(54) Titre: NOUVELLE FORME POLYCRYSTALLINE D'UN PROMEDICAMENT DU TENOFOVIR, SON PROCÉDE DE PREPARATION ET SON APPLICATION

(54) Title: NEW POLYCRYSTALLINE FORM OF TENOFOVIR PRODRUG, AND PREPARATION METHOD AND APPLICATION THEREFOR

(57) Abrégé/Abstract:
Specifically, the present invention relates to a new crystalline form of 9-[(R)-2-[[[(S)-[[1-((isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate represented by formula (I), characterised in that the XRPD pattern of the crystalline form at least includes 2θ ± 0.20° being: 5.08, 12.44, 13.18, 22.37, 23.37 and 28.56 diffraction peaks. The crystalline form of the present invention has features such as high bioavailability, significant efficacy, good stability, high yield and high purity, and contributes to the selection and design of a drug administration route and the determination of process parameters of a pharmaceutical preparation, thereby improving drug production quality.
Abstract:

The present invention relates to a new crystalline form of 9-[(R)-2-[[[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate, and a preparation method and application therefor. Specifically, the present invention relates to a new crystalline form of 9-[(R)-2-[[[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate represented by formula (I), characterised in that the XRPD pattern of the crystalline form at least includes 2θ ± 0.20° being: 5.08, 12.44, 13.18, 22.37, 23.37 and 28.56 diffraction peaks. The crystalline form of the present invention has features such as high bioavailability, significant efficacy, good stability, high yield and high purity, and contributes to the selection and design of a drug administration route and the determination of process parameters of a pharmaceutical preparation, thereby improving drug production quality.
NEW POLYCRYSTALLINE FORM OF TENOFOVIR PRODRUG, AND
PREPARATION METHOD AND APPLICATION THEREOF

FIELD OF THE INVENTION

The present invention relates to the field of medicinal chemistry, and specifically
relates to a new crystal form of tenofovir prodrug 9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate, the preparation method thereof, the pharmaceutical
composition comprising a therapeutically effective amount of this compound and the
medical use thereof.

BACKGROUND OF THE INVENTION

9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]
methoxy]propyl]adenine fumarate (I) has a following structure:

![Structure of 9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]
methoxy]propyl]adenine fumarate (I)](image)

9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]
methoxy]propyl]adenine fumarate (I) is a nucleoside reverse transcriptase inhibitor
and a prodrug of tenofovir (PMPA). PMPA is similar to the natural nucleoside
monophosphate in structure and is rapidly transformed into the active metabolite PMPA
diphosphate (PMPApp) in the body. PMPApp competes with natural 5' deoxyadenosine
triphosphate and is incorporated into the DNA strand of virus. However PMPApp can
not perform 5', 3' phosphodiester bond coupling reaction due to the lack of 3' OH group,
so that DNA strand extension is blocked and the virus replication is ultimately blocked
(Figure 1). It has been proved that PMPA has anti-human immunodeficiency virus (HIV)
activity and anti-hepatitis B virus (HBV) activity.

However, PMPA contains a phosphate group which is usually negatively charged
at physiological pH and the polarity of it is too strong to pass through the biofilm, which
leads to poor oral bioavailability, low tissue distribution coefficient, and a certain
nephrotoxicity. Therefore, in the development of such drugs, it is needed to use the
principle of pro-drug to mask the negative charge of phosphate groups in order to
eliminate the drawback of such drugs. A diester prodrug of PMPA,
tenofovir disoproxil fumarate (TDF), developed by Gilead company has been approved by FDA on 2001 for the treatment of HIV infection.

TDF has significantly improved the pharmacokinetic properties of PMPA to some extent, but it is rapidly hydrolyzed in the body by non-specific esterases that are widely present in plasma, particularly in the presence of carbonate esterases in intestinal epithelial cells to release PMPA. High concentration of PMPA in the plasma is quickly excreted out of the body due to its poor membrane permeability, which leads to difficulty in maintaining adequate concentration in the infected site. In addition, PMPA is the substrate of organic anion transporter (hOAT) in renal proximal tubule epithelial cells, and the high concentration of PMPA in the plasma is easy to accumulate in renal proximal tubular epithelial cells which results in a certain risk of renal toxicity.

