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(54) **USE OF HYDROXAMIC ACID DERIVATIVES FOR THE PREPARATION OF ANTI-TUMOUR MEDICAMENTS**

VERWENDUNG VON HYDROXAMSÄUREDERIVATEN ZUR HERSTELLUNG VON ANTITUMORARZNEIMITTELN

UTILISATION DE DERIVES D'ACIDE HYDROXAMIQUE POUR LA PREPARATION DE MEDICAMENTS ANTITUMORAUX

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(73) Proprietor: **ITALFARMACO S.p.A.**
20126 Milano (IT)

(72) Inventors:
- **LEONI, Flavio**
  I-20153 Milano (IT)
- **MASCAGNI, Paolo**
  I-20058 Villasanta (IT)

(74) Representative: **Pistolesi, Roberto et al**
Dragotti & Associati srl
Via Turati 32
20121 Milano (IT)

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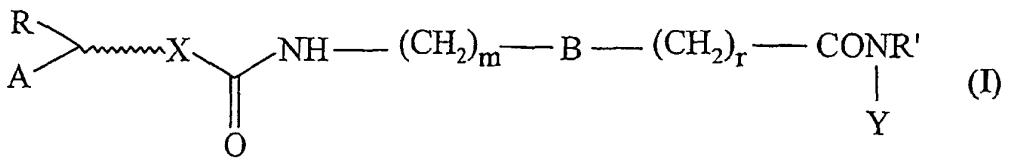
Description

[0001] The present invention relates to the use of a hydroxamic acid derivative containing an amidobenzoic moiety according to claim 1 for the preparation of anti-tumour medicaments.

Technological background

[0002] Hydroxamic acid derivatives containing an amidobenzoic moiety are disclosed in EP 901465 as potential medicaments with anti-inflammatory and immuno-suppressive activity, ascribable to the inhibition of the production of pro-inflammatory cytokines, in particular Tumour Necrosis Factor and interleukin-1-beta.

[0003] Said derivatives are represented by the following general formula (I)



(I)

wherein

R'	is hydrogen or (C_{1-4})alkyl;
A	is adamantyl or a mono-, bi- or tricyclic residue, which can optionally be partially or completely unsaturated, contain one or more heteroatoms selected from the group consisting of N, S or O, and optionally substituted with hydroxy, alkanoyloxy, primary, secondary or tertiary amino, amino (C_{1-4}) alkyl, mono- or di-(C_{1-4})alkyl-amino(C_{1-4})alkyl, halogen, (C_{1-4})alkyl, tri(C_{1-4})alkylammonium-(C_{1-4})alkyl;
~~	is a 1 to 5 carbon atoms chain optionally containing a double bond or a NR' group wherein R' is as defined above;
R	is hydrogen or phenyl;
X	is an oxygen atom or a NR' group wherein R' is either as defined above or absent;
r and m	are independently 0, 1 or 2;
B	is a phenylene or a cyclohexylene ring;
Y	is hydroxy or an amino(C_{1-5})alkyl chain optionally interrupted by an oxygen atom;

with the proviso that a tricyclic group as defined for A is fluorenyl only when, at the same time, X is different from O and Y is different from hydroxy, unless said fluorenyl is substituted with a tri(C_{1-4})alkylammonium(C_{1-4})alkyl group.

[0004] Preferred compounds of formula (I) are those in which R' is hydrogen, A is optionally substituted phenyl or 1- or 2-naphthyl, cyclohexyl, 1- or 2- 1,2,3,4-tetrahydronaphthyl, adamantyl, quinolinyl, isoquinolinyl, 1- or 2-indenyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl.

[0005] Most preferred are the compounds (I) in which A is phenyl or 1- or 2-naphthyl, R is phenyl when A is phenyl or is hydrogen when A is 1- or 2-naphthyl.

[0006] Said compounds can be prepared according to methods disclosed in EP 901465.

