ROUTE DEVELOPMENT, ANTIVIRAL STUDIES, FIELD EVALUATION AND TOXICITY OF AN ANTIVIRAL PLANT PROTECTANT NK0238

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SUPPLEMENTARY MATERIALS

Table S1 Optimization of conditions for synthesis of L-NAC from phosgene[a]

Entry	Amount of phosgene (equiv)	Solvent	Result
1	1.2	THF[e]	turbid[b]
2	1.4	THF	turbid
3	1.8	THF	turbid
4	1.4	DCM[f]	turbid
5[c]	1.4	THF	turbid
6[d]	1.4	DCM	turbid

Note：[a]Reaction conditions: unless otherwise indicated: room temperature (25 ℃). L-tryptophan is 15 mmol (3.06 g), solvent (30 mL), 60min. Phosgene is formed by the reaction of triphosgene with phenanthridine; [b]"turbid" means that the reaction system has not become clear, that is, a large amount of raw materials have not participated in the reaction; [c]Reaction temperature: 64 ℃; [d]Reaction temperature: 50 ℃; [e]THF is the abbreviation of tetrahydrofuran; [f]DCM is the abbreviation of dichloromethane.

Table S2 Screening of quenchers for synthesis of L-NAC from phosgene

Base	Results[a]
N,N-dimethylformamide	Prolonged contact will not cause L-Trp-NCA to decompose
triethylamine	Prolonged contact will cause L-Trp-NCA to decompose
pyridine	Prolonged contact will cause L-Trp-NCA to decompose

Note: [a]Add 100 mg of L-Trp-NCA to each reaction bottle, and add 1 mL of DMF, triethylamine, and pyridine to each reaction bottle for long time.

Table S3 Liquid chromatographic condition of chiral test

Instrument	Agilent 1260 chromatograph
Column	Phenomenex Lux SU Cellulose-1 250×4.6 mm
Mobile phase	90:10 (v/v) CH_3OH/H_2O containing 0.5% $NH_3 \cdot H_2O$
Flow rate	1.0 mL $\cdot min^{-1}$
Column temperature	30℃
UV detection	254 nm
Detection time	45 min

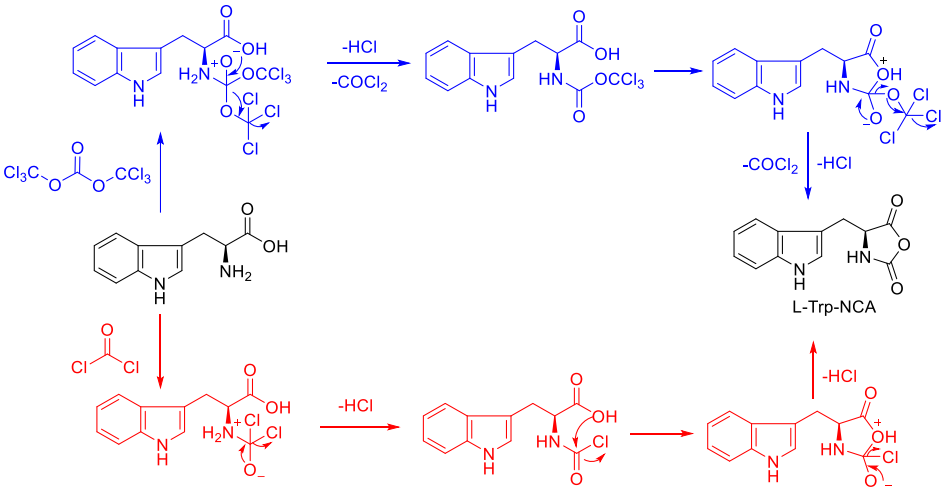


Fig. S1 Mechanisms of reactions of tryptophan with phosgene and triphosgene.

