Title: PHARMACEUTICAL COMBINATION FOR THE TREATMENT OF TUMORS

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 (C₆H₅-C₆) 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-benzylanino, (C₆H₅-C₆) 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.
“Pharmaceutical combination for the treatment of tumors”

******

DESCRIPTION

FIELD OF THE INVENTION

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

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 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 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 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 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's 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)

\[
\begin{align*}
R & \quad A \\
\text{R} & \quad \text{A}
\end{align*}
\]

wherein

\(R_1\) is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by \((\text{C}_1-\text{C}_3)\) alkyl,

A is a \(-\text{CH}_2\text{CO}-\text{o} -\text{SO}_2\text{-}\) group;

R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolinyl, 3,4-dimethoxy-benzylamino, \((\text{C}_1-\text{C}_3)\) 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.

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.

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 μM) and/or with TMZ (10 or 100 μM) 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 ± 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, 100μM), by compound 1 (at the concentrations 10 and 100 μM), by the association of the two compounds (TMZ 100μM + compound 1 100 μM) or by the association of the two compounds (TMZ 100μM + compound 1 10 μM), 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 ± SEM. The statistical analysis was conducted using the ANOVA/Bonferroni one-way test. ** P<0.01, *** P<0.001 vs. control cells.

DETAILED DISCLOSURE OF THE INVENTION

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

![Chemical Structure](image1)

wherein

R₁ is selected from the group consisting of thiethyl, 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.

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

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

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

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

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

\[ \text{N-[(3E)-(2-oxo-3-((pyridine-2-yl)methylene)-2,3-dihydro-1H-indol-5-yl)-2-(6,7-dimethoxy-3,4-dihydroisoquinoln-2-(1H)-yl)-indolin-5-yl-acetamide} \]
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

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

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

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.

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

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

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.

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.

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)

\[
\text{R} \quad \text{A} \quad \text{N} \quad \text{H} \quad \text{R}_1 \quad \text{N} \quad \text{H} \\
\text{I}
\]

wherein

R\(_1\) is selected from the group consisting of thienyl, imidazole and pyridyl,
optionally substituted by (C_1-C_3) alkyl,
A is a –CH_2CO- o –SO_2- group;
R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-
tetrahydroisoquinolinyl, 3,4-dimethoxy-benzylamino, (C_1-C_3)alkyl, benzyl or a
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
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
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
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-2H-indol-2-one commercial compound was reduced to
the corresponding 5-amino-1,3-dihydro-2H-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-1H-indol-5-yl)acetamide
which was condensed with the appropriate amine derivative to provide,
respectively, 2-[(6,7-dimethoxy-3,4-dihydroisoquinol-2-(1H)-yl)]-N-(2-oxo-2,3-
dihydro-1H-indol-5-yl)acetamide and 2-[(3,4-dimethoxybenzyl)amino]-N-(2-oxo-
2,3-dihydro-1H-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.

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

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

**Compound 1:**

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

![Compound 1](image1)

**Compound 2:**

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

![Compound 2](image2)

**Compound 3:**

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

![Compound 3](image3)
Compound 4:
N-[(3Z)-(2-oxo-3-((1H-imidazol-5yl)methylene)-2,3 dihydro-1H-indol-5-yl]-2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2-(1H)-yl)acetamide

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

Compound 6:
N-[(3Z)-2-oxo-3-(thiophene-2-ylmethyldene)-2,3-dihydro-1H-indol-5-yl]methanesulfonamide

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

Compound 10:
N-[(3Z)-2-oxo-3-(thiophene-2-ylmethylidene)-2,3-dihydro-1H-indol-5-yl]-4-methylbenzenesulfonamide

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.


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

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 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 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-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-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 μM), in the absence or in the presence of temozolomide (TMZ, 10 μM or 100 μM). 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 μM and at 100 μM). 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 100μg/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

The results are shown in Figure 2.

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

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

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)

\[ R - A - N - \text{H} - C - \text{H} - C - \text{N} - \text{H} - \text{C} - \text{O} \]

wherein

R\(_1\) is selected from the group consisting of thienyl, imidazolyl and pyridyl, optionally substituted by (C\(_1\)-C\(_3\))alkyl,

A is a -CH\(_2\)CO- or -SO\(_2\)- group;

R is selected from the group consisting of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolyl, 3,4-dimethoxy-benzylamino, (C\(_1\)-C\(_3\))alkyl, benzyl or a pharmacologically 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.

4. The combination of claim 3, wherein R is 6,7-dimethoxy-1,2,3,4-tetrahydroquinolinyl 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-dihydroisoquinol-2-(1H)-yl)-acetamide

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)-indolin-5-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

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

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

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

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

N-[(3E)-3-[(1-methyl-1H-imidazol-2-il)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)

\[
\text{R} - \text{A} - \text{H} - \text{N} - \text{(I)}
\]

wherein

- R₁ is selected from the group consisting of thiethyl, 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 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).

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.
Reagents and Conditions: (a) $\text{H}_2$, Pd/C, EtOH, 20°C; (b) CICOCH$_2$Cl, Acetone, DMF, 20°C; (c) K$_2$CO$_3$, DMF, CH$_3$CN, 82°C; (d) Appropriate carbaldehyde, EtOH, piperidine, 110°C.

Figure 1
Reagents and Conditions: (a) p-toluenesulfonylchloride, H₂O, 20°C; (b) Methansulfonylchloride, H₂O, 20°C; (c) Appropriate carbaldheyde, EtOH, piperidine, 110°C.
U87MG 72 h

Figure 3
Figure 4
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
       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

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>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 ---- -/-</td>
<td></td>
</tr>
</tbody>
</table>

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
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>GIULIA NESI ET AL: &quot;Synthesis of Novel 3,5-Disubstituted-2-oxindole Derivatives As Antitumor Agents against Human Nonsmall Cell Lung Cancer&quot;, 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 -----</td>
<td>1-16</td>
</tr>
<tr>
<td>A</td>
<td>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 -----</td>
<td>1-16</td>
</tr>
<tr>
<td>A</td>
<td>HIREN PATEL ET AL: &quot;Synthesis of hybrid anticancer agents based on kinase and histone deacetylase inhibitors&quot;, 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 -----</td>
<td>1-16</td>
</tr>
<tr>
<td>A</td>
<td>MOHAMED DIWAN M. ABDULHAMEED ET AL: &quot;Combined 3D-QSAR Modeling and Molecular Docking Study on Indolinone Derivatives as Inhibitors of 3-Phosphoinositide-Dependent Protein Kinase-1&quot;, 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 -----</td>
<td>1-16</td>
</tr>
</tbody>
</table>

Form PCT/ISA/210 [continuation of second sheet] (April 2009) page 2 of 3
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BR PI0415773 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2541460 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1898205 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1680401 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2007509173 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 20060123184 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MX PA06004438 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2005090541 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2005040116 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 200604149 A</td>
</tr>
<tr>
<td>FR 2821358 A1</td>
<td>30-08-2002</td>
<td>AT 303380 T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 60205872 D1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 60205872 T2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 1366038 T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1366038 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 2244751 T3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FR 2821358 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 1366038 E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2004110770 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 02068411 A1</td>
</tr>
</tbody>
</table>