A new generation of monophosphamide monoester prodrug overcomes the above-mentioned shortcomings of TDF, which is very stable in plasma and is not easily to be hydrolyzed by esterases. When it is absorbed into the cells, it is immediately transformed into PMPA in the presence of serine protease (cathepsinA) and specific amidase. So it has a better tissue permeability and lymphoid tissue and cell targeting. The monophosphamide monoester prodrug GS7340 (refer to patent application WO2013052094A2) developed by Gilead company has been successfully entered into phase III clinical trial, and the results show that GS7340 has a stronger anti-virus capability and better security in comparision to 30 times the dose of TDF.

9-[(R)-2-[[[(S)-[1-(isoproxy carbonyl)-1-methyl]ethyl]amino]phenoxyphosphinic acid][methoxyl]propyl]adenine fumarate (I), like GS7340, can release the active ingredient PMPA in cells. Its auxiliary group is cleverly designed, which structure is only different from GS7340 in single methyl group, and the removing mechanism and manner of the auxiliary group in cells is also almost the same as those of GS7340. It can be expected that HS-10234 will be more effective than TDF and other prodrugs to play the efficacy of the active ingredient PMPA because of the advantages of absorption and distribution. As the most promising new generation of PMPA prodrugs, HS-10234 will benefit the majority of patients.

It is known for the person skilled in the art that the polymorphic form of a drug has become an essential part of the pharmaceutical research process and the quality control and detection of the finished drug product. The study of drug polymorphism is benefit to selecting the bioactivity of a new drug compound, to improving the bioavailability, improve the clinical curative effect, to selecting and designing the drug administration route, and to determining the parameters of pharmaceutical preparation process, thereby improving the quality of drug production. The bioavailability may be significant different among different crystal forms for the same drug. For one drug, some crystal
forms may have higher biological activity than other crystal forms. To provide a crystal form of tenofovir prodrug with higher bioactivity and more suitable medical application is a technical problem that the medical field has been looking forward to solve.

5 SUMMARY OF THE INVENTION

The object of the present invention is to solve the above technical problem, and to provide a new crystal form of tenofovir prodrug 9-[(R)-2-[[[(S)-[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxypyphosphinyl]methoxy]propyl]adenine fumarate which is named as crystal form A in the present invention.

The XRPD spectrum of crystal form A according to the present invention comprises at least diffraction peaks at 20 ± 0.20° of 5.08, 12.44, 13.18, 22.37, 23.37 and 28.56.

Preferably, the XRPD spectrum of crystal form A of 9-[(R)-2-[[[(S)-[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxypyphosphinyl]methoxy]propyl]adenine fumarate comprises at least diffraction peaks at 20 ± 0.20° of 5.08, 7.42, 10.15, 12.44, 13.18, 22.37, 23.37, and 28.56, more preferably further comprises diffraction peaks at 20 ± 0.20° of 16.35, 18.23, 21.36, 25.00, and 31.68.

Particularly preferred, the XRPD spectrum of crystal form A is as shown in figure 1.

The result of differential thermal analysis of crystal form A of 9-[(R)-2-[[[(S)-[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxypyphosphinyl]methoxy]propyl]adenine fumarate according to the present invention shows a sharp endothermic melting peak at 110.9 °C.

Another object of the present invention is to provide a method for preparing crystal form A of 9-[(R)-2-[[[(S)-[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxypyphosphinyl]methoxy]propyl]adenine fumarate, comprising the following steps of:

3. filtering out the crystal to obtain crystal form A.
Preferably, the organic solvent is selected from the group consisting of acetonitrile, anhydrous methanol, anhydrous ethanol, isopropanol, anhydrous methanol/n-heptane, anhydrous ethanol/n-heptane, isopropanol/n-heptane, anhydrous methanol/methyl tert-butyl ether, anhydrous ethanol/methyl tert-butyl ether, isopropanol/methyl tert-butyl ether, anhydrous methanol/isopropyl ether, anhydrous ethanol/isopropyl ether, isopropanol/isopropyl ether, anhydrous methanol/diethyl ether, anhydrous ethanol/diethyl ether and isopropanol/diethyl ether, more preferably anhydrous methanol/n-heptane.

Preferably, the temperature of heating the organic solvent is generally from 30°C to the reflux temperature, preferably the reflux temperature; the crystallization temperature is preferably -40 ~ 40°C, most preferably 0°C ~ 10°C.