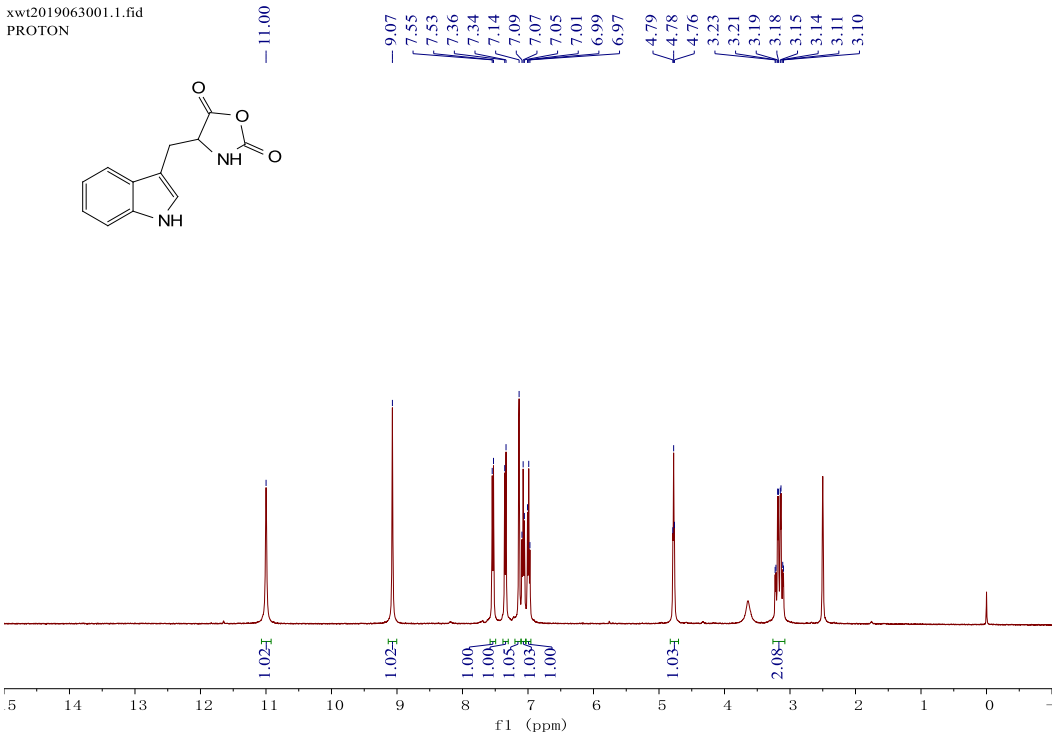


Fig.S2 ^{1}H NMR spectra of L-Trp-NCA. 400 MHz, d_6-DMSO.

