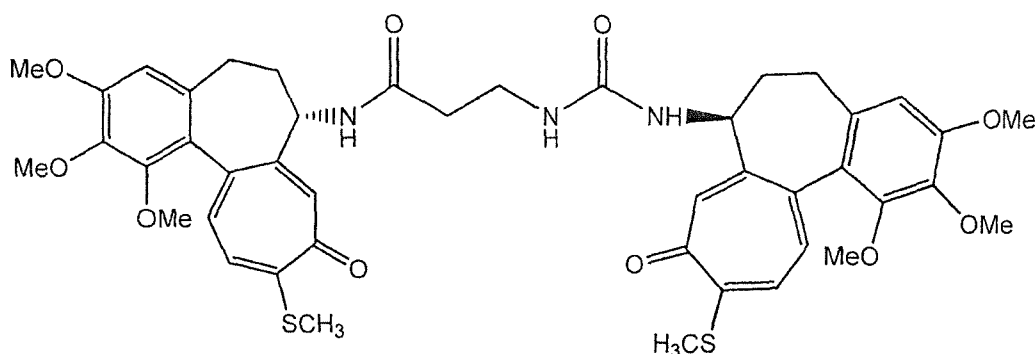


AMORPHOUS FORM OF A THIICOLCHICINE DERIVATIVE

The present invention refers to an amorphous form of a thiocolchicine derivative, IDN 5404, a process for its preparation and pharmaceutical compositions thereof.

IDN 5404 having the following formula (I) is a N-deacetylthiocolchicinoid
5 derivative:



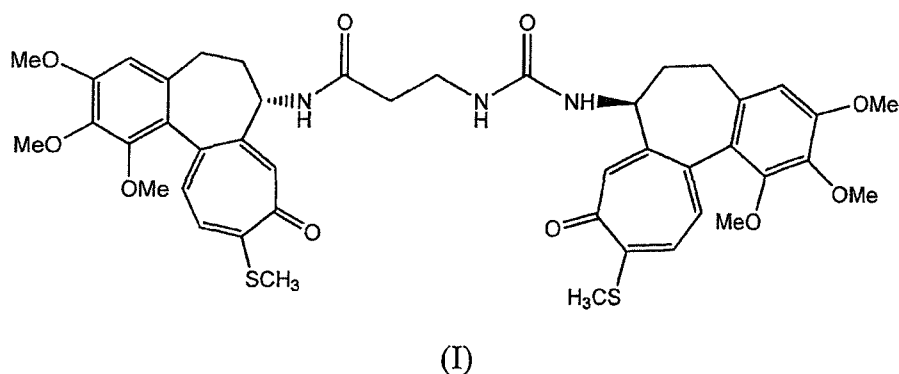
IDN 5404 acts as a vascular disrupting agent, which is a class of compounds able to cause rapid collapse and necrosis of vascular structures. Since endothelial cells of tumours are immature they are much more sensitive to the effects of a vascular disrupting agents than the endothelial cells of normal tissue. IDN 5404 is useful in treating solid tumours, especially if combined with other cytotoxic agents.

IDN 5404 is disclosed in EP 1263719. According to the procedure reported in the example 2 of EP 1263719, the product (Tiocol 54) is first crystallised in EtOAc (as an EtOAc solvate) then is further purified by column chromatography (eluent: Ethyl acetate/hexane or alternatively CH₂Cl₂/EtOH). The patent does not report the way the product is finally recovered from solution and the form of the final product. Usually the products undergoing purification by column chromatography are recovered simply by evaporating the solvent to dryness. In general amorphous materials are produced by this process. Due to the high insolubility and tendency to co-crystallise with solvents of IDN 5404, upon

concentration from the two reported eluting systems, IDN 5404 was obtained either as a EtOAc solvate or EtOH solvate in a crystalline form.

The crystalline form obtained by this process may contain residues of toxic solvents used during the synthetic process, such as dichloromethane and hexane, and it has a very low solubility.

Object of the present invention is an amorphous compound of formula (I):



having the XRPD pattern shown in figure 1.

