

CYANO COMPOUNDS, A PREPARATION METHOD THEREFOR AND USE THEREOF

The present invention claims the right of priority of the following applications:
5 patent application No. 202111168232.4 entitled "CYANO COMPOUNDS, AND
A PREPARATION METHOD AND USE THEREOF" and submitted to the China
National Intellectual Property Administration on Thursday, September 30, 2021
and China patent application No. 202210973184.4 entitled "CYANO
COMPOUNDS, AND A PREPARATION METHOD AND USE THEREOF" and
10 submitted to the China National Intellectual Property Administration on Monday,
August 15, 2022. The entirety of the above-referenced prior applications is
incorporated herein by reference in their entireties.

Technical Field

15 The present invention belongs to the field of medicinal chemistry and
chemical synthesis, in particular, the invention relates to cyano compounds, a
preparation method therefor and use thereof.

Background Art

20 Coronaviruses are single-stranded positive-sense RNA viruses, and some
coronaviruses can spread widely among people and cause severe symptoms. There
are currently 7 known coronaviruses that can infect humans, namely HCoV-229E,
HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV and SARS-
CoV-2. Most of the functional proteins of coronaviruses are encoded by the
25 ORF1ab gene, which is first translated into a polyprotein, and then cleaved into
multiple active proteins by 3CL protease and PL protease. Therefore, inhibiting the
activity of 3CL protease can effectively inhibit the replication of the viruses. 3CL
proteases in different coronaviruses have a high degree of structural homology, so
3CL protease inhibitors have a broad-spectrum anti-coronavirus activity.

30 In addition to coronaviruses, 3CL protease also plays an important role in the
hydrolysis of polyproteins encoded by picornaviruses, and 3CL protease inhibitors
can effectively inhibit the replication of picornaviruses. Enterovirus 71 is a
picornavirus, and is one of the common viruses that cause hand, foot and mouth
disease, and may also cause meningitis, brainstem encephalitis, myocarditis and

other diseases. In recent years, enterovirus 71 has repeatedly broken out in infants and young children, and there is still a lack of effective therapeutic drugs in clinical practice.

Therefore, there remains a need for compounds that can inhibit RNA
5 viruses/picornaviruses including coronaviruses/enterovirus 71.

Summary of the Invention

Based on the crystal structure of 3CL protease, the inventors rationally designed a class of cyano compounds, which can effectively inhibit coronavirus
10 and/or picornavirus 3CL protease activity, effectively inhibit 3CL protease activity of various picornaviruses including enterovirus 71 *in vitro*, effectively inhibit the replication of picornaviruses at a cell level and can be used for preparing a medicament for a disease induced by coronaviruses and/or picornaviruses. The inventors have completed the present disclosure on the basis of
15 this.

The primary object of the present invention is to provide a cyano compound represented by general formula I, a racemate, an enantiomer, a diastereoisomer and a pharmaceutically acceptable salt thereof.

The second object of the present invention is to provide a preparation method
20 of such compounds.

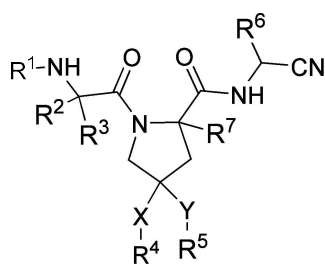
The third object of the present invention is to provide a pharmaceutical composition comprising such compounds.

The fourth object of the present invention is the use of such compounds in the preparation of 3CL protease inhibitors.

25 The fifth object of the present invention is the use of such compounds in the preparation of a medicament for preventing or treating a disease induced by coronaviruses and/or picornaviruses.

In order to accomplish the above objects, the technical solution adopted in the present invention is that:

30 In one aspect, the present invention provides a cyano compound represented by general formula I, a racemate, an enantiomer, a diastereoisomer and a pharmaceutically acceptable salt thereof.



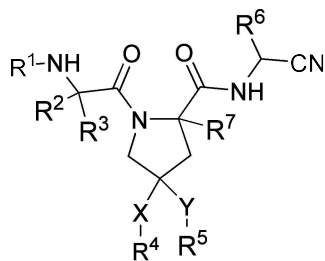
I

In another aspect, the present invention provides a method for preparing the compound of general formula I.

5 The technical solution of the present invention has at least the following technical effects:

The compound of the present invention has a 3CL protease inhibitory activity, can inhibit the hydrolysis of protein complexes expressed by genes of coronaviruses and picornaviruses, and then inhibits virus replication and development, and can be used for the prevention and treatment of diseases caused by coronavirus or picornavirus infection.

The present invention relates to a cyano compound represented by general formula I, a racemate, an enantiomer, a diastereoisomer or a pharmaceutically acceptable salt thereof:



I

wherein

R¹ is selected from -COR⁸ and -SO₂R⁹;

R² and R³ are each independently selected from H, D, C₁-C₁₀ alkyl, adamantyl and C₃-C₇ cycloalkyl, or, R² and R³ and the carbon atom attached thereto together form a 3- to 8-membered carbocyclic ring;

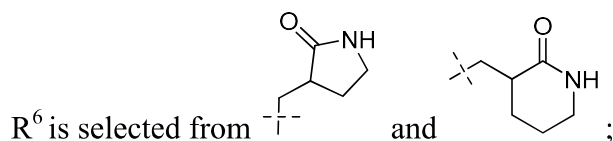
X is selected from O, S, S(=O)₂ and S=O;

Y is absent or selected from O, S, S(=O)₂ and S=O;

R⁴ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ cycloalkyl, C₆-C₂₀ aryl, C₁-C₁₀ alkyl substituted C₆-C₂₀ aryl, C₁-C₁₀ alkoxy substituted C₆-C₂₀ aryl and halogenated C₆-C₂₀ aryl;

R^5 is selected from H, C_1 - C_{10} alkyl and C_3 - C_7 cycloalkyl;

or, R^4 and R^5 are connected to each other to form C_2 - C_6 alkylene, thereby connecting X and Y;



5 R^7 is selected from H and D;

R^8 is selected from H, C_1 - C_{10} alkyl, C_1 - C_{10} alkoxy, C_3 - C_7 cycloalkyl, halogenated C_1 - C_{10} alkyl, halogenated C_3 - C_7 cycloalkyl, $-NR^{13}R^{14}$, C_6 - C_{20} aryl, halogenated C_6 - C_{20} aryl, C_1 - C_{10} alkyl substituted C_6 - C_{20} aryl, halogenated C_1 - C_{10} alkyl substituted C_6 - C_{20} aryl, 5- to 20-membered heteroaryl and halogenated 5- to 20-membered heteroaryl;

R^9 is selected from C_1 - C_{10} alkyl, C_3 - C_7 cycloalkyl, halogenated C_1 - C_{10} alkyl, halogenated C_3 - C_7 cycloalkyl, $-NR^{15}R^{16}$, C_6 - C_{20} aryl, halogenated C_6 - C_{20} aryl, C_1 - C_{10} alkyl substituted C_6 - C_{20} aryl, halogenated C_1 - C_{10} alkyl substituted C_6 - C_{20} aryl, 5- to 20-membered heteroaryl and halogenated 5- to 20-membered heteroaryl;

15 R^{13} and R^{14} are each independently selected from H and C_1 - C_{10} alkyl;

R^{15} and R^{16} are each independently selected from H and C_1 - C_{10} alkyl.

In some embodiments, R^2 and R^3 are each independently selected from H, D, C_1 - C_6 alkyl, adamantyl and C_3 - C_7 cycloalkyl, or, R^2 and R^3 and the carbon atom attached thereto together form a 3- to 8-membered carbocyclic ring.

20 In some embodiments, R^2 and R^3 are each independently selected from H, isopropyl, tert-butyl, cyclopentyl, and adamantyl, or, R^2 and R^3 and the carbon atom attached thereto together form cyclopropyl and cyclopentyl.

In some embodiments, one of R^2 and R^3 is selected from H, and the other is selected from isopropyl, tert-butyl, cyclopentyl, and adamantyl, or, R^2 and R^3 and the carbon atom attached thereto together form cyclopropyl and cyclopentyl.

25 In some embodiments, R^4 is selected from H, C_1 - C_6 alkyl, C_3 - C_8 cycloalkyl, C_6 - C_{10} aryl, C_1 - C_6 alkyl substituted C_6 - C_{10} aryl, C_1 - C_6 alkoxy substituted C_6 - C_{10} aryl and halogenated C_6 - C_{10} aryl;

R^5 is selected from H, C_1 - C_6 alkyl and C_3 - C_7 cycloalkyl;

30 or, R^4 and R^5 are connected to each other to form C_2 - C_6 alkylene, thereby connecting X and Y.

In some embodiments, X is selected from O, S, S(=O)₂ and S=O; Y is absent or selected from O, S and S=O; R⁴ is selected from C₁-C₆ alkyl and C₆-C₁₀ aryl, R⁵ is selected from H; or, R⁴ and R⁵ are connected to each other to form C₂-C₆ alkylene, thereby connecting X and Y.

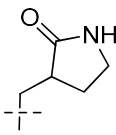
- 5 In some embodiments, R⁴ and R⁵ are connected to each other to form CH₂CH₂ and CH₂CH₂CH₂, thereby connecting X and Y.

In some embodiments, X and Y are each independently selected from O, S and S=O, and R⁴ and R⁵ are connected to each other to form CH₂CH₂ and CH₂CH₂CH₂, thereby connecting X and Y.

- 10 In some embodiments, X and Y are each independently selected from O and S, and R⁴ and R⁵ are connected to each other to form CH₂CH₂, thereby connecting X and Y.

- In some embodiments, both X and Y are selected from S, or both X and Y are selected from O, and R⁴ and R⁵ are connected to each other to form CH₂CH₂, thereby connecting X and Y.
- 15

In some embodiments, X is selected from S, Y is absent, R⁴ is selected from phenyl and isopropyl, and R⁵ is selected from H.



In some embodiments, R⁶ is selected from .

In some embodiments, R⁷ is selected from H.

- 20 In some embodiments, R⁸ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₃-C₇ cycloalkyl, halogenated C₁-C₆ alkyl, halogenated C₃-C₇ cycloalkyl, -NR¹³R¹⁴, C₆-C₁₀ aryl, halogenated C₆-C₁₀ aryl, C₁-C₆ alkyl substituted C₆-C₁₀ aryl, halogenated C₁-C₆ alkyl substituted C₆-C₁₀ aryl, 5- to 10-membered heteroaryl and halogenated 5- to 10-membered heteroaryl.

- 25 In some embodiments, R⁸ is selected from C₁-C₆ alkyl, halogenated C₁-C₆ alkyl, C₁-C₆ alkoxy, -NR¹³R¹⁴, C₃-C₇ cycloalkyl, halogenated C₃-C₇ cycloalkyl, phenyl, halophenyl, C₁-C₆ alkyl substituted phenyl, halogenated C₁-C₆ alkyl substituted phenyl and 5- to 6-membered heteroaryl.

- In some embodiments, R⁹ is selected from C₁-C₆ alkyl, C₃-C₇ cycloalkyl, halogenated C₁-C₆ alkyl, halogenated C₃-C₇ cycloalkyl, -NR¹⁵R¹⁶, C₆-C₁₀ aryl, halogenated C₆-C₁₀ aryl, C₁-C₆ alkyl substituted C₆-C₁₀ aryl, halogenated C₁-C₆
- 30

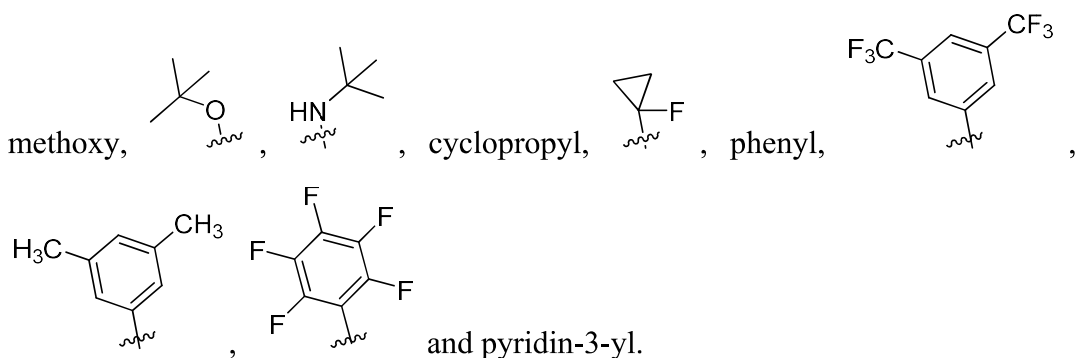
alkyl substituted C₆-C₁₀ aryl, 5- to 10-membered heteroaryl and halogenated 5- to 10-membered heteroaryl.

In some embodiments, R⁹ is selected from C₁-C₆ alkyl, C₃-C₇ cycloalkyl, phenyl, C₁-C₆ alkyl substituted phenyl and halogenated C₁-C₆ alkyl substituted phenyl.

In some embodiments, R¹³ and R¹⁴ are each independently selected from H and C₁-C₆ alkyl.

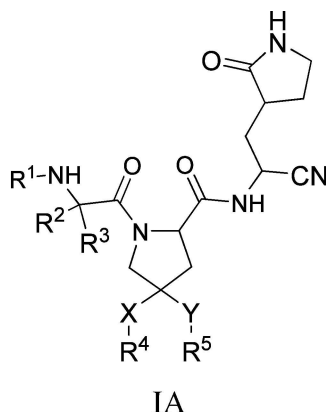
In some embodiments, R¹⁵ and R¹⁶ are each independently selected from H and C₁-C₆ alkyl.

In some embodiments, R⁸ is selected from CH₃, CF₃, CH₂CF₃, CF₂CF₃,



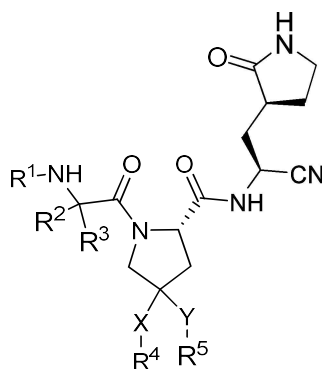
In some embodiments, R⁹ is selected from CH₃, cyclopropyl, phenyl, p-methylphenyl, and p-trifluoromethylphenyl.

In some embodiments, the cyano compound represented by general formula I is selected from the cyano compound represented by general formula IA:



wherein, R¹, R², R³, R⁴, R⁵, X and Y are as defined above.

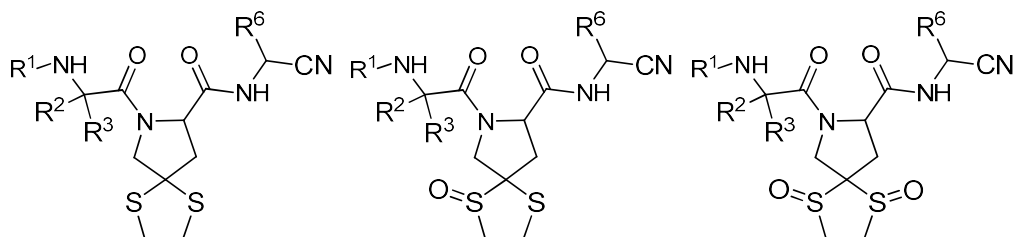
In some embodiments, the cyano compound represented by general formula I is selected from the cyano compound represented by general formula IB:



IB

wherein, each substituent is as defined above.

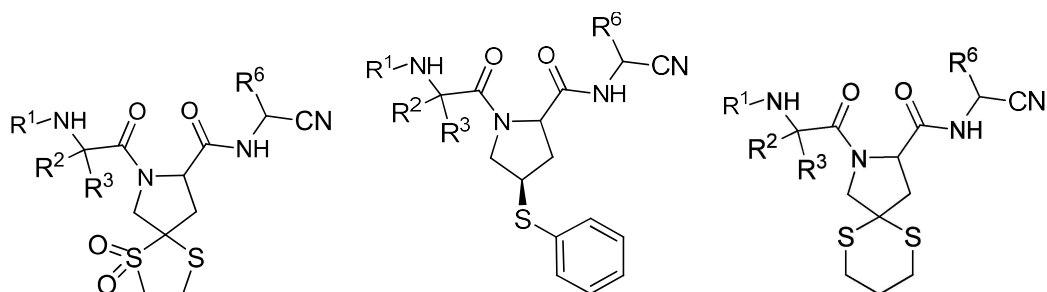
In some embodiments, the cyano compound represented by general formula I
 5 is selected from any one of the cyano compound represented by the following general formula:



I-1

I-2

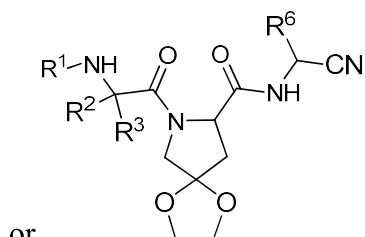
I-3



I-4

I-5

I-6

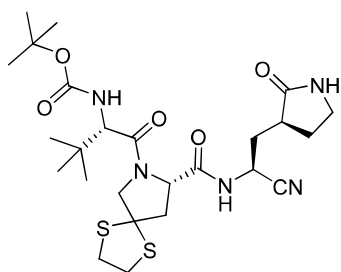


or

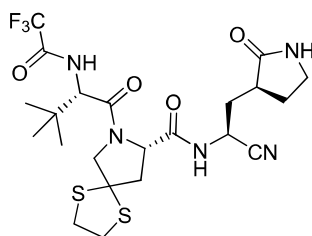
I-7

wherein, R^1 , R^2 , R^3 and R^6 are as defined above.

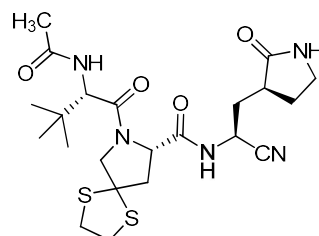
In some embodiments, the compound represented by general formula I of the
 15 present invention is selected from the following compounds:



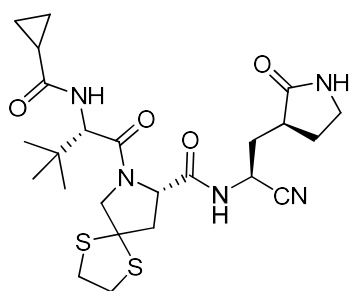
Compound 1



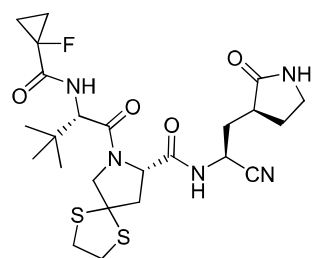
Compound 2



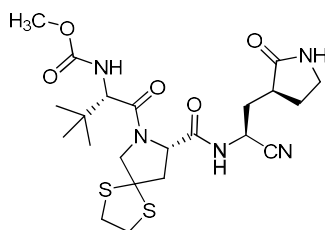
Compound 3



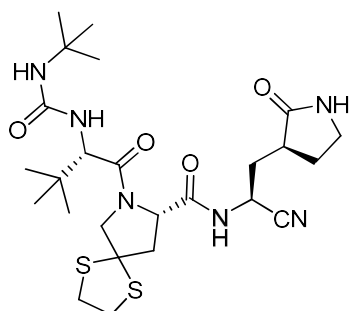
Compound 4



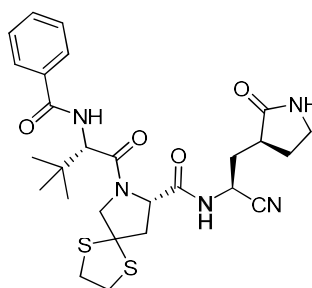
Compound 5



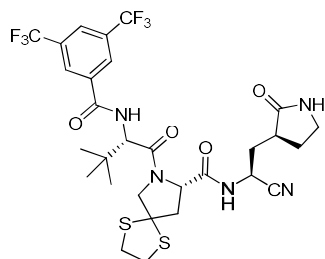
Compound 6



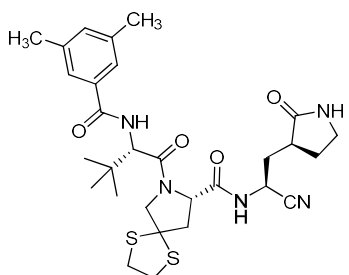
Compound 7



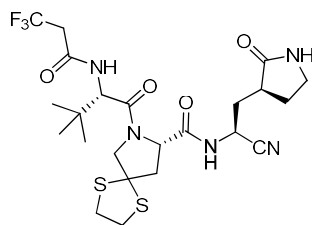
Compound 8



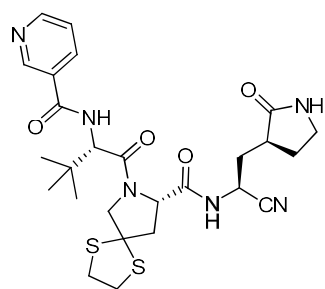
Compound 9



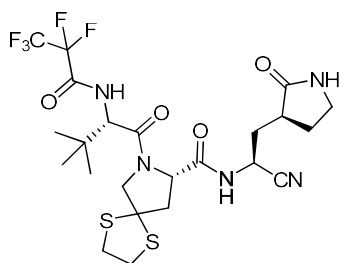
Compound 10



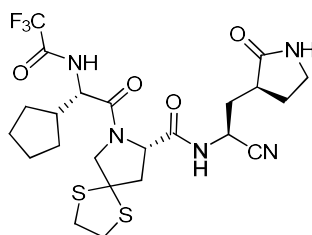
Compound 11



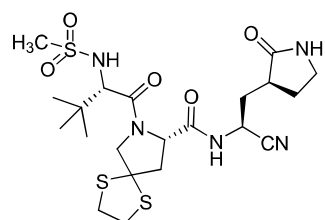
Compound 12



Compound 13

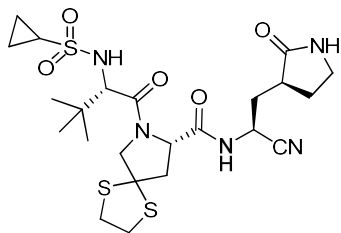


Compound 14

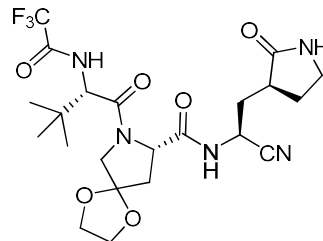
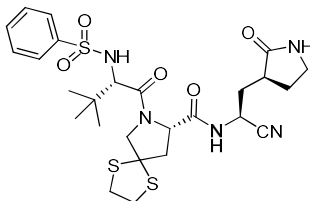