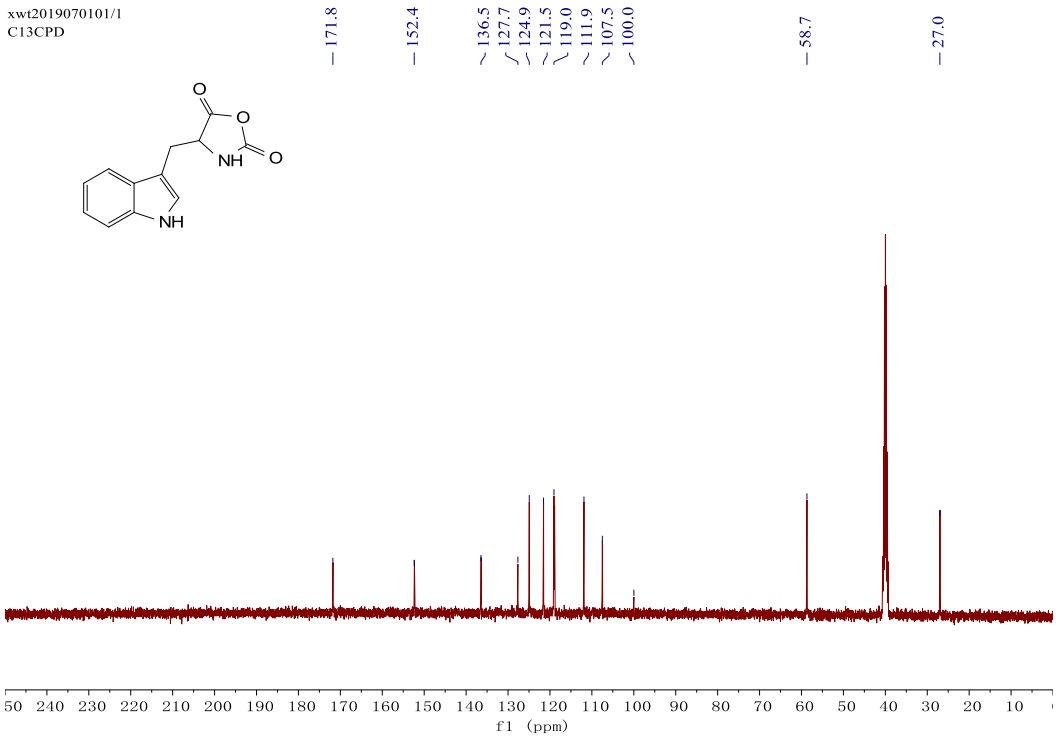


Fig. S3 ^{13}C NMR spectra of L-Trp-NCA. 100 MHz, d_6-DMSO**.**

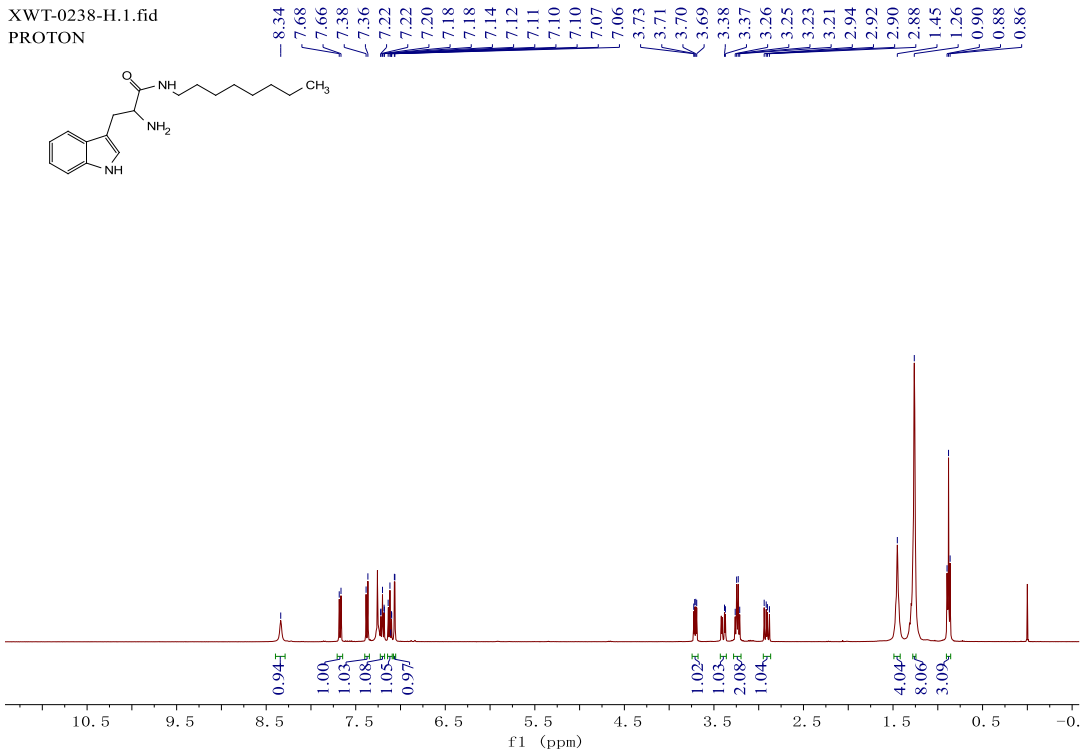


Fig. S4 ^{1}H NMR spectra of NK0238. 400 MHz, $CDCl_3$.

