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(71) Demandeur/Applicant:
NORSK HYDRO ASA, NO

(72) Inventeurs/Inventors:
DUNSAED, CAMILLA BRUNO, NO;
SAGVOLDEN, GEIR, NO;
PETTERSEN, ERIK OLAI, NO;
LARSEN, ROLF OLAF, NO;
MOEN, VIDAR, NO;
BORRETZEN, BERNT, NO

(74) Agent: FETHERSTONHAUGH & CO.

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(71) Applicant *(for all designated States except US)*: NORSK HYDRO ASA [NO/NO]; N–0240 Oslo (NO).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BØRRETZEN, Bernt [NO/NO]; Granlia 3, N–3940 Heistad (NO). MOEN, Vidar [NO/NO]; Meierkollen 33, N–3727 Skien (NO). LARSEN, Rolf, Olaf [NO/NO]; Nustadringen 15, N–3970 Langesund (NO). PETTERSEN, Erik, Olai [NO/NO]; Tokerudberget 10, N–0986 Oslo (NO). SAGVOLDEN, Geir [NO/NO]; Bølerskogen 15, N–0691 Oslo (NO). DUNSÆD, Camilla, Bruno [NO/NO]; Bærumsveien 168, N–1340 Bekkestua (NO).

(74) Agent: SANDBU, Elisabeth, Dahl; Norsk Hydro ASA, N–0240 Oslo (NO).

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CHEMICAL COMPOUNDS

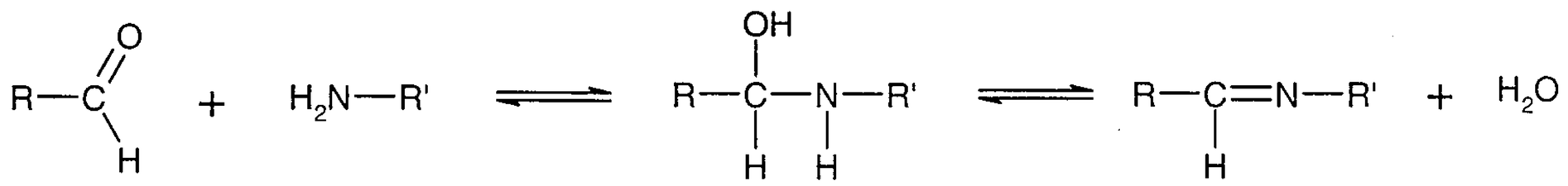
The present invention relates to benzaldehyde derivatives which are useful as anticancer agents. Some of the compounds of this invention are novel *per se*.

Most of the presently used anticancer agents are cytotoxic in their action. Although these agents have shown good results in treatment of some cancers like lymphoma, leukaemia and testicular cancer, they often produce severe and unacceptable side-effects limiting the possibility for an effective treatment. Furthermore, in several types of cancer like in solid tumours (carcinoma), chemotherapy has so far proven to be of limited value since established cytostatic drug seldom improves the prognosis for the patient. The ability of cancer cells to develop resistance against cytotoxic products is also a main reason for the failure in their use in the treatment of solid tumours. There is thus a great need for new anticancer agents having fewer side effects and having a more selective action on malignant cells.

It is known among other from EP-0215395, JP-63264411, JP-8800940, JP-55069510 and EP-0283139 that benzaldehydes and derivatives thereof exhibit a selective anticancer effect.

Aldehydes react with a range of O, S or N nucleofilic entities like hydroxy groups, sulfhydryl groups and amino groups to form carbonyl condensation products like acetals, mercaptals, aminals, etc. However, with primary amines, the reaction normally take the form of Schiff's base (imine) adduct formation. It is well known that *in vivo* Schiff's base formation is involved in key biochemical processes like transamination, decarboxylation and other amino acid modifying reactions mediated by pyridoxal phosphate, the action of aldolase on fructose di-phosphate in the glycolysis and the condensation of retinal with rhodopsin in the process of vision. It is also known that carbonyl condensation reactions are involved in transmembrane signalling events, for example in generating an immune response.

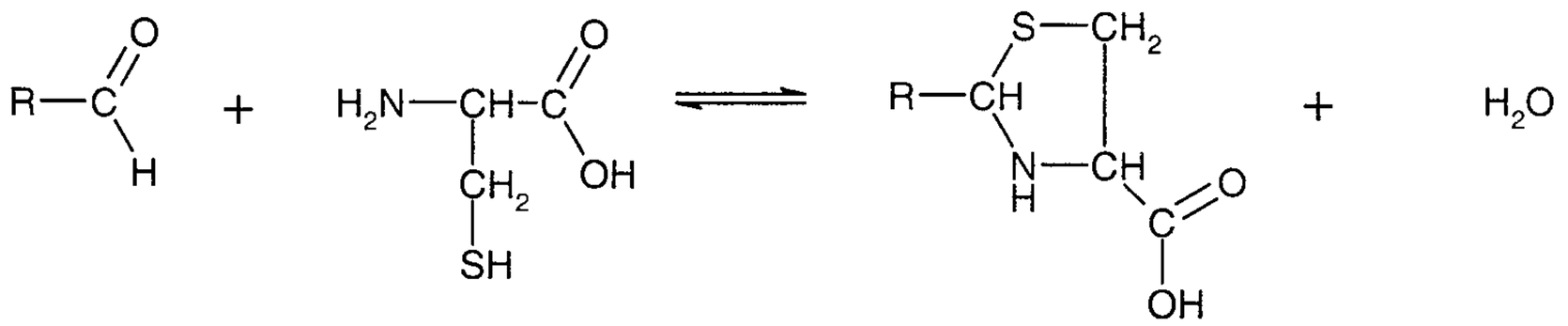
The formation of imines proceeds through a two-stage mechanism: The addition of the amino nucleofile to the carbonyl group to form a carbinolamine (aminohydrin) intermediate followed by a dehydration step to generate the C=N double bond. Both steps are reversible, but are facilitated at different pH values. As a consequence, the reaction occurs according to a characteristic bell-shaped pH/rate profile with the highest over-all reaction rates being found at moderate acidities.



aldehyde amine carbinolamine imine

However, the formation of Schiff's bases are known to take place readily also in physiological conditions, and many carbonyl condensation reactions are well known *in vivo* (E. Schauenstein et. al., Aldehydes in biological systems. London, Pion Ltd. 1977).

The Schiff's base tend to be a reactive species itself and is prone to further reaction resulting in the addition of nucleofilic agents to the double bond. For certain sulfur-containing amines, in particular the amino acids cystein and methionine, and for glutathione, the initially formed Schiff's base can undergo reversible internal cyclization in which the sulfhydryl group adds to the imine to form thiazolidine carboxylate (M. Friedman, The chemistry and biochemistry of the sulfhydryl group in amino acids, peptides and proteins, Oxford, Pergamon Press, 1973).



aldehyde cystein thiazolidine carboxylic acid

Evidence for reactions between carbonyl compounds and free amino groups of proteins to form reversible Schiff's base linkages was reported by G. E. Means and R. E. Feeney (Chemical Modification of Poteins, pp. 125 - 138, San Francisco, Holden-Day, 1971). Aromatic aldehydes are in general more reactive than saturated aliphatic aldehydes, and Schiff's bases can be formed even without removal of the water formed during the reaction. (R. W. Layer Chem. Rev. *63* (1963), 489 - 510). This fact is important when considering formation of Schiff's bases under physiological conditions. Using haemoglobin as a source of amino groups Zaugg et. al. (J. Biol. Chem. *252* (1977), 8542 - 8548) have shown that aromatic aldehydes have a two- to threefold increased reactivity over aliphatic aldehydes in Schiff's base formation. An explanation for the limited reactivity of alkanals could be the fact that in aqueous solution at neutral pH a very large excess of free aldehyde is required to shift the equilibrium in favour of Schiff's base formation (E. Schauenstein et. al., Aldehydes in biological systems. London, Pion Ltd. 1977).

Benzaldehyde readily forms Schiff's base imines with membrane amino groups, and high equilibrium constants have been measured for benzaldehyde reacting with amines (J. J. Pesek and J. H. Frost, Org. Magnet. Res. *8* (1976), 173 - 176; J. N. Williams Jr. and R. M. Jacobs Biochim Biophys Acta. *154*, (1968) 323 - 331).

We have previously shown by radio labelling images that benzaldehyde do not enter the cell, but adhere to the cell membrane (Dornish, J.M. and Pettersen, E.O.: Cancer Letters *29* (1985) 235-243). This is in agreement with an earlier study, showing that benzaldehyde interacted with the membrane proteins of E. coli (K.Sakaguchi et. al. Agric. Biol. Chem., (1979), *43*, 1775-1777). It was also found that pyridoxal and pyridoxal-5-phosphate both protect the cells against the cytotoxic anti-cancer agent cis-DDP. Cis-DDP exerts its action in the nucleus within the cell. While pyridoxal in principle could penetrate the lipophilic cell membrane, this possibility is blocked for pyridoxal-5-phosphate because of the ionic phosphate group on the latter. Pyridoxal-5-phosphate thus have to exert its protective effect by acting from outside the cell membrane. A spectral shift in the absorbance of pyridoxal-5-phosphate to lower wavelengths observed simultaneously is consistent with Schiff's base adduct formation between the aldehyde and cell membrane amino groups (J. M. Dornish and E. O. Pettersen, Cancer Lett. *29*, (1985), 235 -243).

These findings suggest that aldehydes bind to amines and other nucleofilic entities on the cell membrane to form Schiff's bases and other condensation products. It is known that stimulation of cell growth is mediated by a cascade of events acting from outside the cell membrane. In the same way, the derivatives in the present patent application may act by forming adducts with ligands on the cell membrane, triggering impulses inside the cell with significance on cell growth parameters like protein synthesis and mitosis, and on the expression of tumour suppressor genes and immune responses. Since the condensation reactions are reversible, cellular effects can be modulated as a result of a shift in equilibrium involving ligating species. The presence of dynamic equlibria at a chemical level is consistent with the reversible and non- toxic way of action observed with the benzaldehyde derivatives.

Inhibition of the protein synthesis excerted by benzaldehyde derivatives is very well studied *in vitro* in our research group. In solid tumours the reduced protein synthesis may result in a lack of vital proteins which lead to cell death. In normal cells there is a potential capacity for protein synthesis which is higher than in most cancer cells of solid tumours. This is demonstrated by comparison of the cell cycle duration in normal stem cells, which is often below 10h, and thus shorter than that of most cancer cells of solid tumours, which is typically 30-150h (see Gustavo and Pileri in: The Cell Cycle and Cancer. Ed. : Baserga, Marcel Dekker Inc., N.Y. 1971, p 99). Since cells, as an average, double their protein during a cell cycle, this means that protein accumulation is higher in growth-stimulated normal cells than in most types of cancer cells.

Keeping in mind this difference between normal and cancer cells, there is another difference of similar importance: while normal cells respond to growth-regulatory stimuli, cancer cells have reduced or no such response. Thus, while normal cells, under ordinary growth conditions, may have a reserve growth potential, cancer cells have little or no such reserve. If a protein synthesis inhibition is imposed continuously over a long period of time on normal cells as well as on cancer cells, the two different types of cells may respond differently: Normal tissue may make use of some of its reserve growth potential and thereby maintain normal cell production. Cancer tissue however, have little or no such reserve. At the same time the rate of protein accumulation in most cancer cells is rather low (i.e. protein synthesis is only slightly greater than protein degradation). Therefore the protein synthesis

inhibition may be enough to render the tumour tissue imbalanced with respect to protein accumulation, giving as a result a negative balance for certain proteins. During continuous treatment for several days this will result in cell inactivation and necrosis in the tumour tissue while normal tissue is unharmed.

To date, the most tested compound inducing reversible protein synthesis inhibition and displaying anti-cancer activity is 5,6-benzylidene-d_1-ascorbic acid [zilascorb(^{2}H)]. The protein synthesis inhibiting activity of this prior art compound is described in detail by Pettersen et.al. (Anticancer Res., vol. 11, pp. 1077-1082, 1991) and in EP-0283139. Zilascorb(^{2}H) induces tumour necrosis *in vivo* in human tumour xenografts in nude mice (Pettersen et al., Br. J. Cancer, vol. 67, pp. 650-656, 1993). In addition to zilascorb(^{2}H), the closest prior art compound related to cancer treatment is 4,6-O-Benzylidene-D-glucopyranose (Compound 1) . These two compounds are known to possess a general anti-cancer activity and have been tested in clinical trials against a number of cancer diseases. However, no particular cancer afflicted organs or tissues projected as more suitable for treatment with these compounds, and commercial development was not justified.

We have now surprisingly found that benzaldehyde derivatives of sugars of the hexose type, (including 4,6-O-(Benzylidene-d_1)-D-glucopyranose, Compound 2) give an unexpected strong effect on cancer in certain organs or tissues. We cannot yet explain the mechanism for this selectivity, but we believe that this is connected to the affinity of the sugar moiety of the derivatives to certain cells or tissues. For instance Compound 2 (the glucose-derivative of deuterated benzaldehyde) gives an astonishingly better effect on cancer in the liver than zilascorb(^{2}H) (the derivative of deuterated benzaldehyde and ascorbic acid) (see Example 6). Also in experiments on Panc-1 cells originating from human pancreatic adenocarcinoma we have surprisingly found that Compound 2 induces stronger protein inhibition than zilascorb(^{2}H) (see Example 2, Fig. 3). Common for these tissues are the high levels of certain glucose transporters and receptors. There is no evidence in prior art to forsee a better effect by treating liver and pancreatic cancer with Compound 2 as compared to zilascorb(^{2}H). To the contrary, based on earlier known experiments shown in EP-0283139 we would expect that Compound 2 would show similar or little less effect than zilascorb(^{2}H).

We have also found in our experiments that the deuterated analogue of these compounds are substantially more effective than the corresponding proton analogues. This difference in effect is very striking in our experiment on cell adhesion (see Example 3 and also Example 7). When a hydrogen atom is substituted by the twice as heavy deuterium isotope, the kinetic properties of the molecule are altered as the rate in breaking the C-D bond is lowered compared to breaking the C-H bond. It is known among others from M.I. Blake et.al., J. Pharm. Sci. *64* (1975), 367-391 that deuteration of drugs may alter their pharmacological function.

It is also known in the art (EP 0 283 139 and Anticancer Res. 15: 1921-1928 (1995)) that when the acetal proton in 4,6-O-benzylidene-D-glucopyranose is substituted with deuterium (Compound 1 versus Compound 2), this can affect both the protein synthesis and the cell surviving fraction measured *in vitro*. We believe that one possible explanation for this D-isotope effect at a chemical level is related to slower oxidation of deuterated benzaldehyde to inactive benzoic acid, resulting in a longer half-life of the deuterated active ingredient at a cellular level. However, to demonstrate a significant difference in the surviving fraction of NHIK 3025 cells exposed to Compounds 1 resp. 2, drug concentrations of more than 6 mM must be applied. The difference in protein synthesis inhibition was very small when these cells were exposed at 1-10 mM concentration.

The inventors now performed a completely different kind of experiment: The adhesion force between NHIK 3025 cells and the substratum was measured after pre-incubation of the cells in solutions of Compounds 1 and 2 (see Example 3). Even at 1 mM concentration, an astonishing D-isotope effect was shown. Surprisingly, Compound 2 significantly reduced the adhesion force to 1/3 relative to control, whereas Compound 1 did not lead to significant reduction. The inventors believe that Compound 2 may have interfered with the biosynthesis of integrins, reducing the cell's ability to attach to the substratum. Integrins are structural trans-membrane proteins crucial for binding cells to the extracellular matrix and for cell-cell interactions. Inhibiting the function of the integrins could thus directly affect the metastasising ability of cancer cells. The experiment indicate that integrines could be especially sensitive to protein synthesis inhibition. Thus, Compound 2 could well be used for prevention of metastatic processes in cancer development.

In an *in vivo* model where we compare Compounds 1 and 2, cancer cells from the liver-invading human colorectal cancer cell line C170HM2 were injected i.p. in nude mice following treatment with the drugs. The animals treated with Compound 2 had an astonishing less tumour burdon in the liver compared to those treated with Compound 1 (see Example 7).

In the experiments refered to above, we have shown that the D-isotope effect could be more pertinent in cancer treatment with aldehyde derivatives than previously known in the art. The two experiments taken together (Example 3 and Example 7), show that Compound 2 and similar drugs, could be especially beneficial for treatment of primary - and secondary cancers in the liver.

Use of Compound 1 (4,6-O-benzylidene-D-glucopyranose) against *inter alia* cancer of the liver is disclosed in US-4.882.314. According to Experiment 2 in US-4.882.314 a patient having colon carcinoma with metastatic cancer in the liver was cured. However, according to Experiment 5 in said US-patent a patient having primary liver tumour died after four months of treatment.

Later G. Tanum et al., Am. J. Clin. Oncol. (CCT), 13(2), 1990, pp. 161-163 have concluded that Compound 1 is not active in patients with colorectal cancer. Treatment was in this study given daily for 2 months and effect was evaluated by measurement of tumour size.

In an experiment, made by the inventors, on chemically induced liver cancer in Wistar rats treated for 10 days intravenously, it was surprisingly shown that massive necrotic areas had developed in 2 of 5 animals treated with Compound 2 while in none of 5 animals treated with Compound 1 (see Example 6).

The inventors of the present invention further made a study where nude mice injected with C170HM2 cells were treated with Compound 1 and Compound 2. Tumour line C170HM2 is a human colorectal cell line derived from a patient's primary tumour. At termination the liver was exposed, and visible liver tumours were counted and their total cross-sectional

area measured. The effect of Compound 2 on the liver invasion of the human colorectal tumour, C170HM2 was far better than Compound 1 (Example 7).