Compound 15

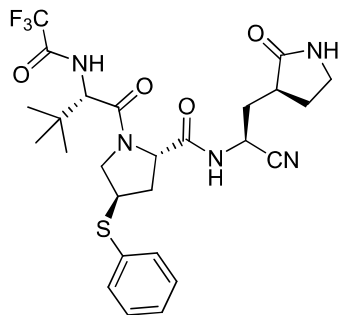
Compound 13



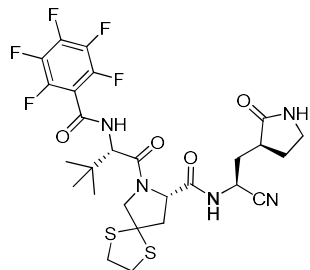
Compound 14



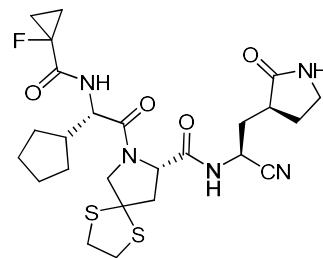
Compound 16



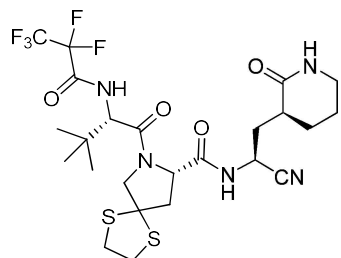
Compound 17



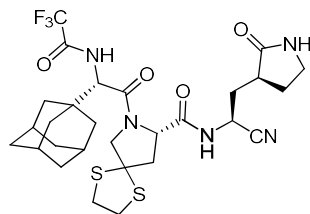
Compound 18



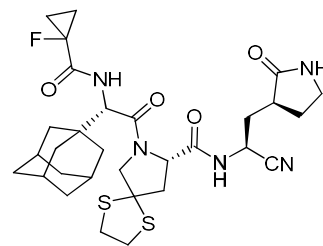
Compound 19



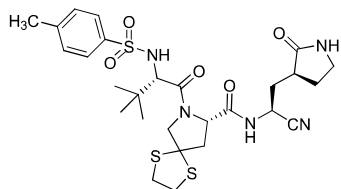
Compound 20



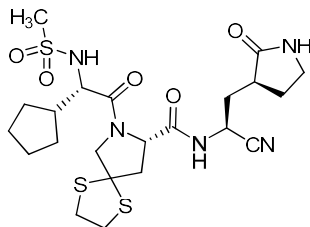
Compound 21



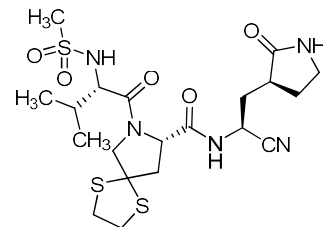
Compound 22



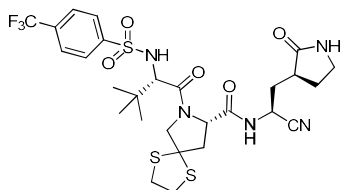
Compound 23



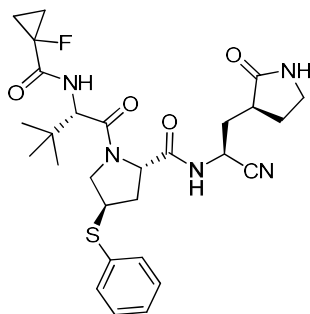
Compound 24



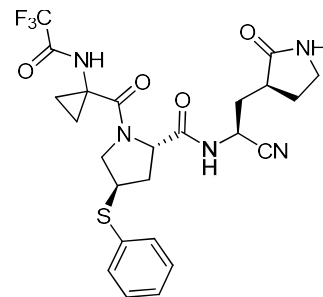
Compound 25



Compound 26



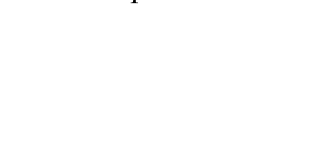
Compound 27



Compound 28

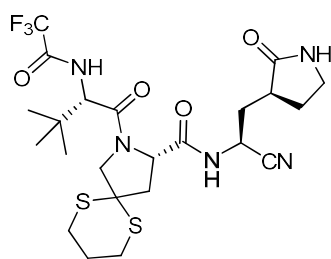


Compound 29

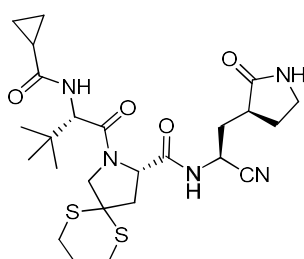


Compound 30

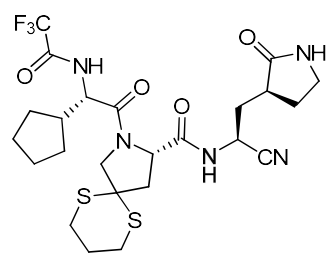




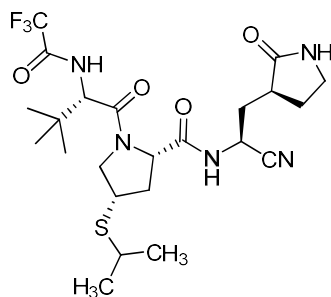
Compound 31



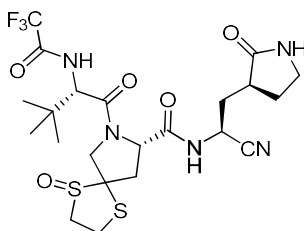
Compound 32



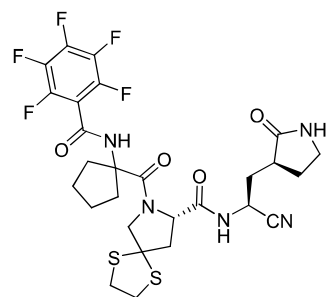
Compound 33



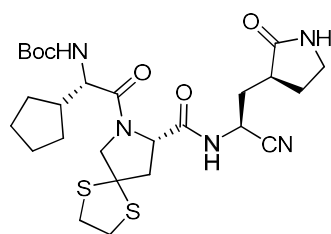
Compound 34



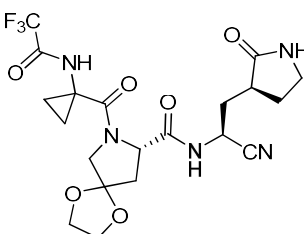
Compound 35



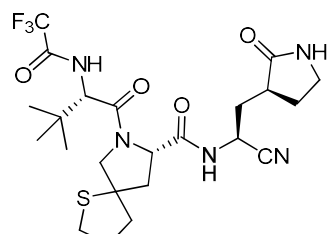
Compound 36



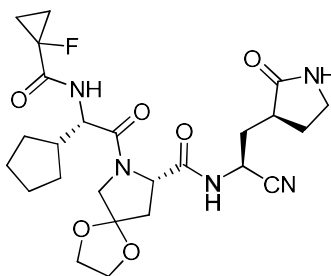
Compound 37



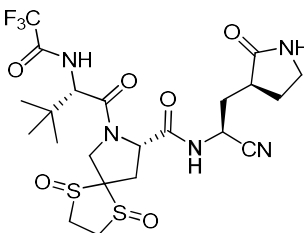
Compound 38



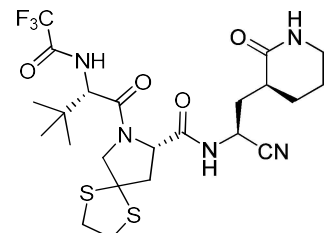
Compound 39



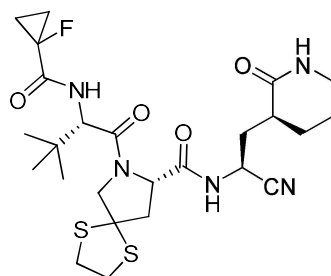
Compound 40



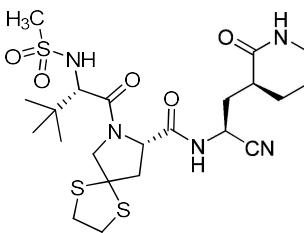
Compound 41



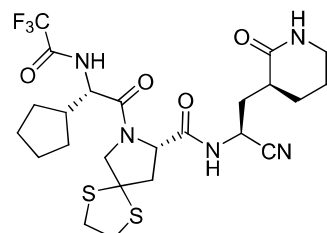
Compound 42



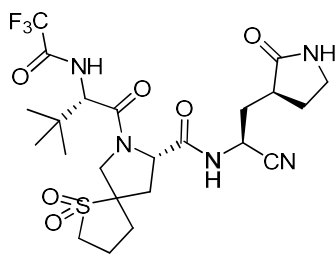
Compound 43



Compound 44



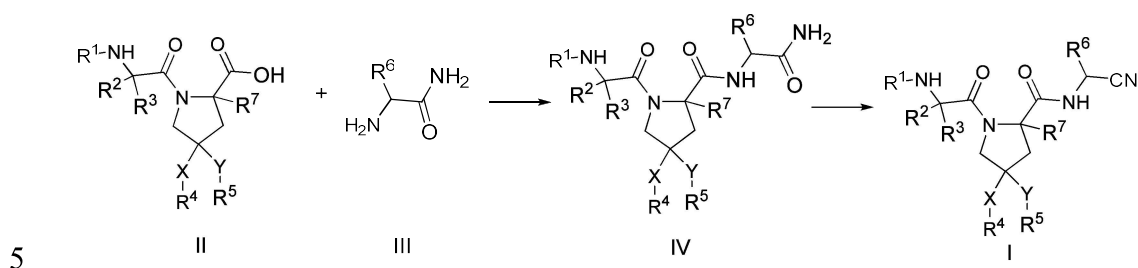
Compound 45



Compound 46

The present invention provides a method for preparing the compound represented by general formula I, and the method is one of following methods:

Method i:



ia) a compound represented by formula IV is obtained via condensation reaction of a compound represented by formula II and a compound represented by formula III;

preferably, the step ia) is that: the compound represented by formula II is reacted with the compound represented by formula III under the action of a condensing agent and a base in a solvent at a temperature of -20°C to 50°C for 0.1-12 h, thereby obtaining the compound represented by formula IV;

wherein, the solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, N,N-dimethylformamide, N,N-dimethylacetamide, ethyl acetate and 1,4-dioxane;

optionally, the condensing agent is one of or a mixture of more than one of the following: 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, 2-hydroxypyridine-N-oxide, 1-propyl phosphoric anhydride, 1H-benzotriazol-1-yloxytripyrrolidinyl hexafluorophosphate, N,N'-carbonyldiimidazole and O-benzotriazole-tetramethyluronium hexafluorophosphate;

optionally, the base is N,N-diisopropylethylamine, triethylamine and N-methylmorpholine;

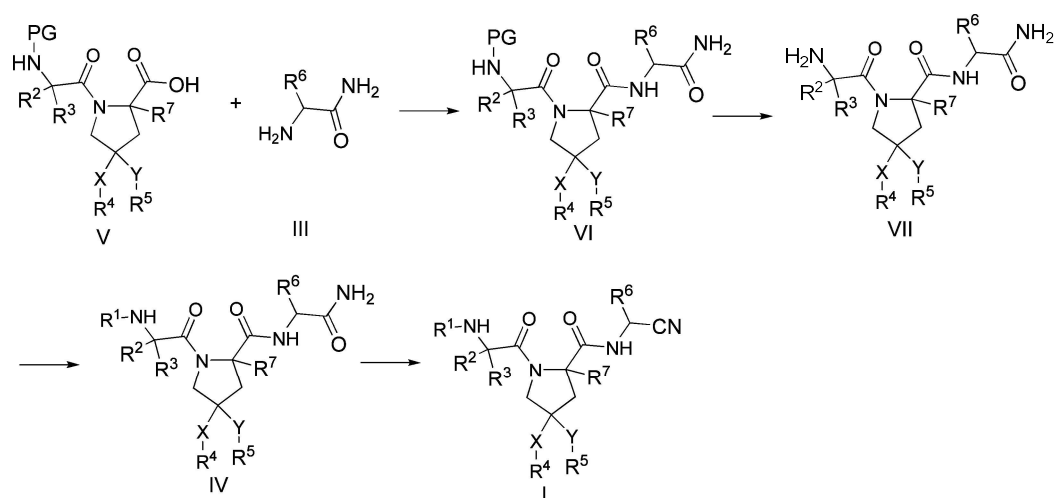
ib) the compound represented by formula IV is dehydrated to obtain the compound represented by general formula I;

preferably, the step ib) is that: the compound represented by formula IV is reacted with a dehydrating agent in an anhydrous solvent at a temperature of -20°C to 50°C for 1-24 h to obtain the compound represented by general formula I;

wherein, the anhydrous solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, toluene, 1,4-dioxane and pyridine;

the dehydrating agent is trifluoroacetic anhydride and methyl N-(triethylammoniumsulfonyl)carbamate; or

10 Method ii:



15 iia) a compound represented by formula VI is obtained by via condensation reaction of a compound represented by formula V and the compound represented by formula III, wherein PG in the compound represented by formula V is an amino protecting group;

preferably, the step iia) is that: the compound represented by formula V is reacted with the compound represented by formula III under the action of a condensing agent and a base in a solvent at a temperature of -20°C to 50°C for 0.1-12 h, thereby obtaining the compound represented by formula VI,

20 wherein, the solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, N,N-dimethylformamide, N,N-dimethylacetamide, ethyl acetate and 1,4-dioxane,

the condensing agent is one of or a mixture of more than one of the following: 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-

25

hydroxybenzotriazole, 2-hydroxypyridine-N-oxide, 1-propyl phosphoric anhydride, 1H-benzotriazol-1-yloxytripyrrolidinyl hexafluorophosphate, N,N'-carbonyldiimidazole and O-benzotriazole-tetramethyluronium hexafluorophosphate;

5 the base is N,N-diisopropylethylamine, triethylamine and N-methylmorpholine;

The amino protecting group PG is tert-butoxycarbonyl, benzyl and p-methoxybenzyl;

10 iib) the compound represented by formula VI is deprotected to obtain a compound represented by formula VII;

preferably, the step iib) is that: at a temperature of -20°C to 50°C, the compound represented by formula VI is reacted with an organic solution of trifluoroacetic acid or hydrogen chloride or Pd/C/H₂ to obtain the compound represented by formula VII;

15 iic) the compound represented by formula IV is obtained by aminoacylation, sulfonylation or condensation reaction of the compound represented by formula VII;

preferably, the step iic) is that:

20 at a temperature of -20°C to 50°C, the compound represented by formula VII is subjected to an aminoacylation reaction with acid chloride or acid anhydride under the condition of adding a base to obtain the compound represented by formula IV; or,

25 at a temperature of -20°C to 50°C, the compound represented by formula VII is subjected to a sulfonylation reaction with sulfonyl chloride or sulfonic anhydride under the condition of adding a base to obtain the compound represented by formula IV; or,

30 at a temperature of -20°C to 50°C, the compound represented by formula VII is subjected to a condensation reaction with a carboxyl compound under the condition of a condensing agent and a base to obtain the compound represented by formula IV;

optionally, the base is N,N-diisopropylethylamine, triethylamine and N-methylmorpholine;

optionally, the condensing agent is one of or a mixture of more than one of the following: 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium

hexafluorophosphate, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, 2-hydroxypyridine-N-oxide, 1-propyl phosphoric anhydride, 1H-benzotriazol-1-yloxytripyrrolidinyl hexafluorophosphate, N,N'-carbonyldiimidazole and O-benzotriazole-tetramethyluronium

5 hexafluorophosphate,

iid) the compound represented by formula IV is dehydrated to obtain the compound represented by general formula I;

preferably, the step iid) is that: the compound represented by formula IV is reacted with a dehydrating agent in an anhydrous solvent at a temperature of -20°C
10 to 50°C for 1-24 h to obtain the compound represented by general formula I;

wherein, the anhydrous solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, toluene, 1,4-dioxane and pyridine;

optionally, the dehydrating agent is trifluoroacetic anhydride and methyl N-(triethylammoniumsulfonyl)carbamate,

15 wherein, each substituent is as defined above.

Another aspect of the present invention further provides a pharmaceutical composition, which comprises one or more selected from a cyano compound of general formula I, a racemate, an enantiomer and a diastereomer thereof, and their pharmaceutically acceptable salts. The pharmaceutical composition may further
20 include one or more pharmaceutically acceptable adjuvants, diluents, carriers, excipients or adjuvants.

Another aspect of the present invention further provides a pharmaceutical composition, which comprises a cyano compound of formula I, or a racemate, an enantiomer, a diastereoisomer or a pharmaceutically acceptable salt thereof, and a
25 pharmaceutically acceptable excipient; optionally, the pharmaceutical composition further comprises ritonavir or a pharmaceutically acceptable salt thereof.

Another aspect of the present invention further provides a pharmaceutical combination, which comprises a cyano compound of general formula I, a racemate, an enantiomer, a diastereoisomer or a pharmaceutically acceptable salt thereof, and
30 ritonavir or a pharmaceutically acceptable salt thereof.

Another aspect of the present invention further provides a pharmaceutical composition, which comprises a cyano compound of general formula I or a pharmaceutically acceptable salt thereof, ritonavir or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Experiments prove that the compound of the present invention has an inhibitory activity on coronavirus 3CL proteases and picornavirus 3CL proteases.

Therefore, another aspect of the present invention provides a coronavirus 3CL protease inhibitor and/or a picornavirus 3CL protease inhibitor, which comprises
5 one or more selected from a cyano compound of general formula I, a racemate, an enantiomer and a diastereomer thereof, and their pharmaceutically acceptable salts, or the above-mentioned pharmaceutical composition.

The present invention further provides the above-mentioned cyano compound, the racemate, the enantiomer, the diastereomer and the pharmaceutically
10 acceptable salt thereof, or their mixture, or use of the pharmaceutical composition in the preparation of a medicament selected from a medicament for inhibiting coronavirus 3CL protease activity, a medicament for preventing and/or treating coronavirus infection, a medicament for inhibiting picornavirus 3CL protease activity, and a medicament for preventing and/or treating picornavirus infection.

15 The present invention further provides a method of inhibiting 3CL protease, the method comprises administering one or more selected from the cyano compound of general formula I, the racemate, the enantiomer and the diastereomer thereof, and their pharmaceutically acceptable salts of the present invention, or the pharmaceutical composition of the present invention to a subject in need thereof.

20 The present invention further provides a method of preventing and/or treating a disease or condition, the method comprises administering one or more selected from the cyano compound of general formula I, the racemate, the enantiomer and the diastereomer thereof, and their pharmaceutically acceptable salts of the present invention, or the pharmaceutical composition of the present invention to a subject
25 in need thereof, the disease or condition is a 3CL protease mediated disease or condition, in particular a disease or condition associated with coronavirus infection and/or picornavirus infection.

The present invention further provides one or more of the cyano compound of general formula I, the racemate, the enantiomer and the diastereomer thereof, and
30 their pharmaceutically acceptable salts described above, use of the pharmaceutical composition thereof in the preparation of a medicament for preventing or treating a related disease caused by coronavirus and/or picornavirus infection.

