

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

(19) World Intellectual Property
Organization
International Bureau



WIPO | PCT



(10) International Publication Number
WO 2016/055454 A1

(43) International Publication Date
14 April 2016 (14.04.2016)

(51) International Patent Classification:

A61K 31/282 (2006.01) A61K 31/4725 (2006.01)
A61K 31/337 (2006.01) A61K 31/506 (2006.01)
A61K 31/395 (2006.01) A61K 31/513 (2006.01)
A61K 31/404 (2006.01) A61K 31/65 (2006.01)
A61K 31/4178 (2006.01) A61P 35/00 (2006.01)

(21) International Application Number:

PCT/EP2015/073017

(22) International Filing Date:

6 October 2015 (06.10.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

MI2014A001746 6 October 2014 (06.10.2014) IT

(71) Applicant: INTERNATIONAL SOCIETY FOR DRUG DEVELOPMENT S.R.L. [IT/IT]; Viale Montenero, 70, I-20135 Milano (IT).

(72) Inventors: RAPPOSELLI, Simona; Via dei Boschi, 118, I-55012 Lucca (IT). MARTINI, Claudia; Via Santa Maria, 65, I-56126 Pisa (IT). CALDERONE, Vincenzo; Via Bagnone, 15, I-54100 Massa (IT). PURICELLI, Guido; Viale Monte Nero, 70, I-20135 Milano (IT).

(74) Agents: CATTANEO, Elisabetta et al.; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria 9, I-20122 Milano (IT).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

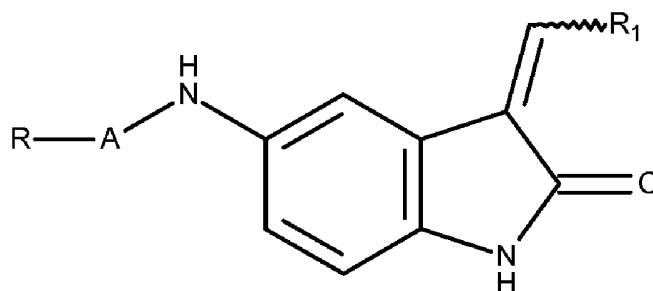
Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

Published:

- with international search report (Art. 21(3))

(54) Title: PHARMACEUTICAL COMBINATION FOR THE TREATMENT OF TUMORS



(I)

(57) Abstract: The invention concerns a combination comprising at least one 2-oxo-indole derivative of formula (I) in which R₁ is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by (C₁-C₃) alkyl, A is a -CH₂CO- or -SO₂- group; R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinyl, 3,4-dimethoxy-benzylamino, (C₁-C₃)alkyl, benzyl or a pharmaceutically acceptable salt thereof and at least one antitumor drug. The combination is for use in the treatment of tumors, in particular glioblastoma multiforme (GBM), breast tumor and pancreatic tumor. The combination is effective in the treatment of resistant tumor forms.

WO 2016/055454 A1

“Pharmaceutical combination for the treatment of tumors”

DESCRIPTION

FIELD OF THE INVENTION

5 The present invention concerns a pharmaceutical combination of at least one 2-oxo-indole derivative of formula (I) and at least one antitumor drug. The combination of the invention is effective in the treatment of tumors, preferably in the treatment of glioblastoma, breast tumor and pancreatic tumor.

STATE OF THE ART

10 Despite the ongoing development of new therapeutic treatments, such as surgery, radiotherapy and chemotherapy, some tumors, including glioblastoma multiforme (abbreviated to GBM), continue to be aggressive and lethal tumors.

In view of the disappointing results achieved by conventional therapies, the search for possible alternative therapies able to significantly improve the outcome
15 of patients in these aggressive tumors is an evident need felt by cancer researchers.

The growing understanding of the complex biological networks (molecular pathways) relevant for the development and progression of the tumor led to the identification of several pharmacological targets and stimulated research to
20 develop new "targeted therapies" aimed at more effective treatment of unresponsive tumors with respect to conventional therapies or at preventing the onset of recurrences.

The process of carcinogenesis is the result of an imbalance between the physiological phenomena of cell division and growth and the normal process of
25 programmed death (apoptosis). In the context of this delicate balance, proteins and signal transduction pathways which regulate growth, cell differentiation and development often undergo genetic alterations which induce oncogenic modifications. The pathway of PI3K/Akt/mTOR is one of the most important intracellular signaling pathways involved in the mechanisms of cell growth and
30 survival. This pathway is based on the cascade sequence of particular phosphorylation reactions, assured by various proteins with protein-kinase action, the main ones being PI3K, Akt and mTOR. This signaling cascade is

constitutively expressed and hyperactive in many types of cancer, due to loss of the functionality of the PTEN factor (tumor suppressor), the amplification or mutation of PI3K, the amplification or mutation of Akt, the activation of receptors of the growth factors or exposure to carcinogenic agents. Once activated, it can be propagated through phosphorylation (activation) of a wide range of downstream effectors, among which the role of mTOR, a protein translocation regulator, is emerging. In particular, it has been observed that this pathway is hyperactive in many tumor forms, such as glioblastoma, multiple myeloma, tumor of the lungs, multiple myeloma, lung cancer, cancer of the head and neck, breast cancer, stomach cancer, acute myeloid leukemia, endometrial cancer, melanoma, kidney cancer, ovarian cancer, prostate cancer and cancer of the colon. Furthermore, the pathway' hyperactivity characterizes numerous tumor forms with fatal prognosis: the phosphorylation of Akt at the level of serine 473 (S473), and therefore the activation of this pathway, has been associated with an unfavorable prognosis in some tumor forms such as NSCLC tumors (non-small cell lung cancer), skin cancer (Dai DL, Martinka M, Li G.J Clin Oncol. 2005 Mar 1;23(7):1473-82), pancreas cancer (Schlieman et al., Incidence, mechanism and prognostic value of activated AKT in pancreas cancer Br J Cancer. 2003 Dec 1;89(11):2110-5; Yamamoto S et al., Prognostic significance of activated Akt expression in pancreatic ductal adenocarcinoma. Clin Cancer Res. 2004 Apr 15;10(8):2846-50), liver cancer (Nakanishi K, et al. Akt phosphorylation is a risk factor for early disease recurrence and poor prognosis in hepatocellular carcinoma. Cancer. 2005 Jan 15;103(2):307-12), prostate cancer (Kreisberg JI et al. Phosphorylation of Akt (Ser473) is an excellent predictor of poor clinical outcome in prostate cancer. Cancer Res. 2004;64(15):5232-6), breast cancer (Perez-Tenorio and Stal, Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer. 2002;86(4):540-5), endometrial cancer (Terakawa et al., Loss of PTEN expression followed by Akt phosphorylation is a poor prognostic factor for patients with endometrial cancer. Endocr Relat Cancer. 2003;10(2):203-82003), stomach cancer (Nam et al., Akt/PKB activation in gastric carcinomas correlates with clinicopathologic variables and prognosis. APMIS. 2003;111(12):1105-13.), brain cancer (Ermoian

et al., 2002), and blood cancer. The hyperactivation of the PI3K/Akt/mTOR pathway is furthermore one of the factors responsible for the onset of resistance to many chemotherapy treatments.

