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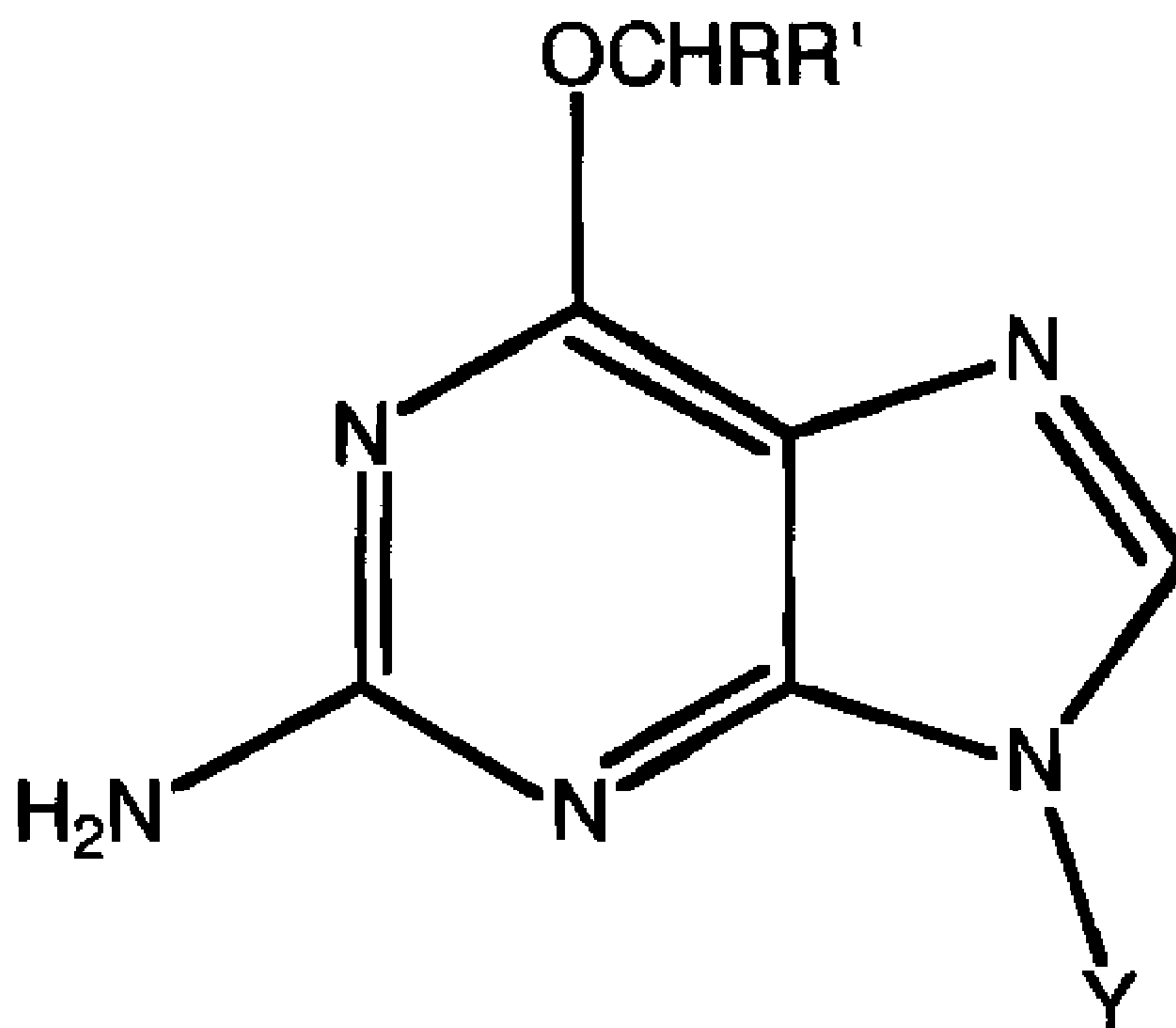
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(54) Titre : DERIVES DE SUBSTITUTION EN O6 DE GUANINE; METHODE DE PREPARATION ET UTILISATION POUR
LE TRAITEMENT DES CELLULES TUMORALES

(54) Title: O6-SUBSTITUTED GUANINE DERIVATIVES, A PROCESS FOR THEIR PREPARATION AND THEIR USE IN
TREATING TUMOUR CELLS



(57) Abrégé/Abstract:

O⁶-hetarylalkyl- or naphthylalkylguanine derivatives of formula (I) wherein Y is H, ribosyl, deoxyribosyl, or R''XCHR''', wherein X is O or S, R'' and R''' are alkyl, or substituted derivatives thereof; R' is H, or alkyl or hydroxyalkyl; R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hereto atom chosen from O, N or S, or a substituted derivative thereof; or (ii) naphthyl or a substituted derivative thereof; and pharmaceutically acceptable salts thereof, exhibit the ability to deplete O⁶-alkylguanine-DNA alkyltransferase (ATase) activity. A process for preparation of the compounds is described. The compounds have utility in combination with alkylating agents in the chemotherapeutic treatment of tumour cells.



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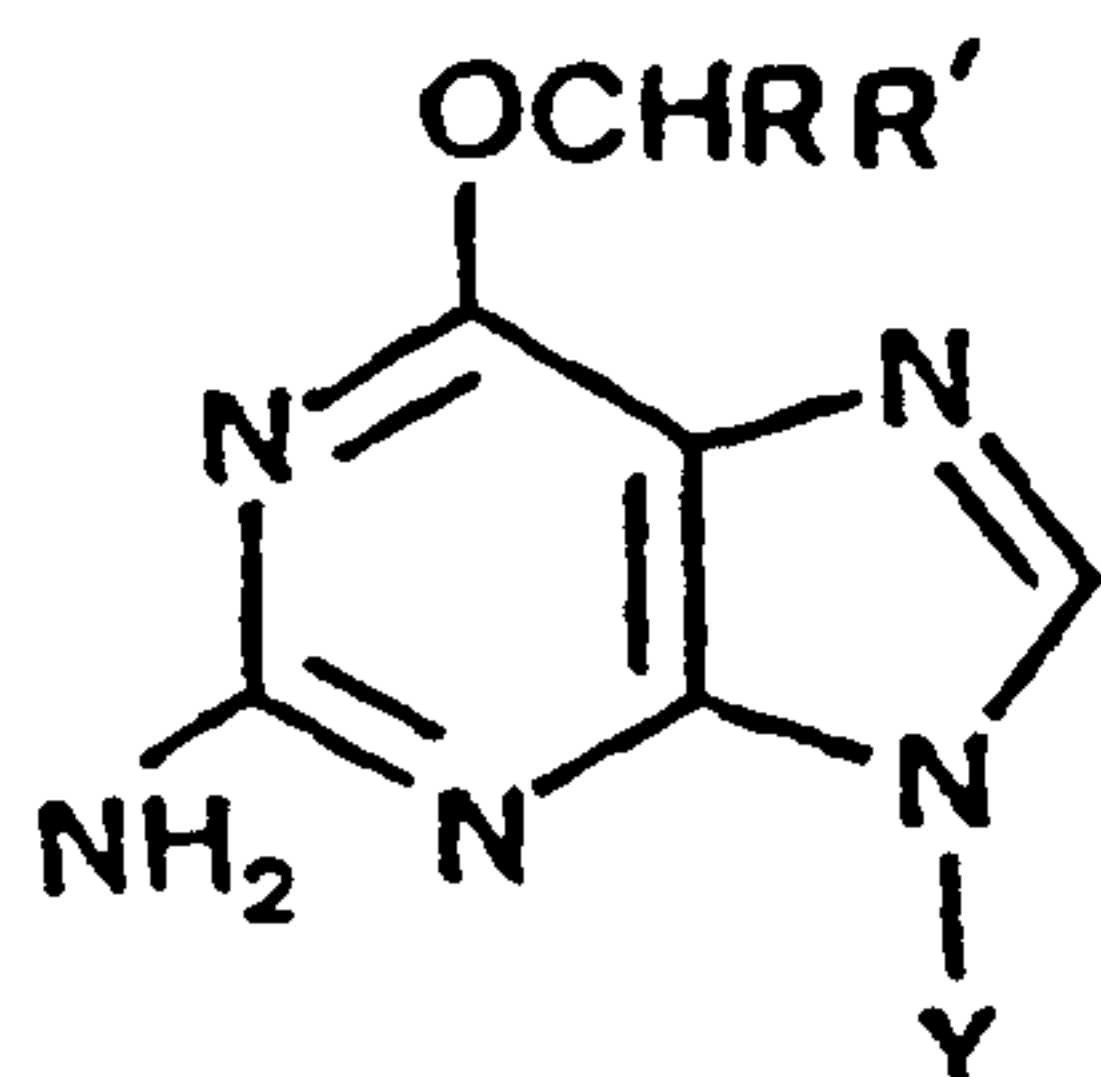


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(21) International Application Number: PCT/IE94/00031 (22) International Filing Date: 8 June 1994 (08.06.94) (30) Priority Data: 930432 8 June 1993 (08.06.93) IE 9410421.3 23 May 1994 (23.05.94) GB (71) Applicant (for all designated States except US): CANCER RESEARCH CAMPAIGN TECHNOLOGY LIMITED [GB/GB]; Cambridge House, 6-10 Cambridge Terrace, Regent's Park, London NW1 4JL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): McMURRY, Thomas, Brian, Hamilton [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). McELHINNEY, Robert, Stanley [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). McCORMICK, Joan, Elizabeth [IE/IE]; The University Chemical Laboratory, Trinity College, Dublin 2 (IE). ELDER, Rhoderick, Hugh [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). KELLY, Jane [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). (74) Agents: PARKES, Andrew, John, Aykroyd et al.; Tomkins & Co., 5 Dartmouth Road, Dublin 6 (IE). (81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), ES, FI, FI (Utility model), GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, SK (Utility model), TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).		(GB). MARGISON, Geoffrey, Paul [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). RAFFERTY, Joseph, Anthony [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). WATSON, Amanda, Jean [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). WILLINGTON, Mark, Andrew [GB/GB]; CRC Dept. of Carcinogenesis, Paterson Institute for Cancer Research, Christie Hospital, Wilmslow Road, Manchester M20 9BX (GB). Published With international search report.	