The amorphous compound as defined above further characterized by at least one of the following characteristics:

- DSC profile characterized by a glass transition with onset at 186.9°C and endset at 194.5°C recorded with a linear heating rate of 10°C/min;

- TG/DTA profile characterized by an endothermic signal between 185.4°C and 195.4°C recorded with a linear heating rate of 10°C/min.

The amorphous compound of the present invention may be obtained by a process comprising the steps of:

- (a) dissolving the crude compound of formula (I) in DMSO;
- (b) removing the possible residual solvents coming from the synthetic process by heating the solution at 65°C under vacuum;
- (c) precipitating the amorphous compound of formula (I) by adding drop wise the solution obtained in step b) to water at 20-25°C under stirring.

In step a) from 1L to 8L of DMSO are preferably used for 1Kg of IDN

5404.

In step c) from 8L to 64L of water are preferably used for 1Kg of IDN

5404.

The amorphous compound of formula (I) as above defined is more soluble
5 in water than other crystalline forms and it is chemically and physically stable.
These properties allow to prepare solid forms containing the compound according
to the invention.

Furthermore the compound obtained by the process reported above is
devoid of any toxic solvent used during the synthetic process such as
10 dichloromethane and hexane.

The only residual solvent present in the amorphous material is DMSO
which is a class 3 solvent (low toxicity).

The above defined amorphous form is not a solvated form differently from
the crystalline forms identified by polymorphic screening which are all solvated
15 forms.

The amorphous form of IDN 5404 has advantageous properties in the
preparation of pharmaceutical compositions such as such as increased solubility,
improved bioavailability, ease of chemical processing and/or ease of
pharmaceutical formulation.

20 Another object of the present invention is therefore a pharmaceutical
composition comprising the amorphous compound of formula (I) as above defined
and a pharmaceutically acceptable diluent and/or carrier.

The pharmaceutically acceptable diluent or carrier is selected with regard to
the intended route of administration and standard pharmaceutical practice. The
25 pharmaceutical formulations of the invention are preferably administered orally or
parenterally.

The term "parenteral" as used herein, includes subcutaneous injections,
intravenous, intramuscular injection or infusion techniques.

The amorphous form of the present invention may be formulated into conventional dosage forms such as, for example, tablets, pills, suspensions, emulsions, granules, capsules and injection preparations.

The preferred dosage forms for the compounds of the invention are injectable preparations. The compound of formula (I) as above defined may be used, alone or in combination with a cytotoxic agent for the treatment of solid tumors.

Example 1

Crude IDN 5404 (1 kg) was dissolved in DMSO (8 L). The solution was heated at 65°C and kept under vacuum for 2 hours in order to remove completely the solvents coming from the synthetic process. The solution was added drop wise to water (64 L) at 20-25°C under stirring causing the precipitation of IDN 5404 as an amorphous solid. The resulting material was filtered and dried under vacuum to afford a quantitative yield of IDN 5404.

Characterisation of the amorphous form:

X-Ray Powder Diffraction (X-RPD)

X-RPD pattern was recorded on a Bruker D2-Phaser Diffractometer. The x-ray generator was operated at 30kV and 10 mA, using the CuK α line as the radiation source. The sample was packed on a suitable slit and the irradiated length was 10 mm. Data were collected between 2 and 50 deg 2-theta with a step size of 0.02 deg 2-theta and a counting time per step of 3 sec. The x-ray powder diffraction pattern of Amorphous (Fig. 1) shows absence of diffraction peaks and a broad noise typical of an amorphous sample.

Differential Scanning Calorimetry (DSC)

The analysis was performed using a Mettler DSC1 System. Heat flow was recorded from 30 to 300°C with linear heating rate (10°C/min) under a 50 ml/min nitrogen flow. About 5 mg of powder was used for the measurement, in closed aluminium crucible (40 μ l volume) with a pinhole.

The DSC profile (Fig. 2) is characterized by a broad endothermic signal with maximum at about 100°C due to release of moisture, and a glass transition with onset at 186.9°C and endset at 194.5°C.