Given the importance of the PI3K/Akt/mTOR axis in the etiopathogenesis of numerous cancer types, logically speaking, in the latest antitumor therapeutic approaches, each of these proteins should be considered an optimal molecular target for the design and synthesis of new drugs. Pharmacological treatments of this type are currently considered more effective antineoplastic therapies than the conventional therapy [Cheng J.Q., Lindsley CW, Cheng GZ, Yang H, Nicosia SV. (2005). The Akt/PKB pathway: molecular target for cancer drug discovery. *Oncogene*;24(50):7482-92], especially in the case of treatment of neoplasias which do not respond to chemotherapy and/or radiotherapy.

Therefore, in line with the need to provide new therapeutic/pharmacological approaches which are alternative and/or complementary to traditional chemotherapy, and on the basis of the latest knowledge on the role of the PI3K/Akt/mTOR pathway in tumor pathologies, new molecules have been synthesized able to interfere with the activity of these cell pathways, in particular with the PI3K-Akt-mTOR pathway.

For said purpose 2-oxo-indole derivative compounds were prepared. These compounds showed to be able to interfere with the activity of this pathway, thus constituting new hypotheses of molecules with antitumor activity vis-à-vis lung tumor (Nesi et al, *ACS Medicinal Chemistry Letters*, "Synthesis of Novel 3,5-disubstituted-2-oxindole derivative as antitumor agents against Non-Small Cell Lung cancer", 2013).

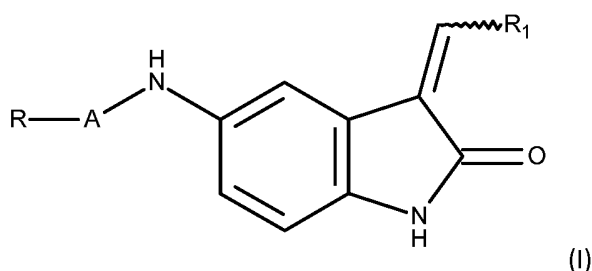
Specifically, the 2-oxo-indole derivative compounds showed a powerful antiproliferative activity associated with inhibition of the phosphorylation of Akt and with the block of the cell cycle at the G1/S phase in human NSCLC (Non-Small Cell Lung Cancer) cells.

In line with the pressing need to provide new therapeutic/pharmacological approaches which are alternative and/or complementary to traditional chemotherapy, the object of the present invention is to provide alternative therapeutic approaches vis-à-vis aggressive tumor forms such as glioblastoma.

SUMMARY OF THE INVENTION

With the intention of identifying new drugs, the inventors of the present invention have surprisingly identified a combination of traditional drugs with molecules able to act against some effectors involved in transduction of the cell signal, which has proved to be more effective than the mono-therapy treatment.

The object of the invention has therefore been achieved by means of a combination comprising at least one 2-oxo-indole derivative of formula (I)



wherein

- 10 R₁ is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by (C₁-C₃) alkyl,

A is a -CH₂CO- or -SO₂- group;

- R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinyl, 3,4-dimethoxy-benzylamino, (C₁-C₃) alkyl, benzyl or a pharmaceutically acceptable salt thereof and at least one antitumor drug.

The idea of the inventors of the present invention was therefore to test a combination comprising one or more molecules able to interfere with the effectors involved in the PI3K-Akt-mTOR pathway in order to improve the activity of the antitumor agent, overcoming the resistance which limits the effectiveness thereof.

- 20 Said combination advantageously had a synergic effect with respect to the activity of the single elements constituting the combination, overcoming the effectiveness limits of the mono-therapy treatment.

The invention will now be described in detail and subsequently exemplified in the experimental part.

25 DESCRIPTION OF THE FIGURES

Figure 1 shows the scheme 1 for preparation of the compounds 1-5.

Figure 2 shows the scheme 2 for preparation of the compounds 6-11.

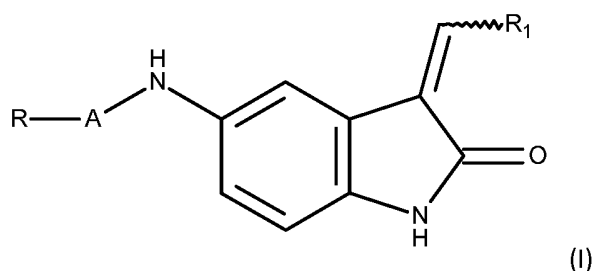
Figure 3 shows the results of the cell viability in the MTS assay of the

combination of compound 9 of the invention and of temozolomide. The U87MG cells were treated with the compound 1 (100 nM, 500 nM, 1 μ M) and/or with TMZ (10 or 100 μ M) for 72h. At the end of the treatments, the cell viability was evaluated using the MTS assay. The values are expressed as a % with respect to the control, and represent the mean value \pm SEM. The statistical analysis was conducted using the ANOVA/Bonferroni one-way test. *P<0.05, ** P<0.01, *** P<0.001 vs. control cells; ## P<0.01, ### P<0.001 vs. compound 9 only; §§§ P<0.001 vs. TMZ only.

Figure 4 shows the results of the cell viability in the WST1 (Water Soluble Tetrazolium Salt 1) assay of the combination of compound 1 of the invention and of temozolomide. The graphs show the inhibitory effect produced by temozolomide (TMZ, 100 μ M), by compound 1 (at the concentrations 10 and 100 μ M), by the association of the two compounds (TMZ 100 μ M + compound 1 100 μ M) or by the association of the two compounds (TMZ 100 μ M + compound 1 10 μ M), on the human cell lines U118-MG and ANGM-CSS of glioblastoma. The cell viability was evaluated using the WST1 (Water Soluble Tetrazolium Salt 1) assay. The values are expressed as a % with respect to the control, and represent the mean value \pm SEM. The statistical analysis was conducted using the ANOVA/Bonferroni one-way test. ** P<0.01, *** P<0.001 vs. control cells.