The present invention further provides a method for preventing or treating a related disease caused by coronavirus and/or picornavirus infection. The method

comprises administering to a patient a therapeutically effective amount of a pharmaceutical formulation comprising one or more of the cyano compound of general formula I, the racemate, the enantiomer and the diastereomer thereof, and their pharmaceutically acceptable salts of the present invention.

5 In some embodiments, the coronavirus is selected from SARS-CoV, MERS-CoV, H229E-CoV, HKU1-CoV, NL63-CoV, OC43-CoV or SARS-CoV-2.

In some embodiments, the related disease caused by the coronavirus infection is selected from respiratory tract infection, pneumonia or a complication thereof.

Definition and Description of Terminology

10 “C₁-C₁₀ alkyl” means a straight or branched saturated hydrocarbon group having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms. Specific examples of the alkyl include, but are not limited to, methyl, ethyl, propyl, butyl, pentyl, hexyl, isopropyl, isobutyl, sec-butyl, tert-butyl, isopentyl, 2-methylbutyl, 1-methylbutyl, 1-ethylpropyl, 1,2-dimethylpropyl, neopentyl, 1,1-dimethylpropyl, 4-methylpentyl,
15 3-methylpentyl, 2-methylpentyl, 1-methylpentyl, 2-ethylbutyl, 1-ethylbutyl, 3,3-dimethylbutyl, 2,2-dimethylbutyl, 1,1-dimethylbutyl, 2,3-dimethylbutyl, 1,3-dimethylbutyl, 1,2-dimethylbutyl, *etc.*; “C₁-C₆ alkyl” means a straight or branched alkyl containing 1-6 carbon atoms, including but not limited to methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, isopentyl, hexyl, *etc.*

20 “Alkylene” can be understood to mean a straight or branched saturated divalent hydrocarbon group. “C₂-C₆ alkylene” can be understood to mean a straight or branched saturated divalent hydrocarbon group containing 2-6 carbon atoms, including but not limited to CH₂CH₂, CH₂CH₂CH₂, CH₂CH₂CH₂CH₂, CH₂CH₂CH₂CH₂CH₂, CH₂CH₂CH₂CH₂CH₂CH₂, *etc.*

25 “C₃-C₈ cycloalkyl” means a cyclic alkyl containing 3-8 ring carbon atoms. “C₃-C₇ cycloalkyl” means a cyclic alkyl containing 3-7 ring carbon atoms, the cycloalkyl in the present disclosure includes but not limited to cyclopropyl, methylcyclopropyl, ethylcyclopropyl, dimethylcyclopropyl, cyclobutyl, methylcyclobutyl, ethylcyclobutyl, cyclopentyl, cyclohexyl, *etc.*

30 “C₁-C₁₀ alkoxy” means a straight branched or cyclic alkoxy containing 1-10 carbon atoms. “C₁-C₆ alkoxy” means a straight, branched or cyclic alkoxy containing 1-6 carbon atoms. The alkoxy in the present disclosure includes but not limited to methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, tert-butoxy, pentyloxy, isopentyloxy, cyclopentyloxy, hexyloxy, cyclohexyloxy, *etc.*

“Aryl” refers to an all-carbon monocyclic or fused polycyclic aromatic ring group having a conjugated π -electron system. The aryl can have 6-20 carbon atoms, 6-14 carbon atoms or 6-12 carbon atoms. “C₆-C₂₀ aryl” can be understood to mean an aryl with 6-20 carbon atoms. Especially a ring with 6 carbon atoms (“C₆ aryl”),
5 such as phenyl; or a ring with 9 carbon atoms (“C₉ aryl”), such as indanyl or indenyl; or a ring with 10 carbon atoms (“C₁₀ aryl”), such as tetrahydronaphthyl, dihydronaphthyl or naphthyl; or a ring with 13 carbon atoms (“C₁₃ aryl”), such as fluorenyl; or a ring with 14 carbon atoms (“C₁₄ aryl”), such as anthracenyl. “C₆-C₁₀ aryl” can be understood to mean an aryl containing 6-10 carbon atoms. Especially
10 a ring with 6 carbon atoms (“C₆ aryl”), such as phenyl; or a ring with 9 carbon atoms (“C₉ aryl”), such as indanyl or indenyl; or a ring with 10 carbon atoms (“C₁₀ aryl”), such as tetrahydronaphthyl, dihydronaphthyl or naphthyl.

“Heteroaryl” is an aromatic ring group containing 5-20 ring atoms, one or more of the ring atoms is a heteroatom selected from N, O or S, and the rest of the
15 ring atoms are carbon. “5- to 20-membered heteroaryl” can be understood to mean a heteroaryl having 5-20 ring atoms, especially 5 or 6 or 9 or 10 or 13 or 14 ring atoms, and containing 1-7 heteroatoms independently selected from N, O and S. “5- to 10-membered heteroaryl” can be understood to mean a heteroaryl having 5-
20 10 ring atoms, especially 5 or 6 or 9 or 10 ring atoms, and containing 1-5 heteroatoms independently selected from N, O and S. “5- to 6-membered heteroaryl” can be understood to mean a heteroaryl having 5 or 6 ring atoms and containing 1-3 heteroatoms independently selected from N, O and S. The heteroaryl described in the present invention can be selected from thienyl, furyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl,
25 oxadiazolyl, triazolyl, thiadiazolyl, and the like, and the benzo derivatives thereof, such as benzofuryl, benzothienyl, benzothiazolyl, benzoxazolyl, benzisoxazolyl, benzoimidazolyl, benzotriazolyl, indazolyl, indolyl, isoindolyl, and the like; or pyridyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, and the like, and the benzo derivatives thereof such as quinolyl, quinazolinyl, isoquinolinyl, and the like; or
30 azocinyl, indoliziny, purinyl, and the like and the benzo derivatives thereof; or cinnoliny, phthalazinyl, quinazolinyl, quinoxalinyl, naphthyridinyl, pteridinyl, carbazolyl, acridinyl, phenazinyl, phenothiazinyl or phenoxazinyl, and the like.

“Halogen” is selected from fluorine, chlorine, bromine and iodine.

“Halo” includes monohalo, polyhalo or perhalo, that is, one, more or all hydrogen atoms are substituted with halogen.

“Substituted” means that one or more hydrogen atoms on a group are substituted with one or more substituents.

5 The term “optional” or “optionally” mean that the subsequently described event or circumstance may or may not occur, and the description includes the occurrence and the non-occurrence of the event or circumstance.

“Therapeutically effective amount” means an amount of a compound of the present invention that (i) treats a particular disease, condition, or disorder, (ii)
10 attenuates, ameliorates, or eliminates one or more symptoms of a particular disease, condition, or disorder, or (iii) delays the onset of one or more symptoms of a particular disease, condition, or disorder described herein. The amount of a compound of the present disclosure which constitutes a “therapeutically effective amount” will vary depending on the compound, the conditions and their severity,
15 the manner of administration, and the age of the mammal to be treated, but can be determined routinely by those skilled in the art according to their own knowledge and the present disclosure.

“Pharmaceutically acceptable salt” refers to salts of pharmaceutically acceptable acids or bases, including salts formed between compounds and
20 inorganic or organic acids, and salts formed between compounds and inorganic or organic bases.

The term “pharmaceutical combination” refers to a combination comprising two or more active ingredients or pharmaceutically acceptable salts thereof. In some embodiments of the present invention, the active ingredients in the
25 pharmaceutical combination or the pharmaceutically acceptable salts thereof can be administered simultaneously. In some embodiments of the present invention, the active ingredients in the pharmaceutical combination or the pharmaceutically acceptable salts thereof can also be administered separately or sequentially.

“Pharmaceutical composition” refers to a mixture of one or more of the
30 compounds or salts thereof according to the present disclosure and a pharmaceutically acceptable adjuvant. An object of the pharmaceutical composition is to facilitate administering the compound according to the present disclosure to an organism.

“Pharmaceutically acceptable adjuvant” refers to those adjuvants which have no significant irritating effect on the organism and do not impair the bioactivity and properties of the active compound. Suitable adjuvants are well known to those skilled in the art, and are such as a carbohydrate, a wax, a water-soluble and/or water-swellable polymer, a hydrophilic or hydrophobic material, gelatin, an oil, a solvent, water, and the like.

Brief Description of the Drawings

FIG. 1 is a graph showing the inhibitory rate-concentration of compound 2 on the *in vitro* activity of the WIV04 strain of SARS-CoV-2.

FIG. 2 is a graph showing the inhibitory rate-concentration of compound 2 on the *in vitro* activity of the B.1.351 strain of SARS-CoV-2.

FIG. 3 is a graph showing the inhibitory effect of compound 2 on the virus titre in the lungs of mice 2 days after infection (FIG. A) and 4 days after infection (FIG. B) in example 26.

FIG. 4 is a graph showing the body weight changes of mice in example 26.

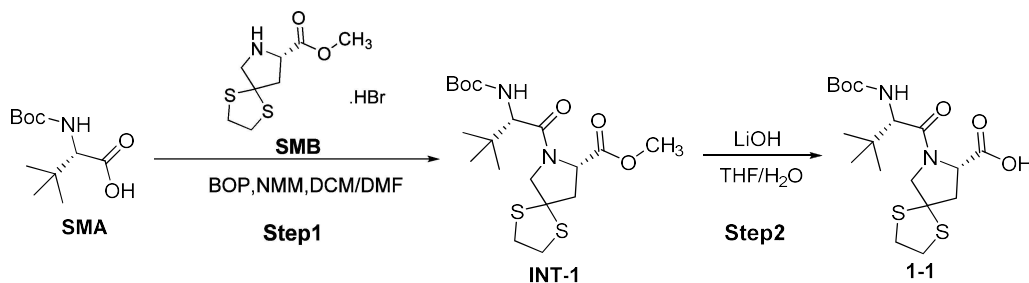
FIG. 5 is a graph showing the inhibitory effect of compound 2 on the virus titre in the brains of mice 4 days after infection in example 26.

Detailed Description of Embodiments

The present disclosure is further described by way of examples below. In the following examples, starting materials are commercially available or can be prepared by methods documented in the literature/organic synthetic methods known in the art.

Example 1

Preparation of compound 1-1:

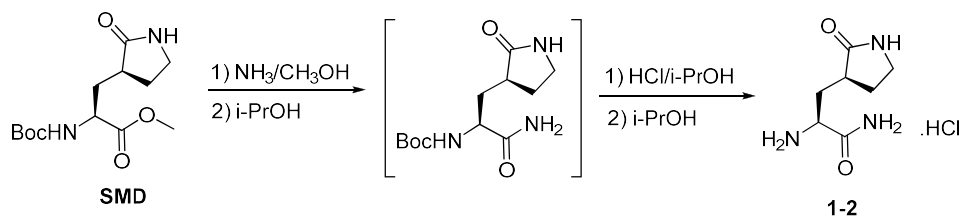


Step 1: The starting material SMA (2.74 g, 11.85 mmol), 35 ml of dichloromethane and 35 ml of DMF were added into a reaction flask and cooled

down to 0°C. The starting material SMB (3.56 g, 11.86 mmol), benzotriazole- 1-
 yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, 6.29 g, 14.22
 mmol) and N-methylmorpholine (NMM, 3.91 ml, 35.56 mmol) were added and
 brought to room temperature for reaction for 10 h. After the reaction was
 5 completed, an appropriate amount of dichloromethane was added, and the organic
 phase was washed successively with 1N aqueous hydrochloric acid solution and
 saturated brine. After the washing, the organic phase was dried over anhydrous
 sodium sulphate, and the organic phase was concentrated to dryness, and 3.71 g of
 INT-1 was obtained by column chromatography; ESI-MS: 433.2 m/z [M+H]⁺; ¹H
 10 NMR (400 MHz, DMSO-*d*₆): δ_H: 6.75 (d, J = 9.2 Hz, 1H), 4.38 (t, J = 8.2 Hz, 1H),
 4.25 (d, J = 10.9 Hz, 1H), 4.11 (d, J = 9.3 Hz, 1H), 3.93 (t, J = 9.3 Hz, 1H), 3.62 (s,
 3H), 3.40-3.31 (m, 4H), 2.70 (dd, J = 13.1, 7.9 Hz, 1H), 2.37 (dd, J = 13.2, 8.4 Hz,
 1H), 1.37 (s, 9H), 0.94 (s, 9H).

Step 2: INT-1 (3.71 g, 8.58 mmol), 37 ml of THF, 37 ml of purified water and
 15 lithium hydroxide monohydrate (0.72 g, 17.16 mmol) were added into a reaction
 flask, and reacted at room temperature for 2 h. After the reaction was completed,
 the reaction liquid was adjusted with concentrated hydrochloric acid to pH = 4, and
 filtered to obtain 3.4 g of compound 1-1; ESI-MS: 419.2 m/z [M+H]⁺; ¹H NMR
 (400 MHz, DMSO-*d*₆): δ_H: 12.68 (s, 1H), 6.71 (d, J = 9.4 Hz, 1H), 4.38-4.19 (m,
 2H), 4.11 (d, J = 9.4 Hz, 1H), 3.88 (d, J = 10.9 Hz, 1H), 3.41-3.29 (m, 4H), 2.69
 20 (dd, J = 13.1, 7.9 Hz, 1H), 2.34 (dd, J = 13.2, 8.9 Hz, 1H), 1.38 (s, 9H), 0.94 (s,
 9H).

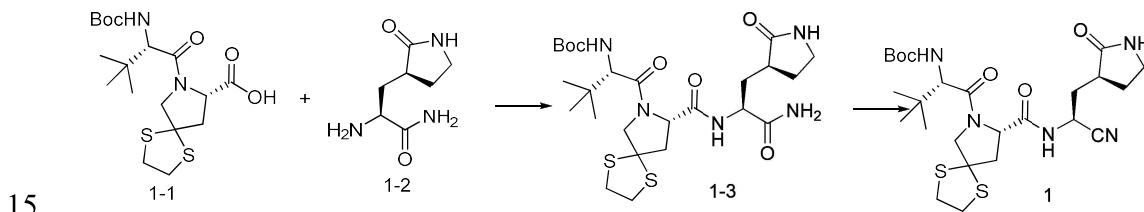
Preparation of compound 1-2:



25 Ammonia-methanol solution (700 ml, 7 mol/L) and starting material SMD
 (100 g, 0.349 mol) were added into a reaction flask, stirred to dissolve, and reacted
 for 36 h at a constant temperature of 25 ± 5°C. After the reaction was completed,
 the reaction liquid was concentrated until the remaining reaction liquid was about
 250 ml. 300 ml of isopropanol was added and further concentrated until the
 30 remaining reaction liquid was about 250 ml (repeated three times). The resulting
 reaction liquid was subjected to nitrogen replacement and cooled down to 10 ± 5°C.

500 ml of hydrogen chloride-isopropanol solution (4 mol/L) was added into the reaction kettle. After the addition, the temperature was raised to $25 \pm 5^\circ\text{C}$, and the temperature was kept at $25 \pm 5^\circ\text{C}$ for 9 hours. After the reaction was completed, the reaction liquid was concentrated under reduced pressure until the remaining
 5 reaction liquid was about 250 ml in volume. 300 ml of isopropanol was added and further concentrated under reduced pressure until the remaining reaction liquid was about 250 ml in volume (repeated twice). 100 ml of isopropanol was added, stirred for 30 ± 5 min and filtered, and the filter cake was rinsed with 50 ml of isopropanol to obtain a wet product; The wet product was dried under vacuum at
 10 $45 \pm 5^\circ\text{C}$ to obtain 66.7 g of compound 1-2, yield: 92%; ^1H NMR (400 MHz, DMSO- d_6): δ_{H} : 8.45 (d, $J = 5.1$ Hz, 3H), 8.25 - 8.04 (m, 1H), 7.95 (s, 1H), 7.67 - 7.49 (m, 1H), 3.85-3.80 (m, 1H), 3.19-3.13 (m, 2H), 2.59 - 2.51 (m, 1H), 2.32-2.27 (m, 1H), 2.05-1.98 (m, 1H), 1.82 - 1.66 (m, 2H); ESI-MS: 172.1 m/z $[\text{M}+\text{H}]^+$.

Preparation of compound 1:

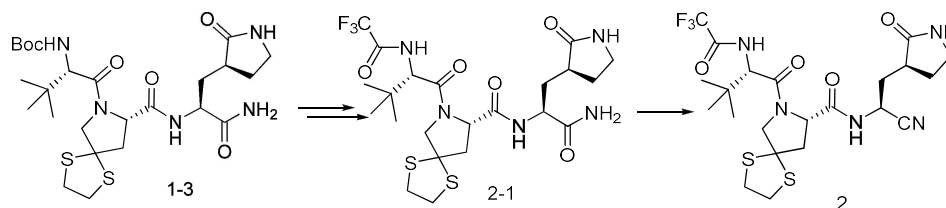


Compound 1-1 (419 mg, 1 mmol) was placed in a two-necked flask, and 5 mL of dichloromethane was added under nitrogen protection, and then 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate (400 mg, 1.1 mmol) was added and stirred at room temperature for 1 h. Compound 1-2 (1
 20 mmol) was dissolved in 1 mL of dichloromethane and added to the above system, then N,N-diisopropylethylamine (0.5 mL, 1 mmol) was added under an ice-water bath, and the ice-water bath was removed. The system was stirred overnight at room temperature. Workup was performed, 50 mL of dichloromethane was added, washed with 1M aqueous hydrochloric acid three times, and washed with saturated
 25 aqueous sodium bicarbonate solution three times. The organic phase was washed with saturated saline solution, dried over anhydrous sodium sulphate, and spin-dried by filtration to afford compound 1-3 (a white solid, 469 mg, yield 82%). ESI-MS: m/z 572.3 $[\text{M}+\text{H}]^+$.

Compound 1-3 (114 mg, 0.2 mmol) and Burgess reagent (1.5 eq) were added
 30 to a two-necked flask, nitrogen was charged and discharged for three time, then dichloromethane dried over molecular sieves was added and stirred at room

temperature overnight. Thin layer chromatography showed that the raw materials was substantially completely reacted. Workup was performed, column chromatography was performed to afford compound 1 (a white solid, 52 mg, yield 47%), ESI-MS: 554.3 m/z [M+H]⁺.

5 Example 2

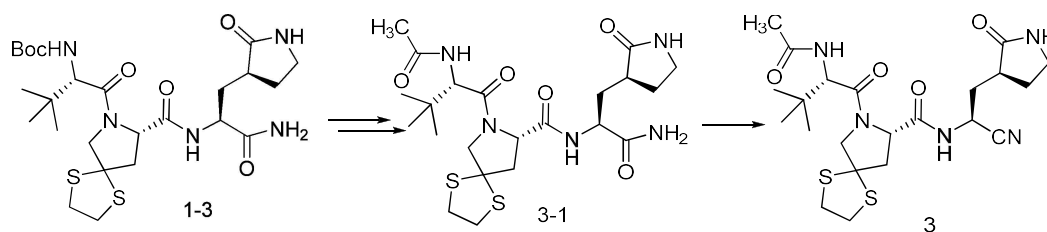


Compound 1-3 (572 mg, 1 mmol) was dissolved in 3 mL of 4M hydrogen chloride/1,4-dioxane solution, or dissolved in 2 mL of dichloromethane, then 2 mL of trifluoroacetic acid was added dropwise and stirred at ambient temperature. Thin layer chromatography showed that the reaction of raw materials was substantially completely, and then the solvent was fully spin-dried. The obtained crude product was dissolved in 2 mL of dichloromethane under nitrogen protection, and triethylamine (3 mmol) was added, then the system was placed in an ice-water bath, and trifluoroacetic anhydride (1.2 mmol) was added dropwise. Thin layer chromatography showed that the raw materials was substantially completely reacted, and then 50 mL of dichloromethane was added and washed three times with 1M aqueous hydrochloric acid solution and three times with saturated aqueous sodium bicarbonate solution. The organic phase was washed with saturated saline solution, dried over anhydrous sodium sulphate, and passed through a column to afford compound 2-1 (a white solid, 265 mg, yield 46%). ESI-MS: m/z 568.3[M+H]⁺.