(54) Title: **O⁶-SUBSTITUTED GUANINE DERIVATIVES, A PROCESS FOR THEIR PREPARATION AND THEIR USE IN TREATING TUMOUR CELLS**

(I)

(57) Abstract

O⁶-hetarylalkyl- or naphthylalkylguanine derivatives of formula (I) wherein Y is H, ribosyl, deoxyribosyl, or R"XCHR'", wherein X is O or S, R'' and R''' are alkyl, or substituted derivatives thereof; R' is H, or alkyl or hydroxyalkyl; R is (i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hereto atom chosen from O, N or S, or a substituted derivative thereof; or (ii) naphthyl or a substituted derivative thereof; and pharmaceutically acceptable salts thereof, exhibit the ability to deplete O⁶-alkylguanine-DNA alkyltransferase (ATase) activity. A process for preparation of the compounds is described. The compounds have utility in combination with alkylating agents in the chemotherapeutic treatment of tumour cells.

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**O⁶-SUBSTITUTED GUANINE DERIVATIVES, A PROCESS FOR THEIR PREPARATION
AND THEIR USE IN TREATING TUMOUR CELLS**

Technical Field

20 The present invention relates to O⁶-substituted guanine
derivatives, a process for their preparation and their use in treating
tumour cells. In particular, it relates to guanine derivatives having
hetarylalkyl or naphthylalkyl substituents in the O⁶ position, these
compounds exhibiting the ability to deplete O⁶-alkylguanine-DNA
25 alkyltransferase (ATase) activity in tumour cells.

Background Art

It has been suggested to use O⁶-alkyl guanine derivatives
possessing O⁶-alkylguanine-DNA alkyltransferase depleting activity
30 in order to enhance the effectiveness of chemotherapeutic alkylating
agents used for killing tumour cells. There is increasing evidence
that in mammalian cells the toxic and mutagenic effects of alkylating
agents are to a large extent a consequence of alkylation at the
O⁶-position of guanine in DNA. The repair of O⁶-alkylguanine is
35 mediated by ATase, a repair protein that acts on the O⁶-alkylated
guanine residues by stoichiometric transfer of the alkyl group to a
cysteine residue at the active site of the repair protein in an
autoinactivating process. The importance of ATase in protecting cells
against the biological effects of alkylating agents

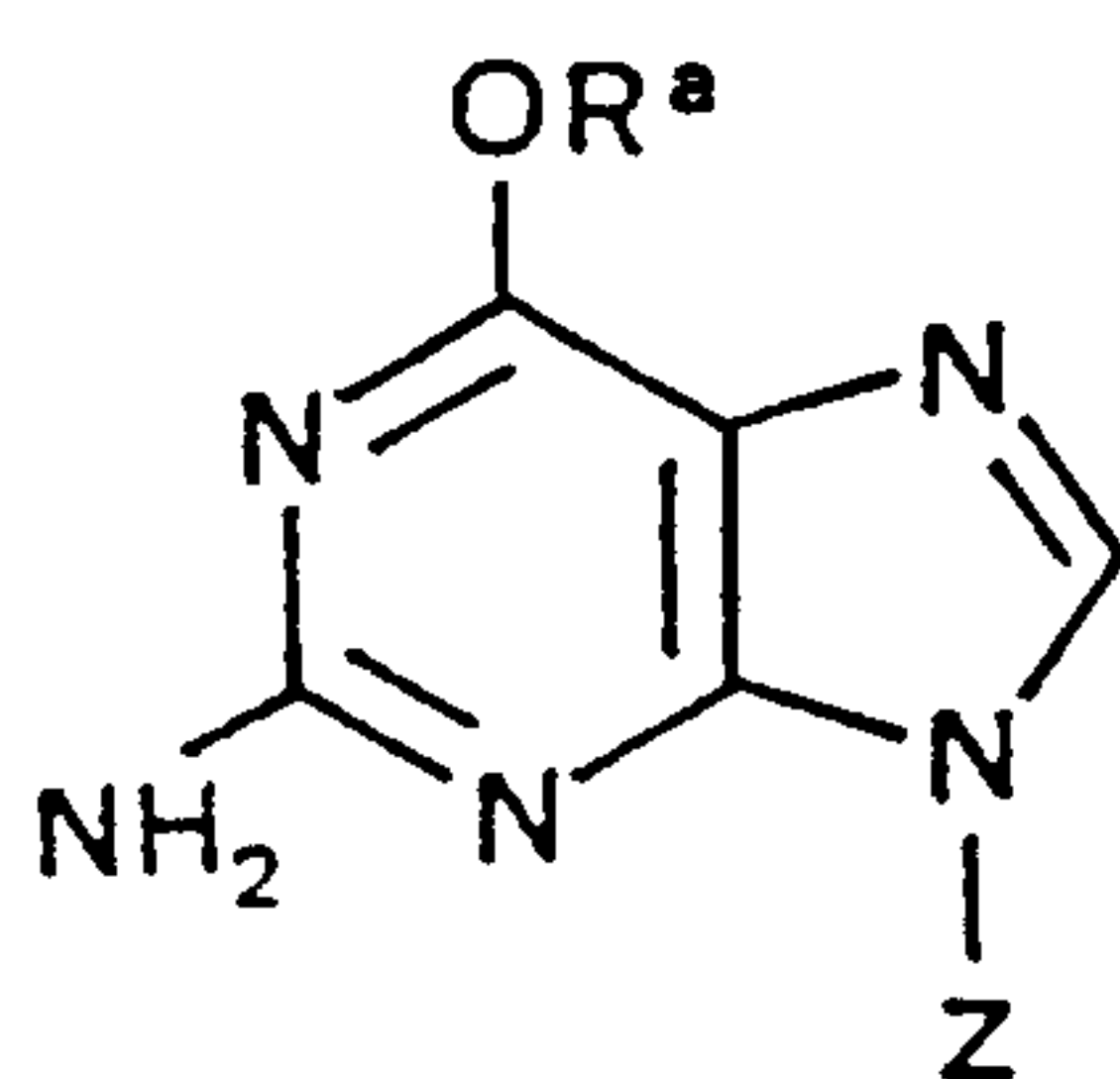
- 2 -

has been most clearly demonstrated by the transfer and expression of cloned ATase genes or cDNAs into ATase deficient cells: this confers resistance to a variety of agents, principally those that methylate or chloroethylate DNA. Whilst the mechanism of cell killing by O^6 -methylguanine in ATase deficient cells is not yet clear, killing by O^6 -chloroethylguanine occurs through DNA interstrand crosslink formation to a cytosine residue on the opposite strand via a cyclic ethanoguanine intermediate, a process that is prevented by ATase-mediated chloroethyl group removal or complex formation.

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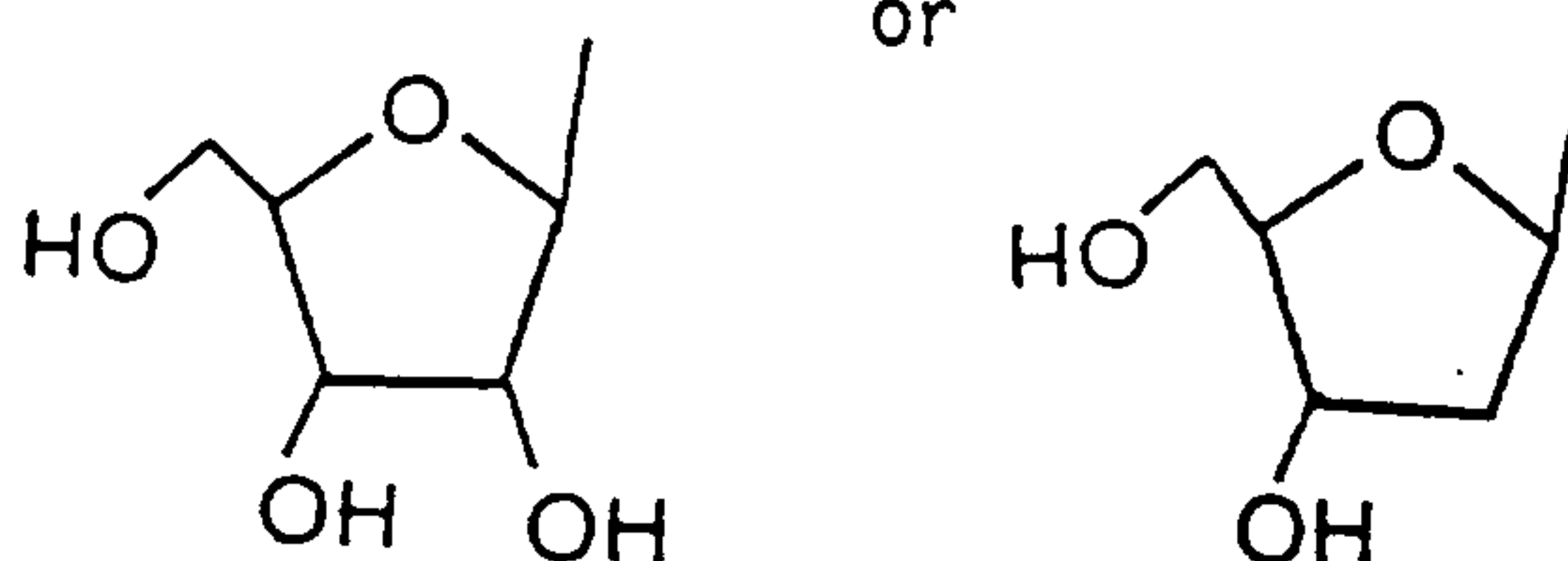
The use of O^6 -methylguanine and O^6 -*n*-butylguanine for depleting ATase activity has been investigated (Dolan *et al.*, Cancer Res., (1986) 46, pp. 4500; Dolan *et al.*, Cancer Chemother. Pharmacol., (1989) 25, pp 103. O^6 -benzylguanine derivatives have been proposed for depleting ATase activity in order to render ATase expressing cells more susceptible to the cytotoxic effects of chloroethylating agents (Moschel *et al.*, J. Med.Chem., 1992, 35, 4486). U.S. Patent 5 091 430 and International Patent Application No. WO 91/13898 Moschel *et al.* disclose a method for depleting levels of O^6 -alkylguanine-DNA alkyl-transferase in tumour cells in a host which comprises administering to the host an effective amount of a composition containing O^6 -benzylated guanine derivatives of the following formula:

25



30

wherein Z is hydrogen, or



35

and R^a is a benzyl group or a substituted benzyl group. A benzyl group may be substituted at the ortho, meta or para position with a

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substituent group such as halogen, nitro, aryl such as phenyl or substituted phenyl, alkyl of 1-4 carbon atoms, alkoxy of 1-4 carbon atoms, alkenyl of up to 4 carbon atoms, alkynyl of up to 4 carbon atoms, amino, monoalkylamino, dialkylamino, trifluoromethyl, hydroxy, hydroxymethyl, and SO_nR^b wherein n is 0, 1, 2 or 3 and R^b is hydrogen, alkyl of 1-4 carbon atoms or aryl. Mi-Young Chae *et al.*, *J. Med. Chem.*, 1994, 37, 342-347 - published after the priority date of the present application - describes tests on O^6 -benzylguanine analogs bearing increasingly bulky substituent groups on the benzene ring or at position 9. Compound No. 6 described therein is O^6 -(2-pyridylmethyl)guanine, which in this application is called O^6 -(2-picolyl) guanine. However in the Results and Discussion at pages 342 - 343 of the Chae *et al.* paper, Compound No. 6 is not highlighted as being of interest but is grouped among "remaining compounds" which "exhibited intermediate activity" (Page 343 Lines 12 - 15 of the text). The authors confirm their earlier observations (*J. Med. Chem.*, 1992, 35 4486) that only allyl or benzyl substituents at the O^6 position of guanine efficiently inactivated ATase (page 343 lines 21 - 23 of the text).

