



(86) **Date de dépôt PCT/PCT Filing Date:** 2007/02/21
(87) **Date publication PCT/PCT Publication Date:** 2007/09/13
(45) **Date de délivrance/Issue Date:** 2019/07/30
(85) **Entrée phase nationale/National Entry:** 2008/09/08
(86) **N° demande PCT/PCT Application No.:** EP 2007/001487
(87) **N° publication PCT/PCT Publication No.:** 2007/101551
(30) **Priorité/Priority:** 2006/03/09 (EP06004820.4)

(51) **Cl.Int./Int.Cl. A61K 36/9066** (2006.01),
A61K 31/12 (2006.01), **A61K 31/685** (2006.01)
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(54) **Titre : COMPLEXES PHOSPHOLIPIDIQUES DE CURCUMINE PRESENTANT UNE BIODISPONIBILITE AMELIOREE**
(54) **Title: PHOSPHOLIPID COMPLEXES OF CURCUMIN HAVING IMPROVED BIOAVAILABILITY**

(57) **Abrégé/Abstract:**

The present invention relates to novel phospholipids complexes of curcumin or extracts containing it having improved bioavailability.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
13 September 2007 (13.09.2007)

PCT

(10) International Publication Number
WO 2007/101551 A3

(51) International Patent Classification:

A61K 36/9066 (2006.01) A61K 31/685 (2006.01)
A61K 31/12 (2006.01)

(21) International Application Number:

PCT/EP2007/001487

(22) International Filing Date:

21 February 2007 (21.02.2007)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

06004820.4 9 March 2006 (09.03.2006) EP

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (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

(88) Date of publication of the international search report:

25 October 2007

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: PHOSPHOLIPID COMPLEXES OF CURCUMIN HAVING IMPROVED BIOAVAILABILITY

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WO 2007/101551 A3

PHOSPHOLIPID COMPLEXES OF CURCUMIN HAVING IMPROVED BIOAVAILABILITY

The present invention relates to novel phospholipids complexes of curcumin or extracts containing it having improved bioavailability.

TECHNOLOGICAL BACKGROUND

Curcumin, [1,7-bis(4-hydroxyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is the major constituent of the spice turmeric extracted from the root of *Curcuma longa* Linn. Curcumin is a polyphenol that has powerful antioxidant and inhibits the expression of the enzyme cyclooxygenase 2 (Cox 2) at least in part *via* interference with activation of the transcription factor NFkB [2, 14]. *In vitro*, curcumin inhibits the growth of cancer cells with an IC₅₀ value of 20-75 µM [6, 16]. In rodent models, curcumin has been shown to prevent cancer in the colon, skin, stomach, duodenum, soft palate, tongue, sebaceous glands and breast [9, 10, 15]. Therefore, curcumin could be considered a promising efficacious and safe cancer chemopreventive agents [18]. In fact, clinical pilot studies have associated curcumin consumption with regression of pre-malignant lesions of bladder, soft palate stomach, cervix and skin [1, 11].

However, concentrations of curcumin achieved in plasma and target tissues are low, probably due, at least in part, to its extensive metabolism by conjugation (glucuronidation and sulfation) and reduction pathways [4, 5, 7, 8].

Preclinical and clinical pilot studies suggest that In a phase I trial plasma and urine concentrations of curcumin in patients, who had ingested 3600 mg curcumin orally, were 11.1 nmol/L and 1.3 µmol/L, respectively [17]. In another study, peak plasma concentrations 1-2 h after oral dosing, reached 0.41-1.75 µM in patients receiving 4 to 8 g curcumin [1]. It is neither practical nor desirable to increase the oral dose of curcumin above that already

investigated.

Therefore, it is highly desirable to find novel curcumin derivatives having improved bioavailability.

Complex compounds of vegetable extracts or of purified components thereof
5 with natural, synthetic or semi-synthetic phospholipids, have been disclosed, e.g., in
EP 209 038, EP 275 005, EP 283 713, EP 1 035 859 and EP 1 140 115. Said
complexes improve the plasma bioavailability of the extract or purified component,
thanks to their lipophilicity. EP 1 140 115 generically mentions ethanol among the
various solvents that can be used of the preparation of said complexes, but does not
10 provide preparation examples which make use of ethanol as the solvent.
Furthermore, the complexes disclosed are phospholipid complexes of
proanthocyanidin A2, which are quite different in the chemical structure with
respect to the phospholipids complexes of curcumin of the present invention.