Compound 2-1 (113 mg, 0.2 mmol) and Burgess reagent (1.5 eq) were added to a two-necked flask, nitrogen was charged and discharged for three time, then dichloromethane dried over molecular sieves was added and stirred at room temperature overnight. Thin layer chromatography showed that the raw materials was substantially completely reacted. Workup was performed, column chromatography was performed to afford compound 2 (a white solid, 41 mg, yield 47%), ¹H NMR (400 MHz, DMSO-*d*₆) δ 9.46 (d, *J* = 8.7 Hz, 1H), 9.05 (d, *J* = 8.6 Hz, 1H), 7.67 (s, 1H), 4.97 (ddd, *J* = 11.0, 8.5, 5.0 Hz, 1H), 4.53 (d, *J* = 8.7 Hz, 1H), 4.34 (dd, *J* = 9.9, 7.1 Hz, 1H), 4.26-4.14 (m, 1H), 3.92 (d, *J* = 10.9 Hz, 1H), 3.50-3.34 (m, 4H), 3.22-3.11 (m, 1H), 3.06 (td, *J* = 9.3, 7.1 Hz, 1H), 2.68-2.58 (m,

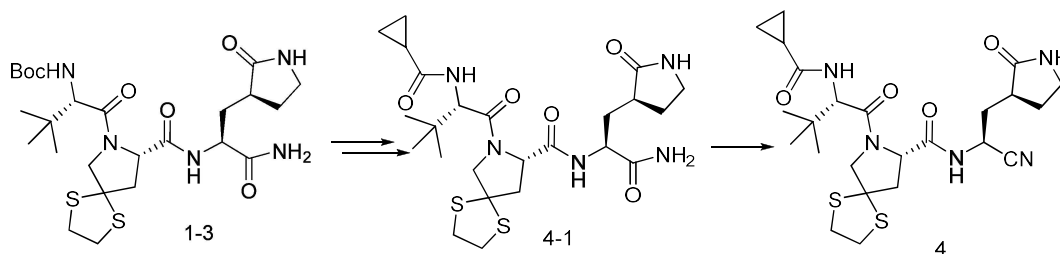
1H), 2.50-2.43 (m, 1H), 2.31 (dd, $J = 13.0, 10.0$ Hz, 1H), 2.23-2.07 (m, 2H), 1.71 (tdd, $J = 14.9, 10.3, 7.4$ Hz, 2H), 0.99 (s, 9H). ESI-MS: 550.3 m/z $[M+H]^+$.

Example 3



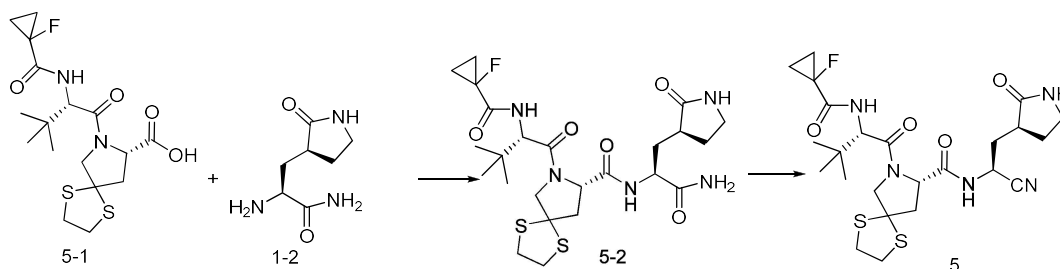
5 According to the same method in example 2, the difference lies in that: acetic anhydride was used instead of trifluoroacetic anhydride to afford compound 3 (a white solid, 45 mg, yield 45%), ESI-MS: 496.3 m/z $[M+H]^+$.

Example 4



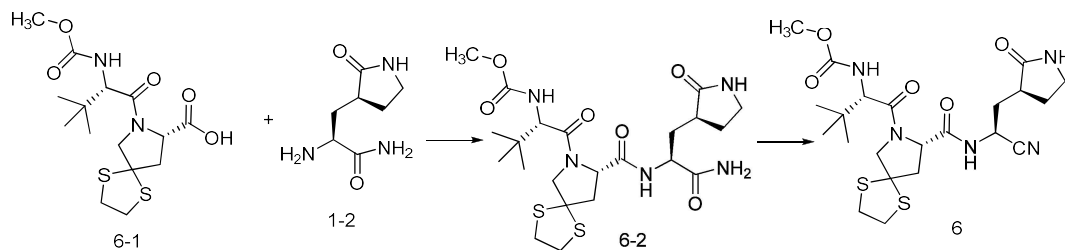
10 According to the same method in example 2, the difference lies in that: cyclopropane formic anhydride was used instead of trifluoroacetic anhydride to afford compound 4 (a white solid, 32 mg, yield 31%), ESI-MS: 522.3 m/z $[M+H]^+$.

Example 5



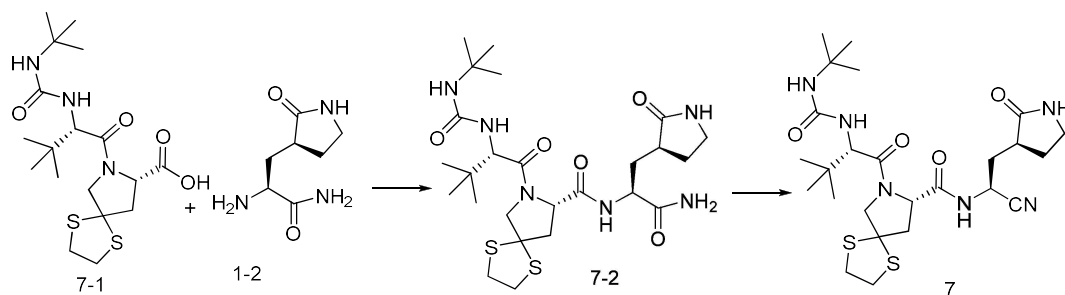
15 According to the same method in example 1, the difference lies in that: 5-1 was used instead of 1-1 to afford compound 5 (a white solid, 22 mg, yield 20%), ^1H NMR (400 MHz, DMSO- d_6) δ 9.03 (d, $J = 8.4$ Hz, 1H), 7.67 (s, 1H), 7.41 (dd, $J = 9.1, 2.6$ Hz, 1H), 4.96 (ddd, $J = 10.6, 8.4, 5.4$ Hz, 1H), 4.58-4.50 (m, 1H), 4.34 (dd, $J = 9.8, 7.2$ Hz, 1H), 4.26-4.21 (m, 1H), 3.89 (d, $J = 11.0$ Hz, 1H), 3.45-3.33 (m, 4H), 3.22-3.03 (m, 2H), 2.64-2.57 (m, 1H), 2.45 (ddd, $J = 10.1, 8.5, 4.5$ Hz, 1H), 2.31 (dd, $J = 12.9, 9.8$ Hz, 1H), 2.20-2.10 (m, 2H), 1.84-1.67 (m, 2H), 1.39-1.31 (m, 2H), 1.24 (t, $J = 9.5$ Hz, 2H), 0.97 (s, 9H). ESI-MS: 540.2 m/z $[M+H]^+$.

Example 6



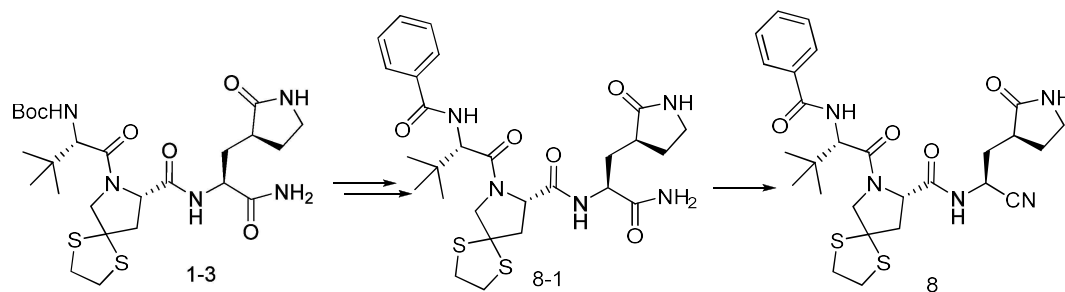
According to the same method in example 1, the difference lies in that: 6-1
was used instead of 1-1 to afford compound 6 (a white solid, 38 mg, yield 37%),
5 ESI-MS: 512.2 m/z [M+H]⁺.

Example 7



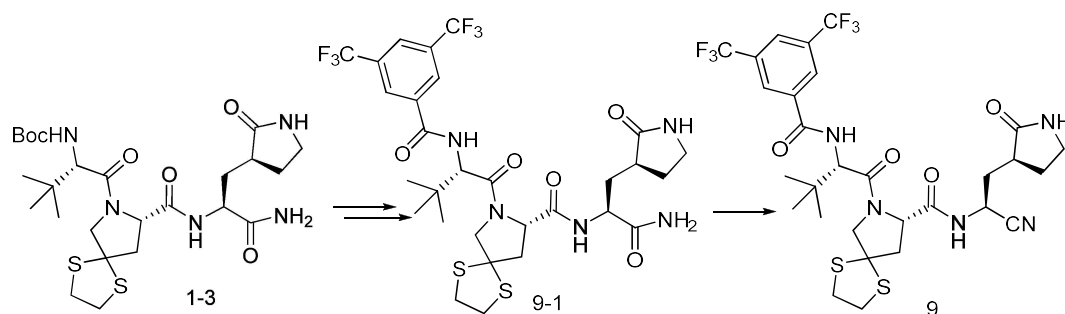
According to the same method in example 1, the difference lies in that: 7-1 was used instead of 1-1 to afford compound 7 (a white solid, 49 mg, yield 44%),
ESI-MS: 553.3 m/z [M+H]⁺.

Example 8



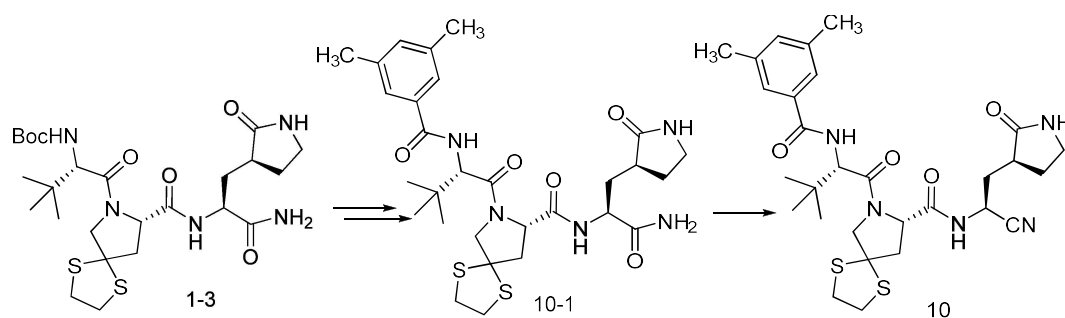
According to the same method in example 2, the difference lies in that: benzoic anhydride was used instead of trifluoroacetic anhydride to afford
15 compound 8 (a white solid, 44 mg, yield 39%), ESI-MS: 558.3 m/z [M+H]⁺.

Example 9



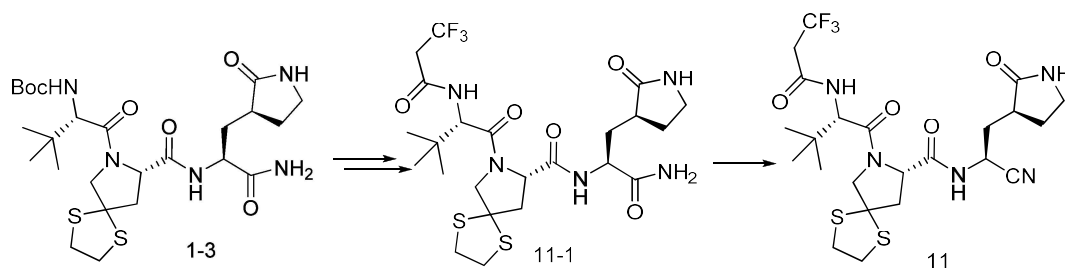
According to the same method in example 2, the difference lies in that: 3,5-bis(trifluoromethyl)benzoyl chloride was used instead of trifluoroacetic anhydride to afford compound 9 (a white solid, 39 mg, yield 28%), ESI-MS: 694.2 m/z [M+H]⁺.

Example 10



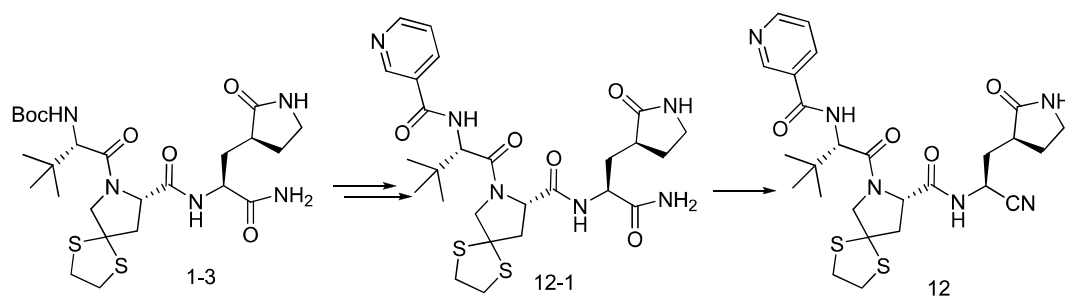
According to the same method in example 2, the difference lies in that: 3,5-dimethylbenzoyl chloride was used instead of trifluoroacetic anhydride to afford compound 10 (a white solid, 31 mg, yield 26%), ESI-MS: 586.2 m/z [M+H]⁺.

Example 11



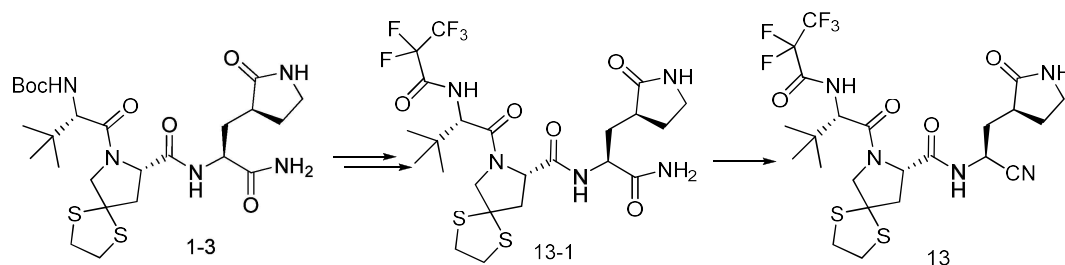
According to the same method in example 2, the difference lies in that: 3,3,3-trifluoropropionic anhydride was used instead of trifluoroacetic anhydride to afford compound 11 (a white solid, 28 mg, yield 25%), ESI-MS: 564.2 m/z [M+H]⁺.

Example 12



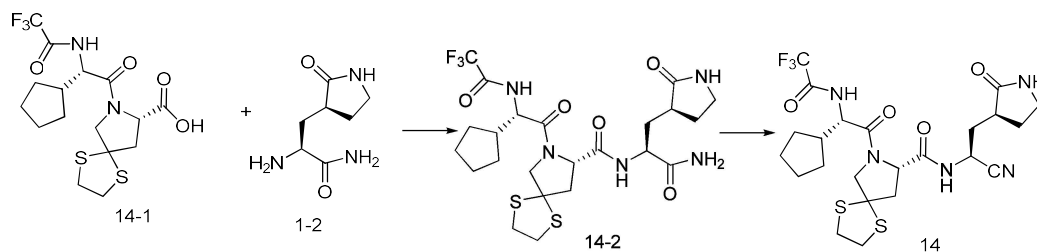
According to the same method in example 2, the difference lies in that: 3-pyridineformyl chloride was used instead of trifluoroacetic anhydride to afford compound 12 (a white solid, 39 mg, yield 35%), ESI-MS: 559.2 m/z $[M+H]^+$.

5 Example 13



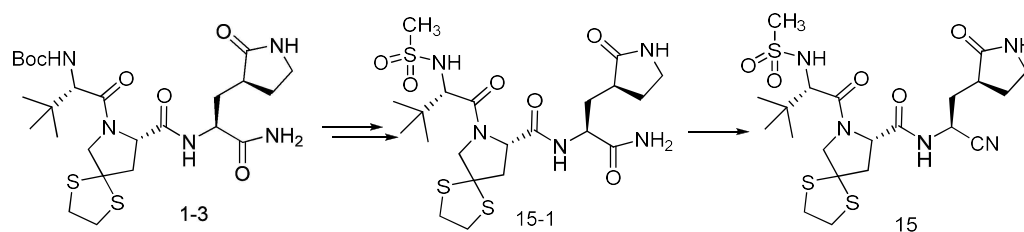
According to the same method in example 2, the difference lies in that: pentafluoropropionyl chloride was used instead of trifluoroacetic anhydride to afford compound 13 (a white solid, 41 mg, yield 34%), ESI-MS: 600.2 m/z $[M+H]^+$.

Example 14



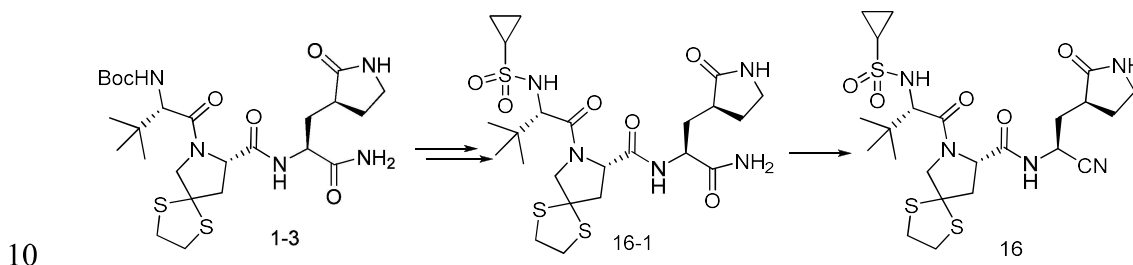
According to the same method in example 1, the difference lies in that: 14-1 was used instead of 1-1 to afford compound 14 (a white solid, 32 mg, yield 29%), ESI-MS: 550.2 m/z $[M+H]^+$.

Example 15



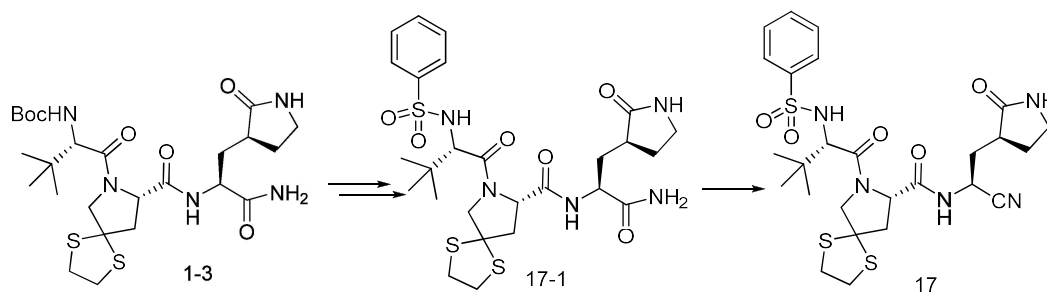
According to the same method in example 2, the difference lies in that: methanesulfonic anhydride was used instead of trifluoroacetic anhydride to afford compound 15 (a white solid, 38 mg, yield 36%), ¹H NMR (400 MHz, Methanol-*d*₄) δ 5.06 (dd, *J* = 11.5, 4.5 Hz, 1H), 4.46 (dd, *J* = 10.3, 7.1 Hz, 1H), 4.27 (dd, *J* = 11.0, 1.6 Hz, 1H), 4.02-3.89 (m, 2H), 3.45 (qt, *J* = 8.6, 4.7 Hz, 4H), 3.30-3.23 (m, 1H), 2.92 (s, 3H), 2.78 (tdd, *J* = 10.3, 8.5, 4.0 Hz, 1H), 2.68 (ddd, *J* = 13.0, 7.2, 1.6 Hz, 1H), 2.51 (dd, *J* = 13.0, 10.3 Hz, 1H), 2.45-2.27 (m, 2H), 1.94-1.77 (m, 2H), 1.05 (s, 9H). ESI-MS: 532.2 *m/z* [M+H]⁺.

Example 16



According to the same method in example 2, the difference lies in that: cyclopropanesulfonyl chloride was used instead of trifluoroacetic anhydride to afford compound 16 (a white solid, 29 mg, yield 26%), ¹H NMR (400 MHz, Methanol-*d*₄) δ 5.06 (dd, *J* = 11.5, 4.5 Hz, 1H), 4.44 (dd, *J* = 10.3, 7.0 Hz, 1H), 4.28 (dd, *J* = 11.0, 1.6 Hz, 1H), 4.00-3.94 (m, 2H), 3.52-3.38 (m, 4H), 3.31-3.21 (m, 1H), 2.79 (tdd, *J* = 10.4, 8.5, 4.1 Hz, 1H), 2.72-2.65 (m, 1H), 2.57 (ddd, *J* = 7.9, 6.2, 3.9 Hz, 1H), 2.51 (dd, *J* = 13.0, 10.4 Hz, 1H), 2.43-2.29 (m, 2H), 1.92-1.74 (m, 2H), 1.34-1.29 (m, 1H), 1.14 (qt, *J* = 6.4, 3.8 Hz, 1H), 1.06 (s, 9H), 1.01 (ddt, *J* = 7.9, 5.8, 2.7 Hz, 2H), 0.97-0.87 (m, 1H). ESI-MS: 558.2 *m/z* [M+H]⁺.