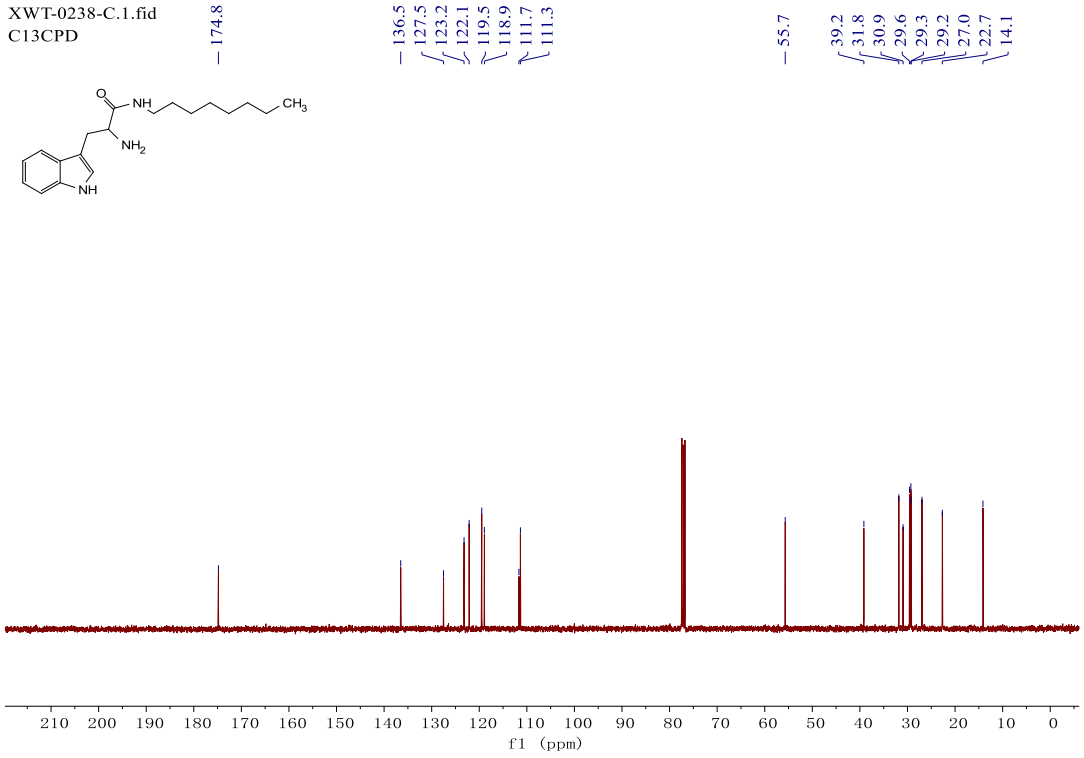


Fig. S5 ^{13}C NMR spectra of NK0238. 100 MHz, $CDCl_3$.