20 DETAILED DISCLOSURE OF THE INVENTION

The invention therefore concerns a combination comprising at least one 2-oxo-indole derivative of formula (I)



wherein

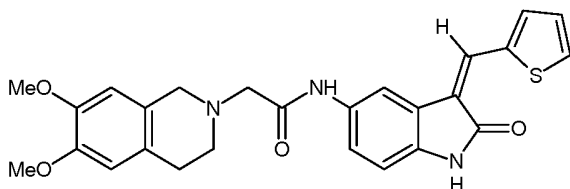
- 25 R₁ is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by (C₁-C₃) alkyl,
 A is a -CH₂CO- or -SO₂- group;
 R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-

tetrahydroisoquinolyl, 3,4-dimethoxy-benzylamino, (C₁-C₃)alkyl, benzyl or a pharmaceutically acceptable salt thereof and at least one antitumor drug.

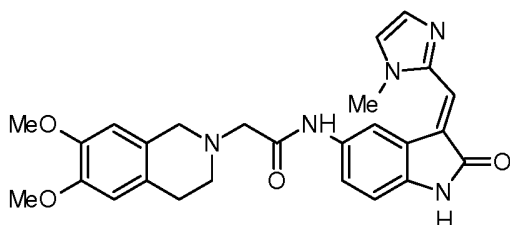
In the oxo-indole derivative of Formula (I), R₁ is selected from the group consisting of thienyl, imidazolyl and pyridyl, preferably it is thienyl or imidazolyl.
 5 A can be a methyl carbonyl or sulfonyl group. When A is -SO₂-, R₁ is preferably imidazolyl.

R is preferably selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl, 3,4-dimethoxy-benzylamino, methyl and benzyl, more preferably it is 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl or benzyl.
 10

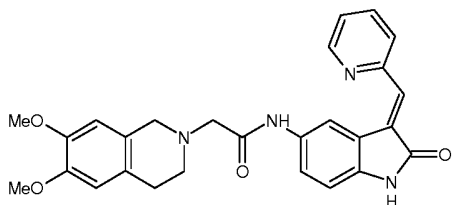
At least one 2-oxoindole derivative of the invention is preferably a compound selected from the group consisting of



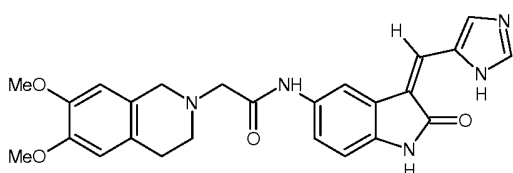
15 N-[(3Z)-(2-oxo-3-(thiophene-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide



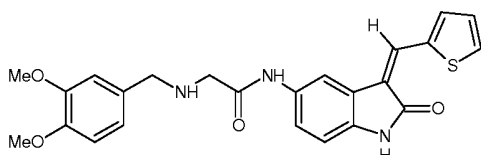
N-[(3E)-(2-oxo-3-((1-methyl-1H-imidazol-2-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide



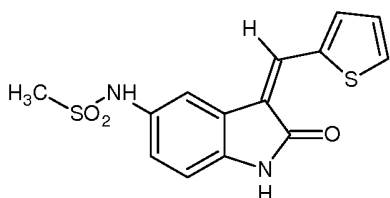
20 N-[(3E)-(2-oxo-3-((pyridine-2-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide



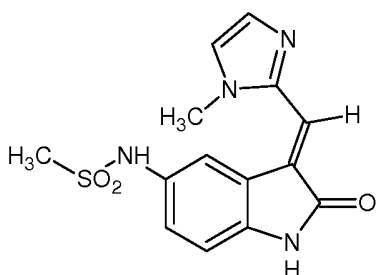
N-[(3Z)-2-oxo-3-((1H-imidazol-5-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)acetamide



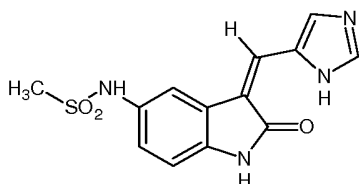
- 5 N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]-2-(3,4-dimethoxybenzylamino)acetamide



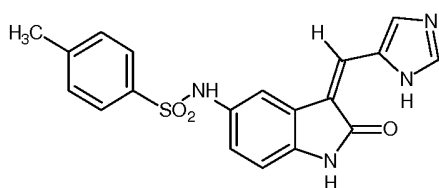
N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



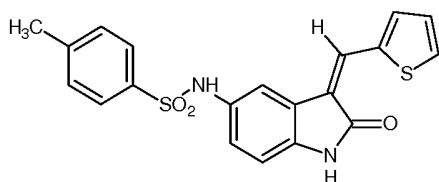
- 10 N-[(3E)-3-[(1-methyl-1H-imidazol-2-yl)methylidene]-2-oxo-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



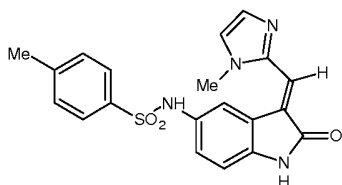
- 15 N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide



- 5 N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide



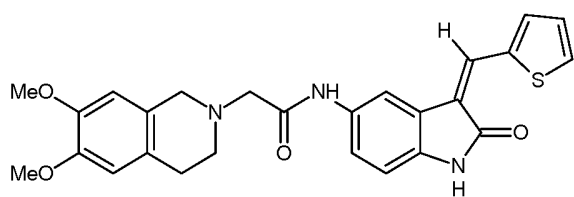
N-[(3E)-3-[(1-methyl-1H-imidazol-2-yl)methylidene]-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide.

- 10 The at least one 2-oxoindole derivative of the invention can be in the form of a pharmaceutically acceptable salt, preferably selected from the group consisting of hydrochlorides, phosphates, sulfates, oxalates, tartrates, maleates, citrates and succinates.

- The compound N-[(3Z)-(2-oxo-3-(thiophene-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl)-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide and the compound N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]-2-(3,4-dimethoxybenzylamino)acetamide are preferably in the form of hydrochlorides.

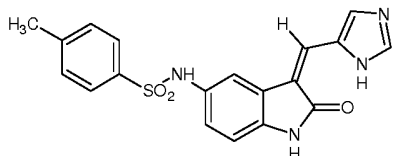
Preferably the at least one 2-oxoindole derivative of the invention is

- 20 N-[(3Z)-(2-oxo-3-(thiophene-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl)-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide



or

N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide, more preferably in the form of hydrochloride



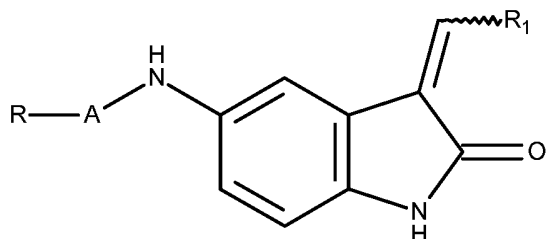
- 5 The at least one antitumor drug is preferably a chemotherapy or radiotherapy agent, more preferably already known for the treatment of a specific tumor. In an advantageous form of the invention, the at least one antitumor agent is selected from temozolomide, 5-fluorouracil, gemcitabine and temozolomide, cisplatin, paclitaxel and doxorubicin.