Fourier-Transform InfraRed Spectroscopy (FTIR)

5 The infrared spectrum was recorded in Attenuated Total Reflectance (ATR) mode using Fourier-Transform spectrometer Perkin Elmer Spectrum One, equipped with Specac ATR Golden Gate accessory. The spectrum is the result of the acquisition and transformation of 16 co-added scans in the 4000-550 cm⁻¹ spectral region at a re.

10 The FTIR-ATR spectrum is shown in Fig. 3 (the 4000-550 cm⁻¹ spectral range). It shows absorption frequencies at 3286, 2936, 2836, 1669, 1606, 1535, 1484, 1403, 1347, 1320, 1283, 1235, 1194, 1135, 1093, 1019, 983, 922, 841, 795, 777, 575 cm⁻¹ ± 2 cm⁻¹.

Thermogravimetry (TG) and Differential Thermal Analysis (DTA)

15 The analysis was performed using a Seiko TG/DTA7200 simultaneous system using open aluminum pans (40 µl volume). The TG/DT signals were recorded from 30 to 300°C with linear heating rate (10°C/min) under a 200 ml/min nitrogen flow. About 10 mg of powder was used for the measurement.

20 The TG/DTA profile (Fig. 4) is characterized by a broad endothermic peak with maximum at about 60°C due to release of residual moisture (weight loss at 100°C = 1.55%), and an endothermic signal between 185.4°C and 195.4°C attributable to a glass transition, immediately followed by an exothermic degradation.

Example 2 (comparative)

25 Crude IDN 5404 (500 mg) was purified by flash chromatography using as eluent AcOEt-Hexane 7:3. The fractions containing IDN 5404 were pooled and the solvent removed until dryness. IDN 5404 (310 mg) was obtained as a crystalline yellow solid with the following characteristics.

The product was analysed by GC to determine the residual organic solvents: AcOEt content is 11.1% (22 ppm of hexane) then it was assumed that the product could be a AcOEt solvate.

5 The TG/DTA and XRPD analysis were performed in the same conditions as example 1.

The TG/DTA profile of IDN5404 (AcOEt solvate) is represented in Fig. 5.

The analysis shows a DT profiles characterized by two intense and not completely resolved endothermic peaks, with onset at about 208°C and two maxima respectively at 221.3°C and 231.2°C.

10 Those peaks, attributable to release of crystallisation solvent followed by melting, are associated to a weight loss of 5.94% from 200°C to 240°C.

The TG profile shows also a progressive weight loss of about 3.1% from 30 to 200°C, followed by a sharp weight loss in coincidence of the first endothermic peak.

15 The total loss of weight from 30°C to 240°C is 9.0%.

The XRPD diffractogram of IDN5404 (AcOEt solvate) is represented in Fig. 6.

The diffractogram is characterized by intense diffraction peaks and sharp peak profile which indicates high crystallinity; the XRPD pattern shows distinctive
20 reflections, expressed as 2-theta degrees values, at: 5.6 - 10.2 - 10.5 - 11.1 - 13.3 - 14.4 - 14.7 - 17.5 - 17.9 - 18.5 - 18.9 - 19.4 - 20.0 - 20.8 - 21.6 - 22.2 - 22.4 - 22.6 - 23.4 - 25.2 - 25.5 - 25.9 - 26.7 - 27.8 - 28.5 - 29.1 - 29.7 - 30.8 - 31.2 - 32.1.

Example 3 (comparative)

Crude IDN 5404 (500 mg) was purified by flash chromatography using as
25 eluent CH₂Cl₂-EtOH 95:5. The fractions containing IDN 5404 were pooled and the solvent removed until dryness. IDN 5404 (315 mg) was obtained as a crystalline yellow solid with the following characteristics:

The product was analysed by GC to determine the residual organic solvents:

EtOH content is 10.7% (269 ppm of CH₂Cl₂) then it was assumed that the product could be a EtOH solvate.

The TG/DTA and XRPD analysis were performed in the same conditions as example 1.

5 The TG/DTA profile of IDN5404 (EtOH solvate) is represented in Fig. 7.

The analysis shows a DT profiles characterized by an endothermic peak with onset at about 198°C and maximum at 210.6°C.

This peak, attributable to melting with release of crystallisation solvent, is associated to a weight loss of 5.34% from 195°C to 230°C.