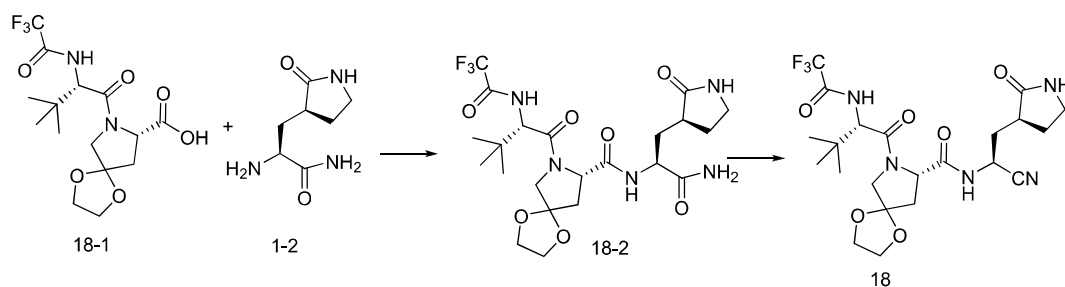
Example 17



According to the same method in example 2, the difference lies in that: benzenesulfonyl chloride was used instead of trifluoroacetic anhydride to afford compound 17 (a white solid, 35 mg, yield 29%), ESI-MS: 594.2 *m/z* [M+H]⁺.

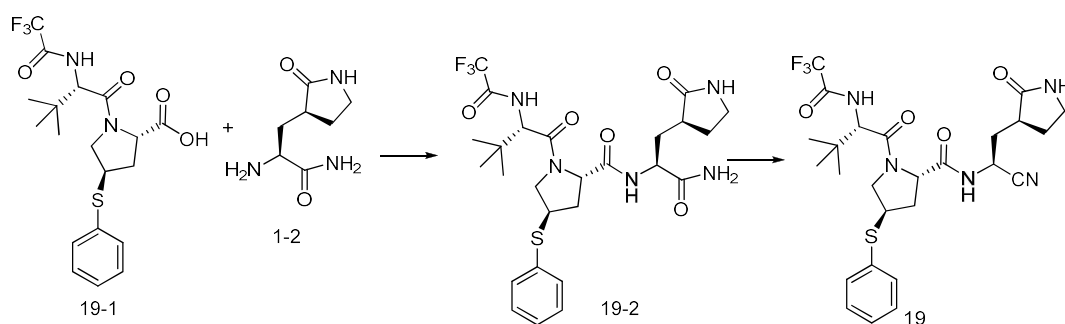
Example 18

25



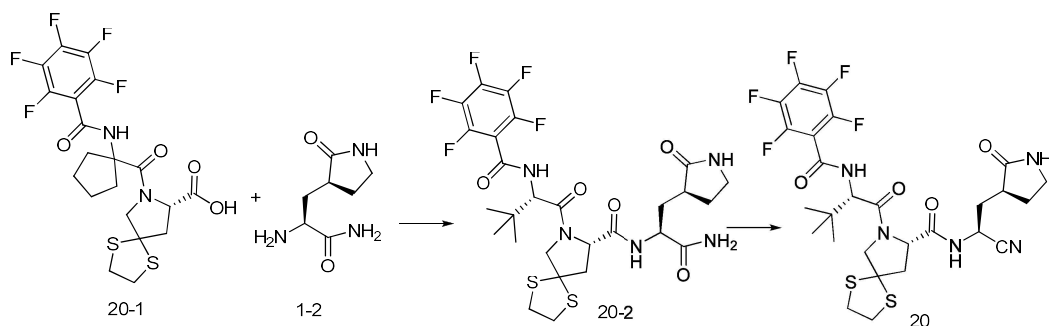
According to the same method in example 1, the difference lies in that: 18-1 was used instead of 1-1 to afford compound 18 (a white solid, 44 mg, yield 42%), ESI-MS: 518.2 m/z [M+H]⁺.

5 Example 19



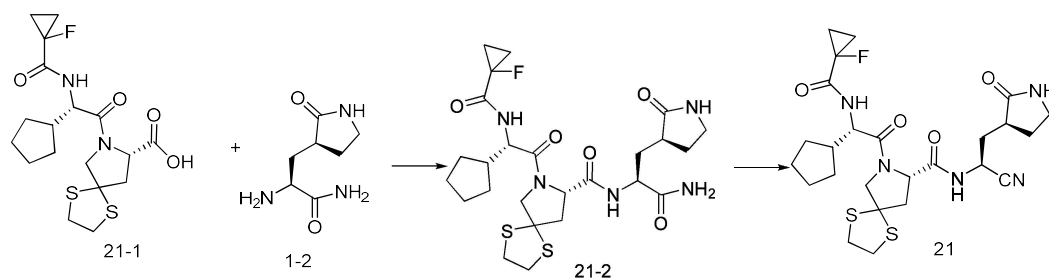
According to the same method in example 1, the difference lies in that: 19-1 was used instead of 1-1 to afford compound 19 (a white solid, 41 mg, yield 36%), ESI-MS: 568.2 m/z [M+H]⁺.

10 Example 20



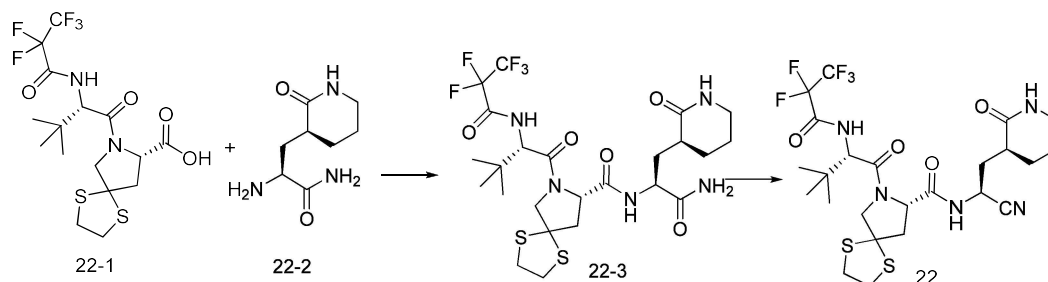
According to the same method in example 1, the difference lies in that: 20-1 was used instead of 1-1 to afford compound 20 (a white solid, 45 mg, yield 35%), ESI-MS: 648.2 m/z [M+H]⁺.

15 Example 21



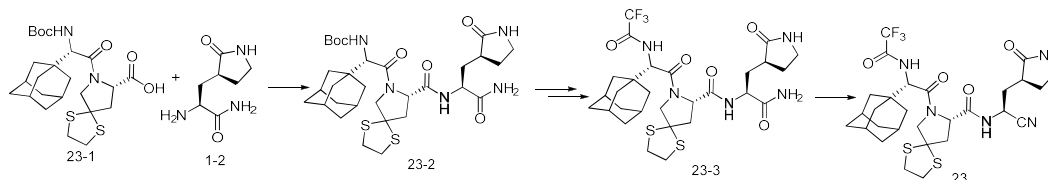
According to the same method in example 1, the difference lies in that: 21-1 was used instead of 1-1 to afford compound 21 (a white solid, 34 mg, yield 31%), ESI-MS: 640.2 m/z $[M+H]^+$.

5 Example 22



According to the same method in example 1, the difference lies in that: 22-1 was used instead of 1-1 and 22-1 was used instead of 1-2 to afford compound 22 (a white solid, 22 mg, yield 18%), ESI-MS: 614.2 m/z $[M+H]^+$.

10 Example 23



Compound 23-1 (497 mg, 1 mmol) was placed in a two-necked flask, and 5 mL of dichloromethane was added under nitrogen protection, and then 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate (400 mg, 1.1 mmol) was added and stirred at room temperature for 1 h. Compound 1-2 (or a corresponding salt thereof, 1 mmol) was dissolved in 1 mL of dichloromethane and added to the above system, then N,N-diisopropylethylamine (0.5 mL, 1 mmol) was added under an ice-water bath, and the ice-water bath was removed. The system was stirred overnight at room temperature. Workup was performed, 50 mL of dichloromethane was added, washed with 1M aqueous hydrochloric acid three times, and washed with saturated aqueous sodium bicarbonate solution three times. The organic phase was washed with saturated saline solution, dried over anhydrous

sodium sulphate, and spin-dried by filtration to afford compound 23-2 (a white solid, 421 mg, yield 65%). ESI-MS: m/z 650.3[M+H]⁺.

Compound 23-2 (325 mg, 0.5 mmol) was dissolved in 1.5 mL of 4M hydrogen chloride/1,4-dioxane solution, or dissolved in 1 mL of dichloromethane, then 1 mL of trifluoroacetic acid was added dropwise and stirred at ambient temperature. Thin layer chromatography showed that the raw materials was substantially completely reacted, and then the solvent was fully spin-dried. The obtained crude product was dissolved in 2 mL of dichloromethane under nitrogen protection, and triethylamine (1.5 mmol) was added, then the system was placed in an ice-water bath, and trifluoroacetic anhydride (0.6 mmol) was added dropwise. Thin layer chromatography showed that the raw materials was substantially completely reacted, and then 25 mL of dichloromethane was added and washed three times with 1M aqueous hydrochloric acid solution and three times with saturated aqueous sodium bicarbonate solution. The organic phase was washed with saturated saline solution, dried over anhydrous sodium sulphate, and passed through a column to afford compound 23-3 (a white solid, 167 mg, yield 52%). ESI-MS: m/z 646.3[M+H]⁺.

Compound 23-3 (130 mg, 0.2 mmol) and Burgess reagent (1.5 eq) were added to a two-necked flask, nitrogen was charged and discharged for three time, then dichloromethane dried over molecular sieves was added and stirred at room temperature overnight. Thin layer chromatography showed that the raw materials was substantially completely reacted. Workup was performed, column chromatography was performed to afford compound 23 (a white solid, 37 mg, yield 29%), ESI-MS: 628.2 m/z [M+H]⁺.

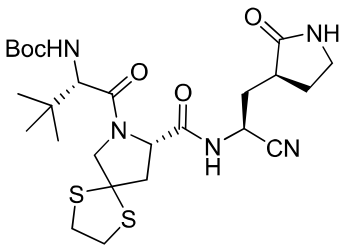
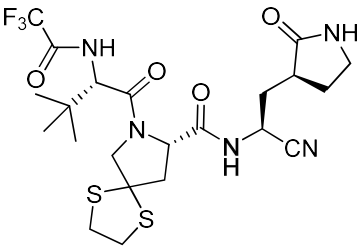
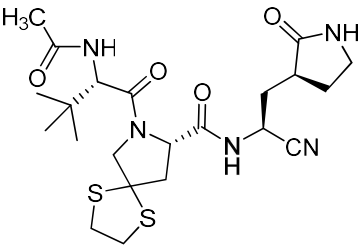
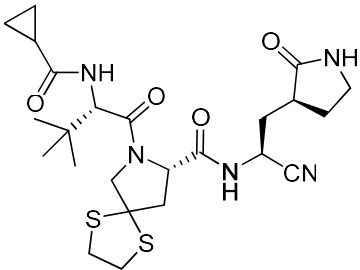
Example 24: Test for inhibitory activity of compounds on SARS-CoV-2 3CL^{pro}

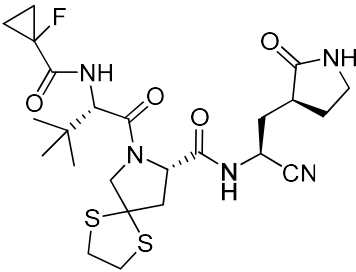
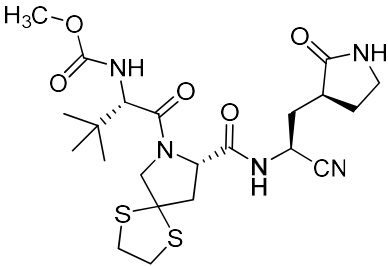
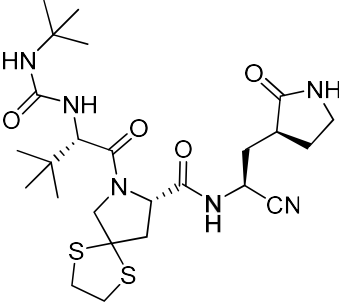
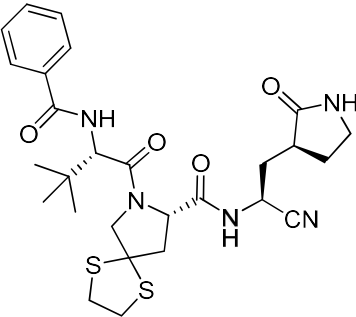
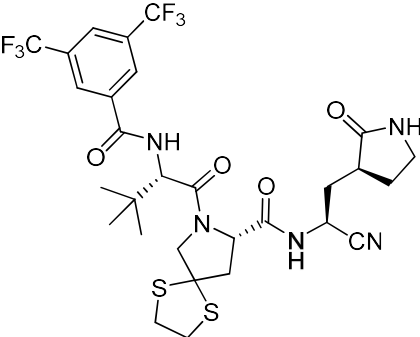
The inhibitory activity of the tested compounds on the enzymatic activity of SARS-CoV-2 3CL^{pro} was evaluated by fluorescence resonance energy transfer method. The volume of the entire enzymatic reaction system is 120 μ L, the final concentration of the protease is 30 nM, and the final concentration of the substrate is 20 μ M. The buffer of the reaction system includes 50 mM Tris pH 7.3, 1 mM EDTA. The SARS-CoV-2 3CL^{pro} protease and different concentrations of the compounds were added to a 96-well plate, incubated at 30°C for 10 min, and then the substrate was added and the plate was quickly put into a microplate reader for

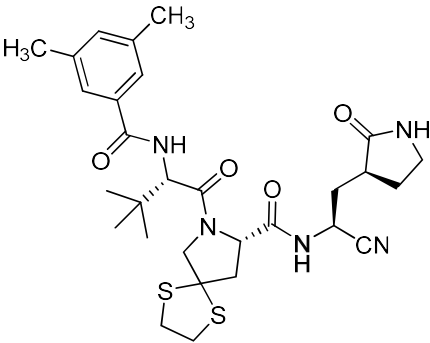
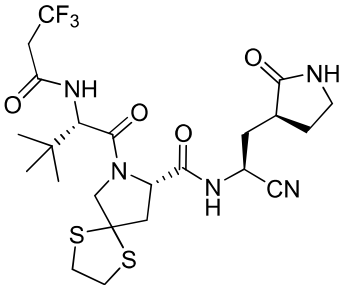
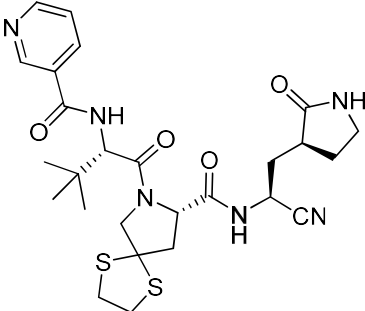
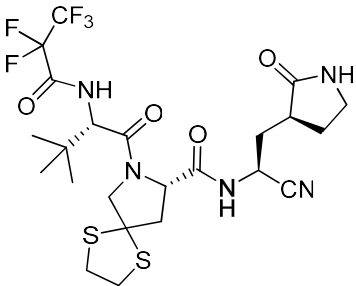
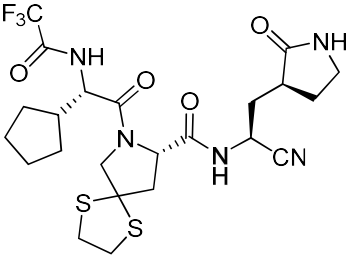
reading. The excitation and emission light were 320 nM and 405 nM, respectively. The test time was 3.5 min, and the fluorescence value was read every 35 s. In the final result, the readings of the first 2 min were used for fitting to obtain the reaction rate, and compared with the control group (DMSO), the inhibition rate was calculated. The IC₅₀ value and the inhibition rate curve were obtained by fitting using the software GraphPad Prism 8.

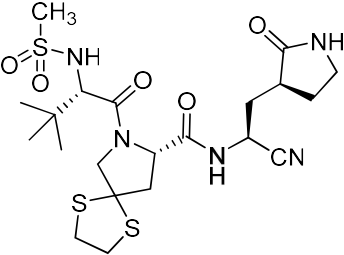
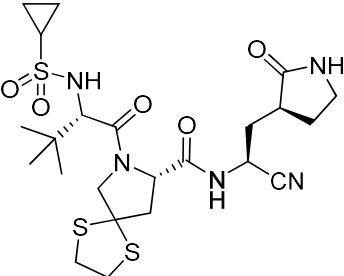
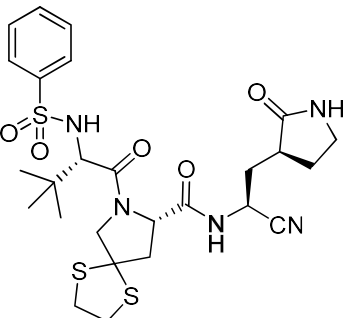
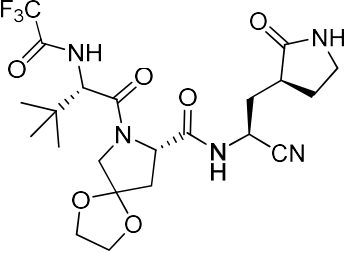
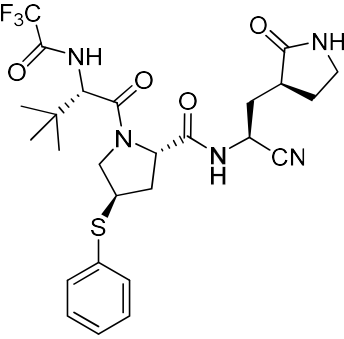
The experimental results are shown in Table 1. The results show that the compounds of the present invention have potent inhibitory effects on SARS-CoV-2 3CL^{pro}. The range of IC₅₀ value is that: A means < 0.1 μM, B means 0.1-1 μM, C means 1-10 μM.

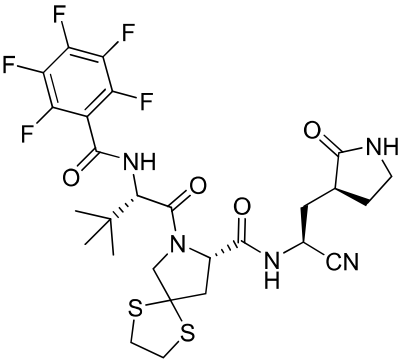
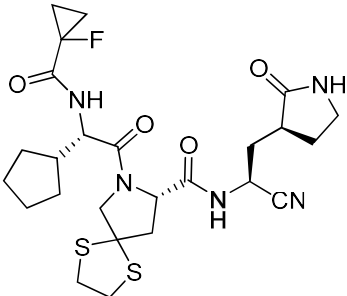
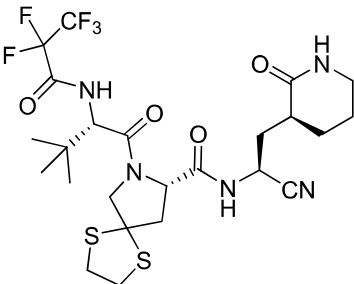
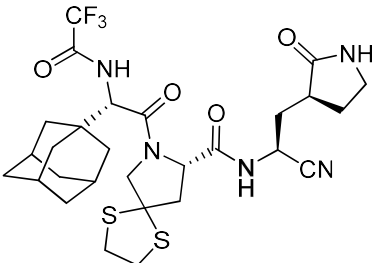
Table 1: Inhibitory effects of compounds **1-23** on SARS-CoV-2 3CL^{pro}

Compound number	Compound structural formula	IC ₅₀ (μM)
1		A
2		A
3		A
4		A

5		A
6		B
7		A
8		C
9		C

10		C
11		B
12		C
13		A
14		A

15		A
16		A
17		C
18		A
19		C

20		B
21		A
22		A
23		A

Example 24-2: Test for inhibitory activity of compound 2 on mutant 3CL protease of SARS-CoV-2 Omicron strain

Experimental principle: The inhibitory effects of the compounds of the invention on the activity of the mutant 3CL protease (P132H) of the Omicron strain were studied by using a method for generate fluorescence resonance energy transfer (FRET) by the reaction of enzyme and substrate.