O^6 -benzylguanine has limitations in its use as an ATase inactivator. It is more stable than would be desirable, resulting in a long survival time in an animal to which it is administered. It has a level of potential toxicity both alone and in combination with chloroethylating agents which is also undesirable and which may be related to the survival time.

The compounds of the present application exhibit different ATase inactivating characteristics from O^6 -benzylguanine and in some cases the activity is up to 8 times greater than that of O^6 -benzylguanine. Different half-life and toxicity characteristics have also been observed. Therefore, it is an object of the present invention to provide novel compounds useful for depleting ATase activity in order to enhance the effects of chemotherapeutic agents such as chloroethylating or methylating anti-tumour agents.

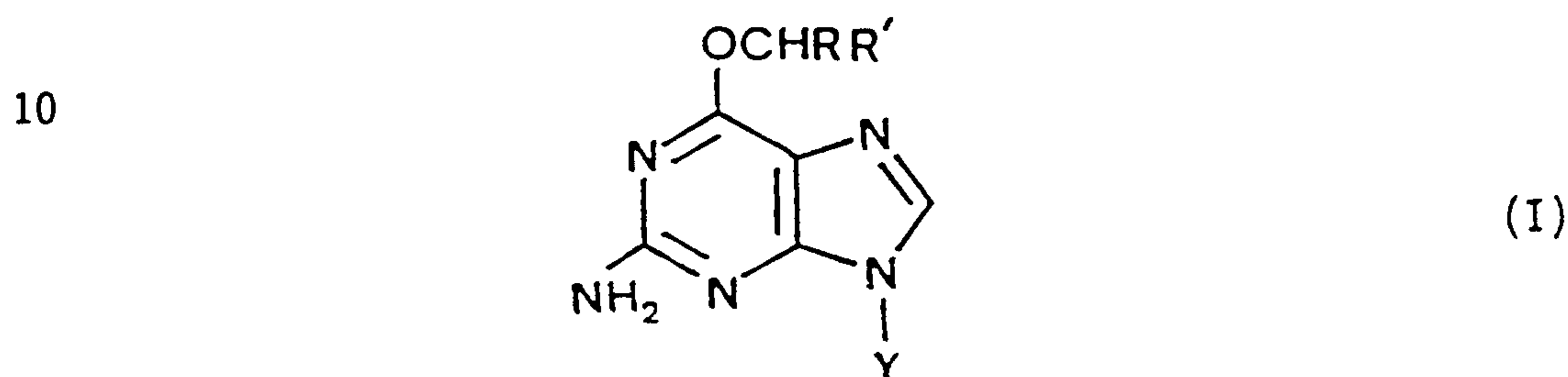
Another object of the invention is to provide pharmaceutical compositions containing compounds which are useful for depleting ATase activity. A further object of the present invention is to provide a

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method for depleting ATase activity in tumour cells. A still further object of the invention is to provide a method for treating tumour cells in a host.

5 Disclosure of Invention

Accordingly, the present invention provides 0⁶-substituted guanine derivatives of formula I:



15 wherein

Y is H, ribosyl, deoxyribosyl, or R"XCHR"',
wherein X is O or S, R" and R"' are alkyl, or
substituted derivatives thereof;

20 R' is H, alkyl or hydroxyalkyl;

R is (i) a cyclic group having at least one 5- or 6-membered
heterocyclic ring, optionally with a carbocyclic or heterocyclic ring
fused thereto, the or each heterocyclic ring having at least one hetero
25 atom chosen from O, N, or S, or a substituted derivative thereof; or

(ii) naphthyl or a substituted derivative thereof;

and pharmaceutically acceptable salts thereof.

30

R may suitably be a 5- or 6-membered heterocyclic ring or a benzo
derivative thereof, in which latter case the 0⁶-alkyl guanine moiety
may be attached to R at either the heterocyclic or the benzene ring.

35 In preferred embodiments, R is a 5-membered ring containing S or O,
with or without a second ring fused thereto.

Preferably, R is a heterocyclic ring having at least one S atom;
more preferably, R is a 5-membered heterocyclic ring having at least one

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S atom; and most preferably, R is a thiophene ring or a substituted derivative thereof.

Alternatively, R may be a heterocyclic ring having at least one O atom, particularly, a 5-membered heterocyclic ring having at least one O atom and more particularly R may be a furan ring or a substituted derivative thereof.

As another alternative, R may be a heterocyclic ring having at least one N atom, particularly R may be a 6-membered heterocyclic ring having at least one N atom and in particular, R may be a pyridine ring. In the definition of Y, the term "substituted derivative" includes substitution by one or more of the following groups: hydroxy, alkoxy, amino, alkylamino, amido or ureido.

15

In the definition of R, the term "substituted derivative" includes substitution of the heterocyclic rings and/or carbocyclic ring(s) by one or more of the following groups: alkyl, alkenyl, alkynyl, halo, haloalkyl, nitro, cyano, hydroxyalkyl, $\text{SO}_n\text{R}''''$ where R'''' is alkyl and $n=0,1$ or 2 , or a carboxyl or ester group of the formula $-\text{COOR}^5$ wherein R^5 is H or alkyl. Halo, haloalkyl, cyano, $\text{SO}_n\text{R}''''$ (as defined above) and $-\text{COOR}^5$ wherein R^5 is alkyl are preferred substituents.

25 An alkyl, alkenyl, or alkynyl group preferably contains from 1 to 20, more preferably from 1 to 10 and most preferably from 1 to 5 carbon atoms. Halo includes iodo, bromo, chloro or fluoro.

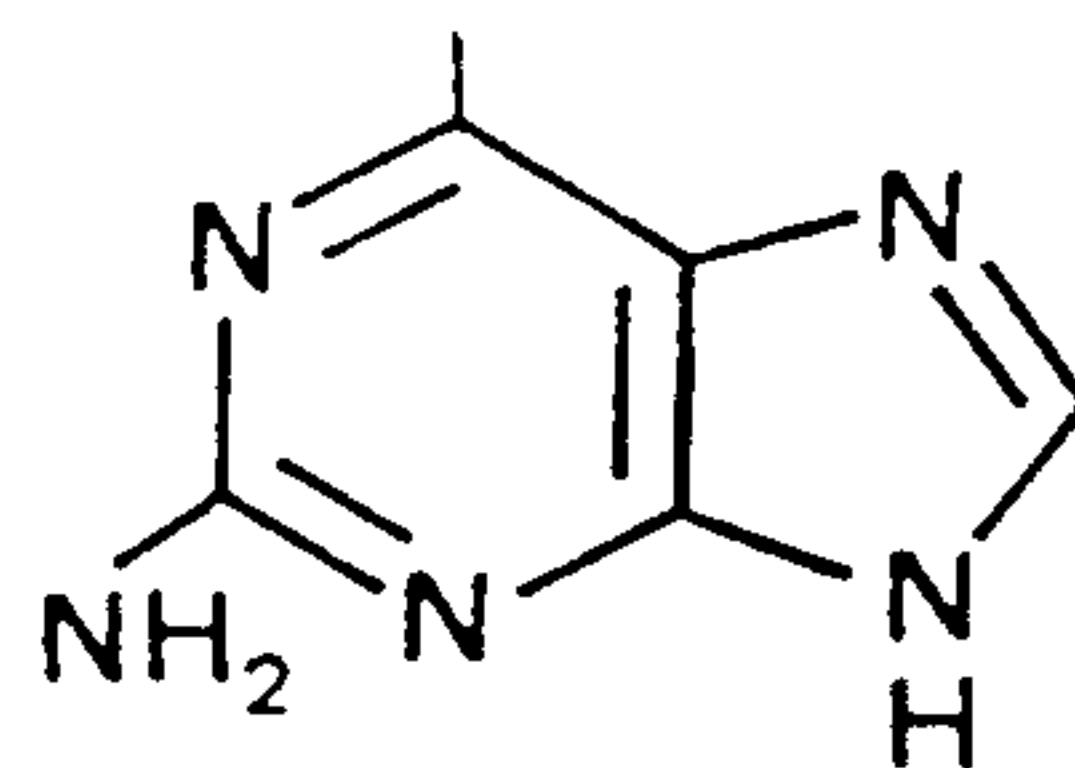
Examples of compounds of the invention (together with Compounds B.4214 and B.4218 not covered by the present application) are shown in Table 1.