DISCLOSURE OF THE INVENTION

15 It has now been found that the phospholipids complexes of curcumin,
providing higher systemic levels of parent agent than unformulated curcumin,
can be prepared in protic solvents.

Therefore, the present invention relates to novel phospholipids
complexes of curcumin having improved bioavailability.

20 In one particular embodiment there is provided a phospholipids complex
of curcumin obtained by reaction of a hydroalcoholic extract of turmeric rhizomes
with phospholipids in an alcoholic solvent followed by isolation by concentration
and drying, wherein the phospholipid to curcumin ratio is in a range from
10:1 w/w to 1:1 w/w.

25 According to the invention, phospholipids of either vegetable or
synthetic origin can be used, particularly preferred are soy phospholipids, such
as phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine.

According to the invention, the complexes are prepared by adding the phospholipid to curcumin dissolved in a protic solvent, more particularly adding the phospholipid to the ethanol solution of the hydroalcoholic extract of turmeric rhizomes, under reflux and with stirring. The resulting suspension
5 is concentrated under reduced pressure to a residue which is dried in oven. The ratio of phospholipids to curcumin is in the range from 10 to 1 w/w, a more preferable mole ratio being 5:1 w/w.

The present invention also relates to pharmaceutical compositions containing as the active principle one of the phospholipids complexes of curcumin according to the invention, in admixture with a suitable
10 pharmaceutical carrier.

The present invention further relates to the use of the phospholipids complexes of curcumin of the invention for the preparation of medicaments having chemopreventive action.

15 The following examples further illustrate the invention.

EXAMPLE 1 - Preparation of extract with a low content of curcumin

Ground dry turmeric rhizomes (1000 g) were extracted with ethanol-water 9:1 mixture (6680 ml). The resulting hydroalcoholic solution was concentrated under reduced pressure and drying was completed in oven at
20 60°C under *vacuum* to yield 69.3 g of an orange paste having a curcumin content of 17.4% w/w and a total curcuminoid content of 29.2% w/w.

EXAMPLE 2 - Preparation of extract with high content of curcumin

Ground dry turmeric rhizomes (1000 g) were defatted with hexane (2500 ml) before being extracted with ethanol-water 95:5 mixture (7500 ml).
25 Curcuminoids were then crystallized by slowly adding hexane (500 ml) to the hydroalcoholic solution and allowing the resulting mixture to stand for 24 hours. A microcrystalline orange solid was filtered and dried at 60°C under vacuum to yield 36.7g of extract having a curcumin content of 71.74% and a

total curcuminoid content of 93.8%w/w.

EXAMPLE 3 - Preparation of the complex from a low-content extract

20 g of the product from Example 1 were dissolved in ethanol (400 ml) and the solution was refluxed. 30 g of soy phospholipids were slowly added in portions, under reflux and with stirring. The resulting suspension was refluxed with stirring for 1 hr, then hot concentrated under reduced pressure and finally dried in oven. 46.7 g of an orange waxy product were obtained, having a curcumin content of 7.54% and a total curcuminoid content of 12.1% w/w.

While the starting extract is insoluble in chlorinated solvent, the resulting product is soluble in CHCl_3 in a 4% w/v concentration thus confirming the complexation of the extract by the phospholipid and the formation of a molecular complex.

The ^1H -NMR spectrum of the complex in CDCl_3 shows the main signals of the phospholipids, typical of complexed phospholipids. On the other hand, the ^1H -NMR spectrum of the complex in $\text{DMSO}-d_6$, which is a solvent preventing the aggregation of the complex, shows the signals of the free starting extract as well as those of the phospholipids.

In the ^{31}P -NMR spectra, the signal of ^{31}P in CDCl_3 is at 1.06 ppm and is 35.3 Hz wide, which is typical of complexed phospholipids. The complex is destroyed in $\text{DMSO}-d_6$ as confirmed by the signal of ^{31}P at 0.12 ppm with 3.80 Hz width typical of the free phospholipid.