The experimental materials are shown in the table below:

Reagent material	Brand	Catalog
------------------	-------	---------

		number:
3CL protease-P132H mutant	Shanghai Profession and Creation Co. , Ltd.	/
Dabcyl-KTSAVLQSGFRKME-Edans (Coronavirus main protease fluorescent substrate)	Beyotime	P9733
DTT (Dithiothreitol)	Invitrogen	P2325
BSA (Bovine Serum Albumin)	Sigma	V900933
EDTA (ethylenediaminetetraacetic acid)	Invitrogen	AM9260G
Tris-HCl (trishydroxymethylaminomethane)	Sangon Biotech	B548127

Experimental instrument and equipment:

Equipment name	Brand	Model
Echo Nanoliter-Scale Sonic Shifting System	Labcyte	Echo 650
Flexstation 3 microplate reader	MolecMar Devices	FLEX3
Centrifuge	Eppendorf	5810

Experimental steps:

- 5 Prepare a reaction buffer containing 20 mM Tris-HCl, 1 mM EDTA, 0.01% BSA, 1 mM DTT, and 100 mM NaCl. The compounds to be tested were diluted to different concentrations in dimethyl sulfoxide (DMSO) with the Echo pipetting system and transferred to a 384-well plate. The mutant 3CL protease was diluted with the reaction buffer, and added to the 384-well plate at an amount of 10
- 10 $\mu\text{L}/\text{well}$, centrifuged at 1000 rpm for 1 min, and then incubated at room temperature for 30 minutes. Then the substrate was added at 10 $\mu\text{L}/\text{well}$, and centrifuged at 1000 rpm for 30 s to start the enzyme reaction. In the reaction system, the final concentration of the enzyme was 50 nM, the final concentration of the substrate was 20 μM , and the concentration of the compound ranged from
- 15 10000 nM to 0.51 nM. Then the Kinetic Reduction Vmax mode on the Flexstation 3 microplate reader was selected, the fluorescence value at 490 nm wavelength was

continuously read every 75 seconds, a total of 35 times, to obtain the reaction rate value (V), and the inhibition rate was calculated, and XLfit software was used to perform four-parameter fitting to obtain the half inhibitory concentration (IC₅₀). The inhibition rate calculation method is as follows:

- 5 Inhibition rate = $(V_{\max} - V_{\text{compound}}) / (V_{\max} - V_{\min}) * 100\%$
 wherein $V_{\max}$ is the reaction rate value for a well containing only the enzyme and the substrate, $V_{\min}$ is the reaction rate value for a well containing only the substrate, and V_{compound} is the reaction rate value for a well containing the compound to be tested, the enzyme and the substrate.

- 10 Experimental results: For the 3CL protease with P132H mutation in the SARS-CoV-2 Omicron strain, compound 2 still maintained a significant inhibitory activity (IC₅₀ of three independent tests was $0.022 \pm 0.00090 \mu\text{M}$).

Table 2: Inhibitory effect of compound 2 on 3CL protease activity of SARS-CoV-2 Omicron strain

3CL protease	Compound 2 IC ₅₀ (μM) (n = 3*)
SARS-CoV-2 (Omicron strain)	0.022 ± 0.00090

- 15 * refers to three independent replicate experiments.

Example 24-3: Test for inhibitory activity of compound 2 on 3CL protease of coronavirus from different sources

Experiment purpose: to study the inhibitory effect of compound 2 on the 3CL protease activity derived from six other coronaviruses capable of infecting humans.

- 20 These six viruses are: SARS-CoV, MERS-CoV, OC43-CoV, H229E-CoV, NL63-CoV, and HKU1-CoV.

Experimental materials:

- 3CL protease: A recombinant full-length coronavirus 3CL protease was made in house according to the coronavirus genome sequence, the genome GenBank
 25 numbers of SARS-CoV, MERS-CoV, H229E-CoV, HKU1-CoV, NL63-CoV and OC43-CoV used therein are AAP13442.1, MT387202.1, AF304460.1, AY597011.2, AY567487.2 and AY903459.1, respectively, and the DNA sequences required for the expression of the 3CL protease proteins of the six coronaviruses were purchased from Nanjing GenScript Biotechnology Co., Ltd.

- 30 The 3CL protease substrate was purchased from Nanjing GenScript Biotechnology Co., Ltd.

The chymotrypsin substrate was purchased from GL Biochemical Co., Ltd.

Other reagents are listed in the table below:

Reagent material	Brand	Catalog number:
Chymotrypsin derived from bovine pancreas	Sigma	C4129
Tris	Sigma	BCBX3837
EDTA	Sigma	0001434776

Experimental steps:

A reaction buffer (containing 50 mM Tris and 1 mM EDTA) was formulated.

5 The compound to be tested was dissolved in DMSO to prepare a 100 mM stock solution, and further 2-fold serially diluted with the reaction buffer, with a total of 11 concentrations. The 3CL protease and different concentrations of the compounds were added to a 96-well plate, incubated at room temperature for 10 minutes, and then the substrate was added and the plate was quickly put into a microplate reader for reading. The volume of the entire enzymatic reaction system was 120 μ L, and the final concentrations of SARS-CoV, MERS-CoV, H229E-CoV, HKU1-CoV, NL63-CoV and OC43-CoV proteases were 30 nM, 80 nM, 30 nM, 20 nM, 30 nM and 10 nM respectively, and the final concentration of the substrate was 10 μ M. The excitation and emission wavelengths were 340 nm and 490 nm, respectively, when reading. The test time was 10 minutes, and the fluorescence value was read every 1 minute. In the final result, the readings of the first 5 min were used for fitting to obtain the reaction rate, the inhibition rate was calculated and the calculation formula is: Inhibition Rate = 1 - (Reaction Rate of Test Group/Reaction Rate of Control Group).

20 Experimental results: The results are shown in Table 3. Compound 2 exhibited a good inhibitory effect on 3CL proteases derived from other six coronaviruses, suggesting that compound 2 may have a broad-spectrum anti-coronavirus activity.

Table 3: Inhibitory effect of compound 2 on 3CL proteases from other coronaviruses

3CL protease source	Compound 2 IC ₅₀ (μ M)
HKU1-CoV	0.0049
OC43-CoV	0.010

SARS-CoV-1	0.024
MERS-CoV	0.060
H229E-CoV	0.13
NL63-CoV	0.85

Example 25-1: Inhibitory effect of compound 2 on SARS-CoV-2 WIV04 and B.1.351 strains at cell level

Vero E6 cells were used in the test, and Vero E6 cells (50,000 cells/well) were added to a 48-well plate, a medium containing gradient concentrations of the compounds was added at 100 μ L/well, and SARS-CoV-2 was added one hour later, the multiplicity of infection (MOI) was 0.01. After co-incubating for 1 hour, the supernatant was aspirated, the residue was washed and the medium containing gradient concentrations of the compounds was added again at 200 μ L/well, and incubated at 37°C for 24 hours. After 24 hours, the cell supernatant was collected, the viral RNA in the supernatant was extracted, and the virus copy number in the supernatant was determined by real-time fluorescent quantitative PCR. The compound inhibition rate was calculated according to the virus copy number, and the EC₅₀ of the compound was calculated by prism 6.0.

The test results showed that the compound 2 inhibited the SARS-CoV-2 WIV04 strain with a half effective concentration EC₅₀ of 0.57 +/- 0.04 μ M, and inhibited the SARS-CoV-2 B.1.351 strain with EC₅₀ of 0.73 +/- 0.06 μ M, and the EC₅₀ curves are shown in FIGs 1 and 2.

Example 25-2: Inhibitory effect of compound 2 on SARS-CoV-2 Vero E6 original strain (WIV04), Delta strain (B.1.617.2), and Omicron strain (B.1.1.529) at cell level

Experiment purpose: In this experiment, the method of determining the virus copy number in the culture supernatant by real-time fluorescent quantitative PCR in Vero E6 cells was used to study the inhibitory effect of compound 2 on SARS-CoV-2 original strain (WIV04 strain), Delta strain (B.1.617.2), and Omicron strain (B.1.1.529) in cell replication. Since Vero E6 cells highly expressed the efflux transporter P-gp, 0.5 μ M of a P-gp inhibitor CP-100356 was added and incubated with the compound.

Experimental materials:

Vero E6 was purchased from ATCC (Catalog number: CRL-1586), SARS-CoV-2 original strain (SARS-CoV-2-WIV04 strain), Delta strain (B.1.617.2), Omicron strain ((B.1.1.529) were obtained from the Microbial Bacteria (Virus) Species Collection Centre of Wuhan Institute of Virology, Chinese Academy of Sciences.

Other reagents are listed in the table below:

Reagent name	Brand	Catalog number:
TaKaRa MiniBEST Viral RNA/DNA Extraction Kit Ver.5.0	Takara	9766
TaKaRa PrimeScript™ RT reagent Kit with gDNA Eraser	Takara	RR047A
TaKaRa SYBR® Premix Ex Taq™ II	Takara	RR820A
Fetal Bovine Serum	Gibco	R2768
DMEM medium	Gibco	C11995500BT
CCK8	Beyotime	C0039
Chloroquine Phosphate	SIGMA	C6628-50G
Trypsin	BIOSHARP	BL512A

Experimental instruments:

Biosafety cabinet (AC2-3S1, ESCO, Singapore)

10 Carbon dioxide incubator (Thermo Scientific HERAcell 150i, Thermo Scientific, USA)

Pure water equipment (ShenYuan SYS ultrapure water machine, Chengdu)

StepOne Plus Real-time PCR system (4376600, ABI, USA)

TC20™ Automated Cell Counter (1450102, BIO-RAD, USA)

15 T100™ Thermal Cycler (1861096, BIO-RAD, USA)

Centrifuge (Micro21/21R Thermo, Thermo Scientific, USA)

Experimental steps:

The Vero E6 cells were digested with trypsin and placed in a culture medium (90% DMEM, 10% fetal calf serum), inoculated into a 48-well plate at 50,000 cells per well, and cultured overnight. The compound to be tested was dissolved in DMSO to prepare a 40 mM stock solution, and further serially diluted with a medium containing 0.5 μM of Pgp inhibitors to obtain the required concentration.

The final concentration of the compound to be tested in the experiment ranged from 1 μ M to 0.004 μ M. The cell supernatant was removed, the diluted compound (containing Pgp inhibitor 0.5 μ M) was added to each well, incubated for 1 h. Different strains of SARS-CoV-2 were added in a biosafety level 3 (BSL-3) laboratory, and the multiplicity of infection (MOI) was 0.01 or 0.001. After incubation for 1 h, the supernatant was removed, the residue was washed with PBS, the diluted compound (containing Pgp inhibitor 0.5 μ M) was added at 200 μ L/well, and the supernatant was collected 24 or 72 hours after infection. The virus RNA in the supernatant was extracted and real-time fluorescence quantitative PCR was used to determine the virus copy number in the supernatant, the compound inhibition rate was calculated according to the virus copy number, and GraphPad Prism 8 was used to calculate the IC₅₀ of the compound.

In the cytotoxicity test, Vero E6 cells were digested and placed in a culture medium (90% DMEM, 10% fetal bovine serum), inoculated into a 96-well plate at 20000 cells per well, and cultured overnight. The compound to be tested was dissolved in DMSO to prepare a 40 mM stock solution, and further serially diluted with a culture medium or a culture medium containing 0.5 μ M Pgp inhibitor to obtain the concentration required for the test. The final concentration of the compound to be tested in the experiment ranged from 500 μ M to 1.95 μ M. The cell supernatant in the 96-well plate was removed, the medium for the compound to be tested (as a monotherapy or containing 0.5 μ M of a Pgp inhibitor) was added at 100 μ L/well and incubated for 24 h; the CCK8 detection kit was used to determine cell viability, and the inhibition rate and median cytotoxic concentration (CC₅₀) were calculated.

Test results: As shown in Table 4, when combined with the P-gp inhibitor CP-100356, compound 2 can dose-dependently inhibit the replication of delta strain in Vero E6 cells with an IC₅₀ of 0.040 μ M. In the original strain, compound 2 combined with the P-gp inhibitor also exerted a strong inhibitory effect, with an IC₅₀ of 0.027 μ M. In addition, compound 2 combined with the P-gp inhibitor can significantly inhibit the replication of Omicron strain in Vero E6 cells, with an IC₅₀ of 0.12 μ M. Compound 2 alone and in combination with the P-gp inhibitor had no obvious cytotoxicity on the proliferation of Vero E6 cells, CC₅₀ > 500 μ M.

Table 4: Inhibitory effect of compound 2 combined with P-gp inhibitor on SARS-CoV-2 replication in Vero E6 cells

Experiment	Compound 2 + 0.5 μ M CP-100356	
	IC ₅₀ (μ M)	Select Index SI SI = (CC ₅₀ /IC ₅₀)
Vero E6 original strain (WIV04)	0.027 (mean, n = 2)	> 18519
Vero E6 delta strain (B.1.617.2)	0.040 (mean, n = 2)	> 12500
Vero E6 Omicron strain (B.1.1.529)	0.12	> 4167
Vero E6 monotherapy CC ₅₀	> 500 μ M	
Vero E6 combined with P-gp inhibitor CC ₅₀	> 500 μ M	

Example 26: *In vivo* antiviral effect of compound 2 on SARS-CoV-2 delta strain in hACE2-K18 transgenic mice

Experiment purpose: This study evaluated the antiviral activity of compound 2 on the SARS-CoV-2 delta strain in K18 transgenic mice stably expressing human angiotensin-converting enzyme 2 (ACE2) (K18-hACE2).

Experimental materials:

7-8 week-old K18-hACE2 transgenic mice were purchased from Jiangsu Jicui Gempharmatech Co., Ltd. The SARS-CoV-2 delta strain virus was obtained from the Microbial Bacteria (Virus) Species Collection Centre of Wuhan Institute of Virology, Chinese Academy of Sciences.

Ritonavir was purchased from Shanghai Desano Inc.

Vero E6 cells were purchased from ATCC (Catalog number: CRL-1586).

Other reagents are listed in the table below:

Reagent name	Brand	Catalog number:
Qiagen 74106 RNeasy Mini Kit	Qiagen	74106
TaKaRa PrimeScript™ RT reagent Kit with gDNA Eraser	Takara	RR047A
TaKaRa SYBR® Premix Ex Taq™ II	Takara	RR820A
Fetal Bovine Serum	Gibco	10099-141C

DMEM medium	Gibco	C11995500BT
Tissue fixative	BOSTER	AR1068
Aquacide II (sodium methylcellulose)	Millipore	17851
DMEM medium (powder, high sugar)	Gibco	12100046
Purple crystal	Sinopharm Group	71012314

Experimental instruments:

Biosafety cabinet (AC2-3S1, ESCO, Singapore)

Carbon dioxide incubator (Thermo Scientific HERAcell 150i, Thermo Scientific, USA)

Pure water equipment (ShenYuan SYS ultrapure water machine, Chengdu)

StepOne Plus Real-time PCR system (4376600, ABI, USA)

TC20™ Automated Cell Counter (1450102, BIO-RAD, USA)

T100™ Thermal Cycler (1861096, BIO-RAD, USA)

Centrifuge (Micro21/21R Thermo, Thermo Scientific, USA)

Tissue grinder (JXFSTPRP-CL, Shanghai Jingxin Industrial Development Co., Ltd.)

Experimental steps: K18-hACE2 transgenic mice were infected with the delta strain of SARS-CoV-2 by intranasal drops on day 0. 2 hours after infection, the vehicle and compound 2 at 50 mg/kg or 200 mg/kg (combined with cytochrome P450 inhibitor ritonavir at 50 mg/kg) were administered by gavage, BID for 2 days (wherein 1 dose was administrated on day 0, 2 doses on day 1, 1 dose on day 2) or 4 days (wherein 1 dose was administrated on day 0, and 2 doses on the day 1, day 2, and day 3, respectively). The body weight changes of the mice were recorded, and lung and brain tissues were collected at the end point. Among them, the left lung was fixed in formaldehyde, and then used for histopathological examination after embedding, sectioning and H&E staining. The right lung and brain tissue were each divided into two parts. One part was ground and the homogenate was taken to extract RNA which was then reverse-transcribed, and the virus copy number was determined by real-time fluorescent quantitative PCR. The other part was ground and the homogenate was taken to determine the virus titre by plaque test. The plaque test method is as follows: Vero E6 cells were inoculated in a 24-well plate at 12,000 cells per well and cultured overnight. The stock of tissue

homogenate was 10-fold serially diluted in DMEM medium for later use. The cell supernatant was removed, add the diluted tissue homogenate was added and incubated for 1 h, then the supernatant was removed, the medium containing 1% sodium methylcellulose and 2% FBS was added, and cultured for 4 days. Then the
5 medium was removed, paraformaldehyde was used for fixation, 1% (w/v) crystal violet was used for staining, and the number of plaques in each well was counted.

Test results: As shown in Table 5, 2 days after infection, compared with the model group (the average virus copy number was $9.19 \pm 0.30 \log_{10}$ copies/g), in the case of being combined with ritonavir, compound 2 at 50 mg/kg and 200 mg
10 /kg significantly reduced the viral load in the lung, with an average copy number of $7.66 \pm 0.27 \log_{10}$ copies/g and $6.79 \pm 0.30 \log_{10}$ copies/g, respectively, wherein the virus copy number decreased by 2.4 $\log_{10}$ copies/g at the dose of 200 mg/kg. 4 days after infection, a sustained inhibitory effect of compound 2 on viral copy number was observed. In terms of virus titre, as shown in FIG. 3, a significant
15 inhibitory effect of compound 2 was observed. 2 days after infection, the virus replication was completely inhibited at a dose of 200 mg/kg, and no titre was detected. Compared with the model group at a dose of 50 mg/kg, the virus titre decreased by more than 3 $\log_{10}$ PFU/g, and compound 2 showed a sustained inhibitory effect on the virus titre 4 days after infection. In terms of body weight,
20 as shown in FIG. 4, 4 days after infection, the body weight of the mice in the model group lost about 10%, while the body weight of the compound 2 administration group did not decrease significantly, indicating that compound 2 did not show obvious toxicity under continuous administration. We further determined the viral load in the brains of the mice, and 2 days after infection, no obvious
25 infection occurred in each group. 4 days after infection, compared with the model group, compound 2 could significantly reduce the virus copy number in the brains of the mice at doses of 50 mg/kg and 200 mg/kg, especially at the dose of 200 mg/kg, the virus copy number was comparable to that of the uninfected normal group. We further determined the virus titre in the brain 4 days after infection, and
30 the results are shown in FIG. 5. Compared with the model group, no virus titre was determined at the two doses of compound 2, showing a strong inhibitory effect of compound 2. In addition, the histopathological analysis of the lung showed that compared with the model group, compound 2 at a dose of 200 mg/kg significantly

improved lung damage, including reducing the degree of alveolar atrophy or dilation and the degree of alveolar membrane thickening.

Table 5 Viral loads in the lungs and brains of mice 2 and 4 days after infection (mean $\pm$ SD)

Group	Lung tissue (log10 copies/g)		Brain tissue (log10 copies/mg)	
	Day 2	Day 4	Day 2	Day 4
Model group	9.19 $\pm$ 0.30	9.35 $\pm$ 0.30	1.34 $\pm$ 0.78	7.17 $\pm$ 0.27
Compound 2 - 200 mg/kg + Ritonavir - 50 mg/kg	6.79 $\pm$ 0.30	7.48 $\pm$ 0.63	1.04 $\pm$ 0.24	0.99 $\pm$ 0.42
Compound 2 - 50 mg/kg + Ritonavir - 50 mg/kg	7.66 $\pm$ 0.27	7.88 $\pm$ 0.74	0.77 $\pm$ 0.20	2.88 $\pm$ 1.26
Normal group	5.76 $\pm$ 0.32	6.27 $\pm$ 0.18	1.08 $\pm$ 0.06	1.08 $\pm$ 0.06

5

Example 27: Selectivity of compound 2 for kinases

Experiment purpose: The inhibitory activity of compound 2 on 413 kinases was determined on the KinaseProfiler experimental platform to study the selectivity of compound 2 on kinases.

10

Experimental materials:

Full Human Panel [10 μ M ATP] KinaseProfiler is a test product provided by Eurofins, Catalog number: 50-005KP10, this product contains 413 kinases.

Experimental steps:

15

Each kinase selected was tested with the compound using the Eurofins standard KinaseProfiler analysis method and following the relevant standard operating procedures. Protein kinases were detected by radioactive method, while lipid kinases were detected by HTRF method. The ATP concentration in the experiment was 10 μ M. Detailed information on each kinase is available on the Eurofins website at:

20

<https://www.eurofinsdiscoveryservices.com/catalogmanagement/viewItem/Full-Human-Panel-10-uM-ATP-KinaseProfiler/50-005KP10>.

Experimental results: For the 413 kinases, the inhibition rate of compound 2 was less than 30% at a concentration of 10 μ M, showing no significant inhibitory effect, suggesting that compound 2 had excellent selectivity.

Example 28: Selectivity of compound 2 for safety targets

5 Experiment purpose: The effect of compound 2 on 47 safety-related targets was determined on the Safetyscan experimental platform.

Experimental materials:

Safety47 Panel Dose Response SAFETYscan is a test product provided by Eurofins, Catalog number: 87-1003DR. This product contains 78 tests related to 47
10 safety targets.

Experimental steps:

For the 78 tests related to the 47 safe targets, the experimental methods used include: cAMP assay, calcium flux assay, hormone nuclear receptor assay, kinase binding assay, enzyme activity assay, neurotransmitter transporter assay, ion
15 channel assay, and transporter assay. Specific methods for each experiment are available on the eurofins website at the following URL: <https://www.eurofinsdiscoveryservices.com/catalogmanagement/viewItem/Safety47-Panel-Dose-Response-SAFETYscan-DiscoverX/87-1003DR>.

