

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 November 2003 (27.11.2003)

PCT

(10) International Publication Number
WO 03/097101 A1

(51) International Patent Classification⁷: **A61K 47/02**,
47/26

(21) International Application Number: PCT/EP03/04871

(22) International Filing Date: 9 May 2003 (09.05.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
MI2002A001040 16 May 2002 (16.05.2002) IT

(71) Applicant (*for all designated States except US*): **NOVUS-PHARMA S.p.A.** [IT/IT]; Via Ariosto, 23, I-20091 Bresso (IT).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **BERNAREGGI, Alberto** [IT/IT]; Via Ariosto, 23, I-20091 Bresso (IT). **LIVI, Valeria** [IT/IT]; Via Ariosto, 23, I-20091 Bresso (IT).

(74) Agents: **MINOJA, Fabrizio** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: INJECTABLE PHARMACEUTICAL COMPOSITIONS OF AN ANTHRACENEDIONE DERIVATIVE WITH ANTI-TUMORAL ACTIVITY

(57) Abstract: Injectable pharmaceutical compositions containing 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate as active ingredient in the form of a lyophilised powder with a carrier selected from lactose and dextran, mixed with sodium chloride.



WO 03/097101 A1

INJECTABLE PHARMACEUTICAL COMPOSITIONS OF AN
ANTHRACENEDIONE DERIVATIVE WITH ANTI-TUMORAL
ACTIVITY

The present invention relates to injectable pharmaceutical compositions containing 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate (from now on also referred to as "BBR 2778") as active ingredient in the form of a lyophilised powder with a carrier selected from lactose and
5 dextran, mixed with sodium chloride.

PRIOR ART

BBR 2778 is a novel anthracenedione derivative with anti-tumoral activity which acts as a DNA intercalating agent and topoisomerase II inhibitor. Pre-clinical studies demonstrate that its cardiotoxicity is lower than
10 that of other known drugs belonging to the same class. BBR 2778 has proved more active than mitoxantrone against haematological tumours, especially ascitic L1210 leukaemia and YC-8 lymphoma, in a wide range of doses.

Clinical trials on the use of BBR 2778 in the treatment of non-Hodgkin's lymphoma are at an advanced stage.

15 The formulation of BBR2778 in injectable liquid pharmaceutical compositions has proved problematic in terms of stability in solution using common solvents suitable for parenteral administration, especially intravenous administration.

A lyophilised formulation to be reconstituted with a suitable solvent
20 such as saline immediately before use has therefore been considered.

Here again, however, unforeseeable problems arose, partly due to the low solubility of BBR 2778 in water and the need to use sodium chloride solutions in concentrations ranging from 0.9 to 4%, in which the drug is progressively more soluble. However, the presence of sodium chloride
25 requires the use of long lyophilisation cycles due to the low glass transition

temperature observed.

The choice of lyophilisation carrier has also proved critical in terms of the stability of the final formulation and in operational terms.

For example, if mannitol is used as lyophilisation carrier, stable
5 formulations are only obtained if they are stored at temperatures of approx.
5°C or lower; stability studies conducted at 25°C with 60% relative humidity
(RH) showed unacceptable levels of degradation products after only one
month. The characterisation of the finished product in the solid state
demonstrates that BBR 2778 is transformed from a crystalline raw material to
10 an amorphous powder in the lyophilisate, with a consequent reduction in
stability. The choice of carriers with greater protective properties
consequently focused on polymeric substances like polyvinylpyrrolidone
(PVP, Povidone).

Replacing mannitol with Povidone K 17 produced a stable lyophilisate
15 even at 25°C, 60% RH, but Povidone has been removed from the list of
excipients approved for parenteral administration in the USA (Fed. Reg. 8
March 1999, Vol. 64, Num. 64) and requires a long lyophilisation cycle due to
the low glass transition temperature observed (-37°C), which requires primary
drying to be conducted at a temperature below -37°C.

20 Unsatisfactory results were also obtained with the use of other
conventional lyophilisation carriers such as urea, glycine, ammonium chloride
and TRIS in the presence and absence of sodium chloride, and by
lyophilisation of BBR 2778 in the absence of excipients.

DESCRIPTION OF THE INVENTION

25 It has now been found that it is possible to obtain stable lyophilised
formulations of BBR 2778 in the presence of sodium chloride by using lactose
or dextran as lyophilisation carrier.

A first aspect of the invention therefore provides injectable

pharmaceutical compositions containing 6,9-bis[(2-aminoethyl)amino]benzo
[g]isoquinoline-5,10-dione dimaleate (BBR 2778) as active ingredient in the
form of a lyophilised powder with a carrier selected from lactose and dextran,
mixed with sodium chloride, to be reconstituted with a solvent suitable for
5 reconstituting the lyophilisate and suitable for parenteral administration,
which solvent is preferably contained in a separate ampoule.