[0007] Uesato, Shinichi et al. Bioorganic&Medicinal Chemistry Letters (2002), 12(10), 1347-1349 discloses that utilising tranexamic acid as starting material, a series of N-hydroxycarboxamides were synthesized in order to seek new histone deacetylase (HDAC) inhibitors. Further structure optimization involving the replacement of the 1,4-cyclohexylene group with the 1,4-phenylene group yielded the promising HDAC inhibitors which possess a terminal bicyclic aryl amide.

[0008] WO01/38322 also describes compounds and methods for inhibiting histone deacetylase enzymatic activity and for treating cell proliferation diseases and conditions.

Brief description of the drawing

[0009]

Figure 1 illustrates the anti-tumor effect of ITF 2357 by measuring the capacity of the compound to reduce the growth of the murine melanoma B16-BL6.

[0010] It has now been found that the compound 4-[(6-diethylaminomethyl)napht-2-ylmethyloxy-carbamoyl]benzohy-

droxamic acid or an acceptable salt thereof can be used for the treatment of neoplasias of different origin, in particular of melanomas, colon, lung and breast carcinomas, neuroblastomas, sarcomas and various forms of leukaemia (erythroleukaemia, acute promyelocytic leukaemia), at daily single or multiple doses ranging from 1 to 500 mg, depending on the disease and pharmaceutical and toxicological characteristics of the considered compound, which can be administered as suitable formulations through the oral, parenteral or topical route, for example through direct perfusion at the site of the tumour lesion. Moreover, the compound according to claim 1 can be administered in combination with other known antineoplastic agents, according to polychemotherapy protocols.

[0011] The activity of the compound according to claim 1 was evidenced *in vitro,* on cultured tumour cell lines, and *in vivo,* on the experimental model of the murine melanoma B 16-BL6.

[0012] The following examples illustrate the invention in greater detail.

EXAMPLE 1 (In vitro activity)

[0013] The anti-tumour effect of the compound of example 12 of EP 901465, 4-[6-(diethylaminomethyl)naphth-2-ylmethyloxycarbamoyl)] benzohydroxamic acid hydrochloride (ITF 2357), was tested *in vitro* by measuring the capacity of the compound to inhibit the growth of tumour cell lines of different histotype deriving from both solid tumours and haematological tumours. The cell lines used were: A549 (pulmonary carcinoma), MDA-MB435 (breast carcinoma) and KG-1 (myeloid leukaemia).

[0014] A549 and MDA-MB435 cells (respectively grown in E-MEM and D-MEM culture medium, supplemented with 10% of fetal calf serum) were seeded on 96 wells-flat bottom-plates (5000 cells/well) and allowed to adhere for 4 hours at 37°C under 5% CO_2 atmosphere. Thereafter ITF 2357 at various doses was added to each well (4 replicates). After further 36 hours, tritiated thymidine (1 μCi/well) was added to each well and left therein for the following 12 hours. After this time, the cells were washed 3 times with culture medium and then solubilized with 1N NaOH for 30 minutes.

[0015] A liquid scintillation beta counter was used for measurement of the radioactivity contained in each sample, which is directly proportional to DNA synthesis and therefore to cell proliferation. KG-1 cells, grown in RPMI 1640 culture medium, supplemented with 5% fetal calf serum, were seeded in 96 wells-flat bottom-plates (250000 cells/well). ITF 2357 at various doses was immediately added and after 36 hours tritiated thymidine was added to each well (1 μCi/well) and left therein for the following 12 hours. At the end the cells were collected by a cell harvester and the radioactivity contained in each sample which is directly proportionated to DNA synthesis and therefore to cell proliferation was measured by a liquid scintillation beta counter.

[0016] The effect of different doses of ITF 2357 was measured as percent of inhibition of radioactivity incorporation compared with untreated control cells. The concentration capable of inducing a 50% cell growth inhibition (IC_{50}) was then determined by means of linear regression.