Furthermore, it was surprisingly observed by the inventors that Compound 2 gave a substantially better effect on development of liver cancer in rats than zilascorb(H^2). Following initial treatment of young rats with nitrosamine and partial hepatectomy, the animals developed liver cancer in 3 to 6 months. Following a further 11 months of treatment with either zilascorb(^2H) or Compound 2, both the number of animals developing liver cancers as well as the amount of cancer tissue in cancer tumours were reduced several times more in Compound 2-treated as compared to zilascorb(^2H)-treated animals (see Example 6).

Thus, it has now been found that Compound 2 surprisingly gives far better therapeutic effect of cancers in the liver (primary as well as metastatic) as compared to previously known effects.

In an inbred strain of Wistar rats (see Eker R., Acta Path. Microbiol. Scand., 34, (1954) 554-562) the animals develop renal cancers as a result of an autosomal dominant gene. At the age of 11 months animals were operated in order to inspect which had developed cancer (50% had renal cancer). Animals having cancer of 2-4 mm in diameter were included in experiment since, by experience, these small tumours does not have necrotic areas. These were injected daily for 10 days, with either isotonic saline (placebo) or saline containing zilascorb(^2H), the undeuterated analogue of zilascorb(^2H) (5,6-O-benzylidene-ascorbic acid sodium salt) or Compound 2. While tumours of animals given the undeuterated analogue of zilascorb(^2H) as well as placebo had little or no necrosis following 10 days of treatment, tumours of animals treated with zilascorb(^2H) or Compound 2 was half necrotic (see Example 8).

The chemical induced carcinogenesis (like in Example 6) has a similar mechanism as the cancerogenesis induced by certain virus types like *hepatitis* B and C, certain *papilloma* virus, certain *herpes* virus etc. Especially this will be the case in the development of liver cancer in *hepatitis* B and C infected patients. It is therefore presumable that a prophylactic treatment of these patients with products of this invention could prevent or delay the

development of liver cancer. Also the fact that these products show a low toxic profile (for example Compound 2) would make them suitable for such a treatment.

In an immunological recognition process, a fragment of a foreign protein is confined in the groove of the class II MHC protein on the surface of an antigen presenting cell (APC). Attached to this MHC-antibody complex is also the receptor of a T helper cell. To activate a T helper cell, at least two signals must be provided: The primary signal is mediated by the antigen itself, *via* the class II MHC complex and augmented by CD4 co-receptors. The second signal can be provided by a specific plasma-membrane bound signalling molecule on the surface of the APC. A matching co-receptor protein is located on the surface of the T-helper cell. Both signals are needed for the T-cells to be activated. When activated, they will stimulate their own proliferation by secreting interleukin growth factors and synthesising matching cell-surface receptors. The binding of interleukins to these receptors then directly stimulates the T-cells to proliferate.

In the 1980'ties, it was recognised that a cyclodextrin benzaldehyde inclusion complex could stimulate the immune system by augmentation of the lymphokine activated killer cells in a murine model (Y. Kuroki et. al., J. Cancer Res. Clin. Oncol. *117*, (1991), 109 - 114). Studies made *in vitro* have later revealed the nature of the chemical reactions at the APC-donor/T-cell receptor interaction site responsible for the second co-stimulatory signal, and that these take form of carbonyl-amino condensations (Schiff's base formation). Moreover, these interactions can be mimiced by synthetic chemical entities. These findings open up for new therapeutic opportunities for artificially potentiating the immune system. In WO 94/07479 use of certain aldehydes and ketons which forms Schiff's bases and hydrazones with T-cell surface amino groups are claimed. In EP 0609606 A1 the preferred immuno stimulating substance is 4-(2-formyl-3-hydroxyphenoxymethyl) benzoic acid (Tucaresol), a compound originally designed to cure sickle cell anaemia. This substance is administered orally and is systemically bioavailable. The potential of Tucaresol in curing a number of diseases including bacteria-, virus- and protozoal infections, auto-immune related illness and cancer is presently being investigated (H.Chen and J. Rhodes, J.Mol.Med (1996) 74:497-504) and combinational strategies where Tucaresol is administered together with a vaccine to cure chronic *hepatitis* B, HIV and malignant melanoma are currently under development.

By measuring immuno parameters *in vitro*, and assessing effects *in vivo*, a bell shaped dose/response profile was revealed (H.Chen and J. Rhodes, J.Mol.Med (1996) 74:497-504). This otherwise somewhat unusual dose/response relationship can be justified by assuming that at high concentration of the aldehyde drug will saturate the co-stimulatory ligands necessary for the effective binding of APC to the T-cell and therefor will be inhibitory. A dose sufficient for achieving a dynamic equilibrium providing co-stimulation without blocking intercellulary ligating, seems to be optimal.

In general, aldehydes are intrinsically unstable due to oxidation. 4-(2-Formyl-3-hydroxyphenoxymethyl) benzoic acid (Tucaresol) which is disclosed in EP-0609606, is considerably more potent *in vivo* than *in vitro*. This may be because of the drug's susceptibility to oxidation in aqueous solutions *in vitro* (H.Chen and J. Rhodes, J.Mol.Med (1996) 74:497-504). Many aldehydes are too reactive to be administered as such, and benzaldehyde, even proven to be an active anti cancer drug *in vitro*, is highly irritating and unsuitable for direct *in vivo* application. In a biotic system, the aldehyde carbonyl group will react rapidly with nucleofilic entities predominantly present in all body fluids. These unwanted by-reactions could lead to fast drug metabolisation and difficulty in controlling serum level of the active drug. Controlling the drug at a cellular level within a narrow concentration window is crucial for achieving an effective immune potentiation. Tucaresol is orally administered as an unprotected aldehyde, and one might suspect drug deterioration and difficulties in controlling pharmacokinetics.

The benzaldehyde derivatives 4,6-benzylidene-D-glucose and the deuterated analogue (Compound 1 and 2) have proven to possess high bioavailability either administered i.v. or per oz. Bioavailability measured as serum level after oral administration of Compound 2 to BALB mice was 93-99% (C.B. Dunsaed, J.M. Dornish and E.O. Pettersen, Cancer Chemother. Pharmacol. (1995) 35: 464-470). Moreover, the glucose moiety can possess affinity to receptors present at the cell surface, thereby improving drug availability at a cellular level. The free aldehyde can easily be released by hydrolysis of the acetal, making the carbonyl group available for Schiff's base formation at the target ligands.

In the present patent application, the aldehydes are derivatised with biologically acceptable carbohydrates like glucose, galactose and others to form acetals. The sugar moiety will thus contribute by improving stability and enhancing bioavailability of the aldehyde function to the target cells. This surprisingly leads to more effective carbonyl condensation reactions and easier controllable pharmacokinetics by using our compounds as compared with previously known compounds.

In order to compare Compound 2 with Tucaresol, cell inactivation and protein synthesis inhibition were measured in the presence of equal concentrations of the two drugs. As can be seen from Fig. 1 and Fig. 2, it was shown that Compound 2 was more effective than Tucaresol with respect to both measured parameters.

The immune stimulating effect of the invented compounds may also be used in the treatment of certain virus diseases in combination with other anti-viral therapy like anti-viral drugs or vaccines. Many virus types, after the first infection, incorporate with the cell nucleus and are inactive for a long period of time. Oncogenic viruses like *hepatitis* B and C, certain retro virus and certain *papilloma* virus may cause development of cancer. In these latent period it is very difficult to cure the virus infection. These viruses can often be triggered by immune responses to cause viremia, and in this stage make it possible to get rid of the virus infection. The ability of the benzaldehyde derivatives to trigger the immune response may be used in combination with antivirals or vaccines to develop a treatment for these diseases.

It is a main object of the invention to provide new compounds for prophylaxis and/or treatment of cancer.

Another object of the invention is to provide compounds for prophylaxis or treatment of cancer not giving toxic side-effects.

A third object of the invention is to provide compounds for effective and favourable prophylaxis and/or treatment of cancers in the liver (primary liver cancer as well as liver metastases from other cancers like colorectal cancer). Prophylactic treatment might also be

(Amended page 12)

of great importance to prevent the development of liver cancer in persons with *Hepatitis* B or C infection.

A fourth object of the invention is to provide compounds for effective and favourable prophylaxis and/or treatment of cancer in tissues and cells having receptors with affinity to corresponding sugar moieties.

A fifth object of the invention is to provide compounds for effective and favourable prophylaxis and/or treatment of renal cancer.

A sixth object of the invention is to provide compounds for effective and favourable prophylaxis and/or treatment of pancreatic cancer.

These and other objects by the invention are achieved by the attached claims.

The compounds of the present invention are 4,6-O-(benzylidene-d_1)-D-glucopyranose (Compound 2), 4,6-O-benzylidene-L-glucopyranose (Compound 3) and 4,6-O-(benzylidene-d_1)-L-glucopyranose (Compound 4), or a pharmaceutical acceptable salt thereof.

Compounds 3 and 4 are new *per se*.

Detailed description of the invention

The invention is further explained below by examples and tables and by the attaced figures.

Description of the figures

Fig. 1: The data represents an experiment where NHIK 3025-cells were treated with Compound 2 (Δ) or Tucaresol (●) for 20 hours at 37°C while attached to plastic Petri dishes. Surviving fraction means fraction of cells able to form a macroscopic colony following treatment. Each point represents the mean value of colony counts from 5 parallell dishes. The standard errors are shown when exceeding the size of the symbols.

Fig. 2: The rate of protein synthesis relative to untreated control of NHIK 3025-cells treated with Compound 2 (■) or Tucaresol (▲) for 1 hour at 37°C. The rate of protein synthesis was measuered by amount of [^{3}H]-valine incorporated during the first hour after start of drug treatment. Protein synthesis rate was measured relative to the total amount of protein in the cells. Data are representative for one experiment performed in quadruplicate. Standard errors are indicated when exceeding the size of the symbols.

Fig. 3: The rate of protein synthesis relative to untreated control of Panc-1 cells treated with Compound 2 (■) or zilascorb(^{2}H) (▲). The rate of protein synthesis was measuered by amount of [^{3}H]-valine incorporated during the first hour after start of treatment. Protein synthesis rate was measured relative to the total amount of protein in the cells. Standard errors are smaller than the size of the symbols.

Fig. 4: Median adhesion forces for cells exposed to different benzaldehyde derivatives. The cells were exposed to a 1 mM concentration of Compounds 1 and 2.

Fig. 5: Peripheral blood mononuclear cells and Superantigen in Ex Vivo 10 medium were exposed to either benzaldehyde, deuterated benzaldehyde, Compound 2 or zilascorb(^{2}H). The proliferation of peripheral blood mononuclear cells was measured as incorporation of tritiated thymidine at different drug concentrations.

Fig. 6: NMRI mice were infected i.p. with spleen invading Friend erythroleukemia virus. Infected- and uninfected mice were treated i.p. daily with 5 mg/kg of Compound 2. After treatment for 19 days, spleens were dissected out and weighted.

Fig. 7: Fraction of animals having liver tumour tissue at the time of autopsy.

Fig. 8: Mean amount of tumour material per liver in those animals showing a tumour take.

Fig. 9: Growth curves representing the mean body weight per animal for each group. The time scale represent the age of the animals. In order to make the curves clear standard deviations are indicated only in a few of the measurements.

Fig. 10: In this figure both frequency and size of liver tumour are plotted in the same curve, using a logarithmic scale on the size axis.

Fig. 11: The effect of Compound 1 and 2 on the liver invasion of human colorectal tumour, C170HM2 is shown.

Fig. 12: Rate of protein synthesis of human cervix carcinoma cells, NHIK 3025, treated with Compound 1 or Compound 3 as measured by amount of incorporated [^{3}H]-valine during a pulse period of 1h starting either immediately following addition of test compound (closed symbols) or starting 2h later (open symbols).

Fig. 13: Rate of protein synthesis of human cervix carcinoma cells, NHIK 3025, treated with Compound 2 or Compound 4 as measured by amount of incorporated [^{3}H]-valine during a pulse period of 1h starting either immediately following addition of test compound (closed symbols) or starting 2h later (open symbols).

Fig. 14: Cell survival as measured by colony-forming ability for human cervix carcinoma cells, NHIK 3025, after treatment for 20h with either Compound 1 (●) or Compound 3 (○) is shown.

Fig. 15: Cell survival as measured by colony-forming ability for human cervix carcinoma cells, NHIK 3025, after treatment for 20h with either Compound 2 (○) or Compound 4 (▲) is shown.

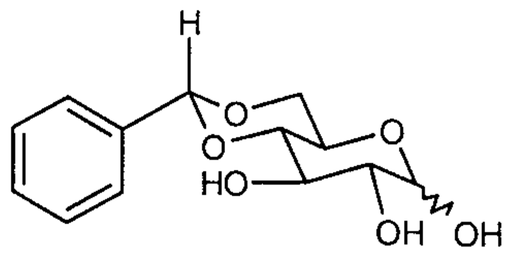
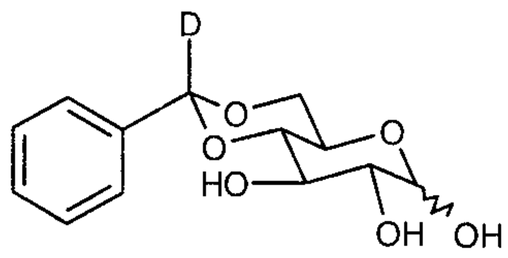
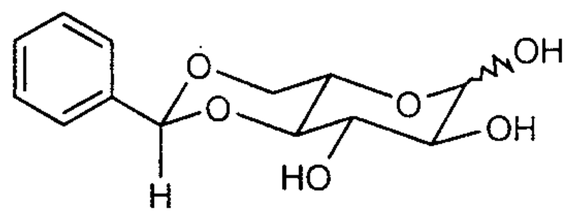
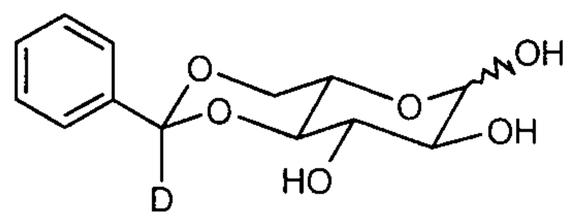
Fig. 16: Cell survival as measured by colony-forming ability for human breast carcinoma cells, T47-D, after treatment for 20h with either L-glucose (●) or Compound 3 (○) is shown.

Description of the tables.

Table 2: Histological observations in normal and cancer tissue. Group 1: Untreated control.

Table 3: Histological observations in normal and cancer tissue. Group 2: Placebo.

Table 4: Histological observations in normal and cancer tissue. Group 3: 85 mg/kg day Compound 2.

Compound No.	Chemical Structure	Name
1		4,6-O-Benzylidene-D-glucopyranose
2		4,6-O-(Benzylidene-d1)-D-glucopyranose
3		4,6-O-Benzylidene-L-glucopyranose
4		4,6-O-(Benzylidene-d1)-L-glucopyranose

5

Preparation

As is well known, aldehydes undergo acid facilitated condensation reactions with alcohols to generate acetals. Water is concomitantly formed as a co-product. The reaction is

10 reversible, and in solution, an equilibrium mixture of aldehyde/alcohol and acetal/water is formed. The position of the equilibrium will mainly be determined by the reactivity and concentration of each species. In order to force the reaction towards completion, one of the products (acetal or water), is normally removed from the reaction mixture.

15 In the present patent application, D(+) or L(-) glucose condensed with benzaldehyde or benzaldehyde equivalents to form the corresponding benzylidene glucose acetals.

Particularly preferred is a re-acetalisation strategy, where benzaldehyde protected as its dimethyl acetal is used instead of benzaldehyde itself. Methanol is then formed as co-product. The reaction mixture is moderately heated at reduced pressure to remove the methanol once it is formed. In most cases, these reaction conditions will drive the equilibrium smoothly in favour of the product acetal.

Acetalisation of sugars will normally lead to mixtures of regio- and stereo isomers. Ring contraction transformations may also occur, leading to mixtures of pyranoses and furanoses, and, in some cases, di-acetalisation adducts are formed. As a consequence, unless protection strategies are applied, complex reaction mixtures are often encountered. However, surprisingly pure product fractions were prepared following appropriate work-up, using liquid chromatography or, as preferred in larger scale synthesis, crystallisation techniques. Identification of the products were achieved by using GC-MS-spectroscopy and various NMR techniques.

The specific reaction conditions, solvent and catalyst maybe subject to variation according to the preferences of the experimentalist. The catalyst may be a mineral acid, e.g. sulphuric acid, an organic acid, e.g. *para*-toluene sulfonic acid, an acidic ion exchanger resin, e.g. Amberlyst 15, a Lewis acid mineral clay, e.g. Montmorillonite K-10 or a resin supported super acid, e.g. Nafion NR 50. The reaction may conveniently be carried out in a dipolar, aprotic solvent such as dimethyl formamide, dimethyl acetamide, dimethyl sulfoxide, N-methyl pyrrolidone, dimethoxyetane or the like. *Para*-toluene sulfonic acid in dimethyl formamide constituted the preferred and most applied reaction condition.

The deuterated compounds may be prepared as described above, but starting with the dimethyl acetal of benzaldehyde-d_1. The preparation of deutero-benzaldehyde may be performed by a modified Rosenmund reduction using D_2 gas in a deuterated solved, as described in EP 0 283 139 B1.

The following examples are illustrative of how the compounds of the present invention may be prepared.