Experimental results: For 47 safety-related targets, compound 2 had no
20 significant inhibitory or activating effect at a concentration of 100 μ M (EC_{50} were all greater than 100 μ M), suggesting that compound 2 had excellent selectivity.

Example 29: Human plasma protein binding assay of compound 2

Experimental materials

Human plasma was purchased from BioIVT, anticoagulated with EDTA K2,
25 and stored at -80°C. 96-well equilibrium dialysis plates were purchased from HTDialysis LLC. Equilibrium dialysis membranes were purchased from Gales Ferry.

Experimental procedure

An alkaline solution with 14.2 g/L of disodium hydrogen phosphate and 8.77
30 g/L of sodium chloride was formulated with ultrapure water, and the alkaline solution can be stored at 4°C for 7 days. An acidic solution with 12.0 g/L of sodium dihydrogen phosphate and 8.77 g/L of sodium chloride was formulated with ultrapure water, and the acidic solution can be stored at 4°C for 7 days. The alkaline solution was titrated with an acidic solution to a pH of 7.4, and the buffer

can be stored at 4°C for 7 days. The pH value of the buffer was measured on the day of the experiment, and the pH value was adjusted if it exceeded the range of 7.4 ± 0.1 .

5 The dialysis membrane was soaked in ultrapure water for 60 minutes to separate the membrane into two pieces, then soaked in 20% ethanol for 20 minutes, and finally soaked in the buffer used for dialysis for 20 minutes.

The frozen plasma was thawed rapidly at room temperature.

The plasma was centrifuged at 3,220 g for 10 min at 4°C to remove clots, and the supernatant was collected into a new centrifuge tube. The pH of the plasma was
10 measured and recorded.

A 10 mM stock solution of the analyte was formulated in DMSO. 2 µL of stock solution (10 mM) was diluted with 98 µL of DMSO to obtain a working solution (200 µM). 3 µL of the working solution was taken and 597 µL of human plasma was added to obtain a final concentration of 1 µM (0.5% DMSO). The
15 mixture was fully vortexed.

120 µL of plasma sample containing the drug was added to one side of the dialysis membrane, and an equal volume of dialysate (phosphate buffer) was added to the other side thereof. The experiment was performed in parallel duplicate. The dialysis plate was sealed, put into the incubation device, and incubated at 37°C, 5%
20 CO₂ and about 100 rpm for 6 hours. After the completion of the incubation, the sealing film was removed and 50 µL was pipetted from the buffer and plasma sides in each well, respectively, into separate wells of a new plate.

50 µL of blank plasma was added to the phosphate buffer sample, and an equal volume of blank phosphate buffer was added to the plasma sample. 300 µL
25 room temperature quencher (containing internal standard acetonitrile (IS, 500 nM labetalol, 100 nM alprazolam and 2 µM ketoprofen)) was added to precipitate proteins. The resulting mixture was vortexed for 5 minutes and centrifuged at 3220 g for 30 min at 4°C, and 100 µL of the supernatant was transferred to a new plate. According to the LC/MS response signal and peak shape of the analyte, the
30 supernatant was diluted with 100 µL or 200 µL of water and mixed evenly and the sample was analysed using LC/MS.

All calculations were performed by Microsoft Excel. The peak area of the analyte of the buffer side and the plasma side was determined. The formula for calculating the plasma protein binding rate of the analyte and the control drug is as

follows: Free rate = (ratio of sample peak area to internal standard peak area of buffer side/ratio of sample peak area to internal standard peak area of plasma side) * 100%, binding rate = 1-free rate, and recovery rate = (ratio of sample peak area to internal standard peak area of buffer side + ratio of sample peak area to internal standard peak area of plasma side)/(ratio of sample peak area to internal standard peak area of initial plasma sample) * 100%. The ratio of sample peak area to internal standard peak area of buffer side represents the free concentration of the compound. The ratio of sample peak area to internal standard peak area of plasma side represents the sum of the free concentration and binding concentration of the compound. The ratio of sample peak area to internal standard peak area of initial plasma sample represents the total concentration of the compound at the beginning of the sample incubation.

Test results:

See Table 6. When 1 μ M compound 2 was incubated at 37°C for 6 hours, the average free rate was 46.63%, the binding rate was 53.37%, and the recovery rate was 88.02%.

Table 6 Human plasma protein binding assay results of compound 2

Compound	Free rate (F%)			Binding rate (%)	Recovery rate (%)
	Repeat 1	Repeat 2	Average value		
Compound 2	48.71	44.55	46.63	53.37	88.02

Example 30: Tissue distribution test of compound 2 by single gavage

Experimental materials:

A total of 60 Balb/c mice (purchased from Shanghai Minchang Biotechnology Co., Ltd.) were used, half male and half female, weighing 18-25 g.

Experimental steps:

The Balb/c mice were administered compound 2 by single gavage at a dose of 100 mg/kg and a volume of 10 mL/kg.

Before administration and 5 min, 0.25, 1.0, 2.0, 3.0, 5.0, 7.0 and 10 h after administration (6 mice at each time point, half male and half female); at the above set time points, 0.2 ml of blood was collected through the retroocular venous plexus, placed in an EDTA-K2 test tube, centrifuged at 11,000 rpm for 5 min, and the plasma was isolated and frozen in a -70°C refrigerator; After the whole blood

collection at the time point of 0.25, 1.0, 3.0, and 7.0 h, the lung tissue was dissected immediately and collected. The residual blood and contents on the surface of the tissue were rinsed with cold saline, blotted dry, labelled, and stored at -70°C for testing. The content of compound 2 in plasma and lung tissue was
5 determined by LC/MS-MS, and the lung blood ratio was calculated.

Test results:

After administration of compound 2 by single gavage to Balb/c mice, the ratio of lung tissue exposure to plasma exposure was 0.62, and the exposure of compound 2 in lung tissue was high.

10 Example 31: Safety pharmacological test of the effect of compound 2 on the cardiovascular system of cynomolgus monkeys by gavage administration

In the cynomolgus monkey repeated dosing toxicity study for 2 weeks, the effect of compound 2 on the cardiovascular system was concomitantly investigated.

Experimental materials:

15 32 cynomolgus monkeys, half male and half female, aged 2.5-5 years old at the time of administration.

Animal source: Yunnan Yingmao Biotechnology Co., Ltd.; Guangxi Xionsen Primate Experimental Animal Breeding and Development Co., Ltd.; Zhongke Lingrui (Zhanjiang) Biotechnology Co., Ltd.

20 The systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MBP) of all conscious animals were measured using an intelligent non-invasive sphygmomanometer BP-98E with a Provantis/v10.2.3.1 electronic data acquisition system (PV-02).

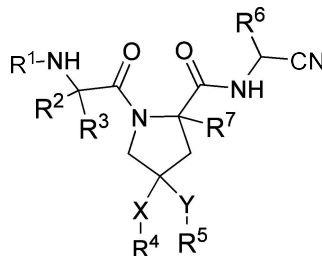
Experimental steps: 32 cynomolgus monkeys (Groups 1 and 4, 5
25 animals/sex/group, Groups 2 and 3, 3 animals/sex/group, 4 groups in total) were randomized and administered compound 2 by nasal feeding (40, 160 and 600 mg/kg/day) or control formulation (98.9% vehicle formulation + 1.1% MTBE, 0 mg/kg/day) twice a day for a total of 14 days, and the animals recovered for 14 days after administration. All animals were included in this study to evaluate the
30 effect of the administration on ECG parameters including heart rate, PR interval, QRS duration, QT interval, and QTcF, and on blood pressure during the pre-dose period, dosing period, and recovery period.

Test results: Under the conditions of this test, cynomolgus monkeys were administered compound 2 (40, 160 and 600 mg/kg/day) by nasal gavage for 14

days, twice a day, and there was no change in the cardiovascular system related to the test product; there was no arrhythmia related to the test product; and there were no changes in ECG parameters or blood pressure related to the test product during the entire test.

CLAIMS

1. A cyano compound represented by general formula I, or a racemate, an enantiomer, a diastereoisomer or a pharmaceutically acceptable salt thereof:



I

characterized in that,

R¹ is selected from -COR⁸ and -SO₂R⁹;

R² and R³ are each independently selected from H, D, C₁-C₁₀ alkyl, adamantyl and C₃-C₇ cycloalkyl, or, R² and R³ and the carbon atom attached thereto together form a 3- to 8-membered carbocyclic ring;

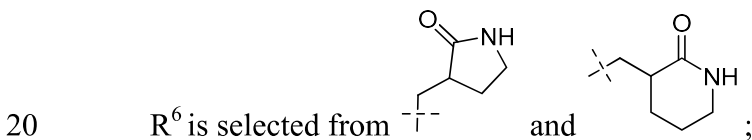
X is selected from O, S, S(=O)₂ and S=O;

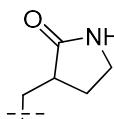
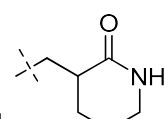
Y is absent or selected from O, S, S(=O)₂ and S=O;

R⁴ is selected from H, C₁-C₁₀ alkyl, C₃-C₈ cycloalkyl, C₆-C₂₀ aryl, C₁-C₁₀ alkyl substituted C₆-C₂₀ aryl, C₁-C₁₀ alkoxy substituted C₆-C₂₀ aryl and halogenated C₆-C₂₀ aryl;

R⁵ is selected from H, C₁-C₁₀ alkyl and C₃-C₇ cycloalkyl;

or, R⁴ and R⁵ are connected to each other to form C₂-C₆ alkylene, thereby connecting X and Y;



R⁶ is selected from  and  ;

R⁷ is selected from H and D;

R⁸ is selected from H, C₁-C₁₀ alkyl, C₁-C₁₀ alkoxy, C₃-C₇ cycloalkyl, halogenated C₁-C₁₀ alkyl, halogenated C₃-C₇ cycloalkyl, -NR¹³R¹⁴, C₆-C₂₀ aryl, halogenated C₆-C₂₀ aryl, C₁-C₁₀ alkyl substituted C₆-C₂₀ aryl, halogenated C₁-C₁₀ alkyl substituted C₆-C₂₀ aryl, 5- to 20-membered heteroaryl and halogenated 5- to 20-membered heteroaryl;

R⁹ is selected from C₁-C₁₀ alkyl, C₃-C₇ cycloalkyl, halogenated C₁-C₁₀ alkyl, halogenated C₃-C₇ cycloalkyl, -NR¹⁵R¹⁶, C₆-C₂₀ aryl, halogenated C₆-C₂₀ aryl, C₁-

C₁₀ alkyl substituted C₆-C₂₀ aryl, halogenated C₁-C₁₀ alkyl substituted C₆-C₂₀ aryl, 5- to 20-membered heteroaryl and halogenated 5- to 20-membered heteroaryl;

R¹³ and R¹⁴ are each independently selected from H and C₁-C₁₀ alkyl;

R¹⁵ and R¹⁶ are each independently selected from H and C₁-C₁₀ alkyl.

5

2. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to claim 1, characterized in that,

10 R² and R³ are each independently selected from H, D, C₁-C₆ alkyl, adamantyl and C₃-C₇ cycloalkyl, or, R² and R³ and the carbon atom attached thereto together form a 3- to 8-membered carbocyclic ring; or

R² and R³ are each independently selected from H, isopropyl, tert-butyl, cyclopentyl, and adamantyl, or, R² and R³ and the carbon atom attached thereto together form cyclopropyl and cyclopentyl; or

15 one of R² and R³ is selected from H, and the other is selected from isopropyl, tert-butyl, cyclopentyl, and adamantyl, or, R² and R³ and the carbon atom attached thereto together form cyclopropyl and cyclopentyl.

20 3. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to claim 1 or 2, characterized in that,

R⁴ is selected from H, C₁-C₆ alkyl, C₃-C₈ cycloalkyl, C₆-C₁₀ aryl, C₁-C₆ alkyl substituted C₆-C₁₀ aryl, C₁-C₆ alkoxy substituted C₆-C₁₀ aryl and halogenated C₆-C₁₀ aryl;

25 R⁵ is selected from H, C₁-C₆ alkyl and C₃-C₇ cycloalkyl;

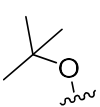
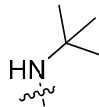
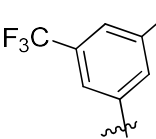
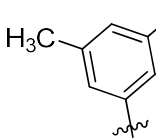
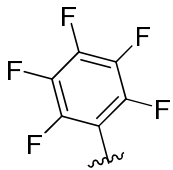
or, R⁴ and R⁵ are connected to each other to form C₂-C₆ alkylene, thereby connecting X and Y; or, R⁴ and R⁵ are connected to each other to form CH₂CH₂ and CH₂CH₂CH₂, thereby connecting X and Y.

30 4. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-3, characterized in that,

R⁸ is selected from H, C₁-C₆ alkyl, C₁-C₆ alkoxy, C₃-C₇ cycloalkyl, halogenated C₁-C₆ alkyl, halogenated C₃-C₇ cycloalkyl, -NR¹³R¹⁴, C₆-C₁₀ aryl,

halogenated C₆-C₁₀ aryl, C₁-C₆ alkyl substituted C₆-C₁₀ aryl, halogenated C₁-C₆ alkyl substituted C₆-C₁₀ aryl, 5- to 10-membered heteroaryl and halogenated 5- to 10-membered heteroaryl, R¹³ and R¹⁴ are each independently selected from H and C₁-C₆ alkyl; or

- 5 R⁸ is selected from C₁-C₆ alkyl, halogenated C₁-C₆ alkyl, C₁-C₆ alkoxy, -NR¹³R¹⁴, C₃-C₇ cycloalkyl, halogenated C₃-C₇ cycloalkyl, phenyl, halophenyl, C₁-C₆ alkyl substituted phenyl, halogenated C₁-C₆ alkyl substituted phenyl and 5- to 6-membered heteroaryl, R¹³ and R¹⁴ are each independently selected from H and C₁-C₆ alkyl; or

- 10 R⁸ is selected from CH₃, CF₃, CH₂CF₃, CF₂CF₃, methoxy, , , , , , ,  and pyridin-3-yl;

- R⁹ is selected from C₁-C₆ alkyl, C₃-C₇ cycloalkyl, halogenated C₁-C₆ alkyl, halogenated C₃-C₇ cycloalkyl, -NR¹⁵R¹⁶, C₆-C₁₀ aryl, halogenated C₆-C₁₀ aryl, C₁-C₆ alkyl substituted C₆-C₁₀ aryl, halogenated C₁-C₆ alkyl substituted C₆-C₁₀ aryl, 5- to 10-membered heteroaryl and halogenated 5- to 10-membered heteroaryl, R¹⁵ and R¹⁶ are each independently selected from H and C₁-C₆ alkyl; or

R⁹ is selected from C₁-C₆ alkyl, C₃-C₇ cycloalkyl, phenyl, C₁-C₆ alkyl substituted phenyl and halogenated C₁-C₆ alkyl substituted phenyl; or

- 20 R⁹ is selected from CH₃, cyclopropyl, phenyl, p-methylphenyl, and p-trifluoromethylphenyl.

5. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-4, characterized in that,

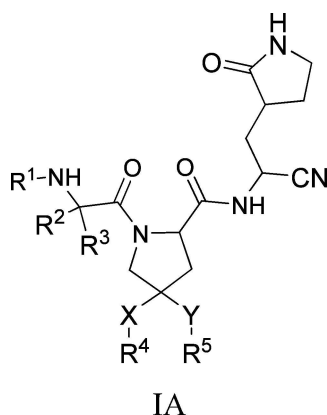
X is selected from O, S, S(=O)₂ and S=O; Y is absent or selected from O, S and S=O; R⁴ is selected from C₁-C₆ alkyl and C₆-C₁₀ aryl, R⁵ is selected from H; or, R⁴ and R⁵ are connected to each other to form C₂-C₆ alkylene, thereby connecting X and Y; or

X and Y are each independently selected from O, S and S=O, and R⁴ and R⁵ are connected to each other to form CH₂CH₂ and CH₂CH₂CH₂, thereby connecting X and Y; or

X and Y are each independently selected from O and S, and R⁴ and R⁵ are
5 connected to each other to form CH₂CH₂, thereby connecting X and Y.

6. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-5, characterized in that,

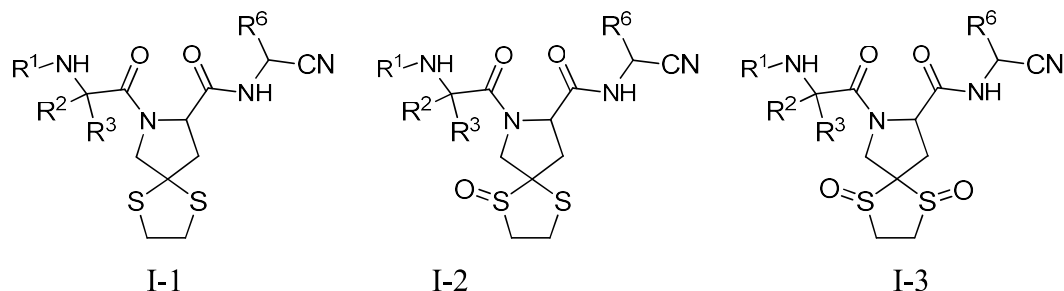
10 the cyano compound represented by general formula I is selected from the
cyano compound represented by general formula IA:

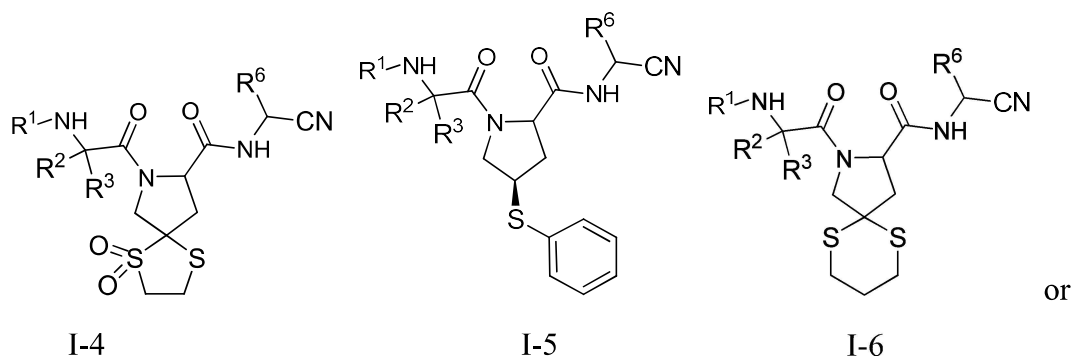


wherein R^1 , R^2 , R^3 , R^4 , R^5 , X and Y are defined as described in any one of
15 claims 1-5.

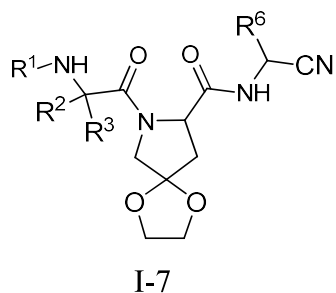
7. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-5, characterized in that,

20 the cyano compound represented by general formula I is selected from the
cyano compound represented by following general formula:





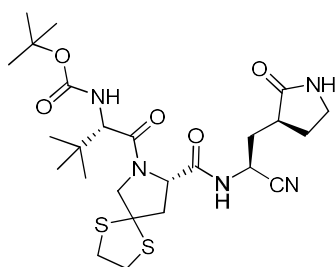
or



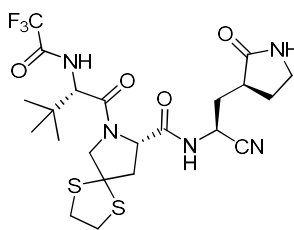
5 wherein R^1 , R^2 , R^3 and R^6 are defined as described in any one of claims 1-5.