EXAMPLE 4 - Preparation of the complex from a high-content extract

20 g of turmeric rhizomes extract having a total curcuminoid content of 93.8% w/w were dissolved in ethanol (800 ml) and the solution was refluxed. 80 g of soy phospholipids were slowly added in portions, under reflux and with stirring. The resulting suspension was refluxed with stirring for 1 hr, then hot concentrated under reduced pressure and finally dried in oven. 97.6 g of an orange waxy product were obtained, having a curcumin content of 13.2% and

a total curcuminoid content of 16.9% w/w.

The solubility of the product in CHCl_3 at a concentration of 4% w/v and the NMR spectra are the same as those of Example 3, confirming the formation of the complex.

5 EXPERIMENTAL SECTION

Tests were carried out to compare curcumin bioavailability afforded by the phospholipid complexes of the invention and by the extract containing uncomplexed curcumin.

Materials and Methods

10 Male Wistar rats (250 g) were fasted overnight and received by oral gavage either extract obtained by Example 2 or complex obtained by the Example 4 at dose of 340 mg/Kg in terms of curcumin.

15 Rats were killed at 15, 30, 60 and 120 min. Whole blood was collected into heparinized tubes, centrifuged immediately at 7000xg for 15 min, plasma was then decanted and stored at -80°C until analysis.

The presence of curcumin and metabolites was verified by negative ion electrospray liquid chromatography/tandem mass spectrometry as previously described (4,5,7).

PHARMACOKINETIC ANALYSIS

20 Peak plasma levels and area under the plasma concentration time curve (AUC) values for parent curcumin after administration of curcumin complexed with phospholipids as described in Example 4 were five-fold higher than the equivalent values seen after treatment with extract of Example 2 (uncomplexed curcumin) (Table 1).

Table 1. Estimated plasma C_{max}, T_{max} and AUC values for unformulated and formulated curcumin.

	C _{max} (nM)	T _{max} (min)	AUC (µg. min/mL)*
<u>Extract of Example 2</u>			
Curcumin	6.5 ± 4.5	30	4.8
Curcumin glucuronide	225 ± 0.6	30	200.7
Curcumin sulfate	7.0 ± 11.5	60	15.5
<u>Complex of Example 4</u>			
Curcumin	33.4 ± 7.1	15	26.7
Curcumin glucuronide	4420 ± 292	30	4764.7
Curcumin sulfate	21.2 ± 3.9	60	24.8

* AUC was calculated using WinNonLin and employing a non-compartmental model.

Results

The results show that the phospholipids complexes of curcumin of the invention provides higher systemic levels of parent agent than uncomplexed curcumin.

The improved bioavailability of phospholipid complex of curcumin increases the potential scope of medical applications for curcumin as a chemopreventive agent.

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CLAIMS

1. A phospholipids complex of curcumin obtained by reaction of a hydroalcoholic extract of turmeric rhizomes with phospholipids in an alcoholic solvent to form a reaction mixture comprising the phospholipids complex of curcumin, followed by isolation of the phospholipids complex of curcumin, wherein the isolation consists of concentration and drying of the reaction mixture, and wherein the phospholipid to curcumin ratio is in a range from 10:1 w/w to 1:1 w/w.
2. The complex of claim 1, wherein the phospholipids are soy phospholipids.
3. The complex of claim 2, wherein the phospholipids are phosphatidyl choline, phosphatidyl serine, or phosphatidyl ethanolamine.
4. The complex of claim 2, wherein the phospholipid to curcumin ratio is 5:1 w/w.
5. A process for preparing the complex of any one of claims 1 to 4, wherein a hydroalcoholic extract of turmeric rhizomes is reacted with phospholipids in an alcoholic solvent, and the complex is then isolated by a step consisting of concentration and drying of the reaction mixture.
6. The process of claim 5, wherein the alcoholic solvent is ethanol.
7. A pharmaceutical composition containing as the active principle the complex as claimed in any one of claims 1 to 4, in admixture with a suitable pharmaceutical carrier.
8. A use of the complex of any one of claims 1 to 4 for preparing chemopreventive medicaments.
9. A use of the complex of any one of claims 1 to 4 as chemopreventive medicaments.