Compound 1: 4,6-O-Benzylidene-D-glucopyranose

This prior art compound was prepared as described for compound 2, starting with undeuterated benzaldehyde dimethylacetal. The identity was confirmed by ^{1}H NMR spectroscopy in DMSO-d_6:

δ rel. to TMS: 7.58-7.29 (5H, m, Ar-H), 6.83 (0.4H, d, OH-1-β), 6.60 (0.6H, d, OH-1-α), 5.61 (1H, s+s, acetal-H), 5.25 (0.4H, d, OH-3-β), 5.21 (0.4H, d, OH-2-β), 5.62 (0.6H, d, OH-3-α), 5.00 (0.6H, H-1-α), 4.82 (0.6H, d, OH-2-α), 4.49 (0.4H, t, H-1-β), 4.18-4.02 (1H, m, H-6'-α+β), 3.89-3.77 (0.6H, m, H-5-α), 3.75-3.57 (1.6H, m, H-6''-α+β and H-3-α), 3.45-3.27 (2.5H, m, H-3-β, H-4-α+β, H-5-β and H-2-α) and 3.11-3.00 (0.4H, m, H-2-β).

Compound 2: 4,6-O-(Benzylidene-d_1)-D-glucopyranose

Benzaldehyde-d_1 was prepared and converted to benzaldehyde dimethylacetal-d_1 as described in EP 0 283 139 B1. The preparation of 4,6-O-(benzylidene-d_1)-D-glucopyranose is also described in EP 0 283 139 B1, but the compound was this time prepared according to an alternative procedure with priority given to achieving high purity:

D(+)-Glucose (706 g, 3.92 mol), benzaldehyde dimethylacetal-d_1 (571 g, 3.73 mol), dry DMF (1.68 kg) and *para*-toluene sulfonic acid (4.5 g, 24 mmol) were mixed in a dry distillation apparatus connected to a vacuum pump through a cold reflux condenser. The mechanical stirred mixture was warmed to max. 69°C at 30 Torr to distil off methanol and after 2 hours, 235 g was collected. The reflux condenser was then shut off and the temperature increased to max 73°C in order to distil off DMF. After 2 more hours, an additional amount of 1385 g was collected and the distillation interrupted.

The residue was cooled to approx. 40°C and ice/water (2.9 L) added within 5 min. The temperature dropped below 0°C and a precipitate was formed, partly as big lumps. The mixture was transferred to a beaker and additional 8-9 L ice/water added in order to make the lumps fell apart and form a suspension. The suspension was filtered on two notches and the two filter cakes left over night on the filters with water jet vacuum connected, each filter cake being flushed with N_2 *via* an inverted funnel. The filter cakes were spread on two

boards and dried at 32°C for 20 hours in a vacuum oven. The vacuum was first set at 13 mbar, then regulated down to 1 mbar.

The crude product was recrystallised (in order to remove di-benzylidene acetals) and water-washed (to remove DMF and glucose) until these contaminants were eliminated. Accordingly, the crude product (500 g) was dissolved in hot dioxane (800 ml) and the solution added *via* a folded filter to boiling chloroform (9 L). The solution was allowed to cool, first to ambient temperature, then in an ice bath overnight. The precipitate was filtered off, dried for 2 hours on the filter (flushing with N_2 as described previously) and dried further overnight at 31°C *in vacuo* on a rotavapor. The product (142 g) was suspended in ice/water (1 L), filtered on a nutch (washing with 200 ml ice/water) and dried on the filter overnight as described previously. It was then grounded, sieved (0.5 mm grid size) and dried *in vacuo* for 5 hours at 31°C on a rotavapor. The product (96 g) once again was suspended in ice/water (500 ml), filtered (washing with 150 ml ice/water) and dried (7 hours under N_2 flush). It was finally grounded on a mortar, sieved (0.5 mm) and dried in a vacuum oven.

The product was a white, finely divided powder of high purity, as analysed on HPLC. The yield was 95 g, 10% of the theoretical. NMR in DMSO-d_6 indicated an α to β anomeric ratio of approx. 7:3.

^{1}H- and ^{13}C NMR (DMSO-d_6), δ rel. to TMS: 7.55-7.28 (5.00H, m, Ar-H), 6.85 (0.27H, d, OH-1-β), 6.58 (0.71H, d, OH-1-α), 5.24 (0.27H, d, OH-3-β), 5.19 (0.28H, d, OH-2-β), 5.61 (0.71H, d, OH-3-α), 4.99 (0.72H, H-1-α), 4.82 (0.71H, d, OH-2-α), 4.48 (0.29H, t, H-1-β), 4.20-4.04 (1.04H, m, H-6'-α+β), 3.88-3.73 (0.78H, m, H-5-α), 3.73-3.56 (1.72H, m, H-6"-α+β and H-3-α), 3.46-3.21 (2.61H, m, H-3-b, H-4-α+β, H-5-β and H-2-α) and 3.09-2.99 (0.28H, m, H-2-β); 137.881, 128.854, 128.042, 126.435 (Ar-C), 100.462 (acetal-C), 97.642 (C-1-β), 93.211 (C-1-α), 81.729 (C-4-α), 80.897 (C-4-β), 75.796 (C-2-β), 72.906 (C-2-α and C-3-β), 69.701 (C-3-α), 68.431 (C-6-α), 68.055 (C-6-β), 65.810 (C-5-β) and 62.032 (C-5-α).

Compound 3: 4,6-O-Benzylidene-L-glucopyranose

L(-)-Glucose (5.0 g, 27.8 mmol), benzaldehyde dimethylacetal (4.66 g, 30.6 mmol) and *para*-toluene sulfonic acid (32 mg, 0.17 mmol) were mixed in dry DMF (20 ml) in a distillation apparatus. A water pump was connected through a short path to remove methanol and DMF *in vacuo*. The colourless suspension dissolved within 1/2 h at 55 °C and the resulting solution stirred at 120 mbar for 1/2 h while gradually increasing the temperature to 65 °C. The vacuum was increased to maximum and the reaction mixture evaporated further for 45 min. The temp. increased to 75 °C at the end of the destillation. The residue was a slightly yellowish sirup, which was neutralised by adding $NaHCO_3$ (29 mg) and allowed to cool.

The crude product was dissolved in methanol (10 ml) and purified on a reversed phase RP-8 column, eluting with methanol/water 1:1. Product fractions were combined and evaporated to remove methanol. The residual solution was further diluted with water and freeze dried. White, fluffy solid from three separate runs were collected to yield a total of 2.42 g, 32.5 % of the theoretical.

GC chromatography of silylated samples indicated the product to consist of two isomers (α and β anomers) in a 35/65 ratio. The ^1H NMR shifts in DMSO-d_6 were similar to those of 4,6-O-benzylidene-D-glucopyranose: 7.51-7.30 (5H, m, Ar-H), 6.86 (0.6H, broad s, OH-1-β), 6.58 (0.3H, broad s, OH-1-α), 5.58 (0.9H, s+s, acetal-H-α+β), 5.23 (0.7H, d, OH-3-β), 5.20 (0.6H, d, OH-2-β), 5.11 (0.4H, d, OH-3-α), 5.00 (0.4H, H-1-α), 4.82 (0.3H, d, OH-2-α), 4.47 (0.7H, d, H-1-β), 4.21-4.08 (1H, m, H-6'-α+β), 3.87-3.73 (0.4H, m, H-5-α), 3.73-3.59 (1.3H, m, H-6"-α+β and H-3-α), 3.46-3.22 (3.7H, m, H-3-β, H-4-α+β, H-5-β and H-2-α) and 3.09-2.99 (0.6H, m, H-2-β).

Compound 4: 4,6-O-(Benzylidene-d_1)-L-glucopyranose

L-Glucose (5.14 g, 28.6 mmol) was warmed in DMF (20 ml) to 95 °C until a clear solution was formed. The reaction flask was then transferred to a water bath at 65 °C and *para*-toluene sulfonic acid (33 mg, 0.17 mmol) was added. Benzylidene dimethyl acetal-d_1

(4.7 ml, 31 mmol) was then added dropwise by syringe over 20 minutes to the stirred glucose solution under a regulated water pump vacuum of 80 mbar. The DMF was then evaporated under vacuum (2 mbar) at 65 °C to give a very pale yellow oil to which was added $NaHCO_3$ (345 mg) followed by stirring for 5 minutes. Warm water (67 °C, 15 ml) was added with stirring (magnetic bead) to the oil at 65 °C and then the flask was shaken in the warm water bath until the oil appeared to have dissolved. The reaction flask was then placed under a stream of cold water for approx. 5 minutes. After only one or two minutes an amorphous mass formed. The aqueous mixture was placed in an ice-water bath and left to stand for 40 minutes. A white precipitate formed during this time which was isolated by vacuum filtration (decanted from the amorphous material), washed with cold spring water (25 ml) followed by cold *iso*-propanol (5 °C, 2 x 5 ml) and dried under a stream of nitrogen to give 1.85 g of a dry white powder. This product was silylated and analysed by gas chromatography and appeared to be 99% pure desired product.

^{1}H NMR, δ (DMSO-d_6) rel. to TMS: 7.55-7.25 (m, 5H, Ar-H), 6.85 (s, 0.48 H, OH-1-β), 6.55 (s, 0.33H, OH-1-α), 5.25 (d, 0.48 H, OH-3-β), 5.20 (d, 0.49 H, OH-2-β), 5.10 (d, 0.35 H, OH-3-α), 4.98 (d, 0.35 H, H-1-α), 4.82 (d, 0.34 H, OH-2-α), 4.48 (d, 0.51 H, H-1-β), 4.20-4.05 (m+m, 0.53 H +0.42 H, H-6' α+β), 3.85-3.73 (m, 0.44 H, H-5-α), 3.72-3.57 (m, 1.27 H, H-6"-α+β and H-3-α), 3.45-3.20 (m, 7.8 H, H-3-β, H-4-α+β, H-5-β and H-2-α) and 3.10-2.98 (m, 0.56 H, H-2-β).

Biological Experiments

Example 1

Biological materials and methods used to demonstrate the effect.

Cell Culturing Techniques

Human cells, NHIK 3025, originating form a cervical carcinoma in situ (Nordbye, K., and Oftebro, R. Exp. Cell Res., 58: 458, 1969, Oftebro R., and Nordbye K., Exp. Cell Res., 58: 459-460, 1969) were cultivated in Eagel's Minimal Essential Medium (MEM) supplemented with 15% foetal calf serum (Gibco BRL Ltd). Human breast carcinoma cells, T-47D, (Keydar, I. et al., Eur. J. Cancer, vol 15, pp. 659-670, 1979) were cultivated in medium RPMI-1640 supplemented with 10% foetal calf serum, 0.2 u/ml insulin, 292 mg/ml L-glutamine, 50 u/ml penicillin, 50 mg/ml streptomycin. The cells are routinely grown as monolayers at 37°C in tissue culture flasks. In order to maintain cells in continuos exponential growth, the cells were trypsinised and recultured three times a week.

Cell Survival

Cell survival was measured as the colony forming ability. Before seeding, the exponentially growing cells were trypsinised, suspended as single cells and seeded directly into 5 cm plastic dishes. The number of seeded cells was adjusted such that the number of surviving cells would be approximately 150 per dish. After about 2 h incubation at 37°C, the cells had attached to the bottom of the dishes. Drug treatment was then started by replacing the medium with medium having the desired drug concentration. Following drug treatment the cells were rinsed once with warm (37°C) Hank's balanced salt solution before fresh medium was added. After 10 to 12 days at 37°C in a CO2-incubator, the cells were fixed in ethanol and stained with methylene blue before the colonies were counted.

It can be seen from Fig. 1 that Compound 2 incuces greater cell inactivation than Tucaresol.

Example 2

Protein Synthesis

The rate of protein synthesis was calculated as described previously (Ronning, O. W. et al., J. Cell Physiol., 107: 47-57, 1981). Briefly, cellular protein was labelled to satuarion during a minimum 2 day preincubation with [^{14}C]valine of constant specific radioactivity (0.5 Ci/mol). In order to keep the specific radioactivity at a constant level, a high concetration of valine (1.0 mM) was used in the medium. At this concetration of valine, the dilution of [^{14}C]valine by intracellular valine and proteolytically generated valine will be negligible (Ronning, O. W., et al., Exp. Cell Res. 123: 63-72, 1979). The rate of protein synthesis was calculated from the incorporation of [^{3}H]valine related to the total [^{14}C]radioactivity in protein at the beginning of the respective measurement periods and expressed as percentage per hour (Ronning, O. W. et al., J. Cell Physiol., 107: 47-57, 1981).

It can be seen from Fig. 2 that Compound 2 induces greater protein synthesis inhibition than Tucaresol.

From Fig. 3 it can be seen that Compound 2 induces stronger protein synthesis inhibition than zilascorb(^{2}H). The cells used in this experiment are Panc-1 cells originating from a human pancreatic adenocarcinoma (Lieber et al., Int. J. Cancer, 15: 741-747, 1975) grown in medium E2a.

Example 3

Cell adhesion measurements

Cell adhesion forces were measured using the manipulation force microscope (G. Sagvolden. Manipulation force microscope. Ph.D. thesis, University of Oslo, 1998, and G. Sagvolden, I. Giaever and J. Feder. Characteristic protein adhesion forces on glass and polystyrene substrates by atomic force microscopy. Langmuir *14*(21), 5984-5987, 1998.). Briefly, NHIK 3025 carcinoma cells were cultured in CO_2-independent medium containing

15% fetal calf serum. The cells were exposed to a 1 mM concentration of Compound 1 or Compound 2 for 20 hours before they were released from the cell culture flasks using trypsin. The cells were kept in suspension, and seeded in medium with Compound 1 or Compound 2 on polystyrene tissue culture substrates 90 minutes after the trypsin reaction had been stopped. The cell-substrate adhesion forces were measured by displacing cells using an inclined atomic force microscope cantilever acing as a force transducer. One cell was displaced at a time and each cell was displaced only once.

The maximal force exerted on each cell was recorded as a function of the time since the cells were seeded on the substrate. The median force of a group of 19 measurements is shown as a function of the mean time for cells exposed to Compound 1 or Compound 2 in Fig. 4, together with the adhesion forces of cells not exposed to these compounds. Compound 2 shows a large effect in reducing the adhesion force at this concentration, while Compound 1 shows no significant response. The effect of the compound is mainly to reduce the adhesion force of the cells, but not the time course of adhesion.

The reduced ability to attach to the substrate may be related to the blocking of integrin-mediated anchorage of the cells. It has been shown that such blocking may induce programmed cell death in both hepatoma and melanoma cancers. (Paulsen JE, Hall KS, Rugstad HE, Reichelt KL and Elgjo K, The synthetic hepatic peptides pyroglutamylglutamylglycylserylasparagine and pyroglytamylglutamylglycylserylaspartic acid inhibit growth of MH1C1 rat hepatoma cells transplanted into buffalo rats and athymic mice. Cancer Res. 52 (1992) 1218-1221. and Mason MD, Allman R, and Quibell M, "Adhesion molecules in melanoma – more than just superglue?" J. Royal Soc. Med. 89 (1992) 393-395.)

The adhesion force between NHIK 3025 cells and the substratum was measured after pre-incubation of the cells in solutions of Compounds 1 and 2. Even at 1 mM concentration, an astonishing D-isotope effect was shown. Surprisingly, Compound 2 significantly reduced the adhesion force to 1/3 relative to control, whereas Compound 1 did not lead to significant reduction. The inventors believe that Compound 2 may have interfered with the biosynthesis of integrins, reducing the cell's ability to attach to the substratum. Integrins are structural trans-membrane proteins crucial for binding cells to the extracellular matrix and

for cell-cell interactions. Inhibiting the function of the integrins could thus directly affect the metastasising ability of cancer cells. The experiment indicate that integrines could be especially sensitive to protein synthesis inhibition. Thus, Compound 2 could well be used for prevention of metastatic processes in cancer development.

Example 4

Experiments with Compound 2 in NMRI mice infected with FRIEND erythroleucaemia virus (FLV).

Virus: Eveline cells were supplied by prof. Gerhard Hunsman, Munich. We have shown that this virus, which originally was used as a source of Friend helper virus, contain a defect virus of the same size as Spleen Focus Forming Virus (SFFV) which induces erythroleukaemia in NMRI mice after a delay of 4-8 weeks.

Mice: NMRI mice came from old Bomholt Farm, Denmark, and were purchased *via* SIFF. The mice were received on May 6th and entered into the experiment on May 11th. They were then infected with 50 microlitres supernatant from Eveline culture, intraperitonally. After 24 hours the treatment was started. Compound 2 was dissolved in sterile isotonic glycerol solution in a concentration corresponding to 5 mg per kg when giving 50 microlitres intraperitonally.

The experiment was set up as follows:

10 mice uninfected control

10 mice infected control

5 mice uninfected, treated with Compound 2

10 mice infected, treated with Compound 2

The mice were given injections intraperitonally once daily for 19 days. From June 1st till June 16th, when they were sacrificed, no treatment was given. On June 16th, the mice were sacrificed. Blood was withdrawn (for future analysis). The spleen was removed and

weighed (see table 1 below). One bit of the spleen was frozen in nitrogen for the purpose of cutting thin slices and one bit was formaline fixated.

Table 1:

uninfected controls	infected controls	Comp. 2, uninfected	Comp. 2, infected
125	154	266	151
160	240	143	153
94	214	106	168
146	212	153	145
118	165	117	149
120	171	157	127
115	190	63.824	131
103	204		170
130	203		127
147	148		148
125.8	190.1	157	146.9
20.5	24.5	63	15.228

The results can also be seen in Fig. 6.

As one can see, there is a significant difference in spleen weights in infected animals compared to uninfected controls. Weights of uninfected animals treated with Compound 2 is above the weights of uninfected controls, even if this is not significant. One notices that infected animals which were treated with Compound 2 actually have a lower average spleen weight compared to uninfected animals which were treated similarly (here, it is assumed that the outcome stems from one animal in the control group having a comparatively big spleen).