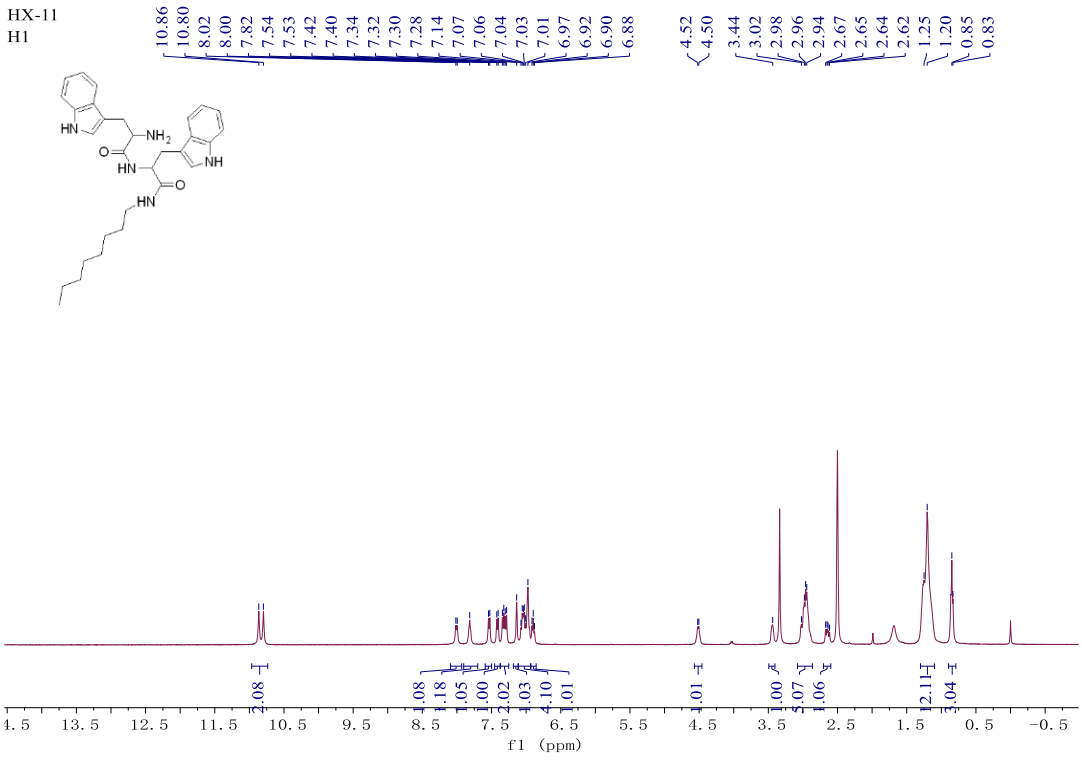


Fig. S6 ^{1}H NMR spectra of by-product. 400 MHz, d_6-DMSO.

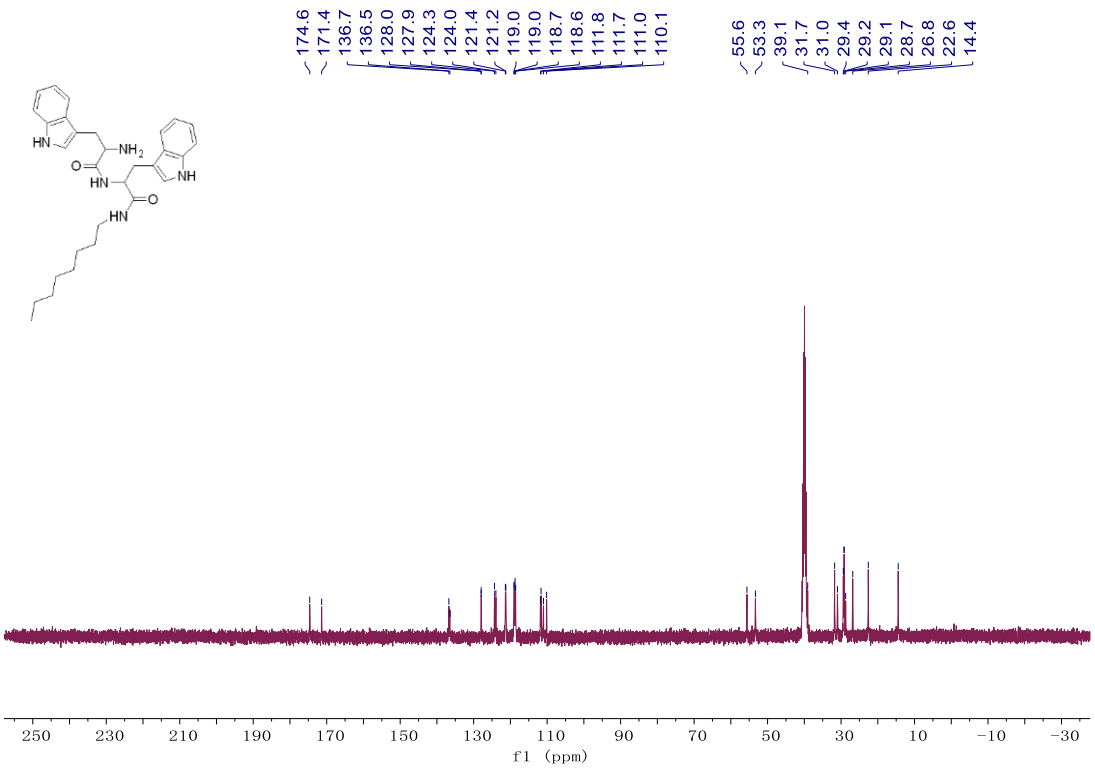


Fig. S7 ^{13}C NMR spectra of by-product. 100 MHz, d_6-DMSO.