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- 6 -

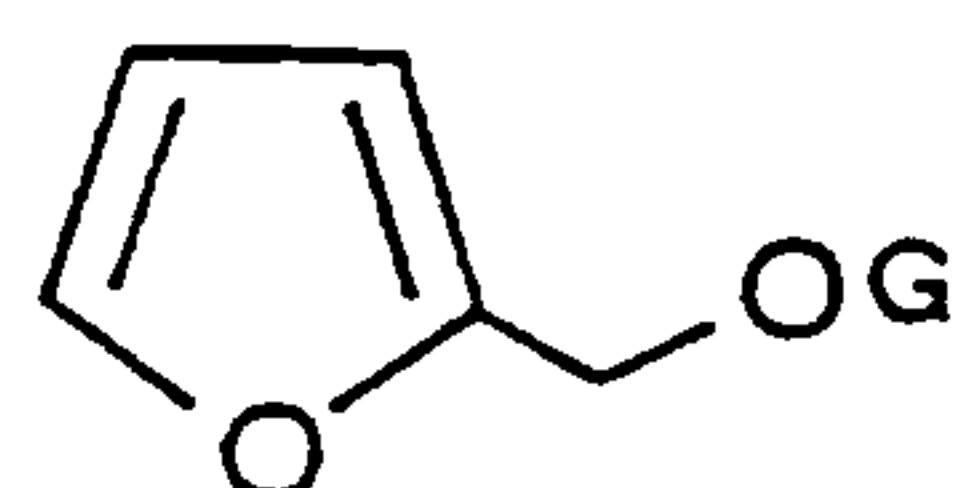
TABLE 1

G =



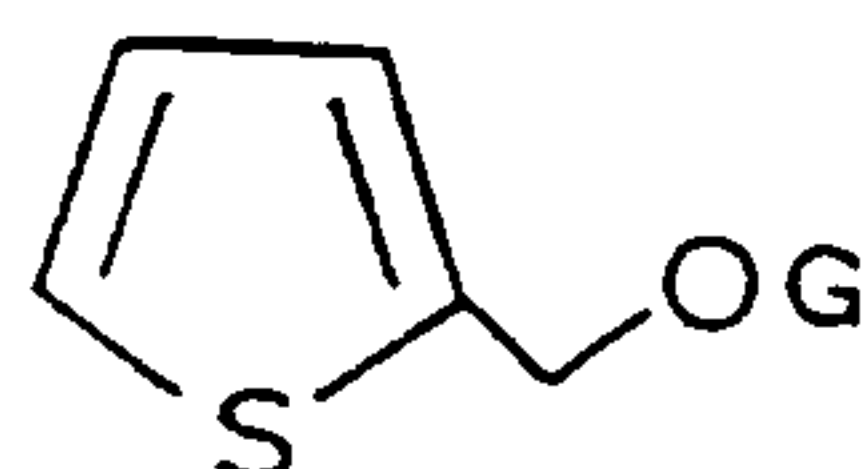
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B.4203

 $\underline{O}^6$ -furfurylguanine

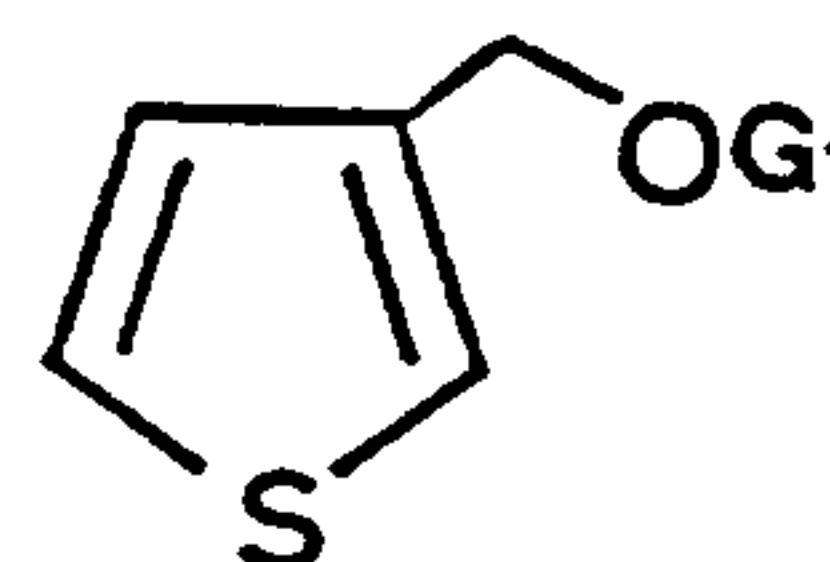
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B.4205

 $\underline{O}^6$ -thenylguanine

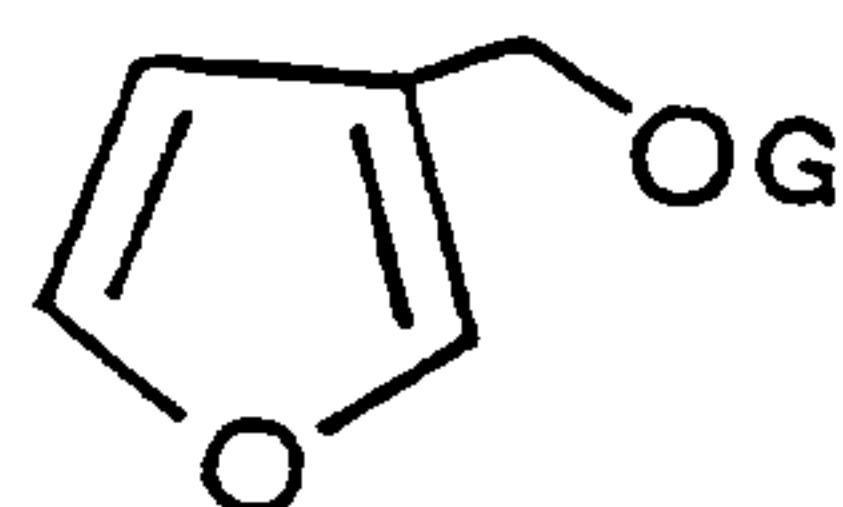
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20 B.4206

 $\underline{O}^6$ -(3-thienylmethyl)guanine

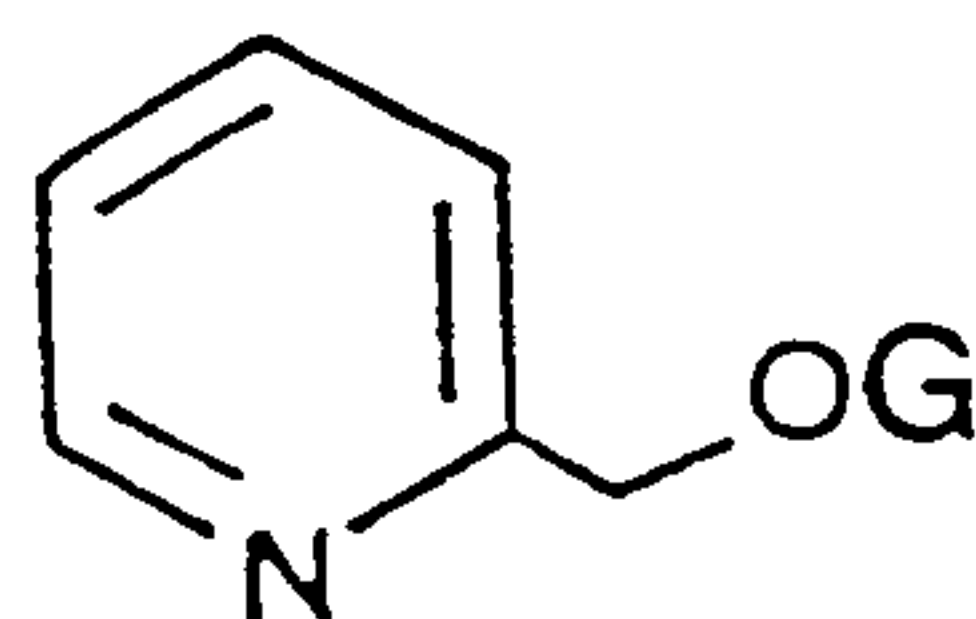
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B.4209

 $\underline{O}^6$ -(3-furylmethyl)guanine

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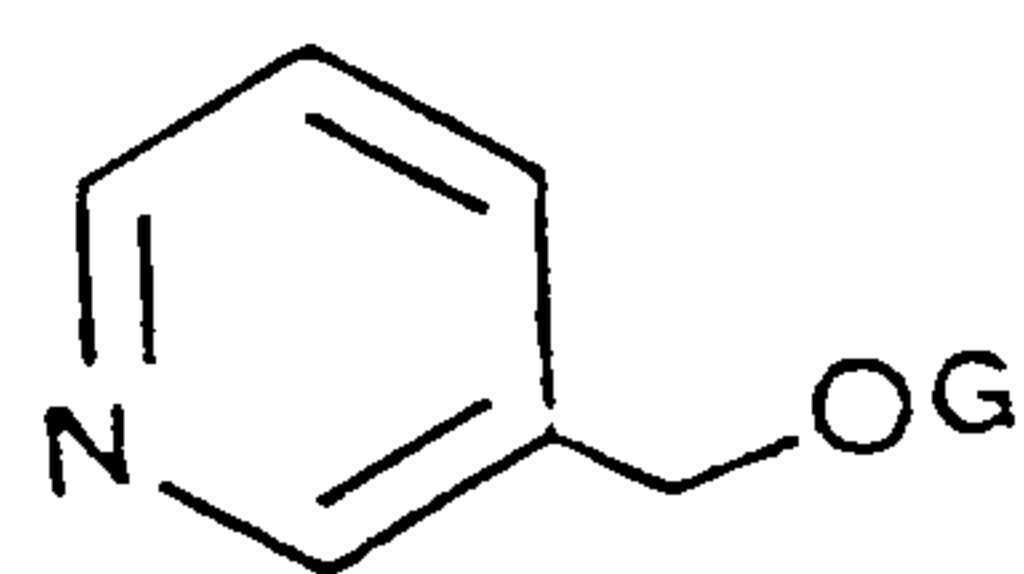
B.4210

 $\underline{O}^6$ -(2-picolyl)guanine

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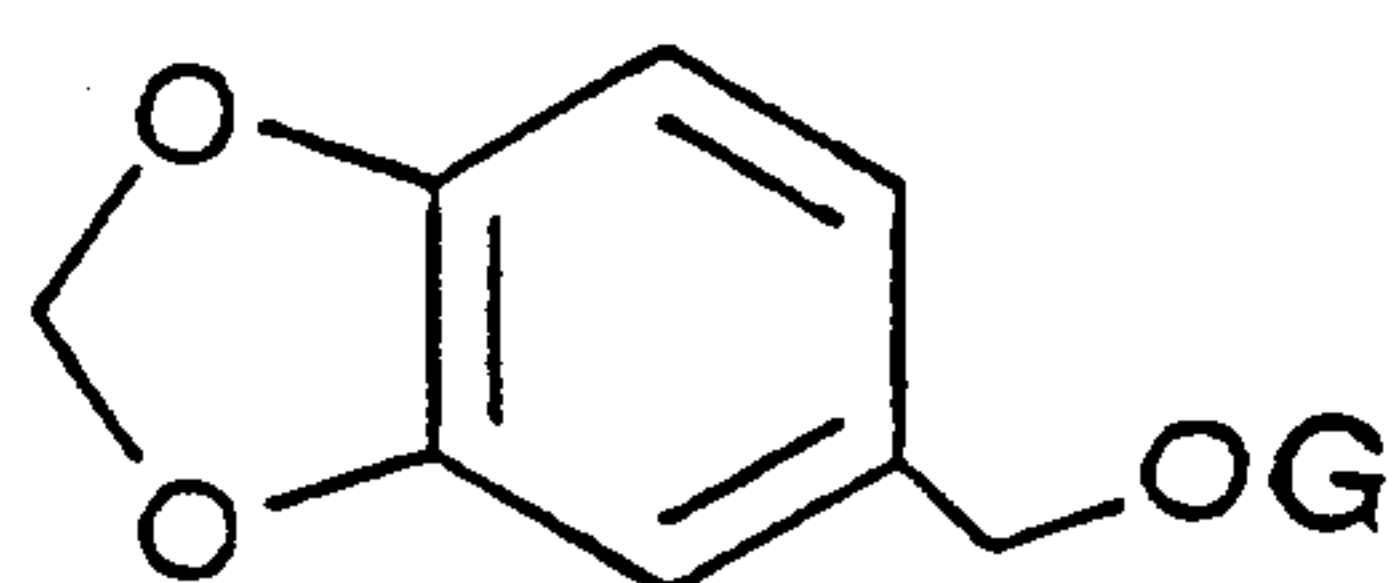
- 7 -