A histological examination revealed that the uninfected controls have a normal spleen anatomy. All animals in the infected untreated group have invasion of pathological leukaemia cells in the red pulpa. The spleens, from the uninfected Compound 2 - treated animals, have hypertrofic germinal centra, which are interpreted as an expression of immune stimulation. One does not find leukemic changes in spleens from the Compound 2 - treated infected group.

The results are encouraging taking into consideration the aggressive nature of FLV in mice and also when one compares the effect with that seen with azidothymidine and other anti-virus treatment.

Example 5

Proliferation of peripheral blood mononuclear cells

The inventors performed an experiment where peripheral blood mononuclear cells were exposed to Superantigen together with benzaldehyde, deuterated benzaldehyde, Compound 2 or zilascorb(^{2}H). Superantigen is used as a very active standard for proliferation of T-cells and is presented *via* antigen presenting cells to T-cells.

The experiment demonstrated (see figur 5) that by adding benzaldehyde, deuterated benzaldehyde or Compound 2, the proliferation of peripheral blood mononuclear cells was increased significantly in a bell-shaped, dose-dependent manner, whereas very little effect was observed with zilascorb(^{2}H). The fact that we are able to increase the proliferation signal from the Superantigen indicates that the compounds act by additional co-stimulatory effects on the T-cells.

Example 6

Tumour effects in nitrosamine-induced hepatic rat cancers

In the present experiment we tested whether 11 months treatment with Compound 2 or zilascorb(^{2}H) could prevent cancer development in livers of rats following partial hepatectomy diethylnitrosamine (DENA) and phenobarbital.

Materials and methods:

We used Wistar Kyoto rats supplied by Versuchtierzucht Institut, Hannover. Partial hepatectomy (70% removed) was performed at the Radium Hospital in 4 weeks old rats before intraperitoneal treatment with diethylnitrosamine (DENA) and 4 weeks feeding with phenobarbital. Liver carcinomas appear in most animals within 10 months after this carcinogenic initiation. Six and one half months after the carcinogenic initiation, i.e. before any cancer had developed, treatment was started at the National Institute of Occupational Health.

56 animals were divided randomly into 4 groups, 14 animals in each, and subjected to the following daily i.v. drug doses:

Group 1: Control, no injections

Group 2: Placebo, injections of saline only.

Group 3: 85 mg/kg Compound 2

Group 4: 100 mg/kg zilascorb(^{2}H)

Treatment was given in periodic cycles of 5 weeks: first 3 weeks of daily i.v. injection, then 2 weeks pause. A total of 9 complete 5 week cycles of treatment was given. The total period from the first treatment to end of treatment and autopsy was 11 months.

At the time of autopsy, an approximate estimate of the volume of liver tumours were done, but without thorough cutting of the liver material. Following fixation in formalin, Professor Jahn M Nesland, head of Department of Pathology at the Norwegian Radium Hospital, firstly performed an accurate estimate of the tumour mass by cutting and macroscopically inspecting the tissue and secondly performed a histological examination on both tumour tissues (which appeared mainly in the liver) as well as normal tissue from all relevant organs and tissues.

The occurrence of multiple foci as well as of pre-malignant nodules were histologically examined in each animal.

Results:

Tumour drug effects analysed by means of frequency of animals having liver tumours at the time of autopsy, show that there is a significantly lower frequency of animals having liver-tumours in the Compound 2-treated group as compared to the placebo-treated group. Analysis by means of a one-sided Fischers exact test give a p-value of 0.05. If both control groups are pooled together, there are 28 control animals, of which 25 developed liver cancer while there are 14 Compound 2-treated animals, of which 7 developed liver cancer. In this case the number of animals is sufficiently high for a chi-square test, which show that the difference is significant by $p = 0.015$. In the zilascorb(^{2}H)-treated group 11 out of 14 animals developed liver cancer. This is only one less than in the control group, and not significant.

The size of the liver tumours are most easily analysed from Fig. 7 and Fig. 8. Here Compound 2 is seen to have exerted a convincing effect: While 50 % of the animals in the placebo group had liver cancers amounting to more than 10 cm^3 no animals in the Compound 2 group had such big cancers (chi-square test: $p = 0.0038$). Furthermore, while only 3 animals (i.e. 21 %) in the Compound 2 group had cancers amounting to over 1 cm^3, 10 animals (i.e. 71 %) in the placebo group had cancers above this limit (Fishers exact test: $p = 0.0002$). Thus, for Compound 2, the anticancer effect is even more clear when tumour size is taken into consideration than when frequency of cancer development is analysed only.

Fig. 9 represent mean body weight measurement of each group of animals over the whole period from partial hepatectomy and nitrosamine treatment (time 0). Both the body weight measurements and the histological evaluation of normal tissues seem clear in indicating a total absence of side effects. The tooth-edged shape of the body weight growth curves (Fig. 9) are undoubtedly due to the treatment, but not to the drugs. Rather it is the injections itself that affect the animals since animals treated with saline only had exactly the same characteristic body weight variations as those treated with saline and drugs.

That animals treated with 210 iv injections over an 11 months period show signs of body weight affection is not surprising: For each injection the animals were warmed up slightly under an electrical heater bulb, and were afterwards immobilised in a specially constructed

holder so that injection could take place. Although this procedure took place in a quiet room and was performed by a skilled person, trained to calm down the animals, it is not surprising that it may create a biological reaction in the animals.

An aspect of the study concerning side effects is the histological evaluation of normal tissues from various organs of the body. This rather extensive study is easily summed up from tables 2 to 4 (attached). In no cases any abnormalities which could be related to drug treatment was found. It is worth noticing that even the rat tails, within the local region where the i.v. injections were given daily for such a long period of time, were unharmed by the treatment.

There is, however, an interesting conclusion to be drawn from the frequencs of preneoplastic lesions in livers: From tables 2 to 4 all animals developed multiple foci. This lesion is considered to be an early stage in the process of development of malignancy in liver, and is not seen to be influenced by the drug treatment. Furthermore, the appearance of preneoplastic nodules, although limited to few animals, is present in all groups. The present data, therefore, do not indicate any drug effect on this lesion further strengthening the impression that Compound 2 exerts a truly cancer-specific effect in the liver.

In Fig. 10 both frequency and size of liver tumour are plotted in the same curve, using a logarithmic scale on the size axis.

Frequency of tumours developed in liver: The number of animals in the control group and in the placebo group that developed liver cancer was 13 and 12 respectively, out of 14 animals in each group (tables 2 and 3). In group 3 (85 mg/kg Compound 2) only 7 animals out of 14 developed liver cancer (table 4). In testing the difference between groups 2 (placebo) and 3 statistically the number of cases is too low for a chi-square test. However, a one-sided Fishers exact test can be done, and shows that the two groups are significantly different ($p = 0.05$) with respect to the frequency of liver tumour appearance. This difference is, however, even stronger statistically if we include in the test also the untreated control animals (group 1) in the control group. In that case there is 28 control animals of which 25 developed liver cancer, and a chi-square test is acceptable. In this case the difference between group 3 and the controls is significant by $p = 0.015$.

In group 4 (100 mg/kg zilascorb(^{2}H)) 11 out of 14 animals developed liver cancer. This is only one less than in the placebo group, and by no means significant. Thus, by analysis of the frequency of development of liver cancer we must conclude that the treatment with Compound 2 significantly reduced this development while zilascorb(^{2}H) had no such effect.

Size of developed liver cancers: The size of the liver tumours are most easily analysed from Fig. 10, but are also given in tables 2 to 4. Here Compound 2 is seen to have exerted a convincing effect: While 50 % of the animals in the control- and placebo groups had liver cancers amounting to more than 10 cm^3, no animals in the Compound 2 group had such big cancers (chi-square test: $p = 0.0038$). Furthermore, while only 3 animals (i.e. 21 %) in the Compound 2 group had cancers amounting to over 1 cm^3, 10 and 13 animals (i.e. 71 and 93 %) in the placebo and control groups, respectively, had cancers above this limit (Fishers exact test: $p = 0.0002$ and $p = 0.011$, respectively).

Thus, for Compound 2 the anticancer effect is even more clear when tumour size is taken into consideration than when frequency of cancer development is analysed only. In fact, this effect is so striking that it became obvious even at the time of autopsy, when only a brief macroscopic overview of the livers of the animals were done.

For animals treated with zilascorb(^{2}H) the effect is much weaker than for those treated with Compound 2, even when tumour size is considered. The number of zilascorb(^{2}H) treated animals having more than 1 cm^3 of liver cancer is 7. A significance test against the control and placebo groups, as was performed with the Compound 2 treated animals, show p values of 0.036 and 0.44 respectively. Thus, when comparing with the untreated control, the difference is significant, while, when comparing with the placebo group, it is clearly not significant. One should take into consideration here that 3 of the animals in the placebo group had tumours just below 1 cm^3 and that if the level of comparison had been chosen at 0.5 cm^3 there would have been a significant difference also with the placebo group (see Fig. 10). Nevertheless, the present analysis indicates that the effect of zilascorb(^{2}H) is weak, and probably at the limit of significance. Furthermore, for animals having tumours 3 above about 5 cm^3 there is no difference between the zilascorb(^{2}H) treated and the control or placebo animals.

In a separate experiment where zilascorb(^{2}H) and Compound 2 were given to rats for only 10 days, histological examinations of liver tumours indicated no change in the zilascorb-treated animals, while there was increased tumour necrosis in 2 of 5 Compound 2-treated animals.

Table 2: Histological observations in normal and cancer tissue. Group 1: Untreated control

Animal no.	Tissue outside of liver								Liver		
	Brain Pituiary	Hart, Aortha Thymus	Lungs Thyro Pancr	Testes Prost	Ventr. Colon	Spleen Bone marrow	Tail Paw*	Kidnesy L| R necros*	Pre-neopl nodul	Foci	Tumour necros
3								0 | +	0	MF	no
11	NF	NF		NF			NF	NF	0	MF	(+)
22	NF	NF	NF	NF		NF	NF	NF	0	MF	+
24	NF	NF		NF	NF	NF	NF	NF	YES	MF	+
25	NF	NF	NF	NF	NF	NF		NF	YES	MF	no
27			NF			NF	NF	NF	0	MF+C	+
31		NF	NF			NF		NF	0	MF	+
33	NF	NF	NF	NF		NF		NF	0	MF	+
35					NF	NF	NF	CARC ++	0	MF	+
38	NF	NF	NF		NF	NF	NF	NF	YES	MF	TUMOR -
51	NF	NF	NF			NF		NF	0	MF	+
54	NF	NF	NF			NF	NF	CARC no	0	MF	no
56	NF	NF				NF	NF	NF	0	MF	+
60	TU no	NF	NF					NF	0	MF	+

Abbrevations: NF: Histologic examination performed, Normal findings.
MF: Multiple foci.
no: means no necrosis.

* In some animals the paw of the left foreleg was examined nicroscopically.Normal findings were done in all cases.

Table 3: Histological observations in normal and cancer tissue. Group 2: Placebo

Animal no.	Tissue outside of liver								Liver		
	Brain Pituiary	Hart, Aortha Thymus	Lungs Thyro Pancr	Testes Prost	Ventr. Colon	Spleen Bone marrow	Tail Paw*	Kidnesy L| R necros*	Pre-neopl nodul	Foci	Tumour necros
4		NF					NF	NF	0	MF	Tumor
18	CARC	NF	NF		NF			CARC	0	MF	no
19	ADENO	NF	NF	NF	NF	NF		NF	0	MF	no
23	NF	NF	NF			NF	NF	NF	YES	MF	+
26		NF	NF			NF	NF	NF	0	MF	+
34		NF	NF	NF		NF	NF	NF	0	MF	no
39	NF	NF	NF		NF			NF	0	MF	+
44	NF	NF	NF		NF	NF	NF	NF	0	MF	TUMOR -
45									0	MF	+
46	NF (?)	NF	NF			NF	NF	NF	0	MF	no
50		NF	NF	NF	NF				0	MF	no
52	NF	NF	NF	NF	NF	NF	NF		0	MF	+
55	NF	NF	NF	NF	NF	NF		NF	0	MF	+
59		NF	NF		NF					MF	no

Abbrevations: NF: Histologic examination performed, Normal finding.
MF: Multiple foci.
no: means no necrosis.

* In some animals the paw of the left foreleg was examined microscopically.
Normal findings were done in all cases.

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Table 4: Histological observations in normal and cancer tisses. Group 3: 85 mg/kg day Compound 2

Animal no.	Tissue outside of liver								Liver		
	Brain Pituiary	Hart, Aortha Thymus	Lungs Thyro Pancr	Testes Prost	Ventr. Colon	Spleen Bone marrow	Tail Paw*	Kidnesy L| R necros*	Pre-neopl nodul	Foci	Tumour necros
5	NF	NF	NF	NF	NF	NF		NF	0	MF	+
7	NF	NF	NF	NF	NF		NF	NF	0	MF	(+)
13		NF	NF			NF	NF	NF	YES	MF	TUMOR -
14		NF				NF			YES	MF	TUMOR -
*15		NF	NF	NF	NF	NF		NF	YES	MF	+
17	NF	NF	NF		NF	NF	NF		YES	MF	TUMOR -
*20	NF	NF	NF	NF	NF		NF	CARC ++	0	DFF	TUMOR -
29	OLIGL						NF		0	MF	TUMOR -
30	NF		GRANU			NF		NF	0	MF	+
32	NF	NF	NF			NF	NF	NF	0	MF	+
37	NF	NF	NF			NF	NF	NF	0	MF	TUMOR -
42	TUMOR	NF	NF	NF	NF	NF		NF	0	MF	++
49											TUMOR -
53	NF	NF	NF	NF	NF	NF	NF		0	MF	+

Abbrevations:
NF: Histologic examination performed, Normal finding.
MF: Multiple foci.
DFF: Diffuse focal change
OLIGL: Oligodendroglioma
GRANU: Granulomas

*Animal no 15 had a squamous cell carcinoma in the trachea. This was cystic and to some extent necrotic outside of the custic region (+). Animal no 20 had cystic degenerative changes in the liver.

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Example 7

Effect on liver invasive colorectal cancer in nude mice

Material and Procedures

The cell line evaluated, C170HM2, is an established human colorectal cell line (S.A.Watson *et al.*, Eur.J.Cancer 29A (1993), 1740-1745) and was derived originally from a patient's primary tumour. C170HM2 cells were maintained *in vitro* in RPMI 1640 culture medium (Gibco, Paisley, UK) containing 10% (v/v) heat inactivated foetal calf serum (Sigma, Poole, UK) at 37°C in 5% $C0_2$ and humidified conditions. Cells from semi-confluent monolayers were harvested with 0.025% EDTA and washed twice in the culture medium described above.

C170HM2 cells harvested from semi-confluent cell monolayers were re-suspended at $1x10^6$/ml of sterile phosphate buffered saline, pH 7.4 [PBS] and injected in a 1ml volume into the peritoneal cavity of 20 MFl male nude mice (bred within the Cancer Studies Unit at the University of Nottingham). Mice were identified by an electronic tagging system (RS Biotech DL2000 Datalogger). On day 10 following cell injection, the mice were randomly assigned to alter a placebo control group (n=2 mice) or experimental groups (n=6/group);-

Group 1:	Compound 1	5 mg/kg (n=2 mice)
		30 mg/kg (n=2 mice)
		90 mg/kg (n=2 mice)
Group 2:	Compound 2	5 mg/kg (n=2 mice)
		30 mg/kg (n=2 mice)
		90 mg/kg (n=2 mice)

The drugs were dosed intravenously (iv) from day 10 and continue until therapy termination. The experiment was terminated at day 40 post cell implantation. Mice were weighed at regular intervals throughout the pilot study.

At termination the liver was exposed, and visible liver tumours were counted and their total cross-sectional area measured. The tumours were also photographed. No liquefaction of the tumours had occurred, thus they were dissected free from the normal liver tissue, weighed and fixed in formal saline. Peritoneal nodules were dissected free and the cross-sectional area and weight measured. Detailed pathological assessment of the tumours was performed.

The effect of Compound 1 and 2 on the liver invasion of the human colorectal tumour, C170HM2 is shown in Fig. 11.

Example 8

Necrotizing effect on primary rat kidney adenoma cells

In an experiment on inheritable rat kidney adenomas (Eker and Mossige, Nature 189, (1961) 858-859) it was found extensive tumour necrosis after 10 days i.v. injections of 85 mg/kg body weight of Compound 2 in two animals. In animals given saline without drug little or no necrosis was observed.

Example 9

Biological effects of Compounds 3 and 4 compared with Compounds 1, 2 and L-glucose

Protein Synthesis

Fig. 12 show rate of protein synthesis of human cervix carcinoma cells, NHIK 3025, as measured by amount of incorporated [^{3}H]-valine during a pulse period of 1h starting either immediately following addition of test compound (closed symbols) or starting 2h later (open symbols). Test compounds, Compound 1 and Compound 3, were present from time zero to the end of the pulses. Cells were pre-labeled with [^{14}C]-valine for at least 4 days in order to have all cellular protein labeled to saturation. Incorporated amount of [^{3}H] was related to

incorporated amount of [^{14}C] so that protein synthesis was calculated as per cent of the total amount of protein in the cells. Rate of protein synthesis is given as per cent of that in an untreated control. The plotted values for protein synthesis represent mean values from 4 simultaneously and similarly treated wells. Standard errors are indicated by vertical barrs in all cases where they exceed the symbols. The data indicate that Compound 1 induces a protein synthesis inhibition which increases linearly with increasing concentration of drug while little or no effect is seen by Compound 3.