- 10 In a first preferred and advantageous embodiment, the combination of the invention comprises N-[(3Z)-(2-oxo-3-(thiophene-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide and temozolomide.

- 15 In a second preferred and advantageous form, the combination of the invention comprises N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide and temozolomide.

The combination of the invention can be used to treat a tumor.

Therefore the invention concerns a combination comprising at least one 2-oxo-indole derivative of formula (I)



(I)

20

wherein

R₁ is selected from the group consisting of thienyl, imidazole and pyridyl,

optionally substituted by (C₁-C₃) alkyl,

A is a –CH₂CO- or –SO₂- group;

R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl, 3,4-dimethoxy-benzylamino, (C₁-C₃)alkyl, benzyl or a
5 pharmaceutically acceptable salt thereof and at least one antitumor drug for use
in the treatment of tumors.

The tumor for which the combination of the invention is used is preferably glioblastoma multiforme (GBM)), breast tumor and pancreatic tumor.

Advantageously and surprisingly the combination of the invention was effective
10 against tumors resistant to the chemotherapy and/or radiotherapy agent.

In the preferred and advantageous form of the invention, the combination is preferably used in the treatment of glioblastoma multiforme (GBM).

Advantageously, according to the invention, the combination of the invention has shown synergic effects, in particular constituting a valid pharmacological strategy
15 for the treatment of chemoresistant tumor forms.

Experimental part

Example 1: Preparation of the 2-oxoindole derivative compounds

In the following experimental part, all the 2-oxoindole derivative compounds were prepared following the indications provided in "Synthesis of new enzyme
20 inhibitors as potential tools for the antineoplastic therapy"-PhD thesis in Drug
Science and Bioactive Substances- XXIV cycle- G. Nesi.

The derivatives 1-5 were synthesized following the synthetic procedure reported in scheme 1 of Figure 1.

The 5-nitro-1,3-dihydro-2*H*-indol-2-one commercial compound was reduced to
25 the corresponding 5-amino-1,3-dihydro-2*H*-indol-2-one by means of catalytic
hydrogenation using Pd/C as a catalyst. The subsequent reaction with the chloro
acetyl chloride provided 2-chloro-*N*-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)acetamide
which was condensed with the appropriate amine derivative to provide,
respectively, 2-[(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1*H*)-yl)]-*N*-(2-oxo-2,3-
30 dihydro-1*H*-indol-5-yl)acetamide and 2-[(3,4-dimethoxybenzyl)amino]-*N*-(2-oxo-
2,3-dihydro-1*H*-indol-5-yl)acetamide. Lastly, condensation of the appropriate

aromatic carbaldehyde in the presence of pyrrolidine provided the desired products 1-5.

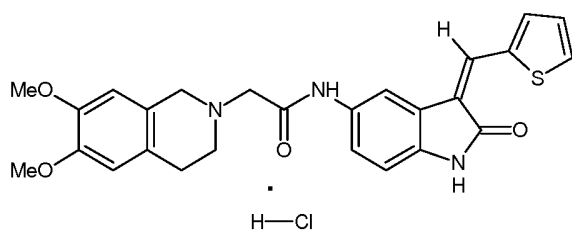
The compounds 6-11 were prepared following the synthetic procedure reported in scheme 2 of Figure 2.

- 5 The condensation reaction of 5-amino-1,3-dihydro-2H-indol-2-one with the tosyl chloride or with the mesyl chloride provided, respectively, *N*-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)-4-methylbenzenesulfonamide and *N*-(2-oxo-2,3-dihydro-1*H*-indol-5-yl)methanesulfonamide. The products obtained were subjected to a condensation reaction with the appropriate aromatic carbaldehydes to provide
- 10 the desired compounds 6-11.

The following 2-oxo-indole derivatives were then prepared:

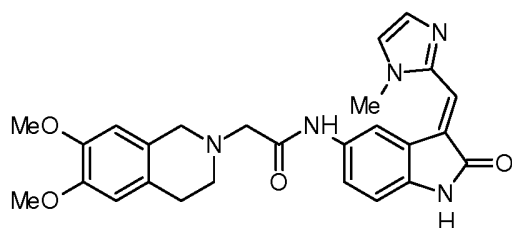
Compound 1:

N-[(3*Z*)-(2-oxo-3-(thiophene-2-ylmethylene)-2,3-dihydro-1*H*-indol-5-yl)]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1*H*)-yl)-acetamide hydrochloride



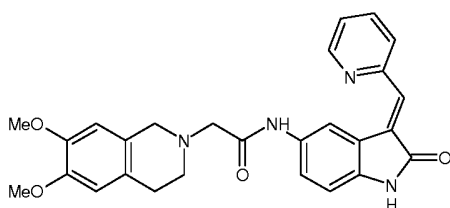
Compound 2:

N-[(3*E*)-(2-oxo-3-((1-methyl-1*H*-imidazol-2-yl)methylene)-2,3-dihydro-1*H*-indol-5-yl)]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1*H*)-yl)-acetamide



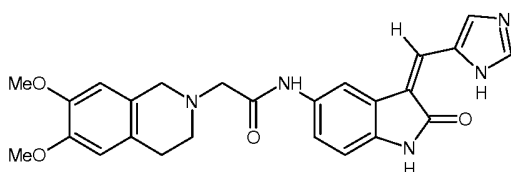
Compound 3:

N-[(3*E*)-(2-oxo-3-((pyridine-2-yl)methylene)-2,3-dihydro-1*H*-indol-5-yl)]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1*H*)-yl)-indolin-5-yl)acetamide



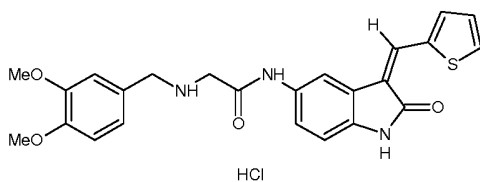
Compound 4:

5 N-[(3Z)-2-oxo-3-((1H-imidazol-5-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)acetamide



Compound 5:

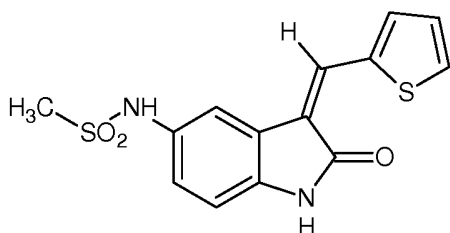
10 N-[(3Z)-2-oxo-3-((1H-imidazol-5-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)acetamide hydrochloride



10

Compound 6:

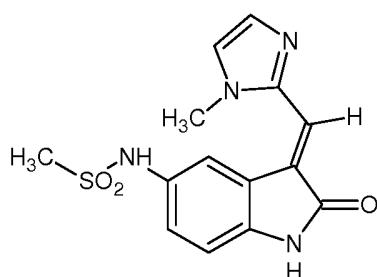
N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



15

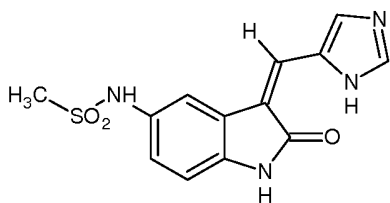
Compound 7:

N-[(3E)-3-[(1-methyl-1H-imidazol-2-yl)methylidene]-2-oxo-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



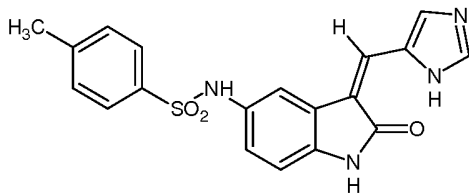
Compound 8:

N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



Compound 9:

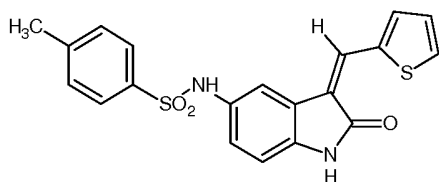
N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide



10

Compound 10:

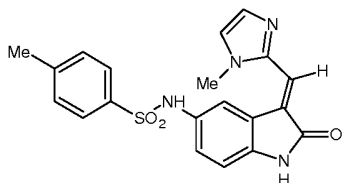
N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide



15

Compound 11:

N-[(3E)-3-[(1-methyl-1H-imidazol-2-yl)methylidene]-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide



The compounds 1 and 9 were used in combination with temozolomide (TMZ) in the treatment of glioblastoma cell lines.

- 5 Temozolomide is a first-line drug for the treatment of glioblastoma; unfortunately, its use in therapy is limited due to the onset of resistance which guarantees survival of the cancer cells (Carmo A, Carneiro H, Crespo I, et al. Effect of temozolomide on the U-118 glioma cell line. *Oncol Lett* 2011;2(6):1165-1170).

Example 2. Combination comprising compound 9 and temozolomide (TMZ)

- 10 Specifically, Glioblastoma Multiforme cells, named U87MG, were used.

The human cells of glioblastoma multiforme U87MG were obtained from the national cancer research institute in Genoa (Italy) and were monitored to determine the DNA profiling. The U87MG cells were cultivated in RPMI medium with the addition of 10% FBS, 2 mM of L-glutamine, 100 U/ml of penicillin, 100
15 mg/ml of streptomycin and 1% of non-essential amino acids at 37°C in 5% CO₂.

The cells were used in the successive experiments up to the fourth detaching passage.

The U87MG cells were grown at 37°C in a humid atmosphere in the presence of 5% CO₂ and were placed in a plate at a density of 3,000 cells/well. After 24
20 hours, the culture medium was replaced with fresh culture medium containing different concentrations of the compound 9 solubilized in DMSO. After 72 hours, the cell viability was assayed by means of MTS assay according to the manufacturer's instructions. Briefly, the assay consists in the reduction of 3-(4,5-

- 25 dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) to soluble formazan by the mitochondrial dehydrogenase. The formazan formed is determined via reading of the absorbance at 490 nM measured with an automated plate reader (Victor Wallac 2, Perkin Elmer). Each experiment was conducted in triplicate. The results were calculated by subtracting the mean background value from the values obtained from each

evaluation and were expressed as a control percentage (non-treated cells). To examine the potential synergic effect of cell growth inhibition, the cells of human Glioblastoma Multiforme (GBM) U87MG were treated for 72 hours with increasing concentrations of compound 9 (100 nM, 500 nM, 1 μ M), in the absence or in the presence of temozolomide (TMZ, 10 μ M or 100 μ M). At the end of the treatments, the cell viability was determined by means of MTS assay. The results obtained are shown in Figure 1.

The results obtained showed that compound 9 alone inhibits cell viability, in a concentration-dependent way, with significant effect starting from 500 nM (Figure 1). The compound 9 combined with temozolomide induces a significant increase in inhibition of the cell viability with respect to the single treatment with compound 9 (at all concentrations tested) or with temozolomide (at 10 μ M and at 100 μ M). Said data show that the combination of compound 9 with temozolomide produces a synergic effect on blocking of the glioblastoma cell proliferation.

Example 3: Combination comprising compound 1 and temozolomide (TMZ)

The cell line ANGM-CSS and U118-MG were used, human glioblastoma multiforme (GBM) lines. The ANGM-CSS cell line (ECACC cat n°08040401) was supplied by Dr. Angelo Notarangelo, Laboratorio di Citogenetica, IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo (FG), Italy. The U118-MG line was purchased from ATCC, Manassas, VA, USA. The culture medium consisted of DMEM:HAMS F12 (1:1) (Sigma-Aldrich) with the addition of 2mM of L-Glutamine (Sigma Aldrich), 10% Fetal Bovine Serum (FBS, Sigma-Aldrich), 100 units/ml penicillin and 100 μ g/ml streptomycin (P/S) at 37°C in an atmosphere with 5% CO₂.

The cytotoxicity was evaluated via a colorimetric assay, using WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolium]-1,3-benzene disulfonate) (Roche, Mannheim, Germany). The inhibition of the cell proliferation is expressed as a percentage of the absorbance at 450nm relative to the non-treated cells in culture. The absorbance was measured by an Enspire microplate reader (PerkinElmer, Wellesley, MA, USA).

The compound 1 was tested on the two cell lines of human glioblastoma multiforme ANGM-CSS and U118MG, which are resistant to temozolomide. In

particular, as regards the cell line ANGM-CSS, it shows an amplification of the genes MET and EGFR (Notarangelo A, Trombetta D, Parrella P et al. Establishment and genetic characterization of ANGM-CSS, a novel, immortal cell line derived from a human glioblastoma multiforme. *Int J Oncol.* 2013;10.3892/ijo.2013.2224), whereas the U118MG presents a PTEN mutation (Verreault M, Wepler SA, Stegeman A et al. Combined RNAi-Mediated Suppression of Rictor and EGFR Resulted in Complete Tumor Regression in an Orthotopic Glioblastoma Tumor Model *PLoSOne.* 2013;8(3):e59597).

The results are shown in Figure 2.

