**Supplementary experimental method**

**1. Synthesis of target compounds**

**1.1 Chemicals**

The required reagents for the experiment were purchased from TCI (Tokyo, Japan). Solvents were purchased from Accela (Shanghai, China). Solvents and reagents were used without further purification and drying.

**1.2 Instruments**

Melting points were determined via X-4 binocular microscope melting point apparatus (Beijing Tektronix Instruments Co., Ltd., China; unadjusted). The 1H, 13C, and 19F nuclear magnetic resonance (NMR) spectra of the target compounds were recorded on a JEOL ECX 500 NMR (JEOL Ltd., Tokyo, Japan) or an AVANCE III HD 400M NMR (Bruker Corporation, Fallanden, Switzerland) in CDCl3 or DMSO-d6 solutions. High-resolution mass spectra (HRMS) were obtained with a Thermo Scientific Q Exactive (Thermo Scientific, Missouri, USA) mass spectrometer.

**1.3 General Procedure for the Synthesis of B**

The ethyl 2- cyanoacetate A (500 mg) and triethyl orthoformate (1.05 g) were added to the round-bottomed flask, acetic anhydride (8 mL) was used as the solvent, and the mixture was refluxed and stirred.25 TLC monitored the progress of the reaction. On completion of the reaction, impurities were removed by filtration. The solvent was evaporated, and the resulting crude material was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=10:1) to give the pure product B in yields of 72 to 85%.

**1.4 General Procedure for the Synthesis of C**

A mixture of 500 mg of intermediate B, 600 mg of 3- chlorodihydrazide pyridine, and 3 mL of *n*-butanol was placed in a 50 mL round-bottomed flask and stirred at reflux for 24 hours. After completion of the reaction (monitored by TLC), impurities were removed by filtration. The solvent was evaporated and the resulting crude material was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=7:1) to give the pure product **C** in 83 to 95% yields1.

**1.5 General Procedure for the Synthesis of D**

A mixture of 1 g of intermediate **C** and 8 mL of triethyl orthoformate was stirred and refluxed in a 100 mL round-bottomed flask. After completion of the reaction (monitored by TLC), impurities were removed by filtration. The solvent was then evaporated and the resulting crude material was purified by column chromatography on silica gel (petroleum ether: ethyl acetate=10:1) to obtain a yellow oily pure products **D** in yields of 78 to 80%.

**1.6 General Procedure for the Synthesis of E**

In a 50 mL round-bottomed flask equipped with a magnetic stirrer, a solution of 1g of intermediate D in 5mL of ethanol was prepared. Excess 80% hydrazine hydrate was added, and the mixture was stirred at room temperature. The progress of the reaction was monitored by TLC. On completion of the reaction, impurities were removed by filtration to obtain intermediate **E** in 60-75% yields2.

**1.7 General Procedure for the Preparation of Target Compounds F1-F45**

The target compounds were prepared by reacting intermediate **E** with different aldehydes in the presence of glacial acetic acid at reflux in ethanol, yielding excellent results. The physical properties, 1H NMR, 13C NMR, 19F NMR and HRMS data of the synthesized compounds can also be found in the **Supplementary Data 2**.

**2. Biological experiment**

**2.1 Extraction and Purification of TMV**

Cut the infected tobacco leaves into small pieces, place them in a mortar, add liquid nitrogen, grind them to powder, and weigh the quality. Add the leaves to a beaker and add 0.2mol/L phosphoric acid buffer (pH=7.2, containing 1% mercaptoethanol by volume) in a ratio of mass to volume (1:1~1.5). Stir for 10 minutes (under ice bath conditions), filter with double-layer gauze, measure, and record the volume of the filtrate (initial extract). Add 8% n-butanol to the initial extraction solution (stirring while adding), stir in an ice bath for 20 minutes, balance the evenly stirred juice (the weight of the centrifuge tube placed in a symmetrical position is equal), and centrifuge it in a centrifuge (4 ℃, 8000 rpm, 20 minutes). Pour out the supernatant into a measuring cylinder and measure the volume of the supernatant. Weigh 4% of the supernatant volume of NaCl and polyethylene glycol, add them to a beaker, and add the supernatant to the beaker. Stir for 5 minutes under ice bath conditions, then evenly divide the juice into centrifuge tubes and centrifuge. The centrifuge parameters are set as above, remove the supernatant and leave sediment. Add 1/5 of the initial extract volume of phosphoric acid buffer (0.01mol/L) to the centrifuge tube containing the precipitate. Use a vortex machine to vortex the precipitate evenly and transfer it to a small beaker. Stir in an ice bath for 1 hour, and evenly separate it into the centrifuge tube. Centrifuge for 20 minutes at 8000rpm, 4 ℃. Take the supernatant, measure the volume, add 4% of the total volume of NaCl and polyethylene glycol, stir in an ice bath for 1 hour, and divide the juice into centrifuge tubes (with the same parameters as above) for centrifugation. Leave the sediment and remove the supernatant. Add 1/10 (initial extract juice volume) of phosphoric acid buffer solution (0.01mol/L) to the centrifuge tube containing the precipitate. Use a vortex machine to vortex the precipitate evenly and transfer it to a small beaker. After stirring in an ice bath for 1 hour, divide the juice into centrifuge tubes for centrifugation (5 minutes, 4 ℃, 8000rpm). Take the clear solution and transfer it to a small centrifuge tube for freezing and preservation.