B.4211

 $\underline{O}^6$ -(3-picolyl)guanine

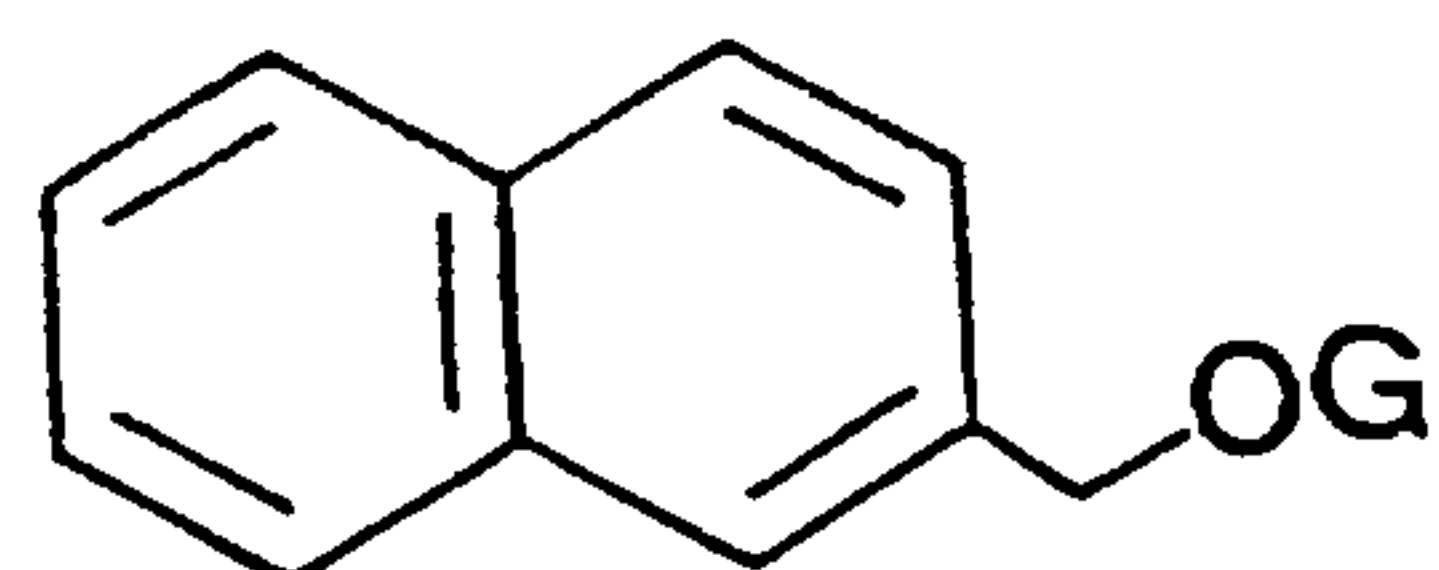
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10 B.4212

 $\underline{O}^6$ -piperonylguanine

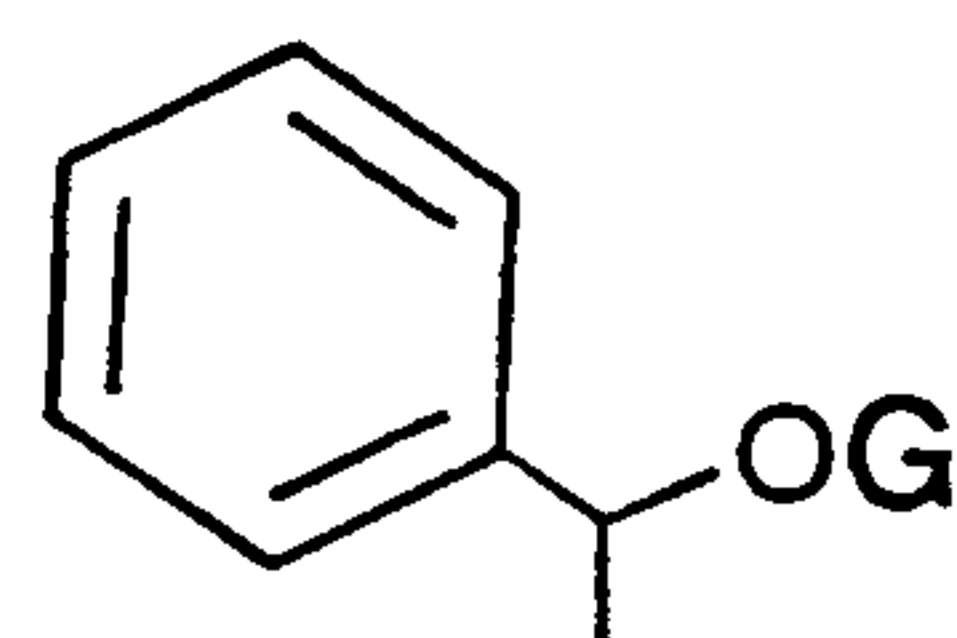
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B.4213

 $\underline{O}^6$ -(2-naphthylmethyl)guanine

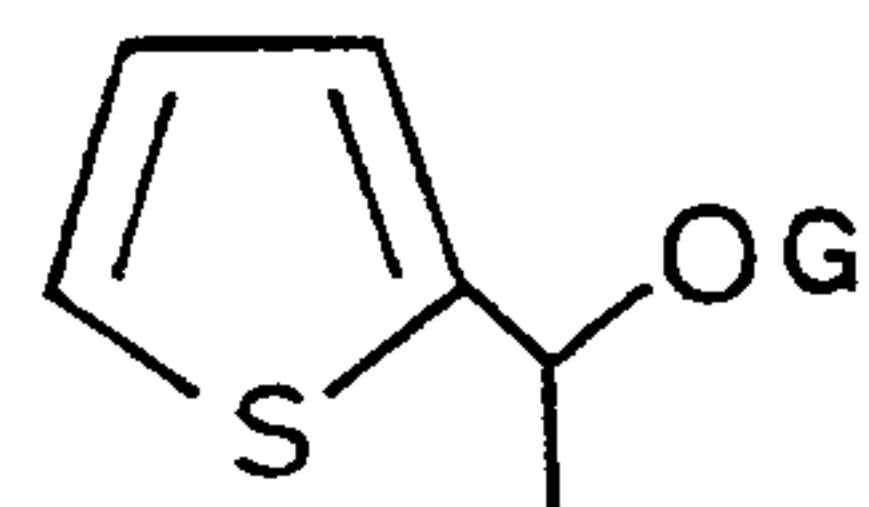
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25 B.4214

DL- $\underline{O}^6$ -(α -methylbenzyl)guanine

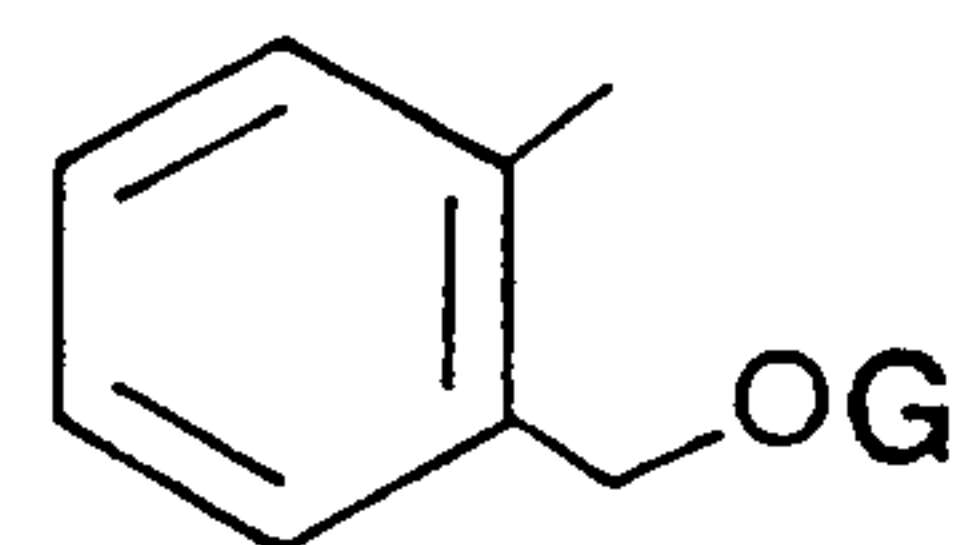
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B.4217

DL- $\underline{O}^6$ -(α -methylthienyl)guanine

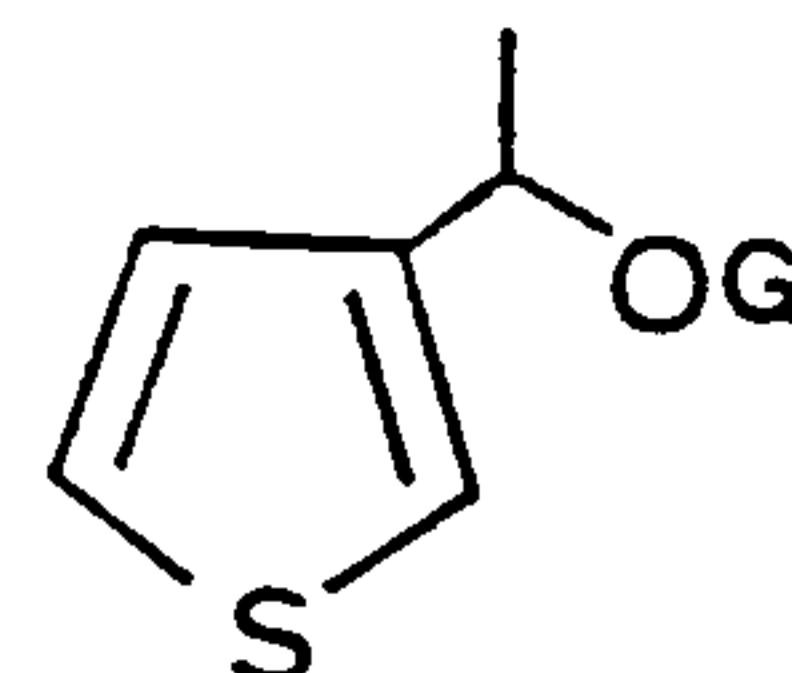
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B.4218