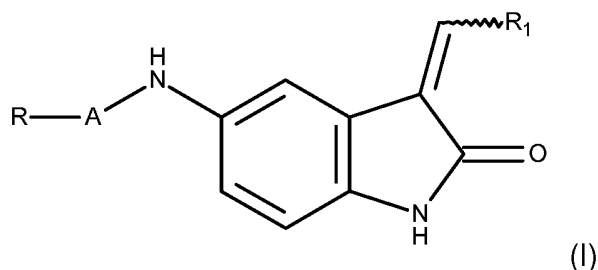
10 The administration of the compound 1 alone (10 μ M and 100 μ M) induces a significant reduction of cell viability in the U118-MG. In this cell line, temozolomide alone (100 μ M) causes a reduction in viability. The association of the compound 1 with temozolomide induces a synergic inhibitory effect on cell growth.

15 In the ANGM-CSS, temozolomide alone (100 μ M) has no significant effect on cell viability, said result was observed also following administration of compound 1 alone at 10 μ M. On the other hand, treatment with compound 1 alone at a concentration 10 times higher (100 μ M) induces a significant reduction in cell viability. The co-administration of compound 1 (10 μ M) and temozolomide (100 μ M) causes a significant reduction in viability which is more marked following
20 co-administration of the compound 1 and temozolomide at 100 μ M.

From the results reported, it is evident that co-administration of the compound 1 together with temozolomide represents a valid pharmacological strategy for the treatment of chemoresistant forms of glioblastoma.

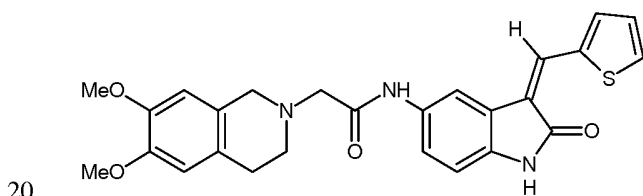
CLAIMS

1. A combination comprising at least one 2-oxo-indole derivative of formula (I)

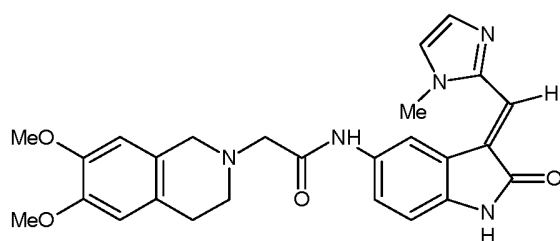


wherein

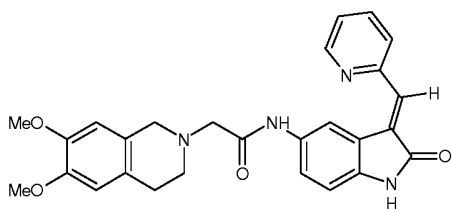
- 5 R_1 is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by (C₁-C₃)alkyl,
 A is a -CH₂CO- or -SO₂- group;
 R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl, 3,4-dimethoxy-benzylamino, (C₁-C₃)alkyl, benzyl or a
 10 pharmaceutically acceptable salt thereof and at least one antitumor drug.
2. The combination of claim 1, wherein R_1 is thienyl or imidazolyl.
3. The combination of claim 1 or 2, wherein R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl, 3,4-dimethoxy-benzylamino, methyl and benzyl.
- 15 4. The combination of claim 3, wherein R is 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl or benzyl.
5. The combination according to claim 1, wherein the at least one 2-oxoindole derivative is a compound selected from the group consisting of



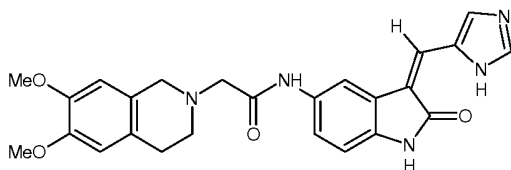
N-[(3Z)-(2-oxo-3-(thiophene-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide



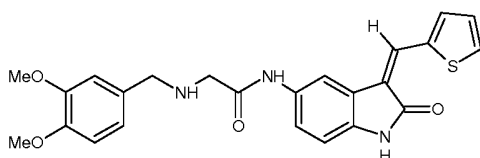
N-[(3E)-2-oxo-3-((1-methyl-1H-imidazol-2-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)-acetamide



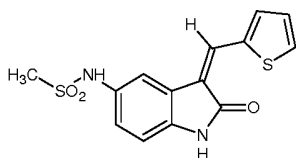
- 5 N-[(3E)-2-oxo-3-((pyridine-2-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)-acetamide



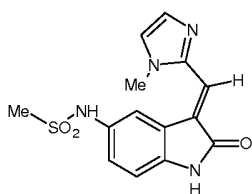
N-[(3Z)-2-oxo-3-((1H-imidazol-5-yl)methylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)-acetamide



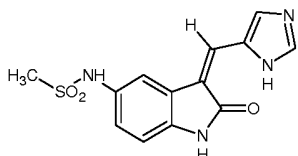
- 10 N-[(3Z)-2-oxo-3-(thiophen-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]-2-(3,4-dimethoxybenzylamino)acetamide



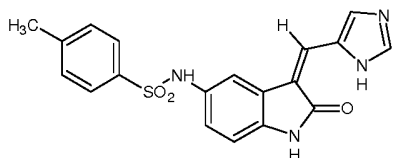
- 15 N-[(3Z)-2-oxo-3-(thiophen-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]methanesulfonamide



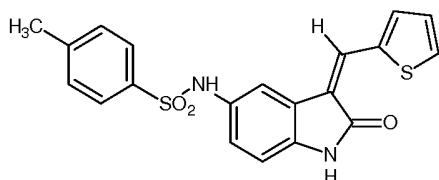
N-((3E)-3-[(1-methyl-1H-imidazol-2-yl)methylidene]-2-oxo-2,3-dihydro-1H-indol-5-yl)methanesulfonamide



5 N-((3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl)methanesulfonamide

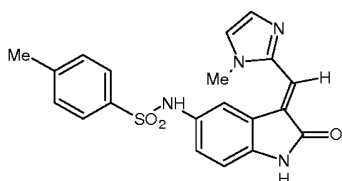


N-((3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl)-4-methylbenzenesulfonamide



10

N-((3Z)-2-oxo-3-(thiophen-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl)-4-methylbenzenesulfonamide



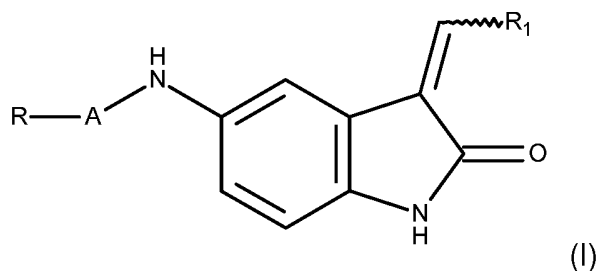
15

N-((3E)-3-[(1-methyl-1H-imidazol-2-yl)methylidene]-2-oxo-2,3-dihydro-1H-indol-5-yl)-4-methylbenzenesulfonamide.