**2.2 Antiviral Bio-assay against TMV.**

Six-leaf-aged tobacco leaves of the same size as were selected, then the entire leaves were sprinkled with uniform silicon carbide, and the leaves were inoculated with TMV virus by rubbing the leaves with a oil painting brush. After 30 minutes, the leaves' silicon carbide was washed with water and dried naturally. The compound solution was applied to the right side of the leaves, and 1% Tween 80 solution was applied to the left side of the leaves as a blank control. Compound solutions were prepared in the same way as in Section 2.8. The virus-inoculated plants were cultured in an artificial climate chamber (28±1°C, light 10000lux). After 3 to 4 days, the number of local lesions was counted, and three repetitions were set for each measurement. The evaluation of protective activity was similar to the above method, but the target compound solution was applied for 24 h before inoculation with the TMV virus. The inactivation activity was evaluated as follows: after mixing TMV virus with the target compound solution for 30 min, it was inoculated on the right side of the leaf, and the left side of the leaf was still inoculated with TMV virus. The inhibition rates were based on a percentage scale (0 = no activity and 100 = total inhibition), and inhibition rates were calculated by the eq 1, where X (%) is the percentage of inhibition rates, A is the lesions on the right leaves and CK is the lesions on the left leaves.

X = ((CK - A) × 100)/CK

**2.3 Protein extraction and digestion**

SDT (4%SDS，100mM Tris-HCl，1mM DTT，pH7.6) buffer was used for sample lysis and protein extraction. The amount of protein was quantified with the BCA Protein Assay Kit (Bio-Rad, USA). Protein digestion by trypsin was performed according to the filter-aided sample preparation (FASP) procedure described by Matthias Mann.[1] The digest peptides of each sample were desalted on C18 Cartridges (Empore™ SPE Cartridges C18 (standard density), bed I.D. 7 mm, volume 3 ml, Sigma), concentrated by vacuum centrifugation and reconstituted in 40 µl of 0.1% (v/v) formic acid.

**2.4 LC-MS/MS analysis**

LC-MS/MS analysis was performed on a timsTOF Pro mass spectrometer (Bruker) that was coupled to Nanoelute (Bruker Daltonics) for 60/120/240 min. The peptides were loaded onto a reverse phase trap column (Thermo Scientific Acclaim PepMap100, 100 μm\*2 cm, nanoViper C18) connected to the C18-reversed phase analytical column (Thermo Scientific Easy Column, 10 cm long, 75 μm inner diameter, 3μm resin) in buffer A (0.1% Formic acid) and separated with a linear gradient of buffer B (84% acetonitrile and 0.1% Formic acid) at a flow rate of 300 nl/min controlled by IntelliFlow technology. The mass spectrometer was operated in positive ion mode. The mass spectrometer collected ion mobility MS spectra over a mass range of m/z 100-1700 and 1/k0 of 0.6 to 1.6, and then performed 10 cycles of PASEF MS/MS with a target intensity of 1.5k and a threshold of 2500. Active exclusion was enabled with a release time of 0.4 minutes.

**2.5 Protein identification**

Protein identification and quantification were performed using MaxQuant software (version 1.5.3.17). The iBAQ arithmetic was used to quantify proteins and classify for a single sample on the absolute abundance of DEP under the mistake check rate to 0.01 for the recognition peptide. The difference in protein expression between two groups was compared using the normalized method. The eliminating identifications reverse database and common contaminants were used to filter protein tables. The treatment and control groups showed different protein accumulation as identified by performing an unpairedt-test between two sets of iBAQ information.

**2.6 Bioinformatic analysis**

Use Uniprot software to analyze the classification of different expressed proteins through the gene ontology (GO) annotations on the Kyoto Encyclopedia of Genes and Genomes (KEGG). The items in GO are related to biological processes (BP), molecular functions (MF), and cellular components (CC). Mapping six differentially expressed proteins (with expression levels greater than 1.5 times) to the GO database (http://www. geneontology.org/), and calculating protein mass for each GO term. Unlabeled proteomics results were used as the target list, and the background list was generated by downloading the GO database.

1. El-Tombary, A.A. (2013). Synthesis, Anti-Inflammatory, and Ulcerogenicity Studies of Novel Substituted and Fused Pyrazolo[3,4-d]pyrimidin-4-ones. Sci Pharm *81*, 393-422. 10.3797/scipharm.1211-21.

2. Wang, Y.Y., Xu, F.Z., Zhu, Y.Y., Song, B., Luo, D., Yu, G., Chen, S., Xue, W., and Wu, J. (2018). Pyrazolo[3,4-d]pyrimidine derivatives containing a Schiff base moiety as potential antiviral agents. Bioorg. Med. Chem. Lett. *28*, 2979-2984. 10.1016/j.bmcl.2018.06.049.