**, **10e**, **10f** and **10g**

| Virus | Curative effect | | | |
|---|---|---|---|---|
| Concentration (mg/mL) | 0.500 | 0.250 | 0.125 | $EC_{50}$ (mg/mL) |
| **10a** [a)] | 50.4 | 39.4 | 35.1 | 0.320 |
| **10e** [a)] | 50.8 | 48.1 | 40.9 | 0.220 |
| **10f** [a)] | 44.1 | 30.1 | 20.0 | 0.525 |
| **10g** [a)] | 50.4 | 37.2 | 32.9 | 0.378 |
| **10a** [b)] | 60.3 | 57.6 | 48.0 | 0.187 |
| **10e** [b)] | 64.8 | 59.8 | 51.0 | 0.149 |
| **10g** [b)] | 49.0 | 33.2 | 22.1 | 0.359 |
| Ningnamycin [a)] | 56.2 | 51.0 | 44.1 | 0.212 |
| Ningnamycin [b)] | 58.2 | 52.1 | 47.9 | 0.201 |

a) $EC_{50}$ values on TMV; b) $EC_{50}$ values on CMV.

## 4 Supporting information available

The synthetic and analytical data for **3a–c**, **4**, **5**, **6a–d**, **7a–c**, **8a–d**, **9a–d**, **10a–l**. This information is available free of charge via the internet at http://www. springerlink. com/ content/121600/

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