Fig. 13 show rate of protein synthesis of human cervix carcinoma cells, NHIK 3025, as measured by amount of incorporated [^{3}H]-valine during a pulse period of 1h starting either immediately following addition of test compound (closed symbols) or starting 2h later (open symbols). Test compounds, Compound 2 and Compound 4, were present from time zero to the end of the pulses. Cells were pre-labeled with [^{14}C]-valine for at least 4 days in order to have all cellular protein labeled to saturation. Incorporated amount of [^{3}H] was related to incorporated amount of [^{14}C] so that protein synthesis was calculated as per cent of the total amount of protein in the cells. Rate of protein synthesis is given as per cent of that in an untreated control. The plotted values for protein synthesis represent mean values from 4 simultaneously and similarly treated wells. Standard errors are indicated by vertical barrs in all cases where they exceed the symbols. The data indicate that both Compound 2 and Compound 4 induces an effective inhibition of protein synthesis at about the same level for both compounds. Both these two deuterated compounds are more effective than the corresponding undeuterated compounds shown in Fig. 12.

<u>Cell Survival</u>

Fig. 14 show cell survival as measured by colony-forming ability for human cervix carcinoma cells, NHIK 3025, after treatment for 20h with either Compound 1 (●) or Compound 3 (○). Cells were treated in open plastic Petri dishes incubated in CO_2-incubators at 37°C. The plotted survival values represent mean values from 5 simultaneously and similarly treated dishes. Standard errors are indicated by vertical barrs in all cases where they exceed the size of the symbols. From the data the dose response curves follow different shapes for the two compounds, indicating that Compound 3 is more

effective than Compound 1 in inactivating cells at low compound-concentrations. The differences in curve shapes indicate different mechanisms of cell inactivation for these two drugs.

Fig. 15 show cell survival as measured by colony-forming ability for human cervix carcinoma cells, NHIK 3025, after treatment for 20h with either Compound 2 (○) or Compound 4 (▲). Cells were treated in open plastic Petri dishes incubated in CO_2-incubators at 37°C. The plotted survival values represent mean values from 5 simultaneously and similarly treated dishes. Standard errors are indicated by vertical barrs in all cases where they exceed the symbols. Compound 4 is more effective than Compound 2 in inactivating cells, particularly in the low-dose region. For example is cell survival down to 50% following treatment with 0.5 mM of Compound 4 and 4 mM of Compound 2 respectively, indicating an 8-fold higher inactivating efficiency of Compound 4 compared to Compound 2 at this particular effect level. At a survival level of 10 % the difference is much smaller.

Fig. 16 show cell survival as measured by colony-forming ability for human breast carcinoma cells, T47-D, after treatment for 20h with either L-glucose (●) or Compound 3 (○). Cells were treated in open plastic Petri dishes incubated in CO_2-incubators at 37°C. The plotted survival values represent mean values from 5 simultaneously and similarly treated dishes. Standard errors are indicated by vertical barrs in all cases where they exceed the symbols. The data indicate that L-glucose has little or no effect on cell survival for concentrations up to at least 10 mM, the highest dose tested. Compound 3 also has little effect on these cells for concentrations up to 2 mM, but induces considerable inactivating effect for higher concentrations and only one of 1000 cells survive 20h in presence of 8 mM of this compound.

Conclusion

Both the two L-glucopyranose derivatives tested (Compounds 3 and 4) inactivate cells more effectively than the corresponding D-glucopyranose derivatives (Compounds 1 and 2). L-glucose alone, however, does not induce any significant cell inactivating effect for

concentrations tested here. Thus, it is in the context of a benzylidene derivative this increased effect of L as compared to D glucose is found.

Administration

The pharmaceutical compositions according to the present invention may be administered in anti-cancer treatment.

For this purpose the compounds according to the present invention may be formulated in any suitable manner for administration to a patient, either alone or in admixture with suitable pharmaceutical carriers or adjuvants.

It is especially preferred to prepare the formulations for systemic therapy either as oral preparations or parenteral formulations.

Suitable enteral preparations will be tablets, capsules, e.g. soft or hard gelatine capsules, granules, grains or powders, syrups, suspensions, solutions or suppositories. Such will be prepared as known in the art by mixing one or more of the compounds of the invention with non-toxic, inert, solid or liquid carriers.

Suitable parental preparations of the compounds according to this invention are injection or infusion solutions.

When administered topically the compounds may be formulated as a lotion, salve, cream, gel, tincture, spray or the like containing the compounds in admixture with non-toxic, inert, solid or liquid carriers which are usual in topical preparations. It is especially suitable to use a formulation which protects the active ingredient against air, water and the like.

The preparations can contain inert or pharmacodynamically active additives. Tablets or granulates e.g. can contain a series of binding agents, filler materials, carrier substances and/or diluents. Liquid preparations may be present, for example, in the form of a sterile solution. Capsules can contain a filler material or thickening agent in addition to the active ingredient. Furthermore, flavour-improving additives as well as the substances usually used

as preserving, stabilising, moisture-retaining and emulsifying agents, salts for varying the osmotic pressure, buffers and other additives may also be present.

The dosages in which the preparations are administered can vary according to the indication, the mode of use and the route of administration, as well as to the requirements of the patient. In general a daily dosage for a systemic therapy for an adult average patient will be about 0.01 -500mg/kg body weight once or twice a day, preferably 0.5-100 mg/kg body weight once or twice a day, and most preferred 1-20 mg/kg weight once or twice a day.

If desired the pharmaceutical preparation of the compound can contain an antioxidant, e.g. tocopherol, N-methyl-tocopheramine, butylated hydroxyanisole, ascorbic acid or butylated hydroxytoluene.

ANNEX

AMENDED CLAIMS

1. Use of 4,6-O-(benzylidene-d_1)-D-glucopyranose, 4,6-O-benzylidene-L-glucopyranose and/or 4,6-O-(benzylidene-d_1)-L-glucopyranose, or a pharmaceutical acceptable salt thereof, for the manufacture of a pharmaceutical preparation for the prophylaxis and/or treatment of liver cancer, renal cancer and pancreatic cancer.

2. Use according to claim 1, for the manufacture of a pharmaceutical preparation for prophylactic treatment of cancers induced by viruses like *hepatitis B and C, oncogene papilloma viruses and other oncogene viruses.*

3. A benzaldehyde derivative useful as a therapeutic agent wherein the benzaldehyde derivative is 4,6-O-benzylidene-L-glucopyranose and/or 4,6-O-(benzylidene-d_1)-L-glucopyranose or a pharmaceutical acceptable salt thereof.

4. A pharmaceutical composition comprising a benzaldehyde derivative according to any preceding claim, and a pharmaceutically acceptable carrier, diluent and/or excipient.

5. A process for manufacture of a pharmaceutical composition, which comprises the step of incorporating a benzaldehyde derivative as defined in any preceding claim, together with a pharmaceutically acceptable carrier, diluent and/or excipient.

6. A benzaldehyde derivative defined as 4,6-O-benzylidene-L-glucopyranose, 4,6-O-(benzylidene-d_1)-L-glucopyranose or a pharmaceutical acceptable salt thereof.

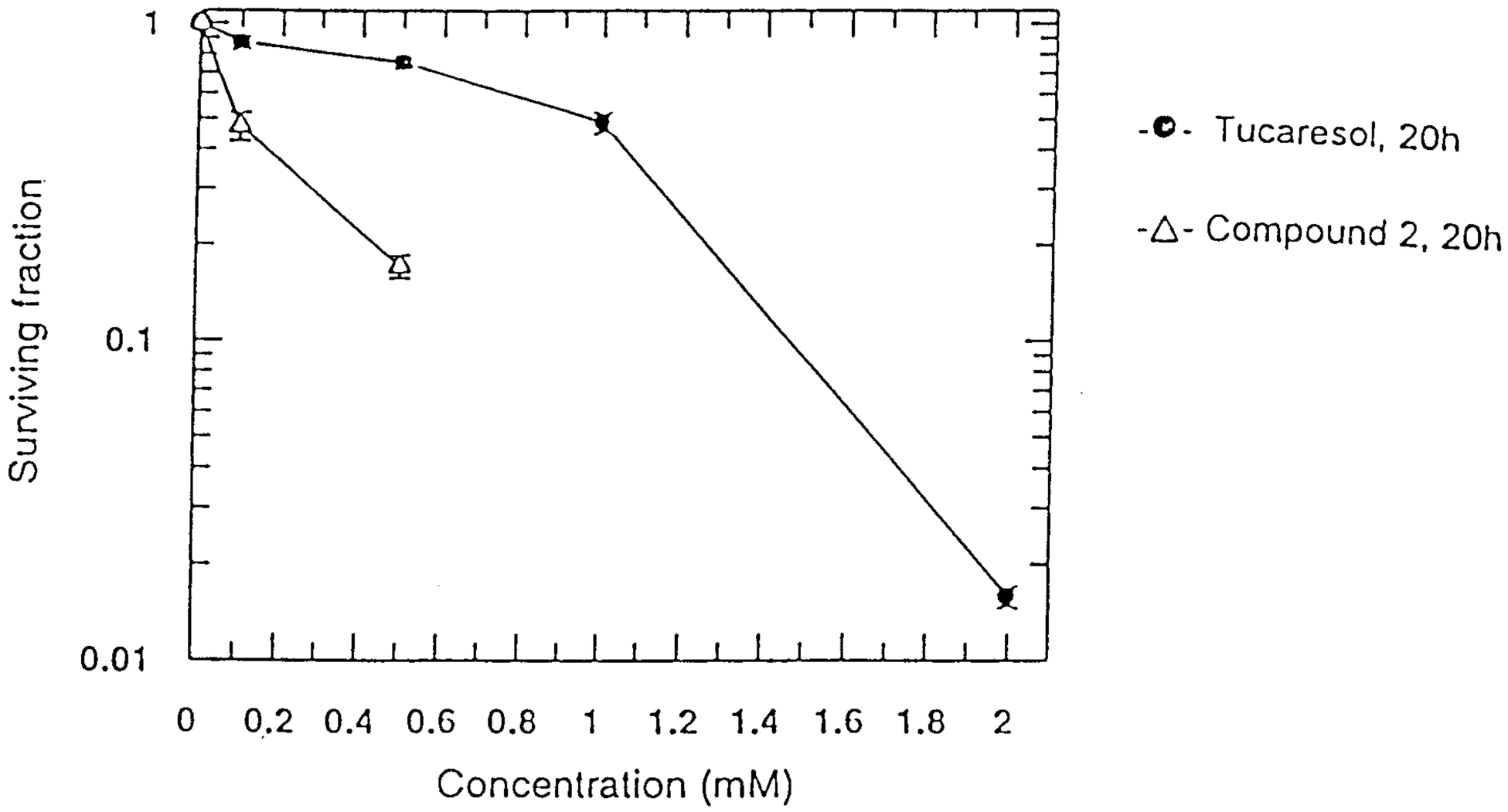
Surviving fraction
1
0.1
0.01
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
Concentration (mM)
-●- Tucaresol, 20h
-△- Compound 2, 20h

Fig. 1

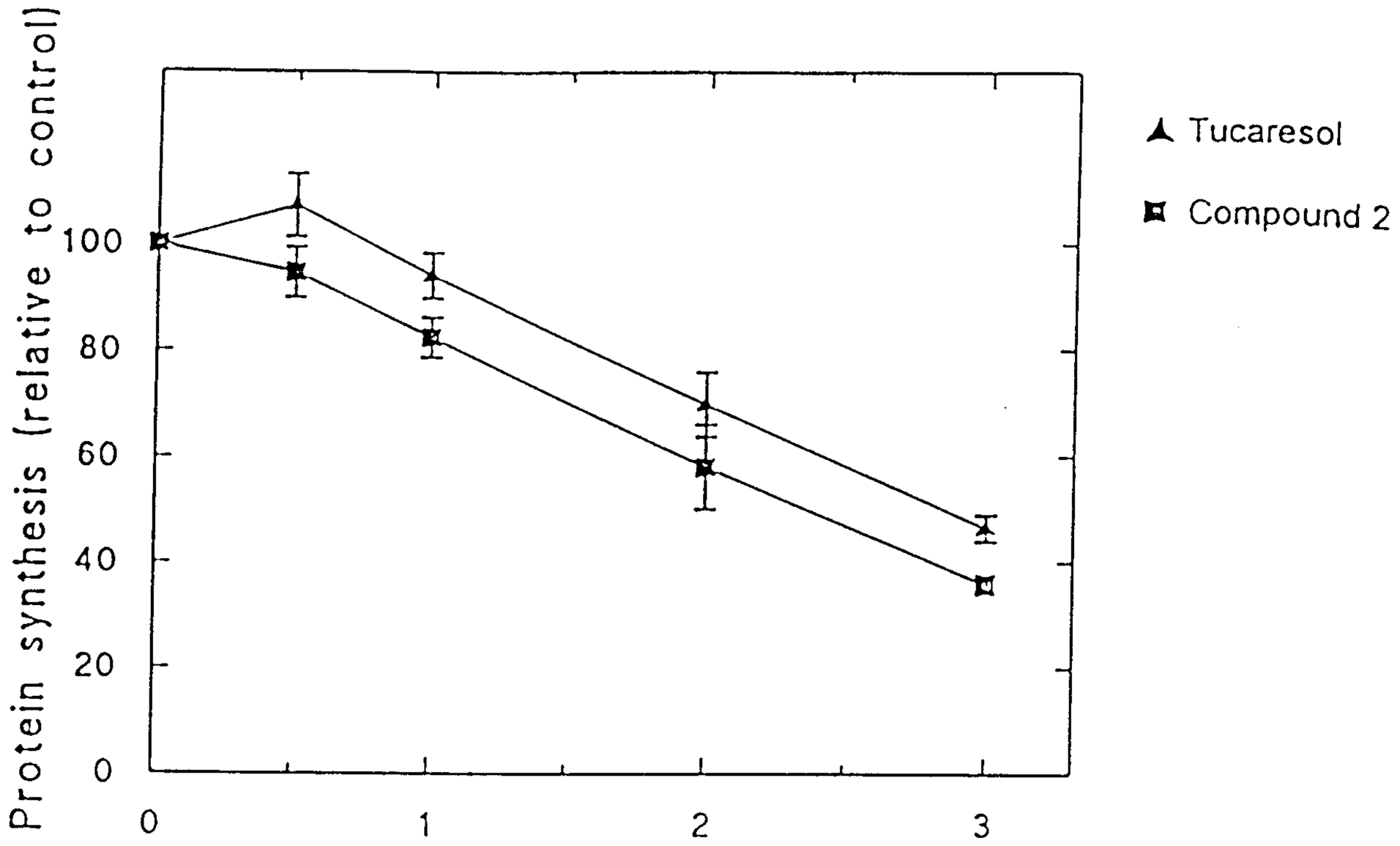
Protein synthesis (relative to contr
100
80
60
40
20
0
0
1
2
3
Concentration (mM)
Tucaresol
Compound 2

Fig. 2

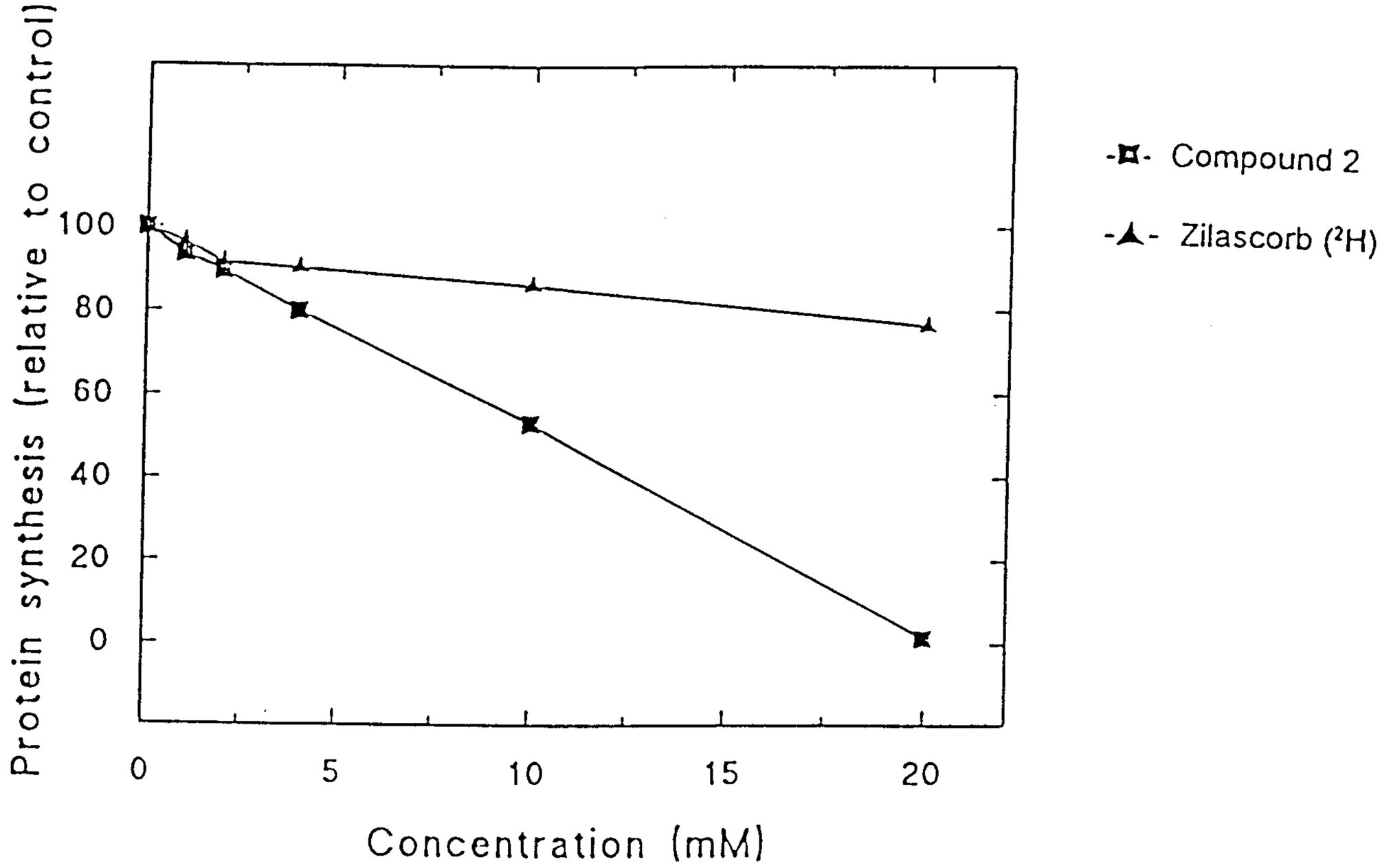
Protein synthesis (relative to contr
100
80
60
40
20
0
0
5
10
15
20
Concentration (mM)
-□- Compound 2
-▲- Zilascorb (²H)