 $\underline{O}^6$ -(2-methylbenzyl)guanine

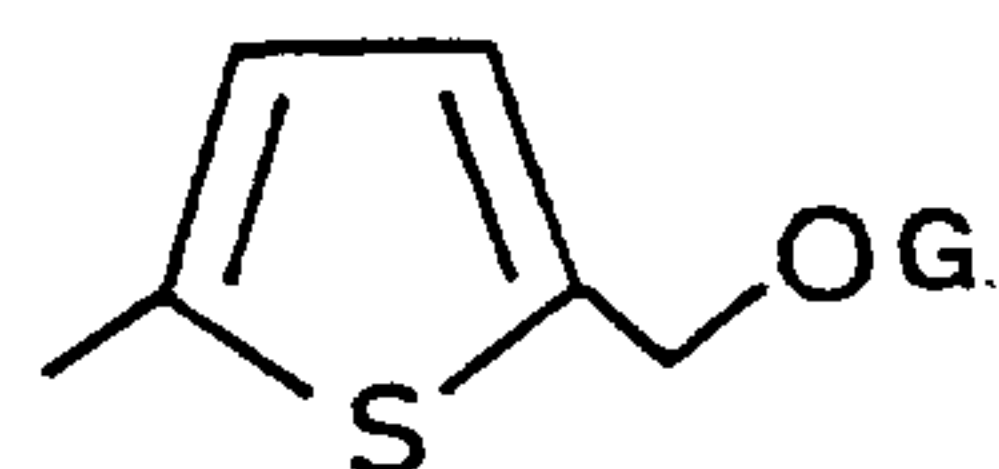
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10 B.4219

DL- $\underline{O}^6$ -[1-(3-thienyl)ethyl]guanine

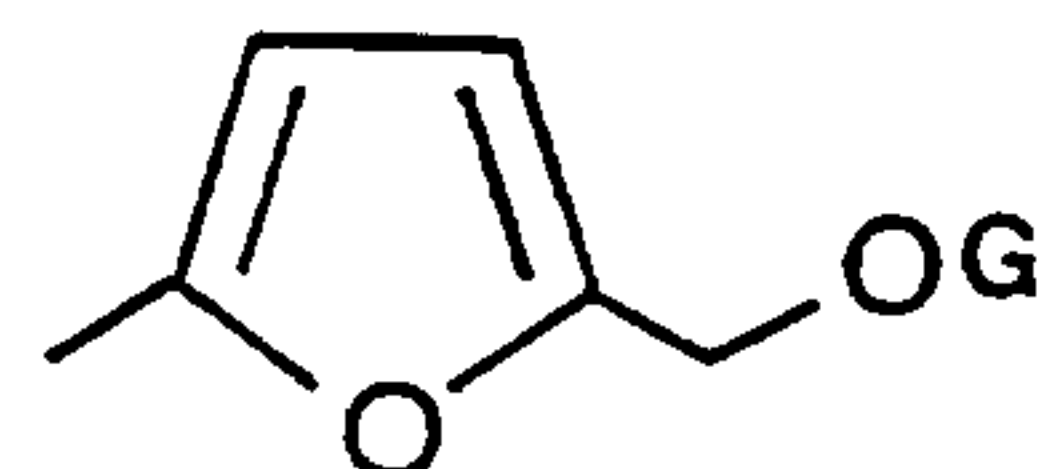
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B.4220

 $\underline{O}^6$ -(5-methylthienyl)guanine

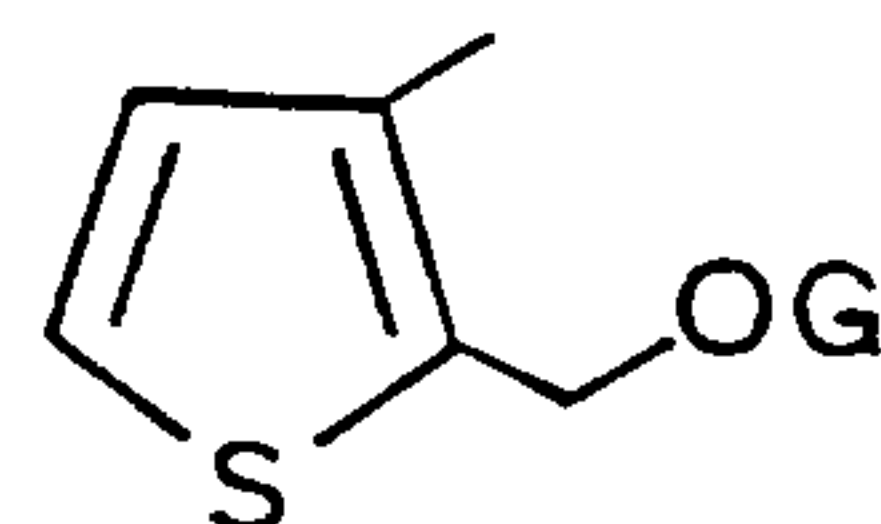
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B.4221

 $\underline{O}^6$ -(5-methylfurfuryl)guanine

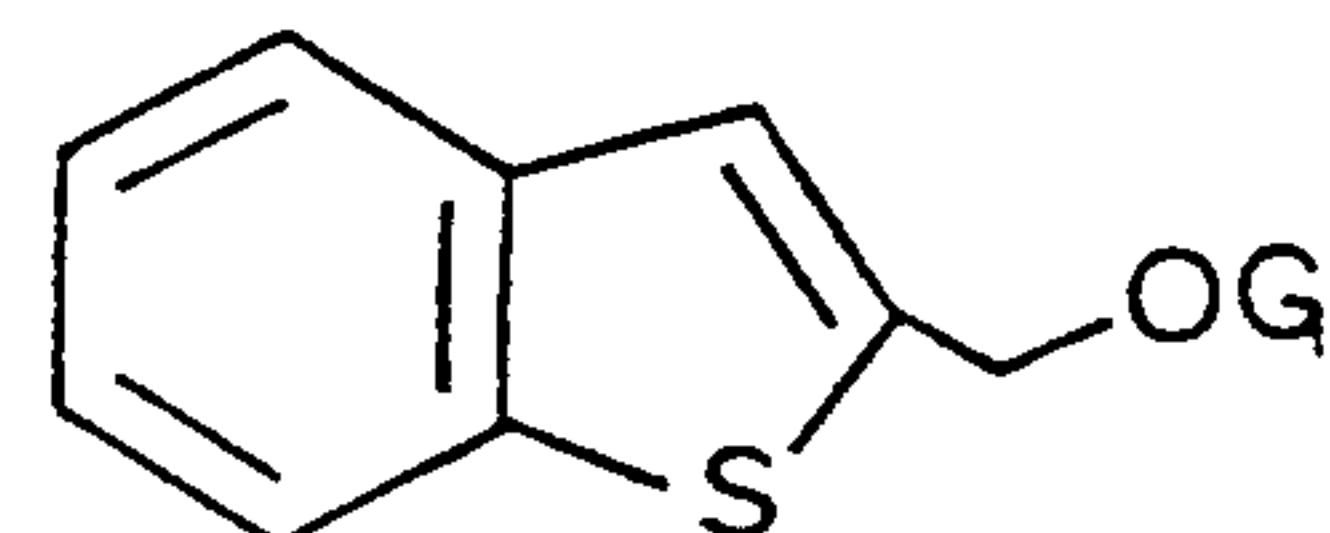
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30 B.4222

 $\underline{O}^6$ -(3-methylthienyl)guanine

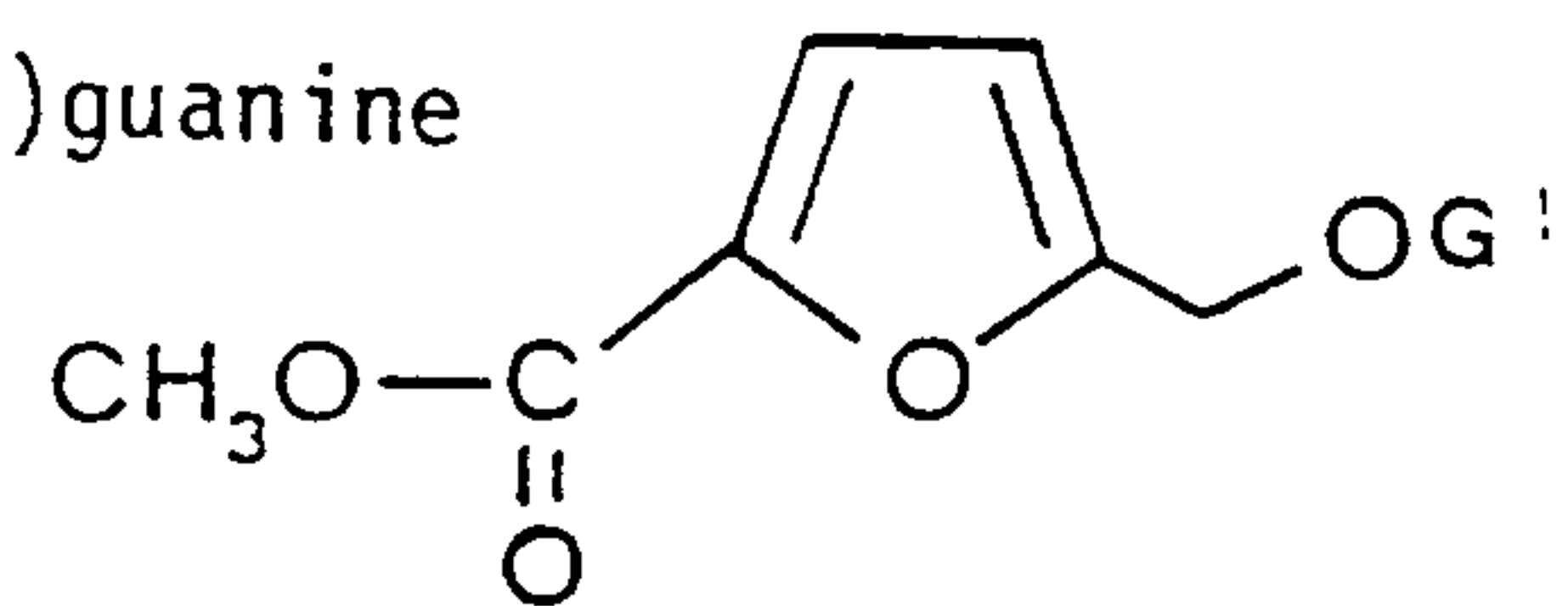
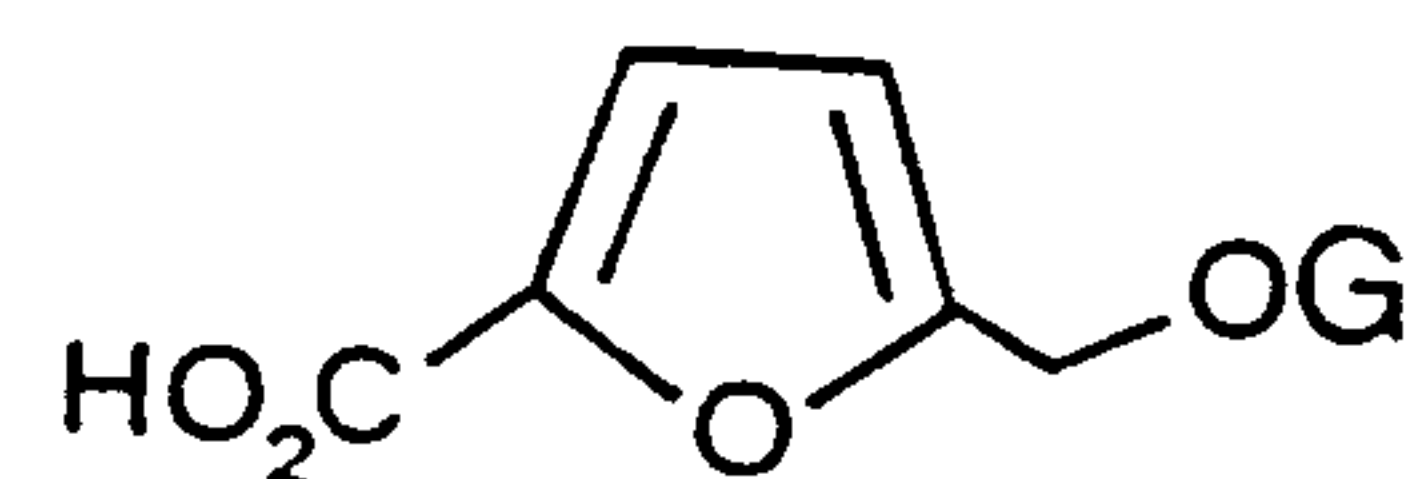
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B.4226