[0017] The results obtained are summarized in the following table:

Cell line	A549	MDA-MB435	KG-1
IC_{50}(nM)	495	73	552

[0018] The results obtained show that ITF 2357 inhibits *in vitro*, at very low dosages (range 10-9M), the growth of the cell lines employed. ITF 2357 in particular inhibits cell growth both of cells from solid tumours (A549 and MDA-MB435) and from leukaemias (KG-1), therefore suggesting its use on tumours of different histotype.

EXAMPLE 2 (In vitro activity)

[0019] Cell lines derived from human solid tumours of different histotype and stabilised *in vitro* were used in the following experiment. In particular, three cell lines from head and neck tumours (KB, Cal27 and Hep2), two cell lines derived from colon carcinomas (HT-29 and LoVo) and four cell lines derived from melanomas (Colo38, Pes41, Pes43 and Anad) were studied.

The cells were grown according to conventional methods in flasks containing synthetic culture medium added with fetal serum, at 37°C under 5% CO_2 atmosphere, then seeded in 96 wells plates and allowed to adhere for some hours. ITF 2357 was added in triplicate at increasing doses to each well and the cells were incubated for further 72 hours. Viable cells were labelled by dyeing with sulforhodamine B and their amount was determined by spectrophotometric evaluation of the dye content in each well. The effect of ITF 2357 was calculated as percentage of inhibition, at each concentration, of the dye incorporation in wells containing ITF 2357 compared with control wells (cells without drug). IC_{50} values of ITF 2357 were calculated by Software Calcusyn (Biosoft) according to dose-response curves.

Cell line	Tumour origin	ITF 2357 IC_{50} (μM)
KB	Head-neck	0.64
Ca127	Head-neck	3.4
Hep-2	Head-neck	1
HT-29	Colon carcinoma	0.7
LoVo	Colon carcinoma	2.5
Colo38	Melanoma	2.38
Pes41	Melanoma	1.6
Pes43	Melanoma	1.4
Anad	Melanoma	5.7

EXAMPLE 3 (In vivo activity)

[0020] The anti-tumour effect of ITF 2357 was studied measuring the capacity of the compound to reduce the growth of the murine melanoma B16-BL6.

[0021] B 16-BL6 tumour is a highly metastatic variant (Sciumbata T. et al. Invasion and Metastasis 1996; 16: 132-143) of the native tumour B16 and it grows subcutaneously in the syngenic mouse C57BL/6 (Gutman M et al. Cancer Biother. 1994; 9(2): 163-170).

[0022] B16-BL6 tumour cells were inoculated subcutaneously in female C57BL/6 mice (10 animals/group, weight 20-22 grams) at the dose of $2x10^5$ cells/mouse. ITF 2357 dissolved in water was administered orally, at the indicated doses, 10 minutes before the inoculum of the tumour cells and then daily for 6 days a week. The tumour growth was expressed as tumour weight, measuring twice a week, by means of a calibre, the two perpendicular diameters of the nodules. The weight was calculated according to the formula: $(\text{diameter 1} \times \text{diameter 2})^2 / 2$ as described in Giavazzi R. et al. Cancer Res. 1986; 46: 1928-1933.

[0023] The results obtained are reported in the enclosed figure.

[0024] It can be observed that ITF 2357 exerts a dose-dependent inhibitory effect on the tumour growth, since it reduced of about 50% the volume of the tumoural nodule after 15 days of treatment.

Claims

1. Use of 4-[6-diethylaminomethyl)naphth-2-ylmethyloxycarbamoyl]benzohydroxamic acid or of an acceptable salt thereof for the preparation of a medicament for the treatment of neoplasias.

2. Use according to claim 1 **characterized in that** said neoplasias are selected from melanomas, colon, lung and breast carcinomas, neuroblastomas, sarcomas and leukaemia.

3. Use according to claim 2 **characterized in that** said leukaemia is selected from etythroleukaemia and acute promyelocytic leukaemia.

4. Use according to claims 1 to 3 **characterized in that** 4-[6-diethylaminomethyl)napbth-2-ylmethyloxycarbamoyl] benzohydroxamic acid or the acceptable salt thereof are administered in combination with other antineoplastic agents.