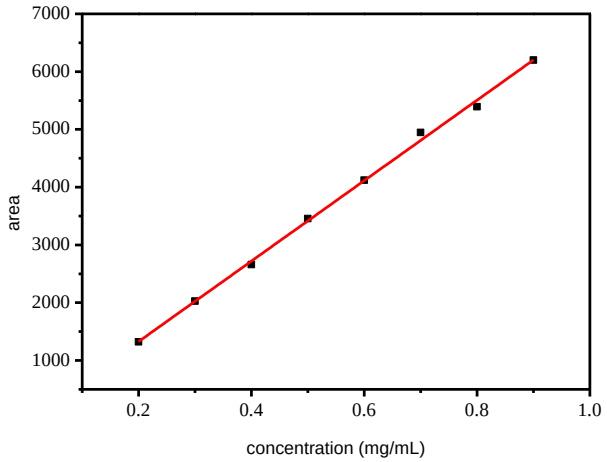
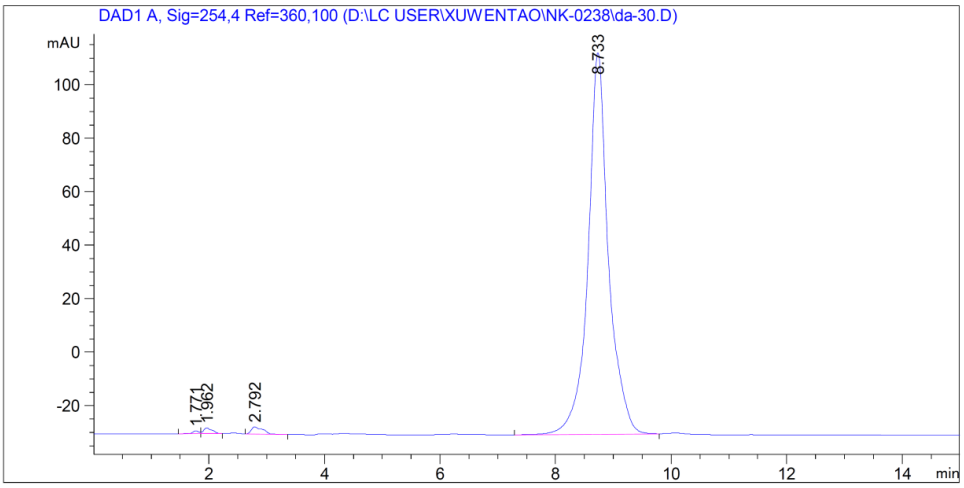


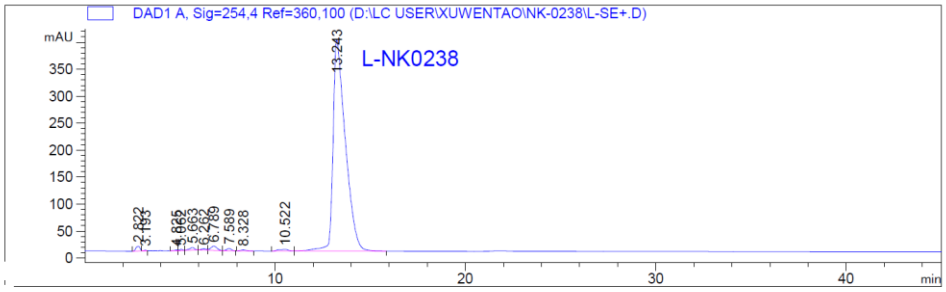
Fig. S8 HPLC standard curve for NK0238. $Y = -65.80 + 6964.61x$.



Signal 1: DAD1 A, Sig=254,4 Ref=360,100

peak #	time [min]	type	width [min]	area [mAU*s]	precentage %
1	1.771	BV	0.1101	7.44480	0.2078
2	1.962	VB	0.1390	21.68966	0.6055
3	2.792	BB	0.1927	38.13806	1.0646
4	8.733	BB	0.3554	3515.06421	98.1221

Fig. S9 HPLC spectrum of NK0238 in scaled-up reaction. HPLC spectrum of NK0238 under optimal conditions: L-tryptophan (30.63 g, 150.00 mmol). The sample concentration is 0.51 mg ·mL^{-1}. According to the standard curve the HPLC purity of NK0238 is 97.8%.