A further aspect of the invention relates to a process for the preparation
of said compositions.

DETAILED DESCRIPTION OF THE INVENTION

10 In the compositions of the invention, the weight ratio between the
carrier and sodium chloride is critical, and is typically between 1:1 and 3:1.

The weight ratio between BBR 2778 and the carrier is preferably
between 1:2 and 1:6. The particularly preferred carrier is lactose.

The unit dose of BBR 2778 will usually be between 25 and 200 mg, and
15 preferably between 50 and 100 mg. The unit dose currently being tested in
clinical trials is 50 mg. For this latter quantity of active ingredient, the
preferred compositions according to the invention will contain 100 to 200 mg
of sodium chloride and 100 to 300 mg of lactose.

If required, the compositions of the invention can also contain other
20 excipients commonly used for parenteral formulations, such as antioxidants,
buffers, local anaesthetics, salts, amino acids and the like.

The vials or ampoules of sterile lyophilised powder will then be
reconstituted at the time of use with sterile solvents constituted by sterile
pyrogen-free water or sterile saline, in volumes of approx. 5 ml to 20 ml,
25 depending on the active ingredient content.

The compositions of the invention are prepared by a process which
comprises lyophilisation of an aqueous solution of BBR 2778, lactose or
dextran and sodium chloride by means of:

- a freezing stage at a temperature below at least -45°C for at least 3 hours;
- a primary drying stage consisting of increasing the temperature of the product to $-35^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in approx. 3 hours and maintaining said temperature for at least 40 hours;
- a secondary drying stage consisting of increasing the temperature of the product to $+30^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in 10 hours and maintaining said temperature for at least 8 hours.

The compositions according to the invention are stable at room temperature for at least 24 months. The lyophilised product is not subject to deliquescence, and maintains its appearance unchanged over time.

A further advantage of the invention is the reduction in lyophilisation times and the consequent reduction in the cost of the process.

The following examples illustrate the invention in greater detail.

15 EXAMPLE 1

Preparation of vials containing lyophilised BBR 2778 in the presence of lactose and sodium chloride.

A solution containing 10 mg/ml of BBR 2778, 20 mg/ml of NaCl and 60 mg/ml of lactose, prepared by dissolving the various components in water for injection at $20\text{-}25^{\circ}\text{C}$, is distributed between type I glass vials under sterile conditions at the rate of 5 ml per vial, after sterile filtration. A lyophilisation stopper is placed on the mouth of the vials.

The pre-stoppered vials are then loaded directly onto lyophilisation shelves and frozen at $-45^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for at least 3 hours.

25 Primary drying is conducted by increasing the temperature of the shelves in the vacuum freeze-dryer from -45°C to $-30^{\circ}\text{C} \pm 3^{\circ}\text{C}$ in 3 hours, and maintaining the temperature at -30°C for 40 hours.

Secondary drying is performed by increasing the temperature of the

- 5 -

shelves from -30°C to $+30^{\circ}\text{C} \pm 3^{\circ}\text{C}$ in 10 hours and then maintaining said temperature of $+30^{\circ}\text{C}$ for a further 8 hours. The freeze-dryer is returned to atmospheric pressure with nitrogen filtered under sterile conditions, and the vials are stoppered by activating the stoppering device. The vials are unloaded
5 in a sterile environment and crimped.

When reconstituted with 5 ml of water for injection, the solution has a pH of between 3.0 and 4.5.

Accelerated stability tests conducted on the lyophilisate at 40°C for 12 months have demonstrated a reduction in BBR 2778 titre within the limits of
10 the specifications approved for the product, and a purity exceeding 95%. The stability is also confirmed at 25°C even after the 12-month observation period.

EXAMPLE 2

Preparation of vials containing lyophilised BBR 2778 in the presence of dextran and sodium chloride.

15 A solution containing 10 mg/ml of BBR 2778, 20 mg/ml of NaCl and 60 mg/ml of dextran 40000, prepared by dissolving the various components in water for injection at $20\text{-}25^{\circ}\text{C}$, is distributed between type I glass vials under sterile conditions, at the rate of 5 ml per vial, after sterile filtration. A lyophilisation stopper is placed on the mouth of the vials.

20 The pre-stoppered vials are then loaded onto trays, which are placed on the shelves of the freeze-dryer. The vials are then frozen in the lyophilisation chamber at $-45^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for at least 3 hours.

Primary drying is conducted by increasing the temperature of the shelves in the vacuum freeze dryer from -45°C to $0^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in 6 hours and
25 maintaining the temperature at 0°C for 30 hours. The temperature of the product during primary drying is maintained at around -30°C .