Patentansprüche

1. Verwendung von 4-[6-Diethylaminomethyl)naphth-2-ylmethyloxycarbamoyl]benzo-hydroxamsäure oder ein annehmbares Salz davon zur Herstellung eines Medikaments zur Behandlung von Neoplasien.

2. Verwendung nach Anspruch 1, **dadurch gekennzeichnet, daß** die Neoplasien ausgewählt sind unter Melanomen, Darm-, Lungen- und Brust-Karzinomen, Neuroblastomen, Sarkomen und Leukämien.

3. Verwendung nach Anspruch 2, **dadurch gekennzeichnet, dass** die Leukämie ausgewählt ist unter Erythroleukämie und akuter promyelotischer Leukämie.

4. Verwendung nach einem der Ansprüche 1-3, **dadurch gekennzeichnet, daß** 4-[6-Diethylaminomethyl)naphth-2-ylmetyloxycarbamoyl]benzohydroxamsäure oder das annehmbare Salz davon zusammen mit anderen anti-neoplastischen Mitteln verabreicht wird.

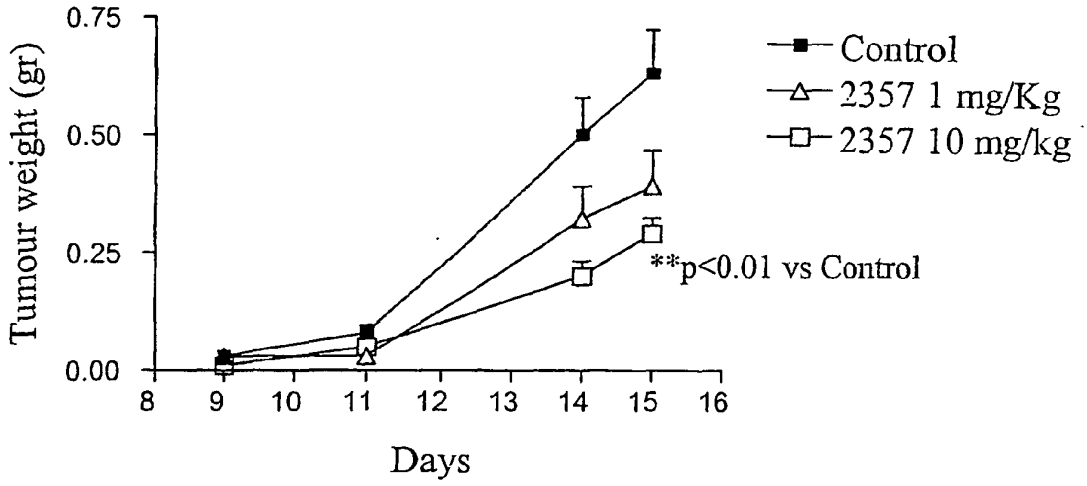
Revendications

1. Utilisation d'acide 4-[6-(diéthylaminométhyl)naphth-2-ylméthyloxycarbamoyl]benzohydroxamique ou d'un sel acceptable de celui-ci pour la préparation d'un médicament pour le traitement de néoplasies.

2. Utilisation selon la revendication 1, **caractérisée en ce que** lesdites néoplasies sont choisies parmi les mélanomes, les carcinomes du colon, des poumons et du sein, les neuroblastomes, les sarcomes et les leucémies.

3. Utilisation selon la revendication 2, **caractérisée en ce que** ladite leucémie est choisie parmi l'érythroleucémie et la leucémie aiguë promyélocytaire.

4. Utilisation selon les revendications 1 à 3, **caractérisée en ce que** l'acide 4-[6-(diéthylaminométhyl)naphth-2-ylméthyloxycarbamoyl]benzohydroxamique ou le sel acceptable de celui-ci sont administrés en combinaison avec d'autres agents anticancéreux.


0.75
0.50
0.25
0.00
Tumour weight (gr)
8
9
10
11
12
13
14
15
16
Days
Control
2357 1 mg/Kg
2357 10 mg/kg
**p<0.01 vs Control

Figure