Fig. 3

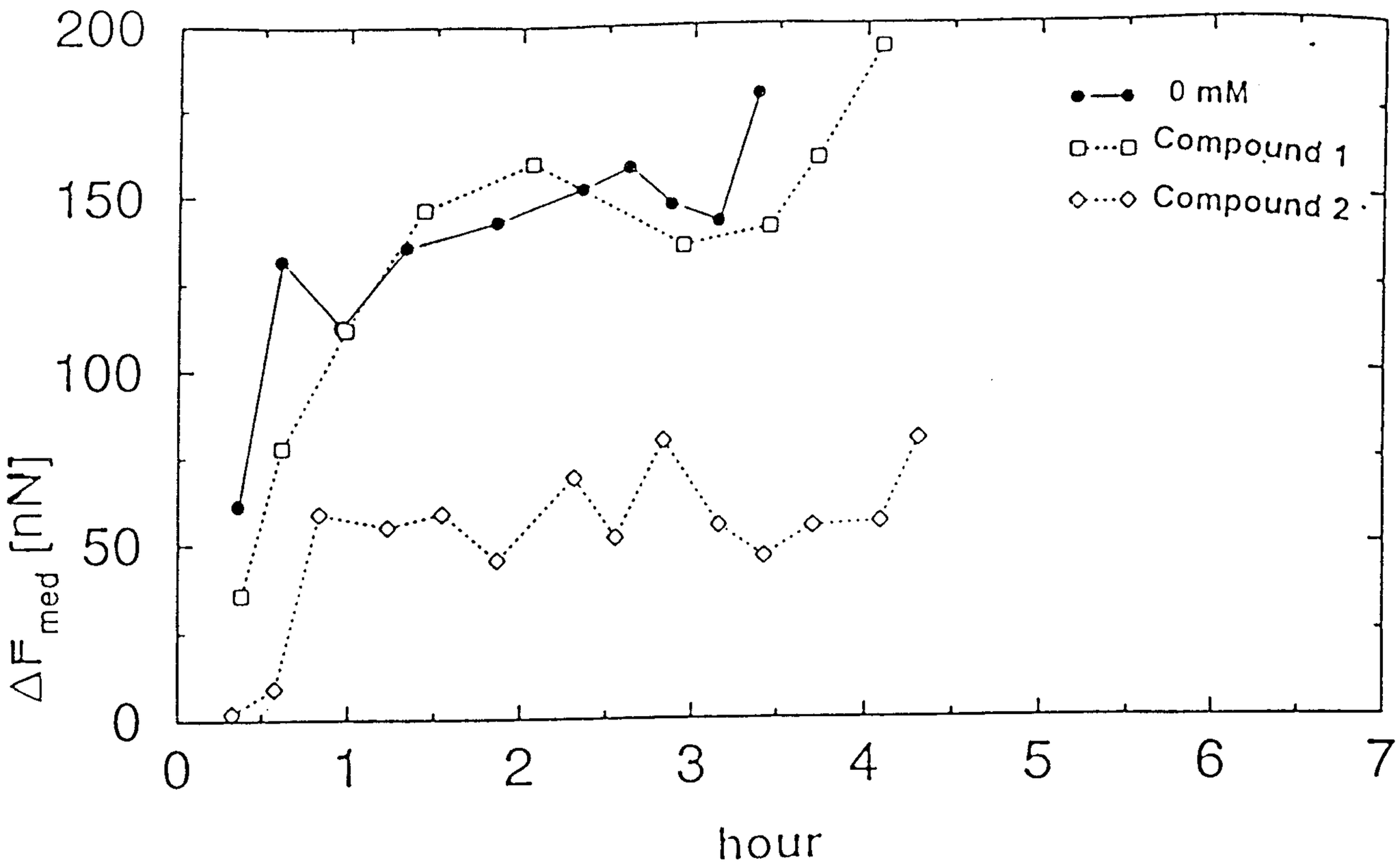
200
150
100
50
0
ΔF_{med} [nN]
0
1
2
3
4
5
6
7
hour
0 mM
Compound 1
Compound 2

Fig. 4

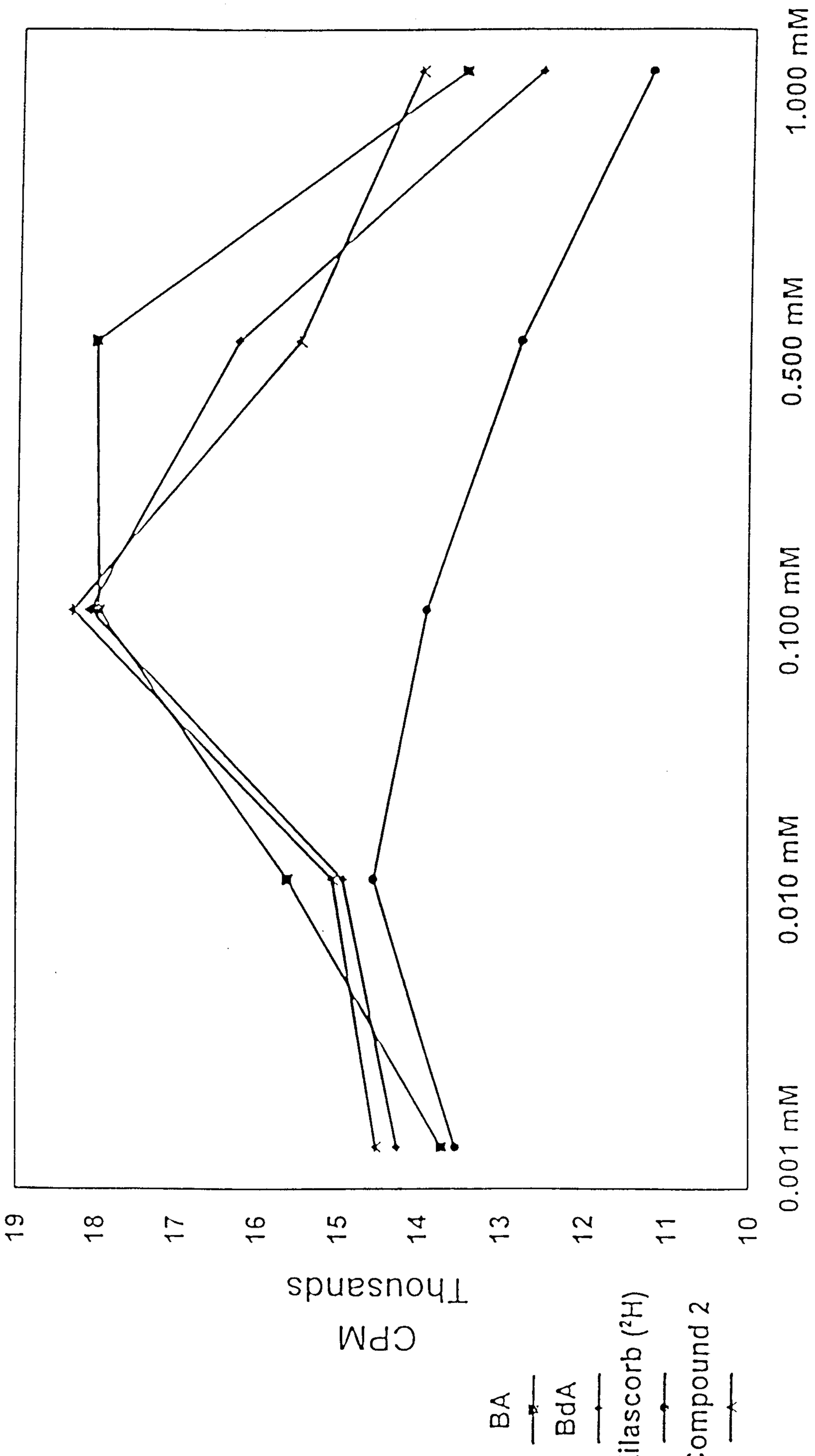
CPM
Thousands
19
18
17
16
15
14
13
12
11
10
0.001 mM
0.010 mM
0.100 mM
0.500 mM
1.000 mM
Drug concentration
BA
BdA
Zilascorb (²H)
Compound 2

Fig. 5

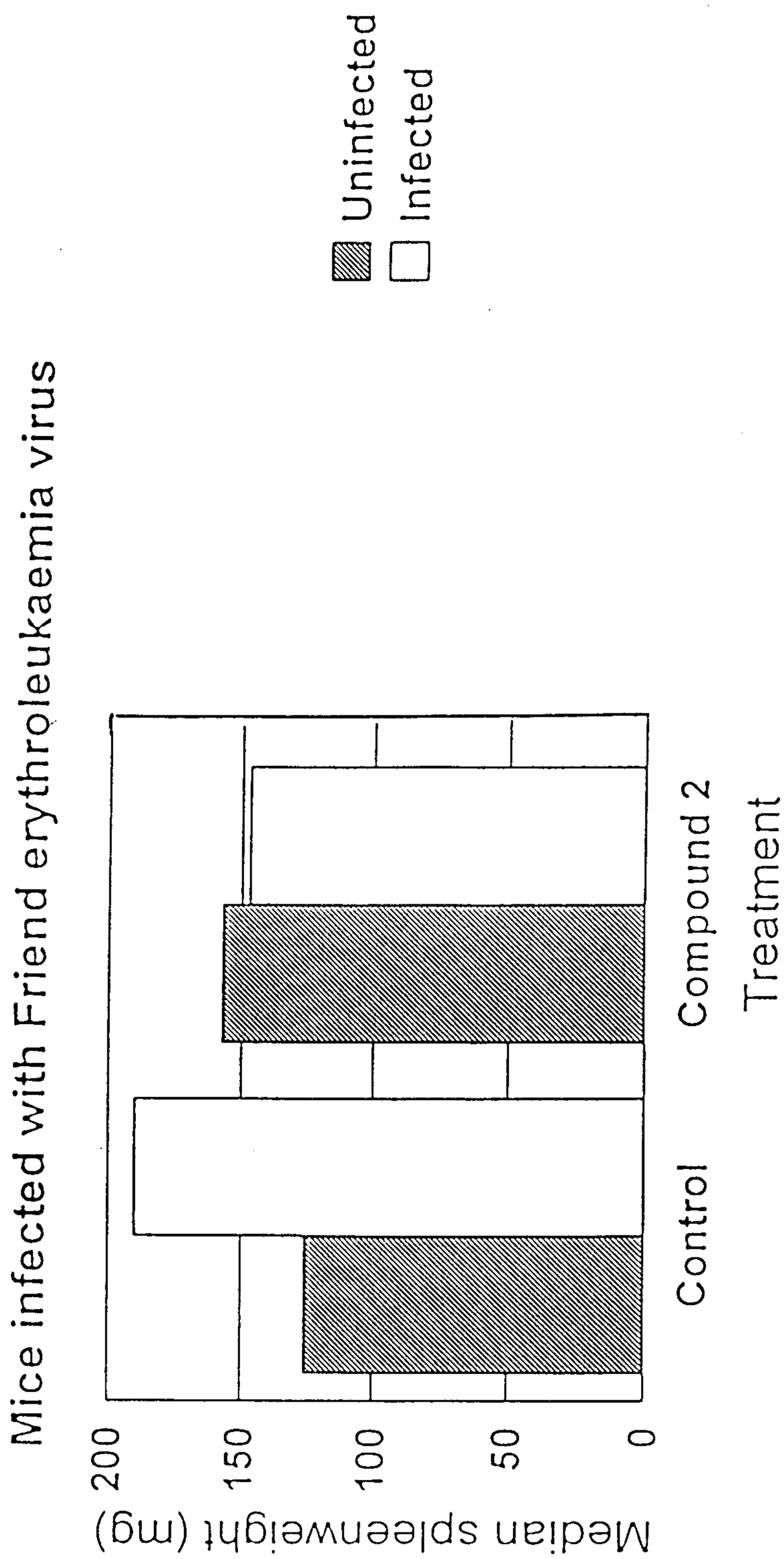
Uninfected
Infected
Mice infected with Friend erythroleukaemia virus
Median spleenweight (mg)
200
150
100
50
0
Control
Compound 2
Treatment

Fig. 6

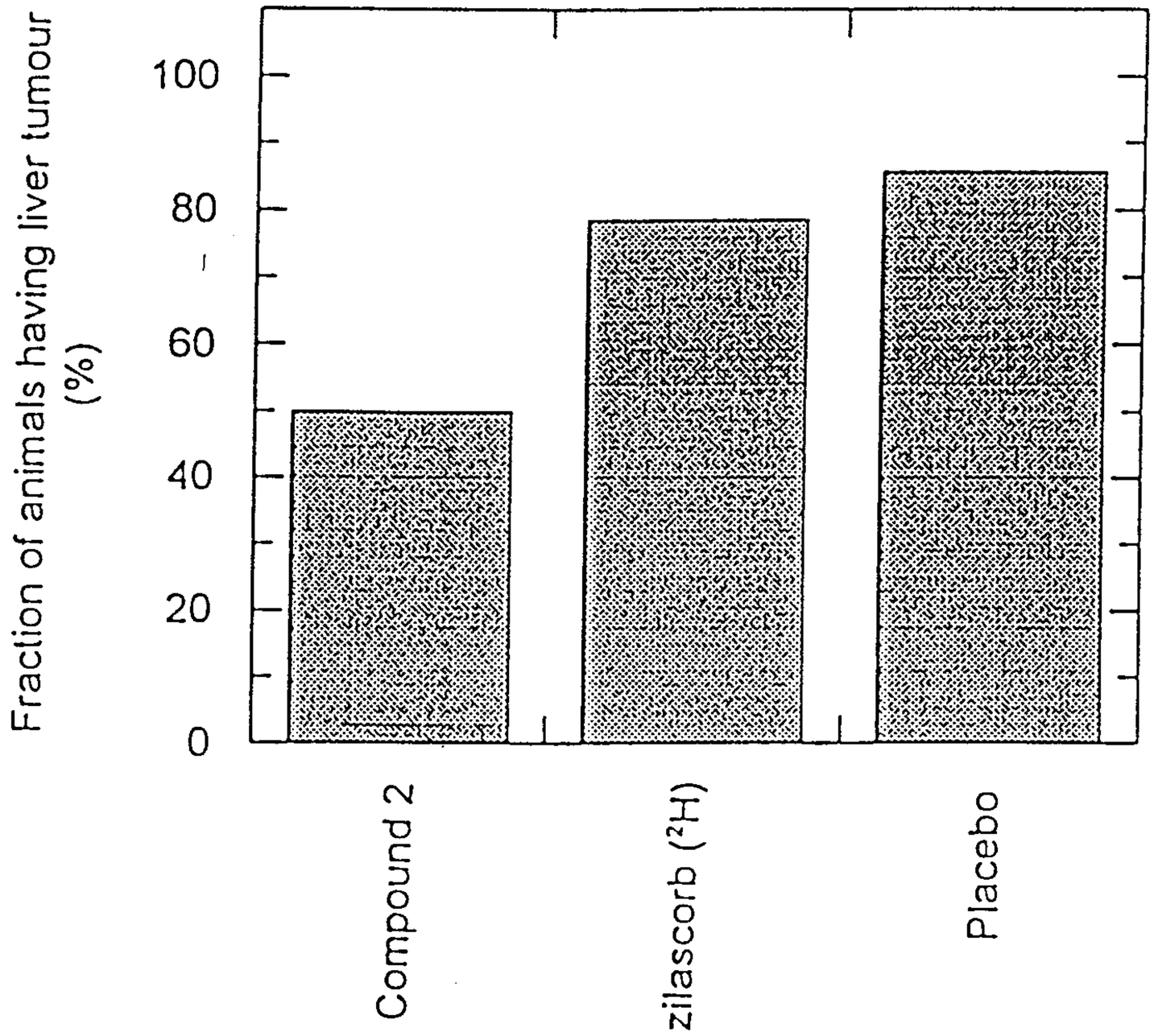
Fraction of animals having liver tumour (%)
100
80
60
40
20
0
Compound 2
zilascorb (^{2}H)
Placebo