Secondary drying is performed by increasing the temperature of the shelves from 0°C to $+30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in 3 hours and then maintaining said

- 6 -

temperature of + 30°C for a further 8 hours. The freeze-dryer is returned to atmospheric pressure with nitrogen filtered under sterile conditions, and the vials are stoppered by activating the stoppering device. The vials are unloaded in a sterile environment and crimped.

- 5 When reconstituted with 5 ml of water for injection, the solution has a pH of between 3.0 and 4.5.

Accelerated stability tests conducted on the lyophilisate at 40°C for 4 months have demonstrated a reduction in BBR 2778 titre within the limits of the specifications approved for the product, and a purity exceeding 96%.

CLAIMS

1. Injectable pharmaceutical compositions containing 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate as active
5 ingredient in the form of a lyophilised powder with a carrier selected from lactose and dextran, mixed with sodium chloride.
2. Compositions as claimed in claim 1, wherein the carrier is lactose.
3. Compositions as claimed in claim 1 or 2, further containing an antioxidant.
- 10 4. Compositions as claimed in any one of claims 1 to 3, wherein the weight ratio between carrier and sodium chloride is between 1:1 and 3:1.
5. Compositions as claimed in any one of claims 1 to 4, wherein the weight ratio between 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate and the carrier is between 1:2 and 1:6.
- 15 6. Compositions as claimed in any one of claims 1 to 5, wherein 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate is present in the quantity of 7-15 mg/ml in the solution to be lyophilised.
7. Compositions as claimed in claim 6 containing 10 to 40 mg/ml of sodium chloride and 20 to 60 mg/ml of lactose in the solution to be lyophilised.
- 20 8. Compositions as claimed in any one of claims 1 to 7, wherein the unit dose of 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate is between 25 and 200 mg.
9. Compositions according to claim 8, wherein the unit dose of 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate is 50 mg.
- 25 10. Compositions as claimed in any one of claims 1 to 9, associated with an ampoule containing a sterile solvent suitable to reconstitute the lyophilisate and suitable for parenteral administration.
11. A process for preparation of the compositions claimed in claims 1-10

- 8 -

which involves lyophilisation of an aqueous solution of 6,9-bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione dimaleate, lactose or dextran and sodium chloride by means of:

- a freezing stage at a temperature below at least -45°C for at least 3
5 hours;
- a primary drying stage consisting of increasing the temperature of the product to $-35^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in approx. 3 hours and maintaining said temperature for at least 40 hours;
- a secondary drying stage consisting of increasing the temperature of the product to $+30^{\circ}\text{C} \pm 5^{\circ}\text{C}$ in 10 hours and maintaining said temperature for
10 at least 8 hours.

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/EP 03/04871

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/02 A61K47/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PATENT ABSTRACTS OF JAPAN vol. 1996, no. 09, 30 September 1996 (1996-09-30) & JP 08 126685 A (SHIONOGI & CO LTD), 21 May 1996 (1996-05-21) abstract ----	
A	WO 01 28521 A (NOVUSPHARMA S P A ;BUGATTI CARLO (IT)) 26 April 2001 (2001-04-26) ----	
A	US 5 587 382 A (GIULIANI FERDINANDO C ET AL) 24 December 1996 (1996-12-24) ----	
A	US 3 590 124 A (HOYT JACK W) 29 June 1971 (1971-06-29) ----	
A	US 4 822 785 A (ASHIZAWA KAZUhide ET AL) 18 April 1989 (1989-04-18) -----	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

18 September 2003

Date of mailing of the international search report

01/10/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Stienon, P

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT, L. 03/04871

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
JP 08126685	A	21-05-1996	NONE	
WO 0128521	A	26-04-2001	IT MI992219 A1	23-04-2001
			AU 1514601 A	30-04-2001
			BR 0014881 A	11-06-2002
			CA 2386143 A1	26-04-2001
			CN 1382039 T	27-11-2002
			CZ 20021388 A3	14-08-2002
			WO 0128521 A2	26-04-2001
			EP 1221940 A2	17-07-2002
			HU 0203473 A2	28-02-2003
			JP 2003512313 T	02-04-2003
			NO 20021867 A	19-04-2002
			SK 5382002 A3	10-09-2002
US 5587382	A	24-12-1996	NONE	
US 3590124	A	29-06-1971	NONE	
US 4822785	A	18-04-1989	JP 2690009 B2	10-12-1997
			JP 63017827 A	25-01-1988
			AT 63814 T	15-06-1991
			CA 1293445 C	24-12-1991
			DE 3770355 D1	04-07-1991
			EP 0253329 A2	20-01-1988
			ES 2039215 T3	16-09-1993
			GR 3002026 T3	30-12-1992