6. The combination of claim 5, wherein the at least one 2-oxoindole derivative is N-((3Z)-2-oxo-3-(thiophen-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl)-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide or N-((3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl)-4-

methylbenzenesulfonamide.

7. The compound according to any one of claims 1 to 6, wherein said pharmaceutically acceptable salt is selected from the group consisting of hydrochloride, phosphate, sulfate, oxalate, tartrate, maleate, citrate and succinate.
8. The combination according to any one of claims 1 to 7, wherein the at least one antitumor drug is a chemotherapeutic agent or radiotherapeutic agent, which is already known for the treatment of a specific tumor.
9. The combination according to any one of claims 1 to 7, wherein the at least one antitumor drug is selected from temozolomide, 5-fluorouracil, gemcitabine and temozolomide, cisplatin, paclitaxel, doxorubicin.
10. The combination according to claim 1 comprising N-[(3Z)-(2-oxo-3-(thiophen-2-ylmethylene)-2,3-dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)-acetamide and temozolomide.
11. The combination according to claim 1 comprising N-[(3Z)-3-(1H-imidazol-5-ylmethylidene)-2-oxo-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide and temozolomide.
12. A combination comprising at least one 2-oxo-indole derivative of formula (I)



- wherein
- R_1 is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by (C₁-C₃) alkyl,
- A is a -CH₂CO- or -SO₂- group;
- R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoliny, 3,4-dimethoxy-benzylamino, (C₁-C₃)alkyl, benzyl or a pharmaceutically acceptable salt thereof and at least one antitumor drug for use in the treatment of tumors.
13. The combination according to claim 12, wherein the tumor is glioblastoma

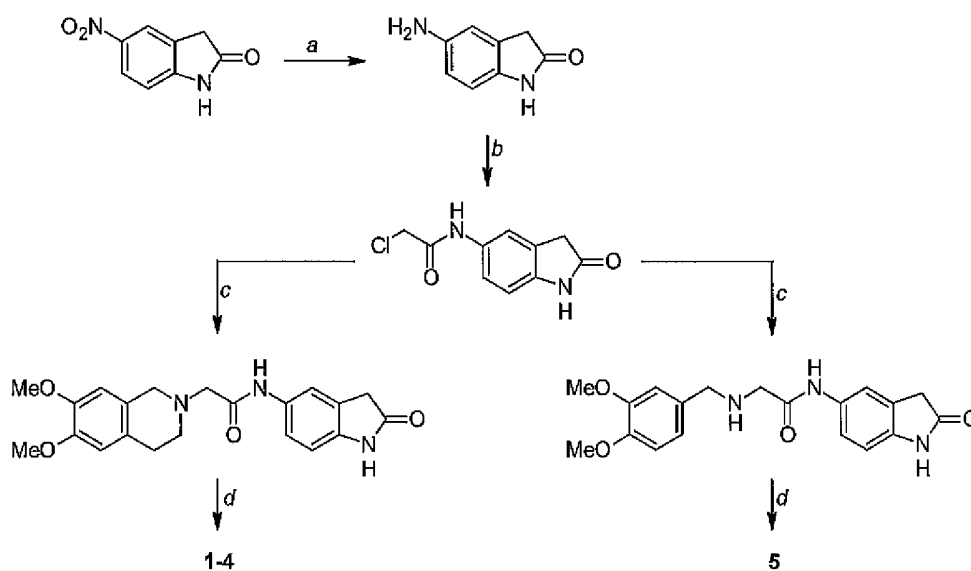
multiforme (GBM), breast tumor, pancreatic tumor.

14. The combination according to claim 13, wherein the tumor is glioblastoma multiforme (GBM).

5 15. The combination according to claim 11, wherein the tumor is a tumor resistant to the chemotherapeutic and/or radiotherapeutic agent.

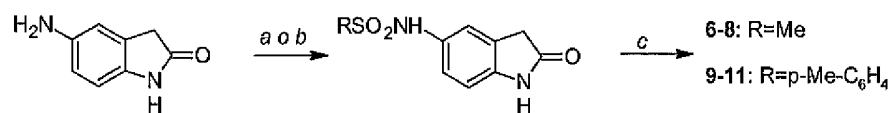
16. The combination according to claim 15, wherein the tumor is glioblastoma.

1/4



Reagents and Conditions: (a) H_2 , Pd/C, EtOH, 20°C; (b) ClCOCH_2Cl , Acetone, DMF, 20°C; (c) K_2CO_3 , DMF, CH_3CN , 82°C; (d) Appropriate carbaldehyde, EtOH, pyrrolidine, 110°C.

Figure 1



Reagents and Conditions: (a) p-toluensulfonylchloride, H_2O , 20°C ; (b) Methansulfonylchloride, H_2O , 20°C ; (c) Appropriate carbaldehyde, EtOH , pyrrolidine, 110°C .