Fig. 7

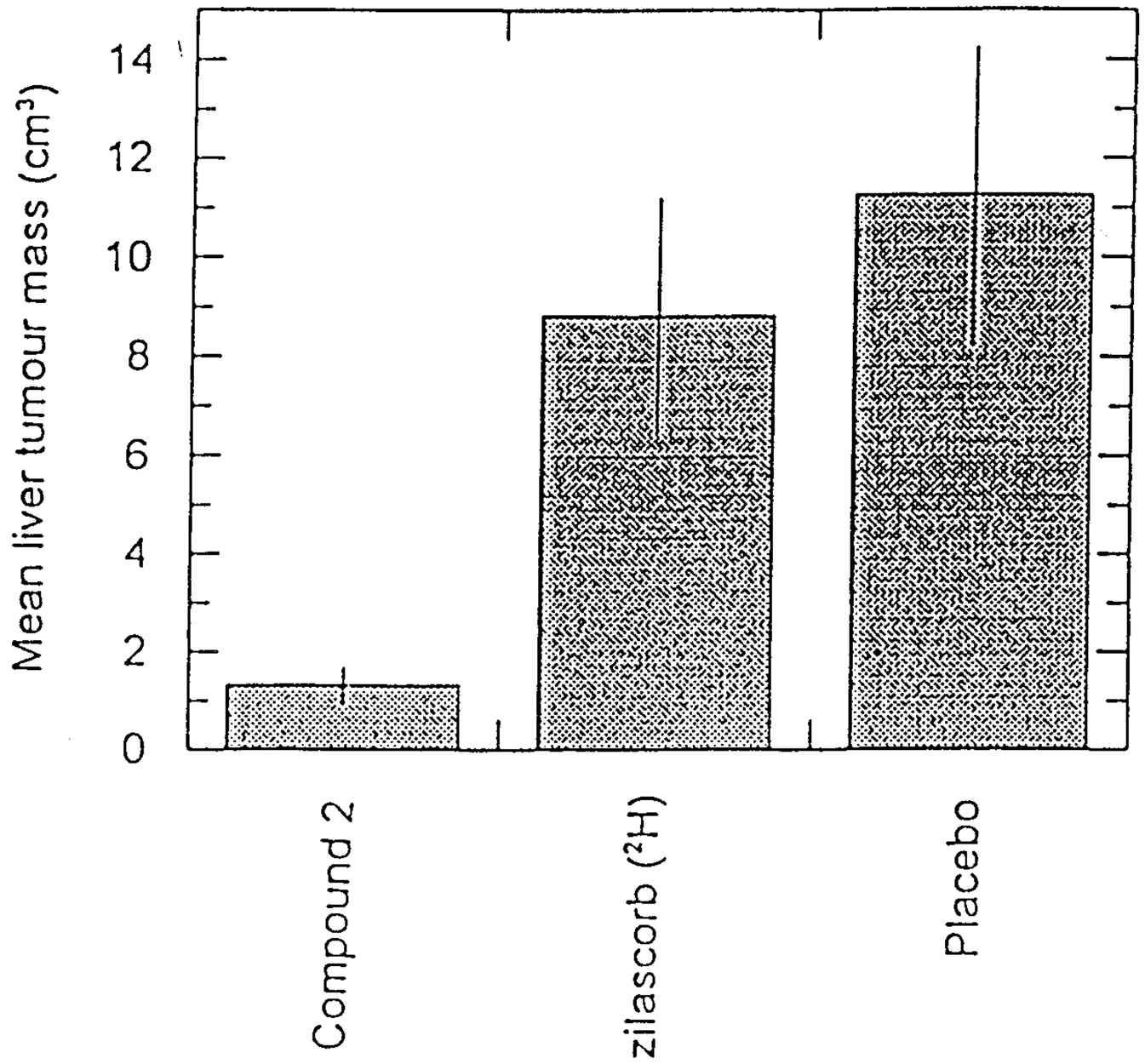
Mean liver tumour mass (cm³)
0
2
4
6
8
10
12
14
Compound 2
zilascorb (²H)
Placebo

Fig. 8

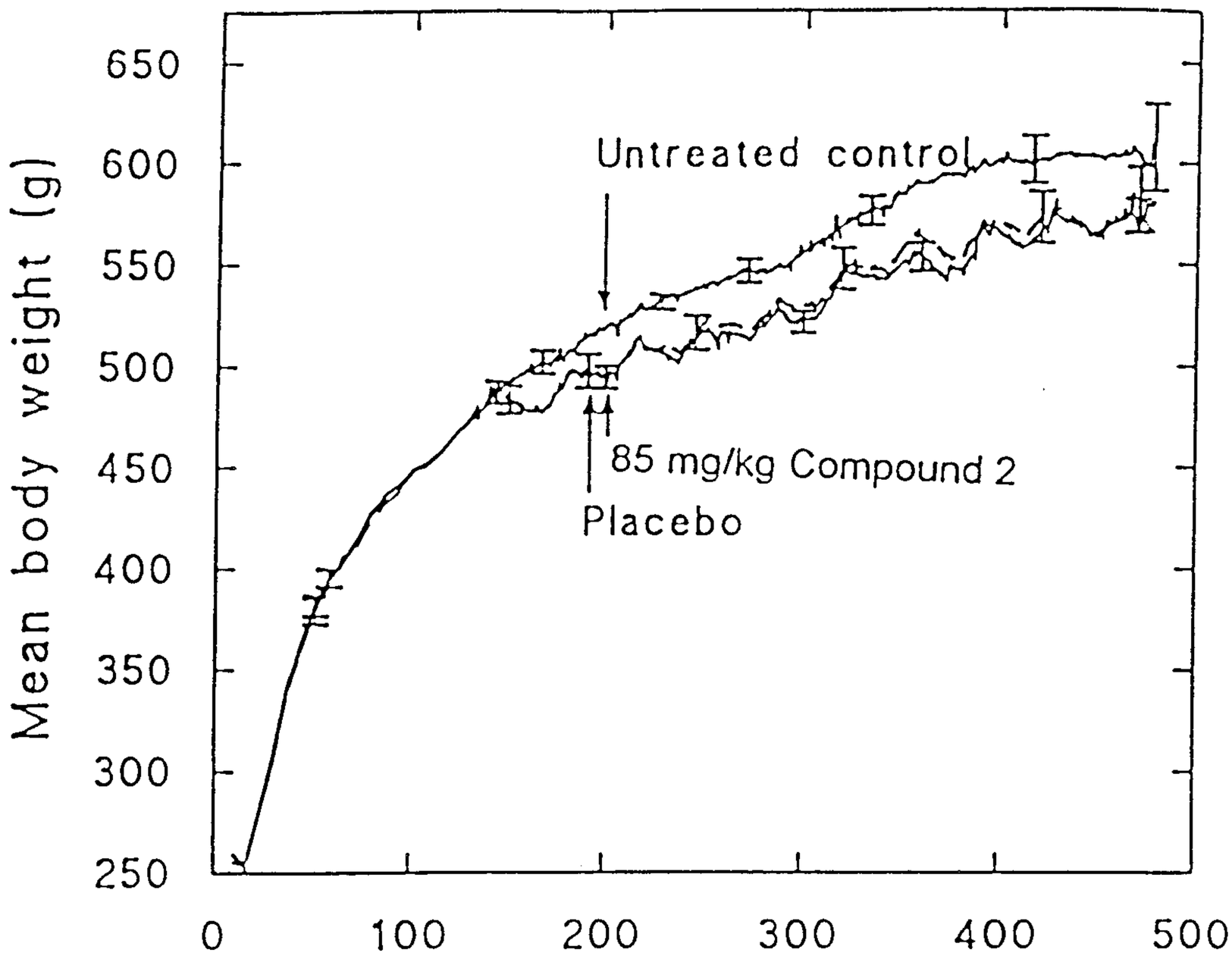
650
600
550
500
450
400
350
300
250
Mean body weight (g)
Untreated control
85 mg/kg Compound 2
Placebo
0
100
200
300
400
500
Time (days)

Fig. 9

10/16

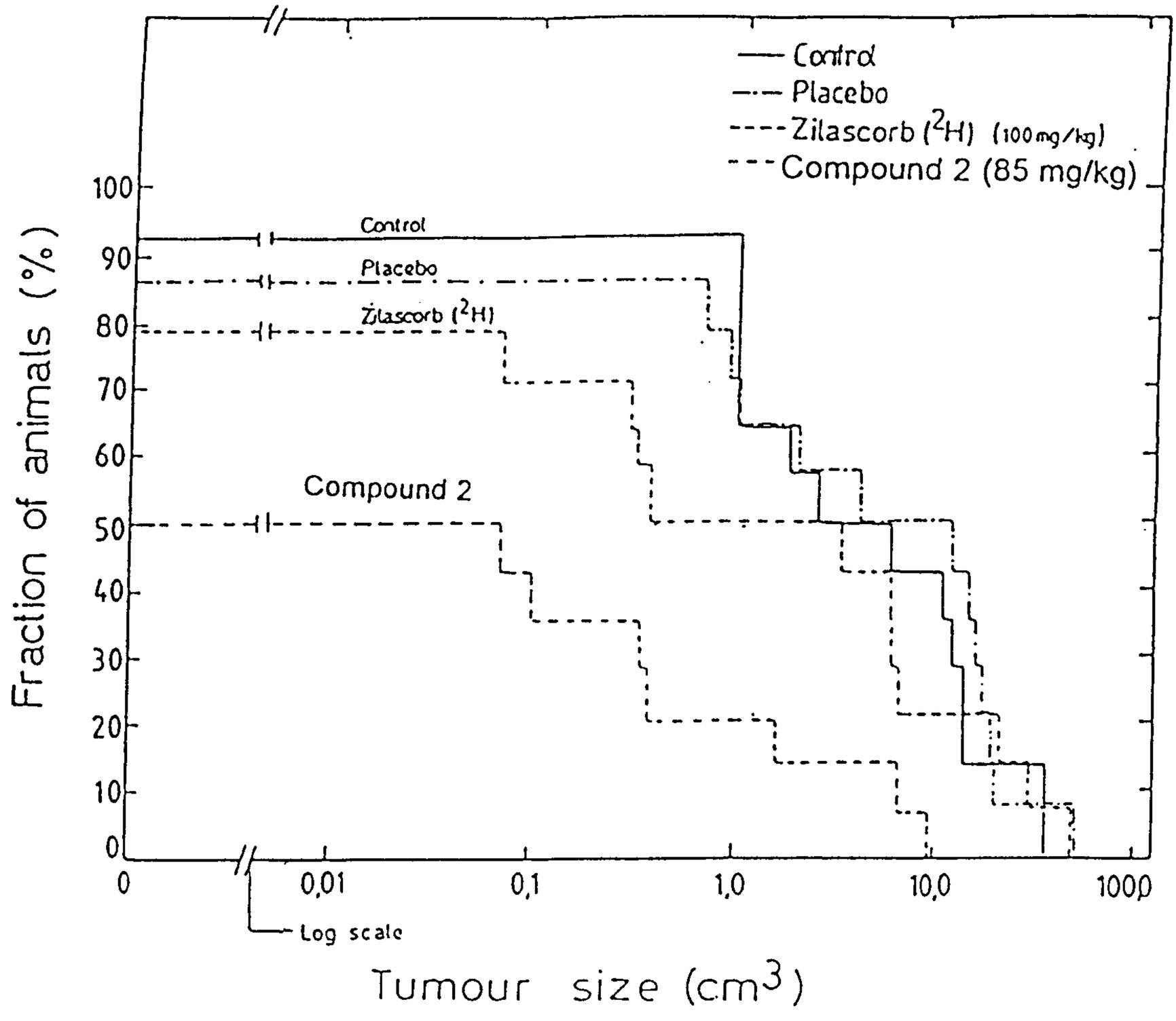
Control
Placebo
Zilascorb (^{2}H) (100 mg/kg)
Compound 2 (85 mg/kg)
Fraction of animals (%)
100
90
80
70
60
50
40
30
20
10
0
Control
Placebo
Zilascorb (^{2}H)
Compound 2
0
0,01
0,1
1,0
10,0
100
Log scale
Tumour size (cm^3)

Fig. 10

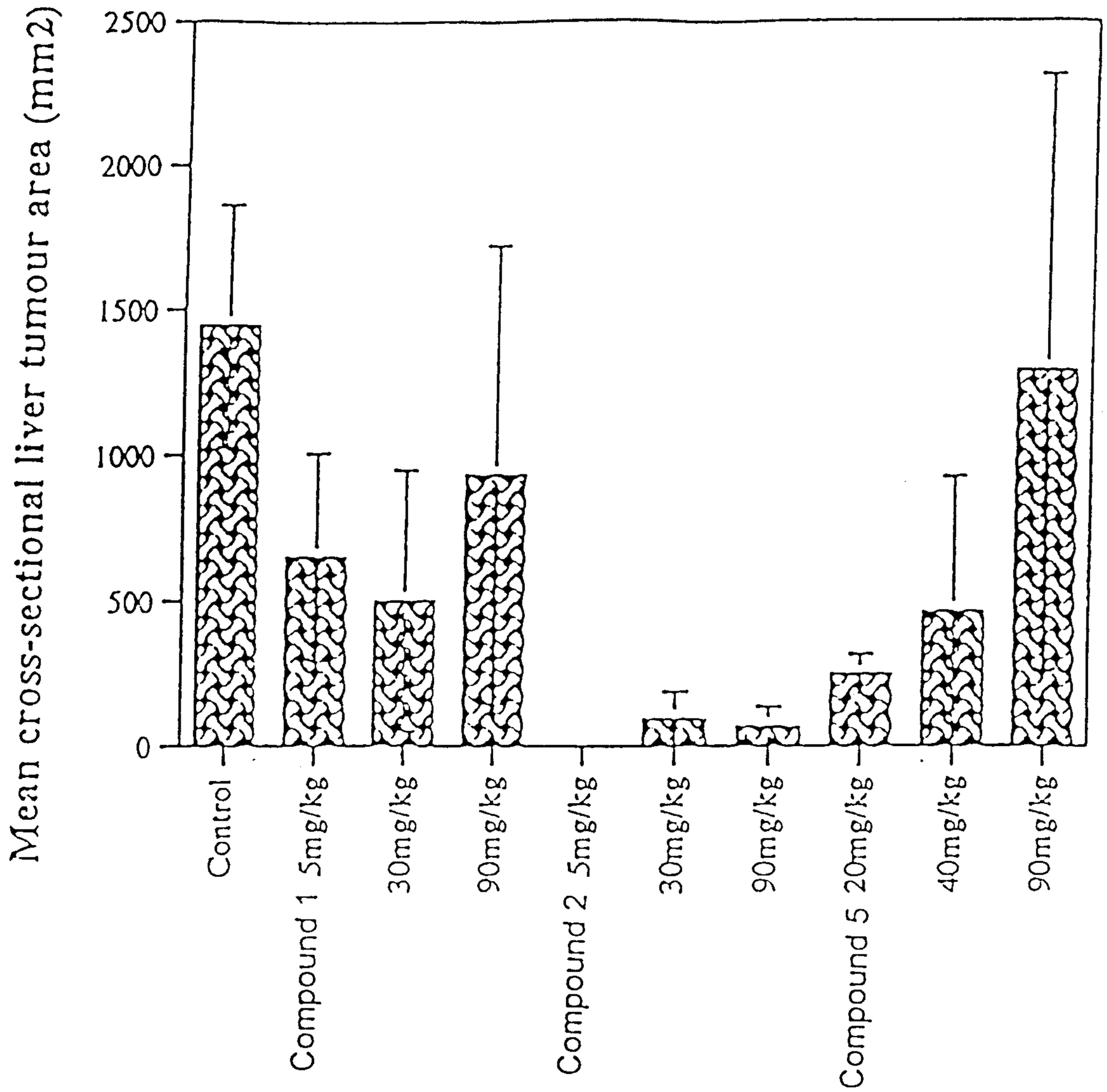
Mean cross-sectional liver tumour area (mm2)
2500
2000
1500
1000
500
0
Control
Compound 1 5mg/kg
30mg/kg
90mg/kg
Compound 2 5mg/kg
30mg/kg
90mg/kg
Compound 5 20mg/kg
40mg/kg
90mg/kg
Group

Fig. 11

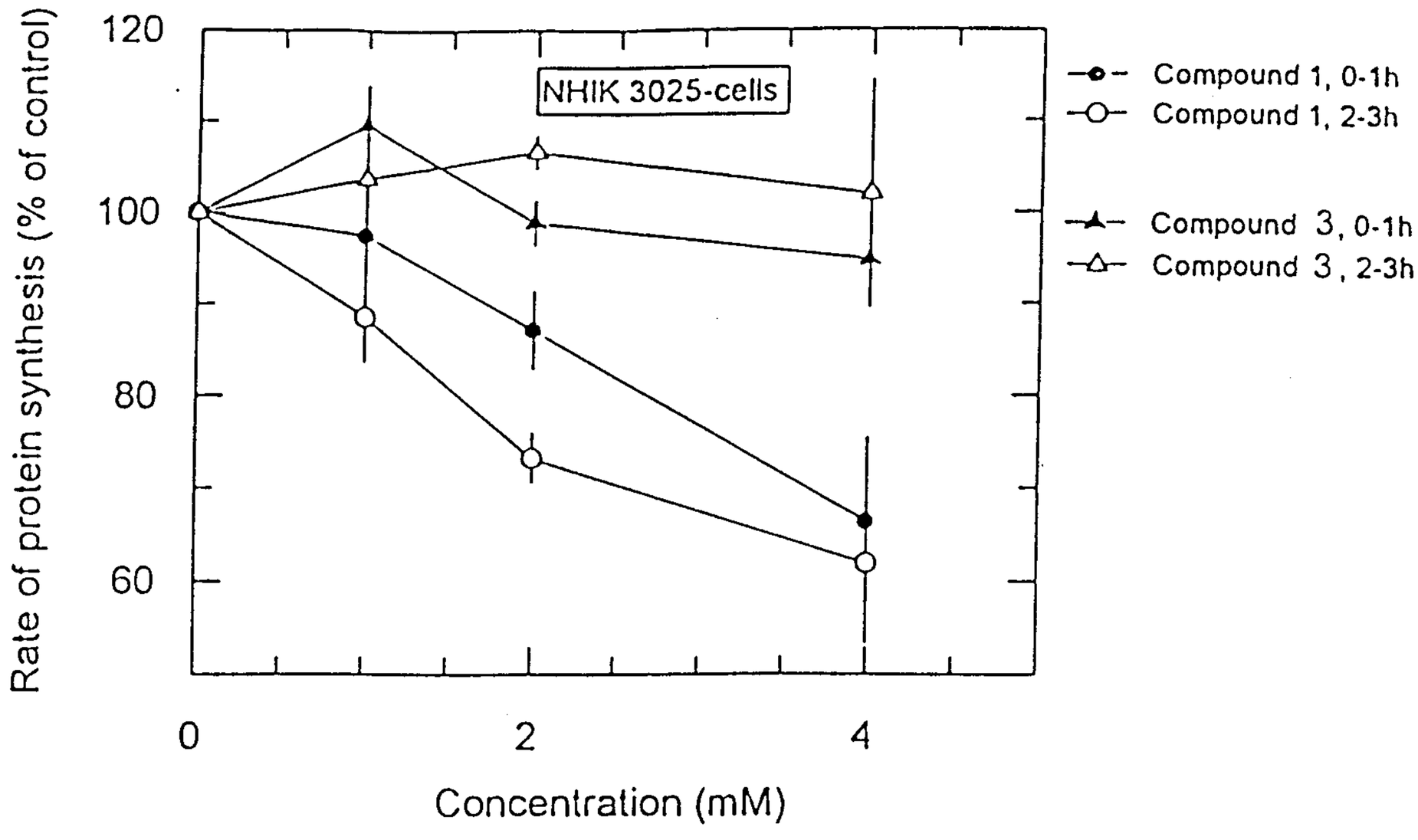
Rate of protein synthesis (% of control)
120
100
80
60
0
2
4
Concentration (mM)
NHIK 3025-cells
Compound 1, 0-1h
Compound 1, 2-3h
Compound 3, 0-1h
Compound 3, 2-3h