A further object of the present invention is to provide a pharmaceutical composition comprising an effective amount of said crystal form A, optionally, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier.

The composition according to the present invention is administered via a suitable route comprising oral route and injection route etc, preferably oral route. Suitable dosage forms include tablets, capsules, dispersions and suspensions, preferably tablets.

Another object of the present invention is to provide a use of said crystal form A and the pharmaceutical composition comprising crystal form A in the preparation of a medicament for the treatment of AIDS or hepatitis B virus.

The new crystal form A according to the present invention has the advantages of high bioavailability, remarkable efficacy, good stability, high yield and high purity etc. The new crystal form according to the present invention is benefit to selecting and designing the drug administration route, and to determining the parameters of pharmaceutical preparation process, thereby improving the quality of the drug production.

DESCRIPTION OF THE DRAWINGS

Figure 1 is the XRPD spectrum of the new crystal form of 9-[(R)-2-[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphiny]met hoxy]propyl]adenine fumarate according to the present invention.

Figure 2 is the DSC spectrum of the new crystal form of 9-[(R)-2-[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphiny]met
hydroxy]propyl]adenine fumarate according to the present invention.

**DETAILED DESCRIPTION OF THE INVENTION**

5 In order to illustrate the technical solution of the present invention and the effect obtained thereby, the present invention will be further described with reference to the specific examples below, but it will be appreciated that the scope of the present invention is not limited to these specific examples.

10 Example 1

5.0 g of 9-[(R)-2-[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]met hydroxy]propyl]adenine fumarate, 20.0 ml of anhydrous methanol and 5 ml of n-heptane were placed in a reaction flask and then heated to reflux until the solid was completely dissolved. The heating was stopped and the solution was cooled to 0 ~ 10°C and stirred for 2h to precipitate a crystal. The solid was filtered out to obtain crystal form A.

After testing and verification, its X-ray powder diffraction spectrum was as shown in Figure 1, and its DSC spectrum was consistent with Figure 2, which demonstrated that the resulting crystal form was crystal form A.

Example 2

5.0 g of 9-[(R)-2-[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]met hydroxy]propyl]adenine fumarate and 20.0 ml of anhydrous ethanol were placed in a reaction flask and then heated to reflux until the solid was completely dissolved. The heating was stopped and the solution was cooled to 0 ~ 10°C and stirred for 2h to precipitate a crystal. The solid was filtered out to obtain crystal form A.

After testing and verification, its X-ray powder diffraction spectrum was consistent with Figure 1, and its DSC spectrum was consistent with Figure 2, which demonstrated that the resulting crystal form was crystal form A.

Example 3

5.0 g of 9-[(R)-2-[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]met hydroxy]propyl]adenine fumarate, 20.0 ml of isopropanol and 5 ml of methyl tert-butyl ether were placed in a reaction flask and then heated to reflux until the solid was completely dissolved. The heating was stopped and the solution was cooled to 0 ~ 10°C and stirred for 2h to precipitate a crystal. The solid was filtered out to obtain crystal form A.

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After testing and verification, its X-ray powder diffraction spectrum was consistent with Figure 1, and its DSC spectrum was consistent with Figure 2, which demonstrated that the resulting crystal form was crystal form A.

Experimental Example: Stability Study
The stability of the new crystal form prepared by the method of Example 1 of the present invention was studied. The results showed that the new crystal form A of the present invention did not undergo transformation in the stability test and did not undergo chemical degradation, which was stable at room temperature and in line with the drug and preparation requirements. The details were shown in the table below:

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Appearance</th>
<th>Product Purity</th>
<th>Crystal Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>30°C±2°C /RH 65%±5%</td>
<td>0 month off-white powder</td>
<td>98.97%</td>
<td>Crystal form A</td>
</tr>
<tr>
<td></td>
<td>3 months off-white powder</td>
<td>98.95%</td>
<td>Crystal form A</td>
</tr>
<tr>
<td></td>
<td>6 months off-white powder</td>
<td>98.92%</td>
<td>Crystal form A</td>
</tr>
</tbody>
</table>

Experimental Example: Flowability Study
The flowability of the new crystal form prepared in Examples 1, 2 and 3 of the present invention was studied. The results showed that the new crystal form A of the present invention had good flowability.