Figure 2

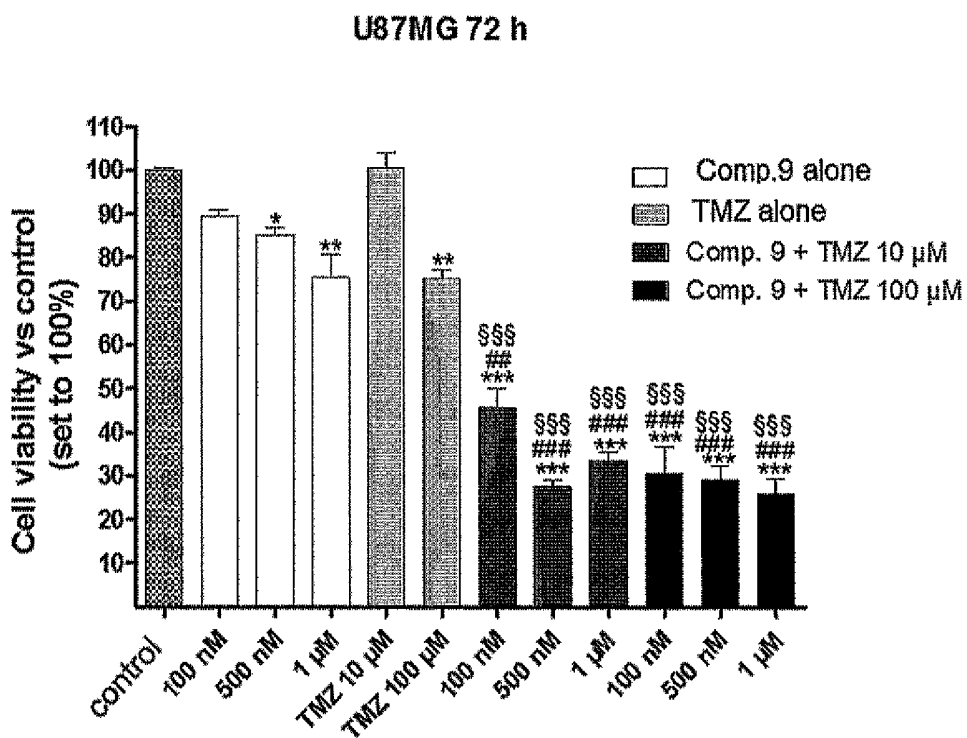


Figure 3

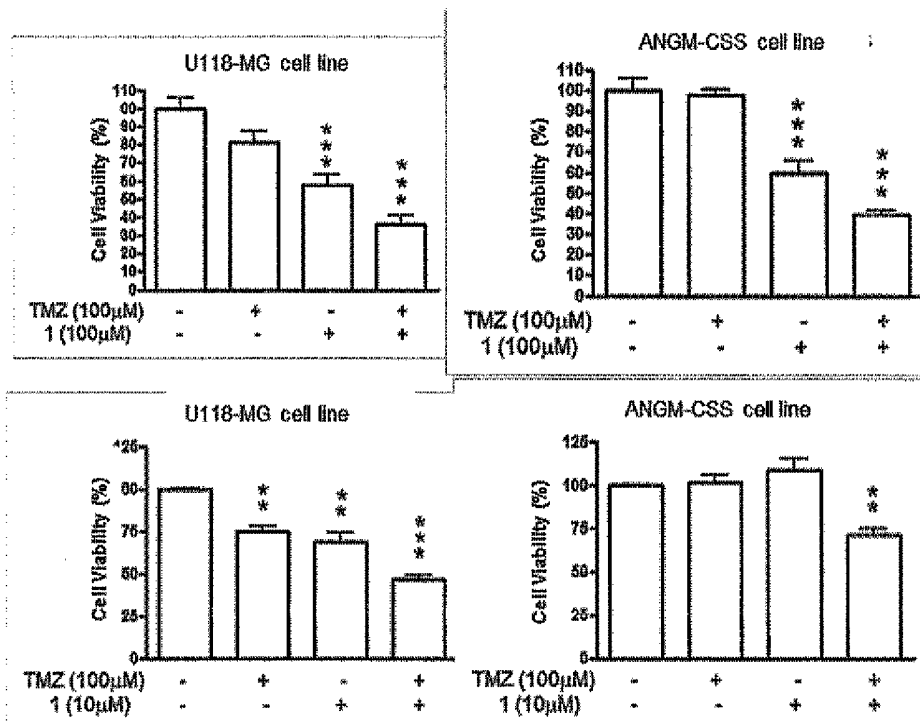


Figure 4

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2015/073017

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A61K31/282 A61K31/337 A61K31/395 A61K31/404 A61K31/4178
 A61K31/4725 A61K31/506 A61K31/513 A61K31/65 A61P35/00
 ADD.
 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)
 A61K A61P

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 practicable, search terms used)
 EPO-Internal, WPI Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2005/040116 A2 (SCHERING AG [DE]; ARNAIZ DAMIAN [US]; BRYANT JUDI [US]; CHOU YUO-LING) 6 May 2005 (2005-05-06) the whole document page 22, paragraph 2-3 page 39, line 9 page 77, lines 4-7; example 15 ----- <div style="text-align: center;">-/--</div>	1-16

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&" document member of the same patent family</p>
---	---

Date of the actual completion of the international search 5 January 2016	Date of mailing of the international search report 14/01/2016
--	---

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Jakobs, Andreas
--	--

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/073017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GIULIA NESI ET AL: "Synthesis of Novel 3,5-Disubstituted-2-oxindole Derivatives As Antitumor Agents against Human Nonsmall Cell Lung Cancer", ACS MEDICINAL CHEMISTRY LETTERS, vol. 4, no. 12, 12 December 2013 (2013-12-12), pages 1137-1141, XP055190819, ISSN: 1948-5875, DOI: 10.1021/ml400162g cited in the application the whole document	1-16
A	FR 2 821 358 A1 (AVENTIS PHARMA SA [FR]) 30 August 2002 (2002-08-30) the whole document example 6; compounds 5-1,5-4,5-12	1-16
A	JEFFREY C. HENISE ET AL: "Irreversible Nek2 Kinase Inhibitors with Cellular Activity", JOURNAL OF MEDICINAL CHEMISTRY, vol. 54, no. 12, 23 June 2011 (2011-06-23), pages 4133-4146, XP055190816, ISSN: 0022-2623, DOI: 10.1021/jm200222m the whole document	1-16
A	HIREN PATEL ET AL: "Synthesis of hybrid anticancer agents based on kinase and histone deacetylase inhibitors", MEDCHEMCOMM, vol. 5, no. 12, 18 June 2014 (2014-06-18), pages 1829-1833, XP055190821, ISSN: 2040-2503, DOI: 10.1039/C4MD00211C compounds 9,10 the whole document	1-16
A	MOHAMED DIWAN M. ABDULHAMEED ET AL: "Combined 3D-QSAR Modeling and Molecular Docking Study on Indolinone Derivatives as Inhibitors of 3-Phosphoinositide-Dependent Protein Kinase-1", JOURNAL OF CHEMICAL INFORMATION AND MODELING, vol. 48, no. 9, 22 September 2008 (2008-09-22), pages 1760-1772, XP055190840, ISSN: 1549-9596, DOI: 10.1021/ci800147v the whole document compound 24	1-16

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/073017

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ISLAM ET AL: "Indolinone based phosphoinositide-dependent kinase-1 (PDK1) inhibitors. Part 1: Design, synthesis and biological activity", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 17, no. 14, 19 June 2007 (2007-06-19), pages 3814-3818, XP022119960, ISSN: 0960-894X, DOI: 10.1016/J.BMCL.2007.04.071 the whole document compound 3j -----	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/073017

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005040116 A2	06-05-2005	AU 2004284084 A1 BR PI0415773 A CA 2541460 A1 CN 1898205 A EP 1680401 A2 JP 2007509173 A KR 20060123184 A MX PA06004438 A US 2005090541 A1 WO 2005040116 A2 ZA 200604149 A	06-05-2005 26-12-2006 06-05-2005 17-01-2007 19-07-2006 12-04-2007 01-12-2006 20-06-2006 28-04-2005 06-05-2005 28-11-2007

FR 2821358 A1	30-08-2002	AT 303380 T DE 60205872 D1 DE 60205872 T2 DK 1366038 T3 EP 1366038 A1 ES 2244751 T3 FR 2821358 A1 PT 1366038 E US 2004110770 A1 WO 02068411 A1	15-09-2005 06-10-2005 08-06-2006 19-12-2005 03-12-2003 16-12-2005 30-08-2002 30-11-2005 10-06-2004 06-09-2002