Fig. 12

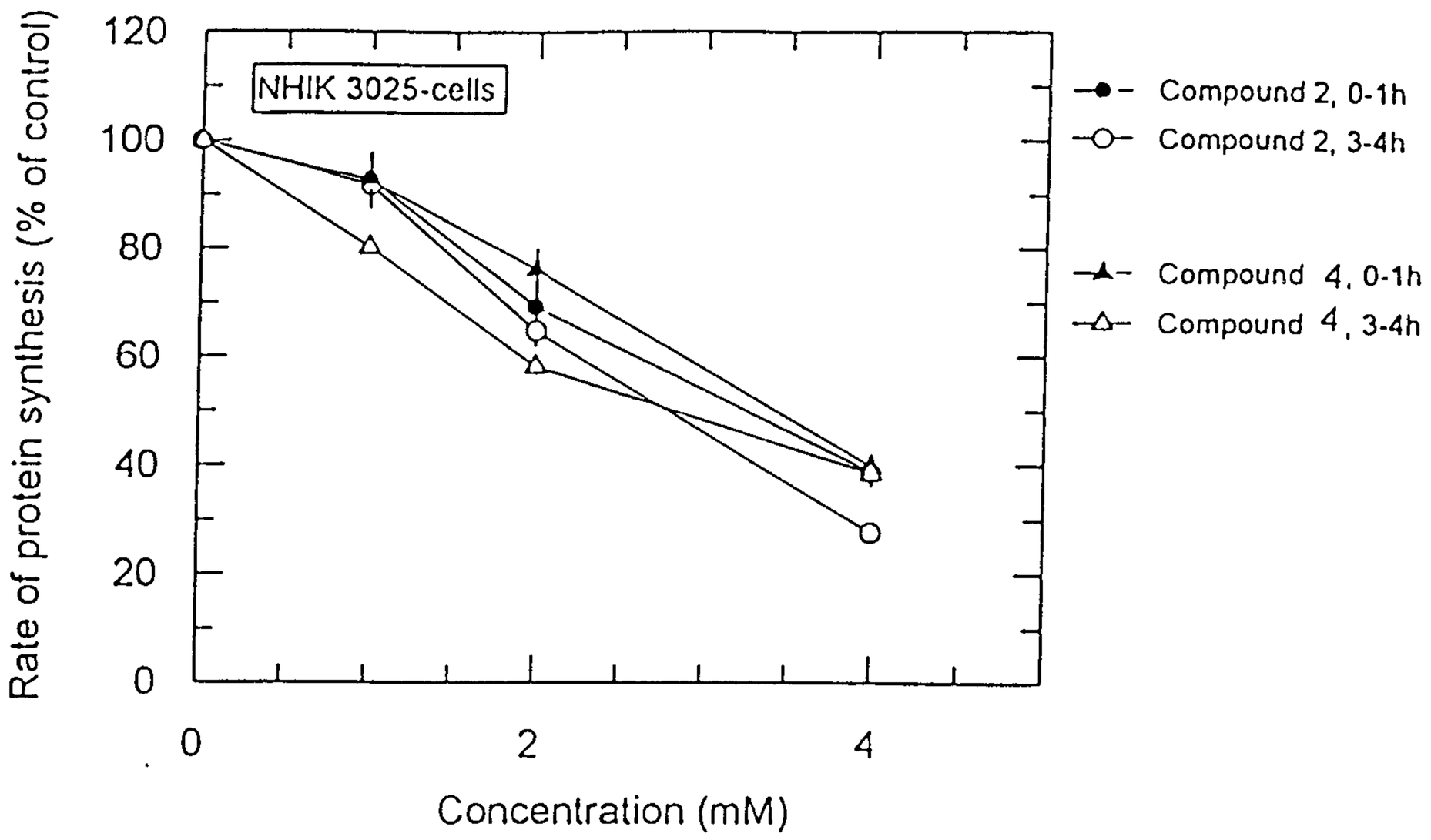
Rate of protein synthesis (% of control)
120
100
80
60
40
20
0
NHIK 3025-cells
0
2
4
Concentration (mM)
Compound 2, 0-1h
Compound 2, 3-4h
Compound 4, 0-1h
Compound 4, 3-4h

Fig. 13

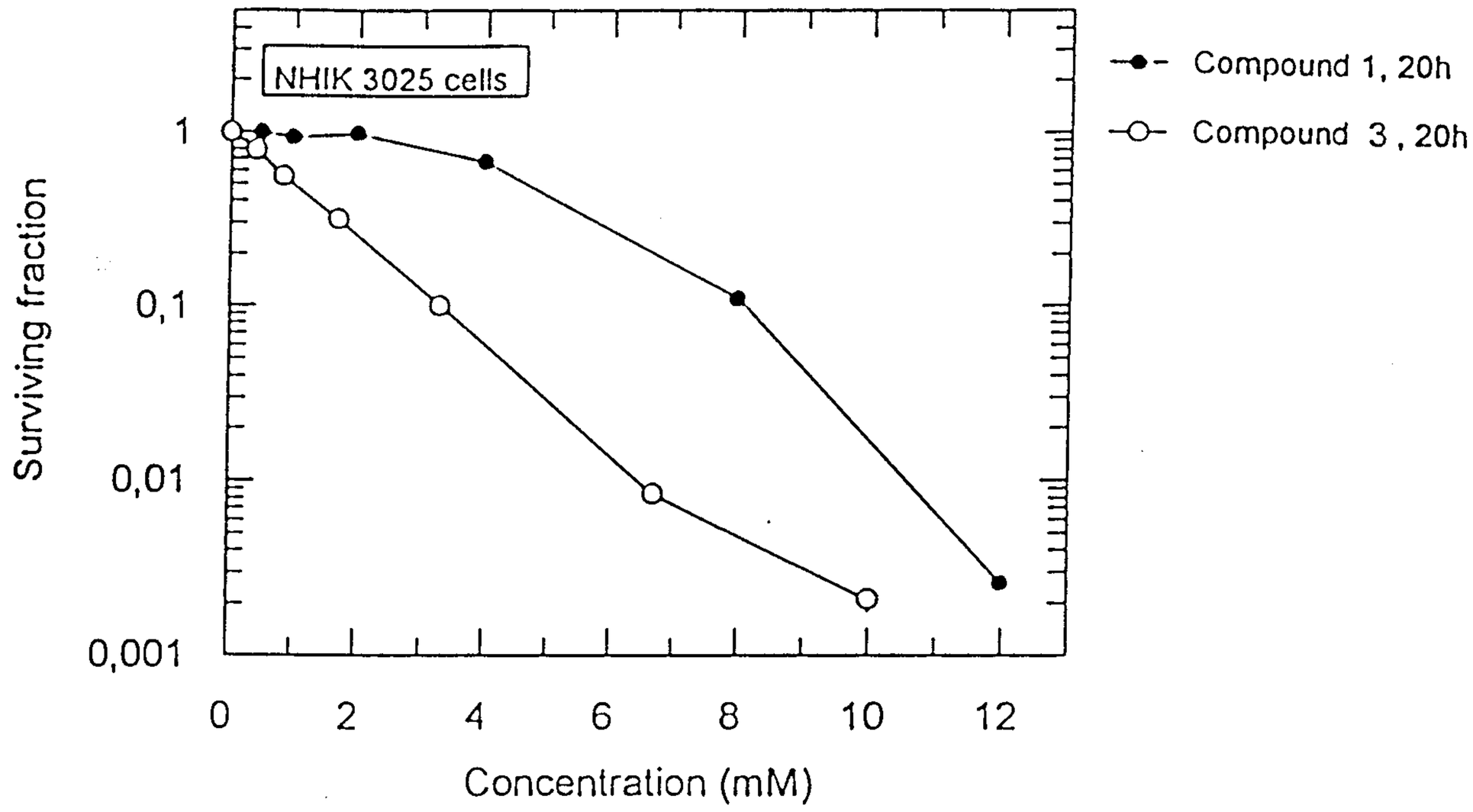
NHIK 3025 cells
Surviving fraction
1
0,1
0,01
0,001
0
2
4
6
8
10
12
Concentration (mM)
Compound 1, 20h
Compound 3 , 20h

Fig. 14

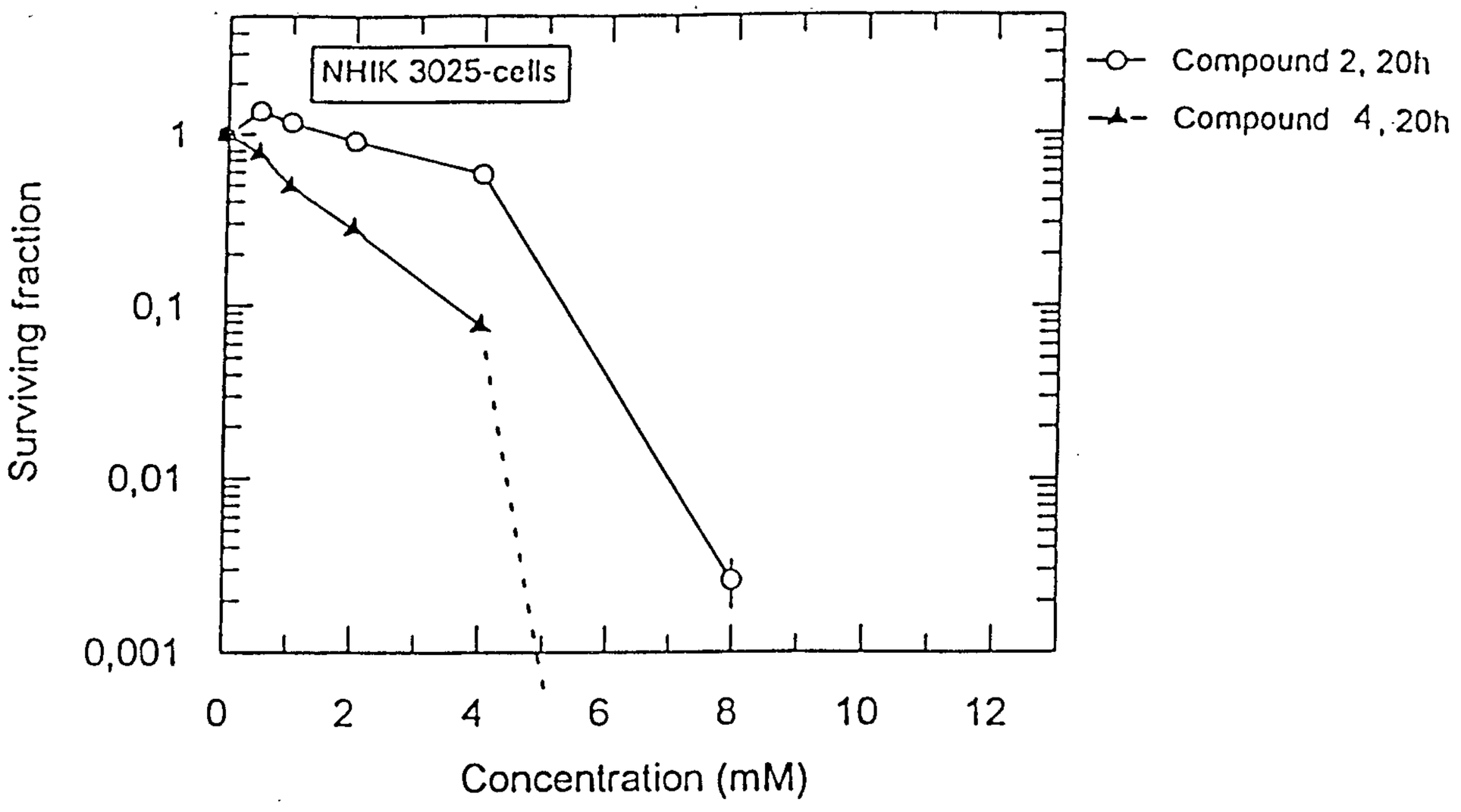
NHIK 3025-cells
Compound 2, 20h
Compound 4, 20h
Surviving fraction
1
0,1
0,01
0,001
0
2
4
6
8
10
12
Concentration (mM)

Fig. 15

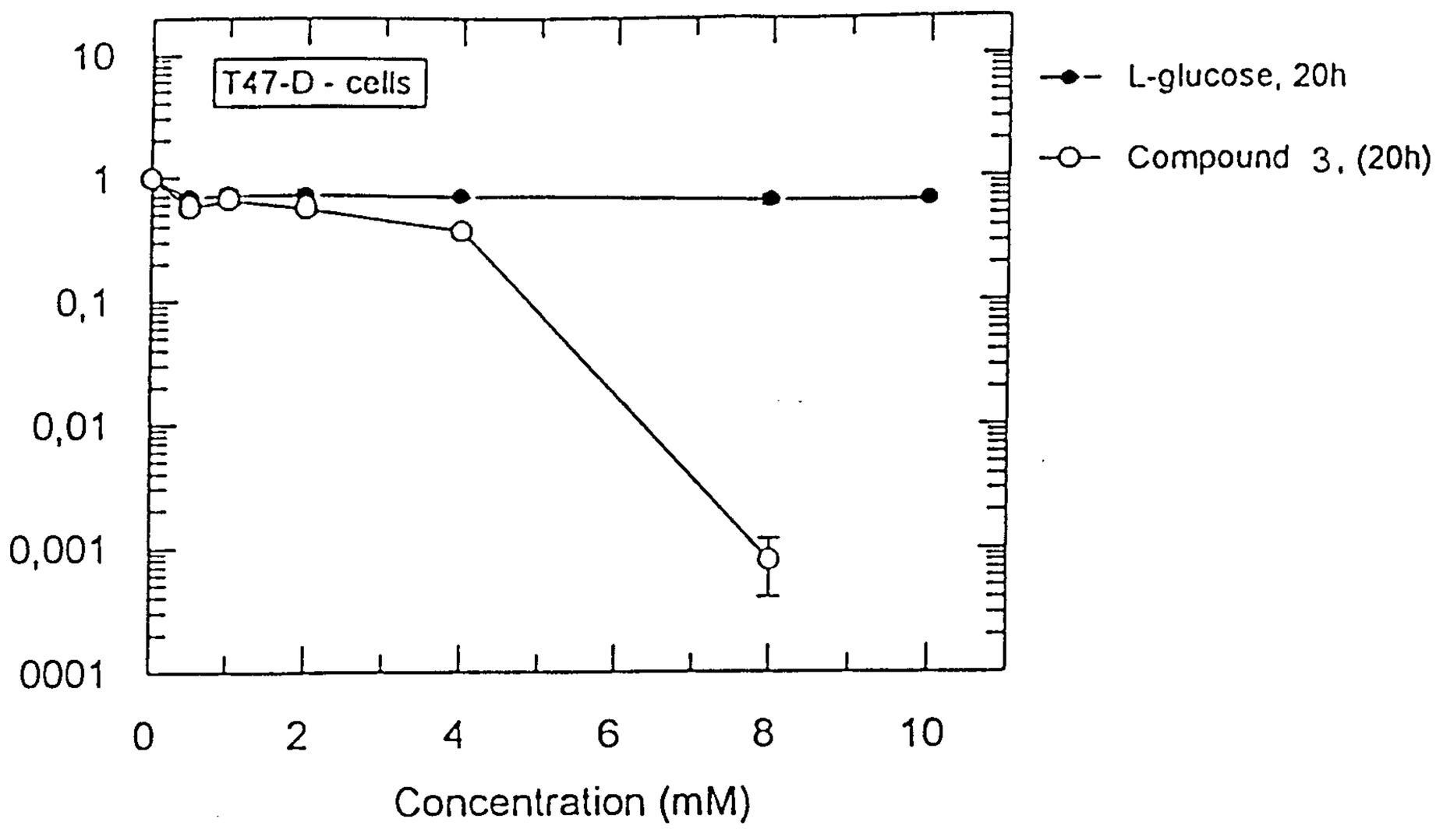
T47-D - cells
L-glucose, 20h
Compound 3, (20h)
10
1
0,1
0,01
0,001
0001
0
2
4
6
8
10
Concentration (mM)

Fig. 16