10 The TG profile shows also a progressive weight loss of about 6.7% from 30 to 195°C.

The total loss of weight from 30°C to 230°C is 12.1%.

The XRPD diffractogram of IDN5404 (EtOH solvate) is represented in Fig. 8.

15 The diffractogram is characterized by intense diffraction peaks and sharp peak profile which indicates high crystallinity; the XRPD pattern shows distinctive reflections, expressed as 2-theta degrees values, at: 6.3 - 10.4 - 10.6 - 11.2 - 12.5 - 13.3 - 14.4 - 14.8 - 16.9 - 17.8 - 18.8 - 19.3 - 19.7 - 20.3 - 20.9 - 21.8 - 22.5 - 23.0 - 23.3 - 24.9 - 25.5 - 26.0 - 27.1 - 27.9 - 28.9 - 29.4 - 29.7 - 32.2.

20 Stability data

The amorphous form of compound (I) has been found to be chemically stable at 25 ± 2°C / 60 ± 5% relative humidity for at least three years and at 40 ± 2°C/ 75 ± 5% relative humidity for at least 6 months, as none impurity has departed from its initial T₀ value. The analyses were performed by HPLC.

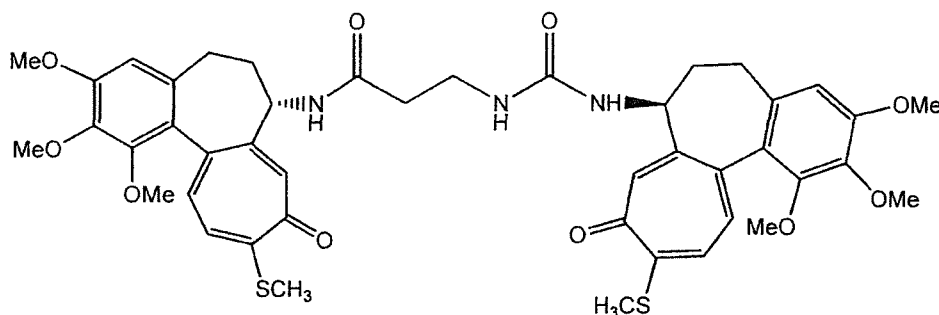
25 The amorphous form of compound (I) has also been found physically stable at 25 ± 2°C / 60 ± 5% relative humidity for at least three years and at 40 ± 2°C/ 75 ± 5 relative humidity for at least 6 months, as it maintained the characteristics features reported in Fig. 1-4.

The crystalline IDN 5404 obtained in Example 2 shows a variation in the chemical composition of 7% after one month at $40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ relative humidity.

5 The crystalline IDN 5404 obtained in Example 3 shows a variation in the chemical composition of 4.1% after one month at $40 \pm 2^\circ\text{C}$ / $75 \pm 5\%$ relative humidity.

CLAIMS

1. Amorphous compound of formula (I):



(I)

having the XRPD pattern shown in figure 1.

2. The amorphous compound according to claim 1 having a DSC profile characterized by a glass transition with onset at 186.9°C and endset at 194.5°C recorded with a linear heating rate of 10°C/min.
3. The amorphous compound according to claim 1 having a TG/DTA profile characterized by an endothermic signal between 185.4°C and 195.4°C recorded with a linear heating rate of 10°C/min.
4. A process of preparing the amorphous compound of formula (I), as defined in claim 1, comprising the steps of:
- (a) dissolving the crude compound of formula (I) in DMSO;
 - (b) removing the possible residual solvents coming from the synthetic process by heating the solution at 65°C under vacuum;
 - (c) precipitating the amorphous compound of formula (I) by adding drop wise the solution obtained in step b) to water at 20-25°C.
5. A pharmaceutical composition comprising the amorphous compound of formula (I) according to any one of the claims 1-3 and a pharmaceutically acceptable carrier and/ diluent.
6. The pharmaceutical composition according to claim 5 for parenteral or oral

administration.

7. The pharmaceutical composition according to claim 6 in form of injectable preparation.

5

8. The amorphous compound of formula (I) according to any one of claims 1-3, for use in the treatment of solid tumors.