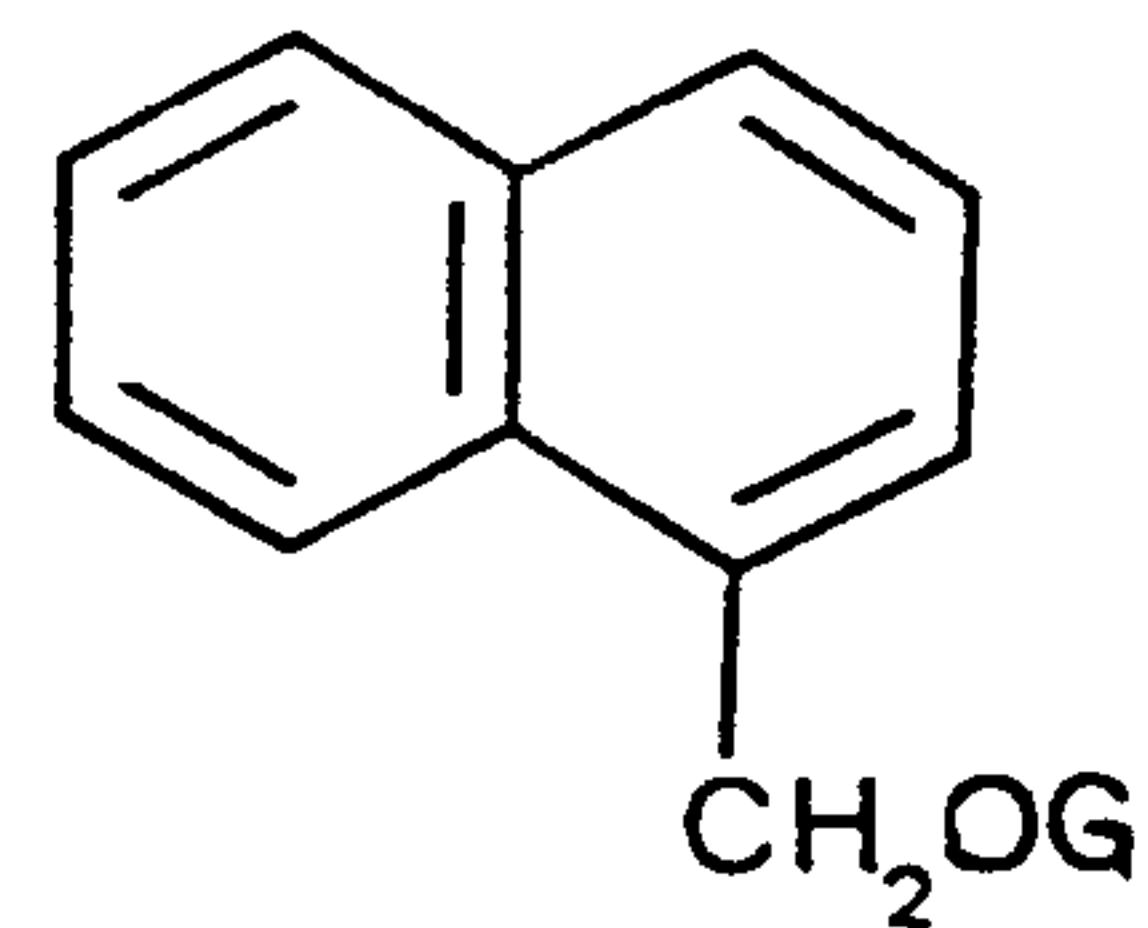
 $\underline{O}^6$ -(2-benzo[b]thienylmethyl)guanine

B.4229 $\underline{0}^6$ -(5-methoxycarbonylfurfuryl)guanine

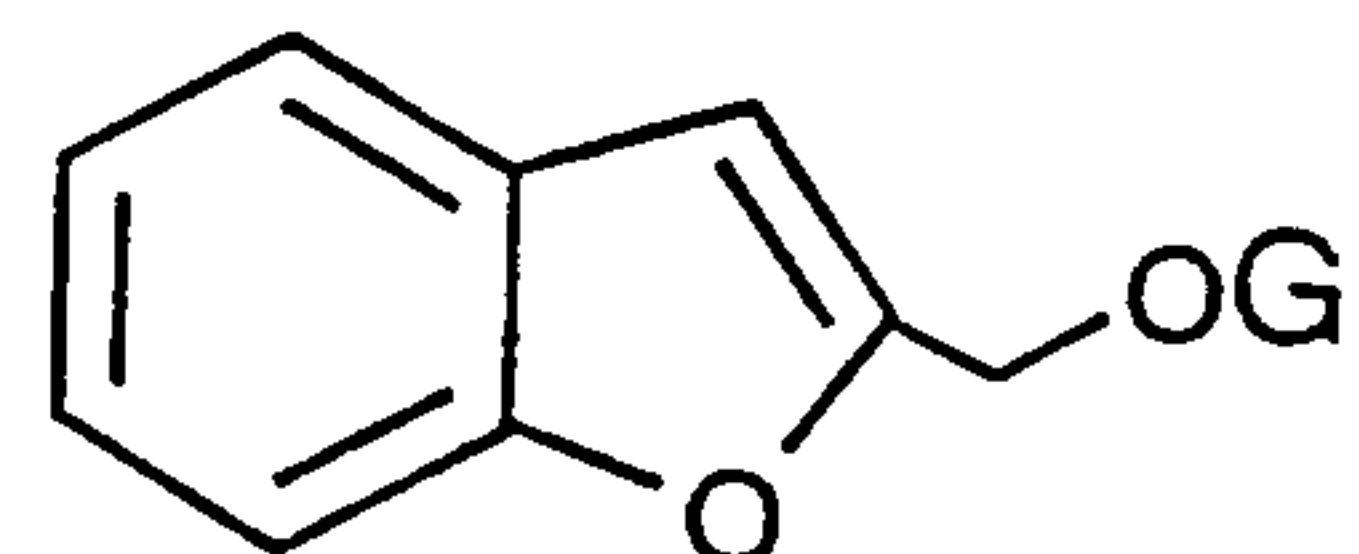
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10 B.4234 $\underline{0}^6$ -(5-carboxyfurfuryl)guanine

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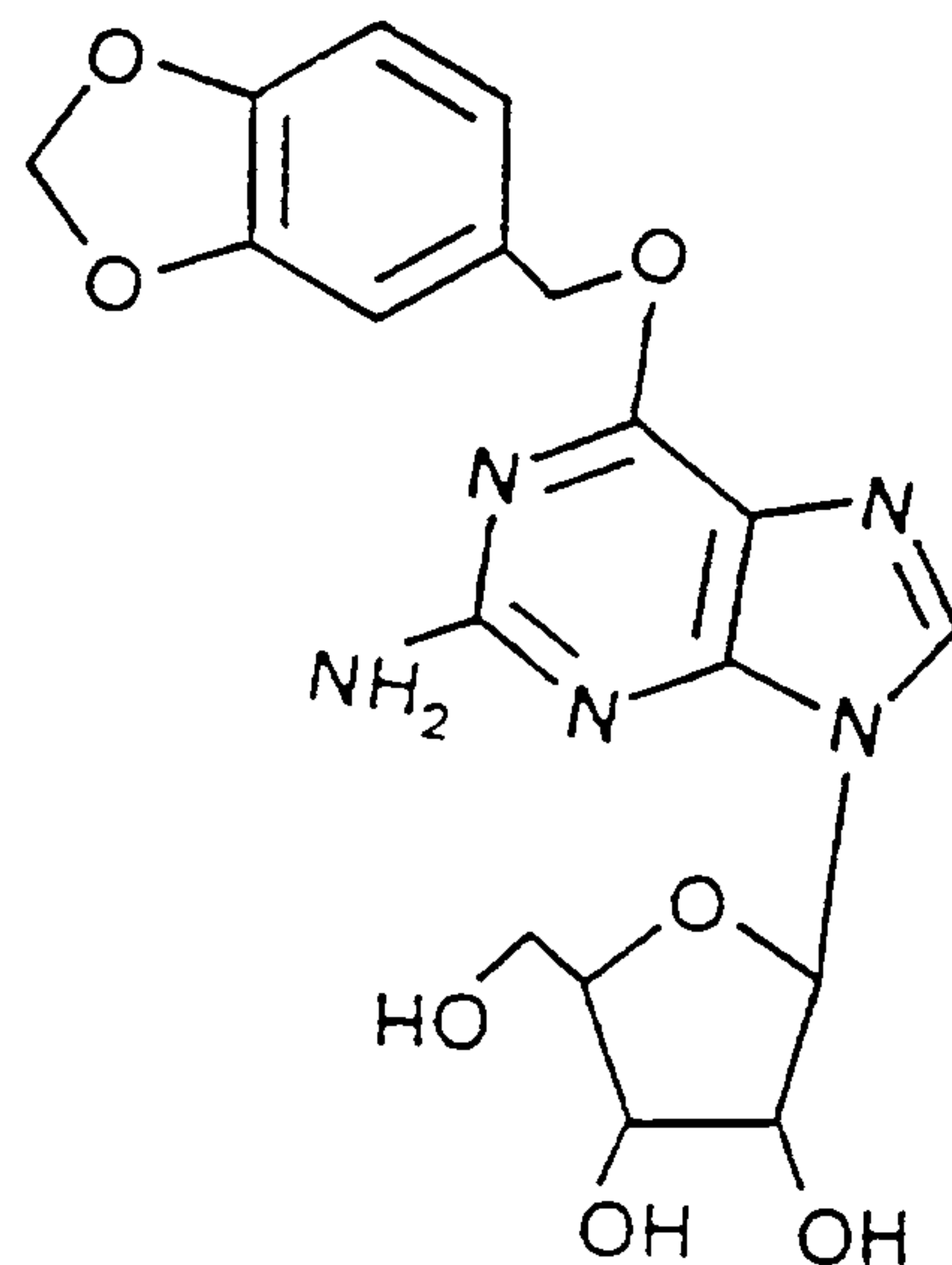
B.4265 $\underline{0}^6$ -(1-naphthylmethyl)guanine