<table>
<thead>
<tr>
<th></th>
<th>Example 1</th>
<th>Example 2</th>
<th>Example 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repose Angle</td>
<td>36°</td>
<td>35°</td>
<td>34°</td>
</tr>
</tbody>
</table>

Experimental Example: Determination of Absolute Bioavailability
The absolute bioavailability of the new crystal form A prepared according to the present invention as measured by intravenous administration and oral administration in rats was as high as 81%. The results showed that the new crystal form prepared according to the present invention had high bioavailability.
What is claimed is:


   \[
   \text{(I)}
   \]

characterized in that the XRPD spectrum of the crystal form comprises at least diffraction peaks at 20 ± 0.20° of 5.08, 12.44, 13.18, 22.37, 23.37, and 28.56.

2. The new crystal form of 9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate of formula (I) according to claim 1, characterized in that the XRPD spectrum of the crystal form comprises at least diffraction peaks at 20 ± 0.20° of 5.08, 7.42, 10.15, 12.44, 13.18, 22.37, 23.37, and 28.56, preferably further comprises diffraction peaks at 20 ± 0.20° of 16.35, 18.23, 21.36, 25.00, and 31.68.

3. The new crystal form of 9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate of formula (I) according to claim 1, characterized in that the result of differential thermal analysis of the crystal form shows a sharp endothermic melting peak at 110.9 °C.

4. The new crystal form of 9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate of formula (I) according to any one of claims 1-3, wherein the crystal form has an X-ray diffraction spectrum substantially consistent with figure 1.

5. A preparation method of the new crystal form of 9-[(R)-2-[[[(S)-[[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate of formula (I) according to any one of claims 1-3, comprising the following steps of:

   1) dissolving any forms of
hoxy]propyl]adenine fumarate into an organic solvent under heating;
2) cooling the solution of 9-[(R)-2-[(S)-[[1-(isopropoxycarbonyl)-1-methyl]ethyl]amino]phenoxypyrophosphinyl]meta
hoxy]propyl]adenine fumarate to precipitate a crystal;
3) filtering out the crystal to obtain the target crystal form.

6. The preparation method according to claim 5, characterized in that the organic
solvent is selected from the group consisting of acetonitrile, anhydrous methanol,
anhydrous ethanol, isopropanol, anhydrous methanol/n-heptane, anhydrous
ethanol/n-heptane, isopropanol/n-heptane, anhydrous methanol/methyl tert-butyl ether,
anhydrous ethanol/methyl tert-butyl ether, isopropanol/methyl tert-butyl ether,
anhydrous methanol/isopropyl ether, anhydrous ethanol/isopropyl ether,
isopropanol/isopropyl ether, anhydrous methanol/diethyl ether, anhydrous
ethanol/diethyl ether and isopropanol/diethyl ether, preferably anhydrous
methanol/n-heptane.

7. The preparation method according to claim 5, characterized in that the temperature of heating the organic solvent is generally from 30°C to the reflux
temperature, preferably the reflux temperature.

8. The preparation method according to claim 5, characterized in that the cooling
temperature for crystallization is -40 ~ 40°C, preferably 0°C ~ 10°C.

9. A pharmaceutical composition comprising the new crystal form of
hoxy]propyl]adenine fumarate of formula (I) according to any one of claims 1-4,
optionally further comprises a pharmaceutically acceptable carrier.

10. The pharmaceutical composition according to claim 9, characterized in that the composition is administered via a suitable route such as oral route and injection route,
preferably oral route.

11. The pharmaceutical composition according to claim 9, characterized in that the
composition may be formulated into tablets, capsules, dispersions and suspensions,
preferably tablets.

hoxy]propyl]adenine fumarate of formula (I) according to any one of claims 1-4 or the
pharmaceutical composition according to any one of claims 9-11 in the preparation of a
medicament for the treatment or prevention of AIDS or hepatitis B virus.
Figure 2

**DSC (mW/mg)**

- **Onset:** 104.4°C
- **Peak:** 110.9°C, 2.46mW/mg/min
- **Peak:** 109.2°C, 3.69mW/mg/min
- **Peak:** 112.9°C, -8.76mW/mg/min

**Temperature (°C)**

- **Corresponding**
  - 50
  - 100
  - 150
  - 200
  - 250
  - 300

**DDSC (mW/mg/min)**

- 25
- 20
- 15
- 10
- 5
- 0
- -5
- -10
- -15