8. The cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to claim 1, characterized in that,

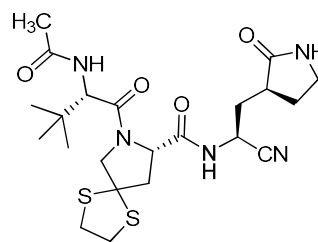
10 the compound represented by general formula I is selected from the following compounds:



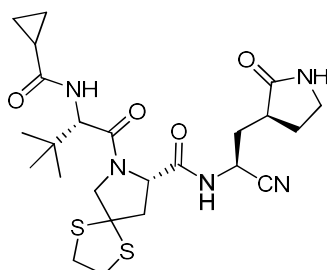
Compound 1



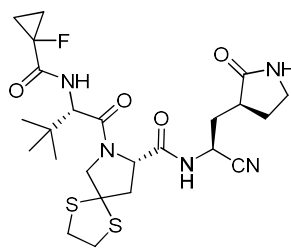
Compound 2



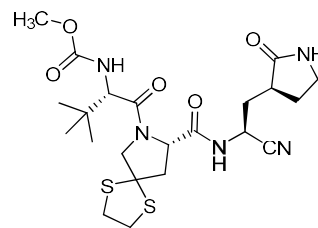
Compound 3



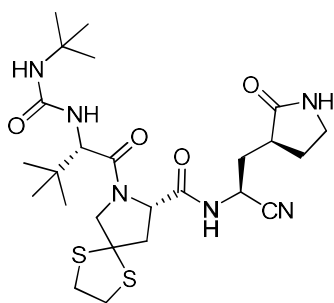
Compound 4



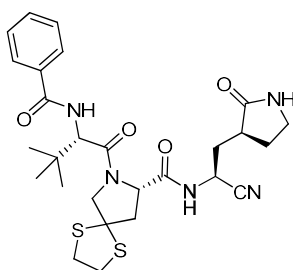
Compound 5



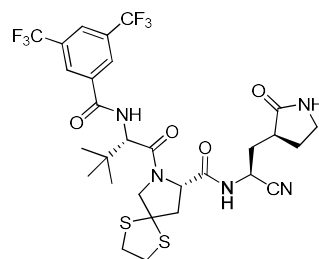
Compound 6



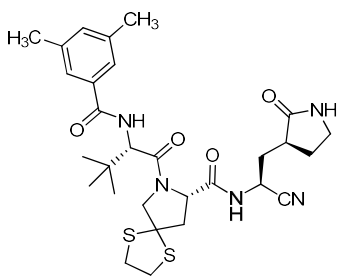
Compound 7



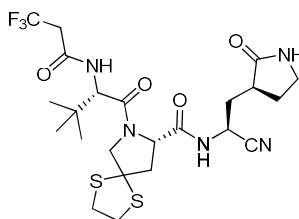
Compound 8



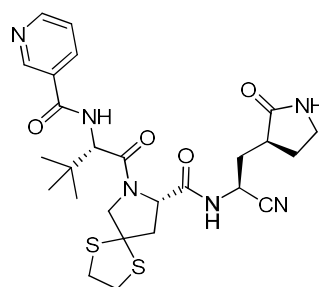
Compound 9



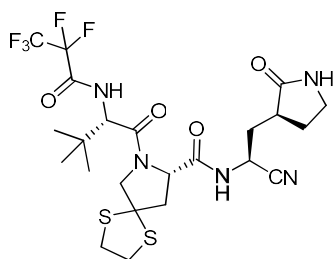
Compound 10



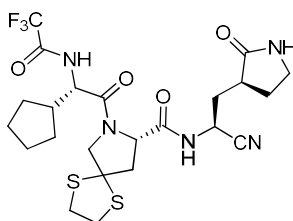
Compound 11



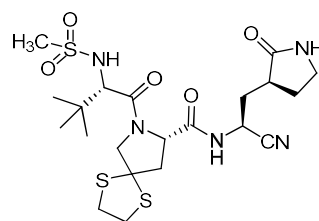
Compound 12



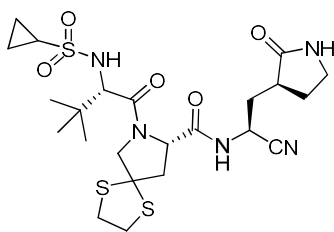
Compound 13



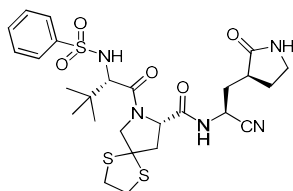
Compound 14



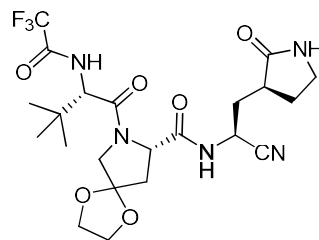
Compound 15



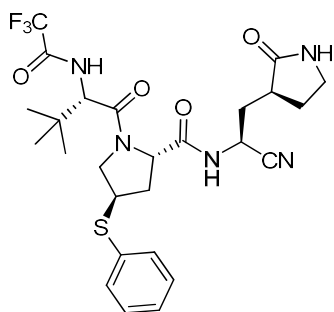
Compound 16



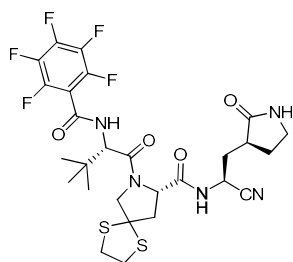
Compound 17



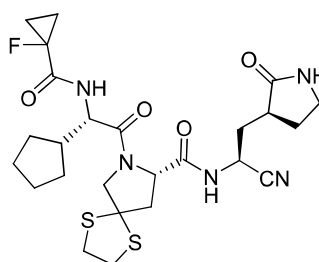
Compound 18



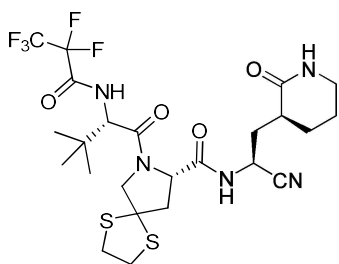
Compound 19



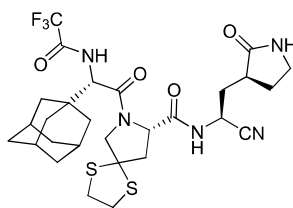
Compound 20



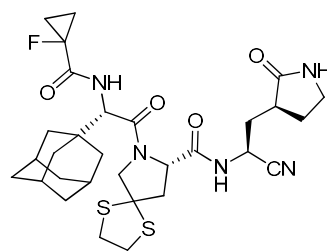
Compound 21



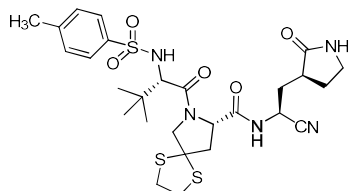
Compound 22



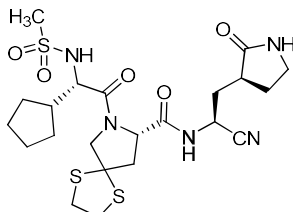
Compound 23



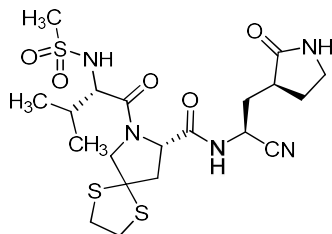
Compound 24



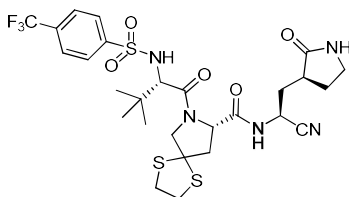
Compound 25



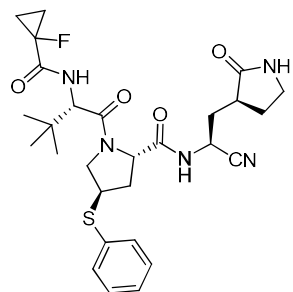
Compound 26



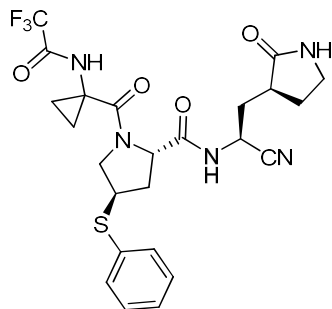
Compound 27



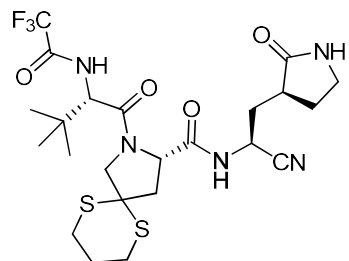
Compound 28



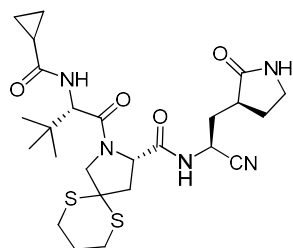
Compound 29



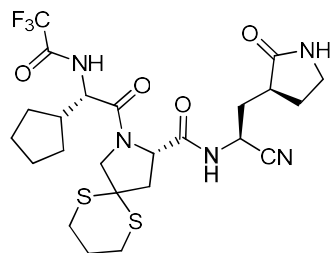
Compound 30



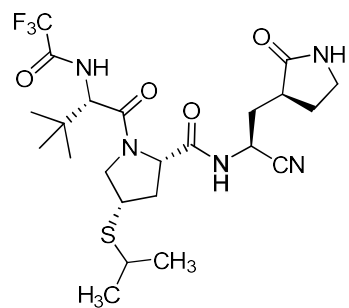
Compound 31



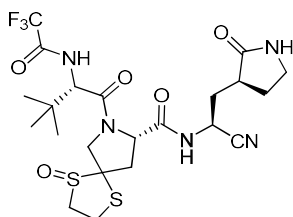
Compound 32



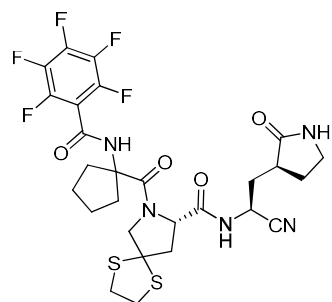
Compound 33



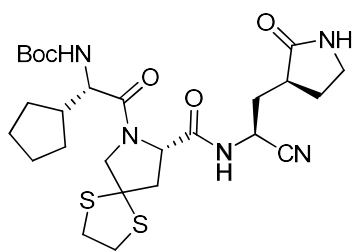
Compound 34



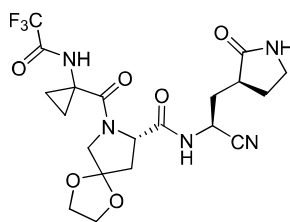
Compound 35



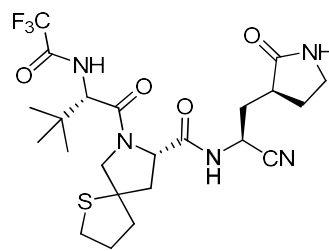
Compound 36



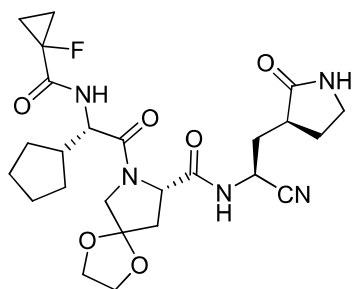
Compound 37



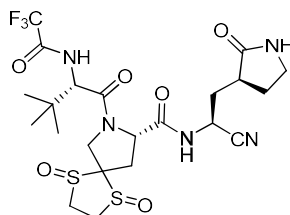
Compound 38



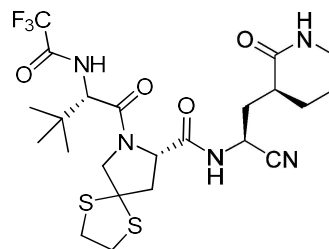
Compound 39



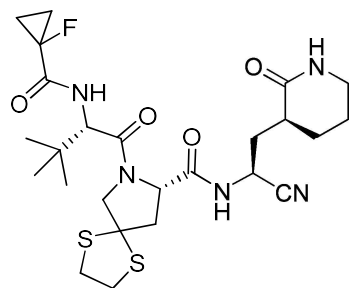
Compound 40



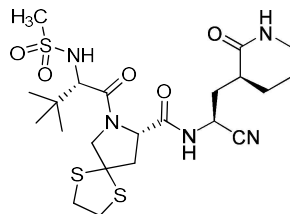
Compound 41



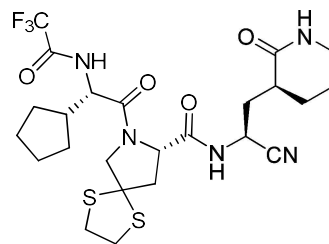
Compound 42



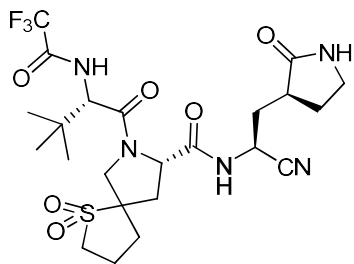
Compound 43



Compound 44



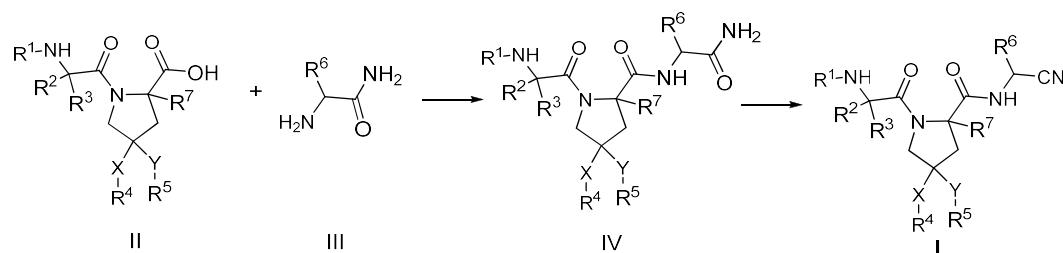
or
Compound 45



Compound 46

9. A method for preparing the compound represented by general formula I, characterized in that the method is one of the following methods:

5 Method i:



ia) a compound represented by formula IV is obtained via a condensation reaction of a compound represented by formula II and a compound represented by formula III;

5 preferably, the step ia) is that: the compound represented by formula II is reacted with the compound represented by formula III under the action of a condensing agent and a base in a solvent at a temperature of -20°C to 50°C for 0.1-12 h, thereby obtaining the compound represented by formula IV,

optionally, the solvent is one of or a mixture of more than one of the
10 following: tetrahydrofuran, dichloromethane, N,N-dimethylformamide, N,N-dimethylacetamide, ethyl acetate and 1,4-dioxane,

optionally, the condensing agent is one of or a mixture of more than one of the following: 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium
hexafluorophosphate, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride,
15 1-hydroxybenzotriazole, 2-hydroxypyridine-N-oxide, 1-propyl phosphoric anhydride, 1H-benzotriazol-1-yloxytripyrrolidinyl hexafluorophosphate, N,N'-carbonyldiimidazole and O-benzotriazole-tetramethyluronium hexafluorophosphate;

optionally, the base is N,N-diisopropylethylamine, triethylamine and N-
20 methylmorpholine;

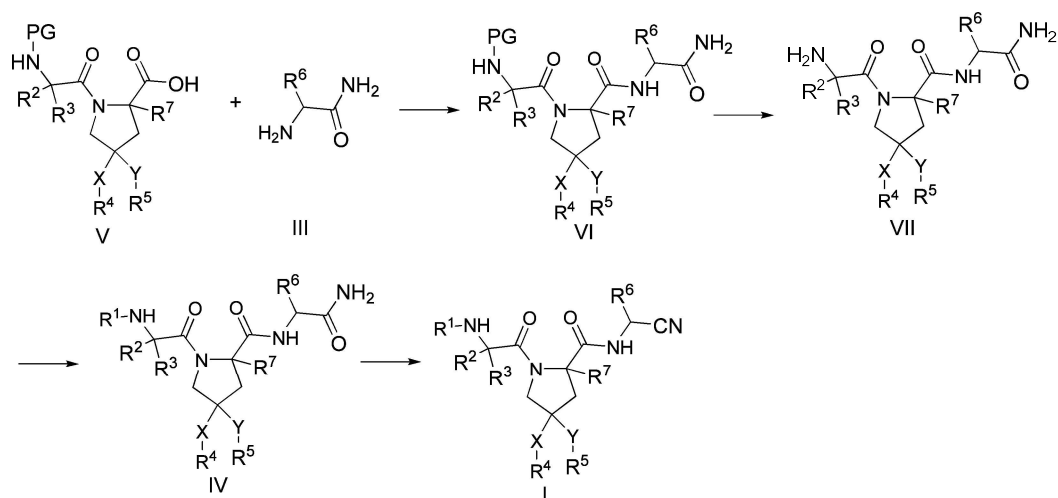
ib) the compound represented by formula IV is dehydrated to obtain the compound represented by general formula I;

preferably, the step ib) is that: the compound represented by formula IV is reacted with a dehydrating agent in an anhydrous solvent at a temperature of -20°C
25 to 50°C for 1-24 h to obtain the compound represented by general formula I;

optionally, the anhydrous solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, toluene, 1,4-dioxane and pyridine;

optionally, the dehydrating agent is trifluoroacetic anhydride or methyl N-(triethylammoniumsulfonyl)carbamate; or

30 Method ii:



5 iia) a compound represented by formula VI is obtained by via a condensation reaction of a compound represented by formula V and the compound represented by formula III, wherein PG in the compound represented by formula V is an amino protecting group;

preferably, the step iia) is that: the compound represented by formula V is reacted with the compound represented by formula III under the action of a condensing agent and a base in a solvent at a temperature of -20°C to 50°C for 0.1-12 h, thereby obtaining the compound represented by formula VI;

10 optionally, the solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, N,N-dimethylformamide, N,N-dimethylacetamide, ethyl acetate and 1,4-dioxane,

optionally, the condensing agent is one of or a mixture of more than one of the following: 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, 2-hydroxypyridine-N-oxide, 1-propyl phosphoric anhydride, 1H-benzotriazol-1-yloxytripyrrolidinyl hexafluorophosphate, N,N'-carbonyldiimidazole and O-benzotriazole-tetramethyluronium hexafluorophosphate;

15

20 optionally, the base is N,N-diisopropylethylamine, triethylamine and N-methylmorpholine;

optionally, the amino protecting group PG is tert-butoxycarbonyl, benzyl and p-methoxybenzyl;

25 iib) the compound represented by formula VI is deprotected to obtain a compound represented by formula VII;

preferably, the step iib) is that: at a temperature of -20°C to 50°C, the compound represented by formula VI is reacted with an organic solution of trifluoroacetic acid or hydrogen chloride or Pd/C/H₂ to obtain the compound represented by formula VII;

- 5 iic) the compound represented by formula IV is obtained by aminoacylation, sulfonylation or a condensation reaction of the compound represented by formula VII;

preferably, the step iic) is that:

- 10 at a temperature of -20°C to 50°C, the compound represented by formula VII is subjected to an aminoacylation reaction with acid chloride or acid anhydride under the condition of adding a base to obtain the compound represented by formula IV;

- 15 at a temperature of -20°C to 50°C, the compound represented by formula VII is subjected to a sulfonylation reaction with sulfonyl chloride or sulfonic anhydride under the condition of adding a base to obtain the compound represented by formula IV;

- 20 at a temperature of -20°C to 50°C, the compound represented by formula VII is subjected to a condensation reaction with a carboxyl compound under the condition of a condensing agent and a base to obtain the compound represented by formula IV;

optionally, the base is N,N-diisopropylethylamine, triethylamine and N-methylmorpholine;

- 25 optionally, the condensing agent is one of or a mixture of more than one of the following: 2-(7-azabenzotriazole)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride, 1-hydroxybenzotriazole, 2-hydroxypyridine-N-oxide, 1-propyl phosphoric anhydride, 1H-benzotriazol-1-yloxytripyrrolidinyl hexafluorophosphate, N,N'-carbonyldiimidazole and O-benzotriazole-tetramethyluronium hexafluorophosphate;

- 30 iid) the compound represented by formula IV is dehydrated to obtain the compound represented by general formula I;

preferably, the step iid) is that: the compound represented by formula IV is reacted with a dehydrating agent in an anhydrous solvent at a temperature of -20°C to 50°C for 1-24 h to obtain the compound represented by general formula I;

optionally, the anhydrous solvent is one of or a mixture of more than one of the following: tetrahydrofuran, dichloromethane, toluene, 1,4-dioxane and pyridine;

optionally, the dehydrating agent is trifluoroacetic anhydride or methyl N-(triethylammoniumsulfonyl)carbamate;

5 wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, X and Y are defined as described in any one of claims 1-8.

10 10. A pharmaceutical composition, characterized in that the pharmaceutical composition comprises the cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-8, and a pharmaceutically acceptable excipient; optionally, the pharmaceutical composition further comprises ritonavir or a pharmaceutically acceptable salt thereof.

15 11. A pharmaceutical combination, characterized in that the pharmaceutical composition comprises the cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-8, and ritonavir or a pharmaceutically acceptable salt thereof.

20

12. Use of the cyano compound, or the racemate, the enantiomer, the diastereoisomer or the pharmaceutically acceptable salt thereof according to any one of claims 1-8, the pharmaceutical composition according to claim 10 or pharmaceutical combination according to claim 11 in the preparation of a medicament selected from a medicament for inhibiting coronavirus 3CL protease activity, a medicament for preventing and/or treating coronavirus infection, a medicament for inhibiting picornavirus 3CL protease activity, and a medicament for preventing and/or treating picornavirus infection; optionally, the coronavirus is selected from SARS-CoV, MERS-CoV, H229E-CoV, HKU1-CoV, NL63-CoV, OC43-CoV and SARS-CoV-2.

25

30