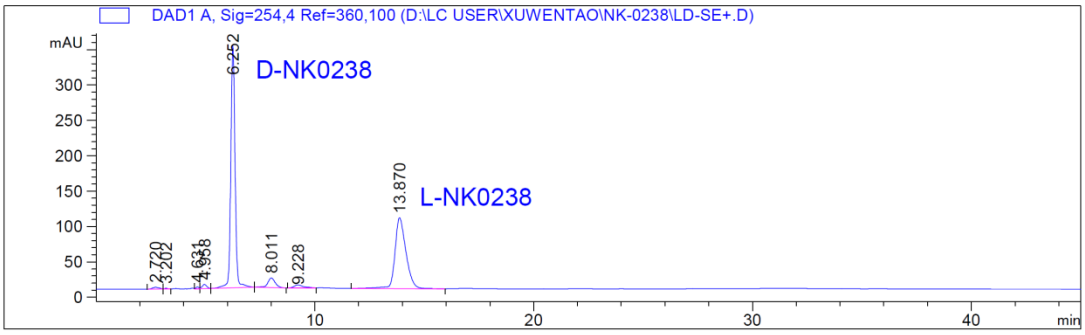


signal 1: DAD1 A, Sig=254,4 Ref=360,100

peak #	time [min]	type	width [min]	area [mAU*s]	percentage %
1	2.822	BV R	0.2495	132.45100	0.7245
2	3.193	VB E	0.1151	9.38173	0.0513
3	4.825	BV	0.2078	15.88547	0.0869
4	5.062	VB I	0.1716	21.52748	0.1178
5	5.663	BB	0.2817	89.62458	0.4903
6	6.262	BV	0.2660	48.18077	0.2636
7	6.789	VB	0.3538	176.38560	0.9649
8	7.589	BB	0.2551	70.21645	0.3841
9	8.328	BB	0.3423	34.37854	0.1881
10	10.522	BB	0.5014	103.23586	0.5647
11	13.243	BB	0.6475	1.75793e4	96.1639

总量 : 1.82806e4

Fig. S10 HPLC spectrum of L-NK0238.



signal 1: DAD1 A, Sig=254,4 Ref=360,100

peak #	time [min]	type	width [min]	area [mAU*s]	percentage %
1	2.720	BV	0.2620	53.47571	0.6159
1	3.202	VB	0.1173	9.50962	0.1095
3	4.631	VV	0.1287	21.30638	0.2454
4	4.958	VB	0.2064	80.63963	0.9288
5	6.252	BB	0.2080	4574.80225	52.6939
6	8.011	BB	0.3607	321.45511	3.7026
7	9.228	BB	0.4324	122.64297	1.4126
8	13.870	BB	0.5192	3498.00610	40.2911

total: 8681.83777

Fig. S11 HPLC spectrum of L-NK0238 and D-NK0238.

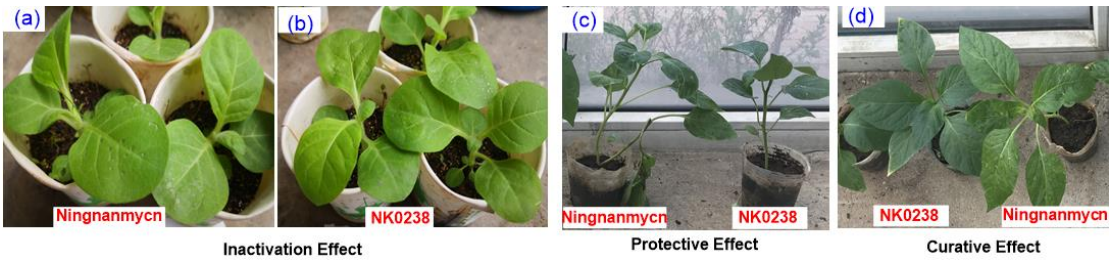


Fig. S12 The inactivation, protective and curative effect against TMV.

PROCEDURES FOR ANTIVIRUS SPECTRUM TEST

The pathogen used for testing:

Tobacco mosaic virus, TMV; *Sugarcane mosaic virus*, SCMV; *Pepper mild mottle virus*, PMMoV.

Calculation of virus concentration:

Take the calculation of tobacco Mosaic virus concentration as an example

The absorbance of 260nm was measured by ultraviolet spectrophotometer, and the virus concentration was calculated according to the formula.

Virus concentration（mg·mL^{-1}）=（A_{260}×diluted multiples）/$E^{0.1\%}_{1cm}{}^{260nm}$

E(Extinction coefficient)，That is, the light absorption (optical density) value of 0.1% (1mg·mL^{-1}) suspension at the wavelength of 260 nm at the optical path of 1cm.

The $E^{0.1\%}_{1cm}{}^{260nm}$of TMV is 3.1.

Plants used for testing:

The following plants were seeded in sterile soil and cultivated in a greenhouse.

TMV: 7–9 leaf stage of tobacco K326; SCMV: 6–7 leaf stage of corn; PMMoV: 6–7 leaf stage of pepper were used for experiment.

Inoculated virus concentration:

TMV (tobacco K326): W/V = 1:64;

TMV (pepper): W/V = 1:32;

SCMV (corn): W/V = 1:64;

PMMoV (pepper): W/V = 1:32.

Treatment method:

Compounds of different concentrations were mixed with virus SAP for 30min, and then inoculated to the test plants by SAP friction inoculation method.

Investigation and calculation:

Investigating the number of necrosis spots and disease grades, and the inactivation effects were calculated statistically.

$$\text{Disease grades} = \frac{\sum(\text{Grade} \times \text{Number of plants of this grade})}{\text{The highest grade} \times \text{The total number of plants}} \times 100 \quad \text{.....(1)}$$

$$\text{Inactivation effect} = \frac{\text{Disease grade of blank control-Disease grade}}{\text{Disease grade of blank control}} \times 100 \quad \text{.....(2)}$$

PROCEDURES FOR PROTECTIVE AND CURATIVE EFFECT AGAINST TMV

The pathogen used for testing: *Tobacco mosaic virus*, TMV.

Plants used for testing:

Pepper were seeded in sterile soil and cultivated in a greenhouse.

Experiment operation:

Protective effect: Compounds of different concentrations were sprayed 24 h before virus inoculation; **Curative effect:** Compounds of different concentrations were sprayed 24 h after inoculation. Inoculated by SAP friction inoculation method; Each treated 30 plants.

Investigation and calculation:

Investigating the number of necrosis spots and disease grades, disease grade of blank control.The prevention and treatment effects were calculated statistically.

$$\text{Disease grades} = \frac{\sum(\text{Grade} \times \text{number of plants of this grade})}{\text{The highest grade} \times \text{The total number of plants}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\text{Control effect} = \frac{\text{Disease grade of blank control-Disease grade}}{\text{Disease grade of blank control}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

PROCEDURES FOR ANTI-TMV EFFECTS AT DIFFERENT SPRAYING DATES

The pathogen used for testing:

Tobacco mosaic virus, TMV.

Plants used for testing:

Tobacco K326 was seeded in sterile soil and cultivated in a greenhouse, the experiment was did in the test field.

Treatment method:

Spraying date 1: Spraying at seedling stage, inoculating virus 3 days later; Spraying date 2: Inoculating virus at seedling stage, then spraying after the seedling recovering from transmitting; Spraying date 3: Inoculating virus at seedling stage, then spraying at rosette stage.

Investigation and calculation:

7 days after spraying at rosette stage.

$$\text{Disease grades} = \frac{\sum(\text{Grade} \times \text{number of plants of this grade})}{\text{The highest grade} \times \text{The total number of plants}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$\text{Control effect} = \frac{\text{Disease grade of blank control-Disease grade}}{\text{Disease grade of blank control}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

PROCEDURES FOR FIELD TRIALS

The pathogen used for testing:

Tobacco mosaic virus (TMV), panax notoginseng virus Y, gladiolus mosaic virus, banana bunchy top virus.

Plants used for testing:

Tobaco Yunyan 85, panax notoginseng, gladiolu, banana.
Drug formulations: moroxydine hydrochloride–cupric acetate (20% WP); amino-oligosaccharins (5% aqueous solution); NK0238 (1% microemulsion).

Treatment method:

In the early stage of the disease, spraying method is used. Spraying drugs every seven days, spraying 3 times in total. The disease grade was investigated before the first spraying, and the incidence of each treatment was investigated 10 days after the last spraying.

Calculation:

$$\text{Disease grades} = \frac{\sum(\text{Grade} \times \text{number of plants of this grade})}{\text{The highest grade} \times \text{The total number of plants}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$\text{Control effect (\%)} = 1\text{-}\left(\frac{I_0 \times I'}{I'_0 \times I} \times 100 \right) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

I_0 : Disease grade of blank control before spraying
I_0': Disease grade of blank control after spraying
I: Disease grade before spraying
I′: Disease grade after sprayi