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B.4266 $\underline{0}^6$ -(2-benzofuranylmethyl)guanine

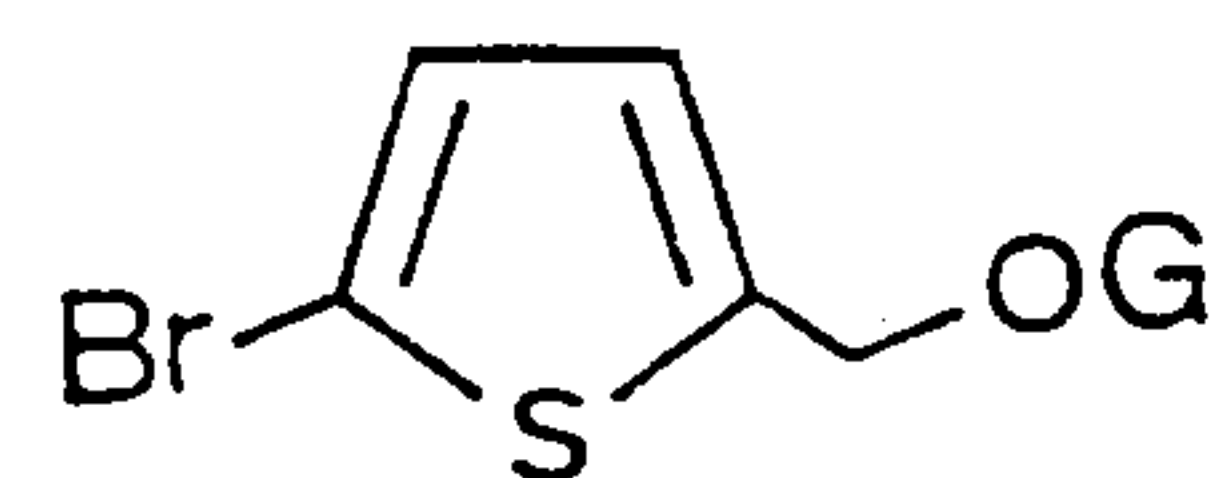
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B.4268 $\underline{0}^6$ -piperonylguanosine

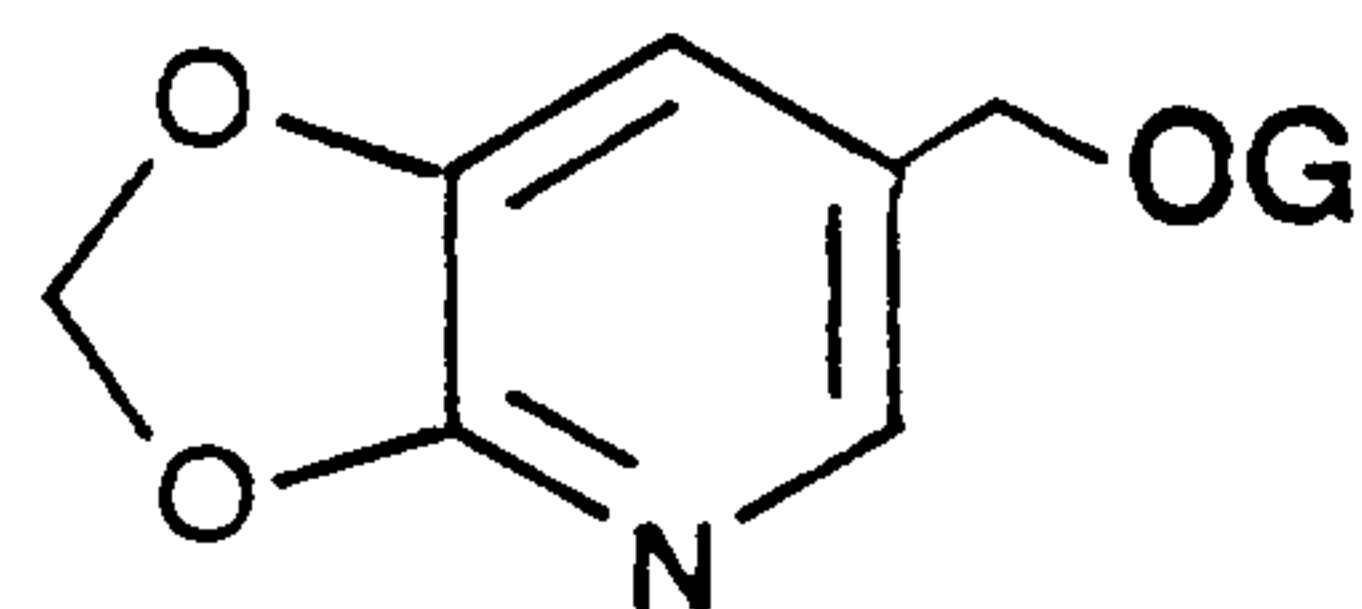
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B.4269

 $\underline{0}^6$ -(5-bromothienyl)guanine

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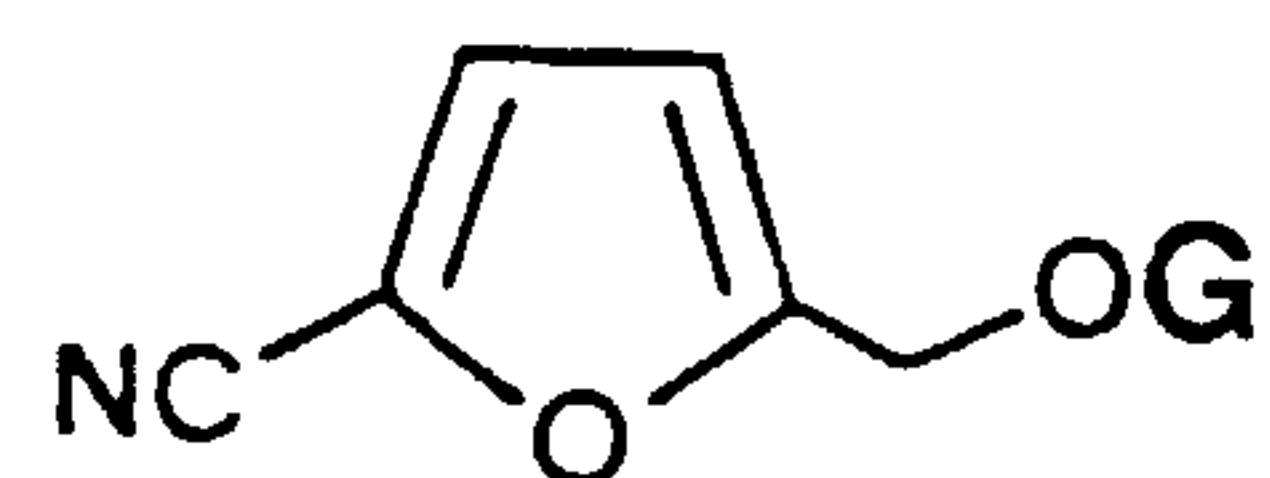
B.4271

 $\underline{0}^6$ -(5-azapiperonyl)guanine

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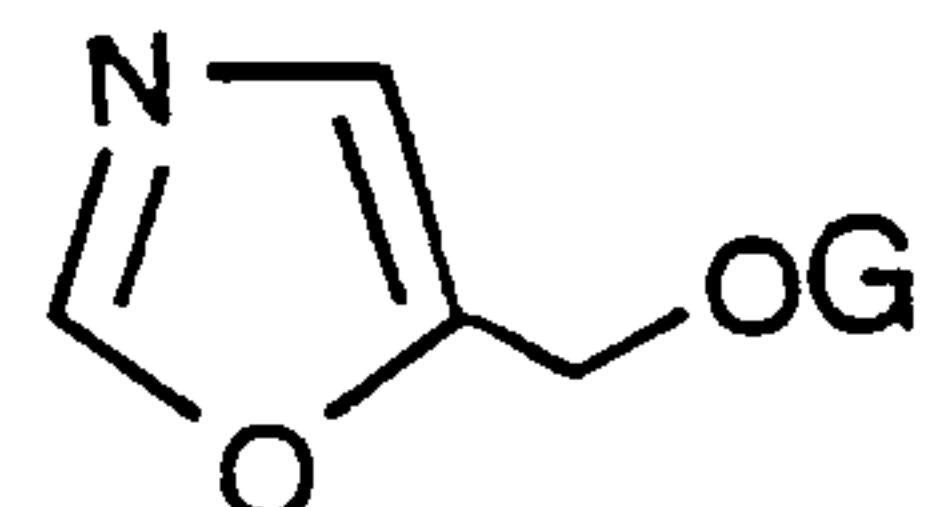
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B.4273

 $\underline{0}^6$ -(5-cyanofurfuryl)guanine

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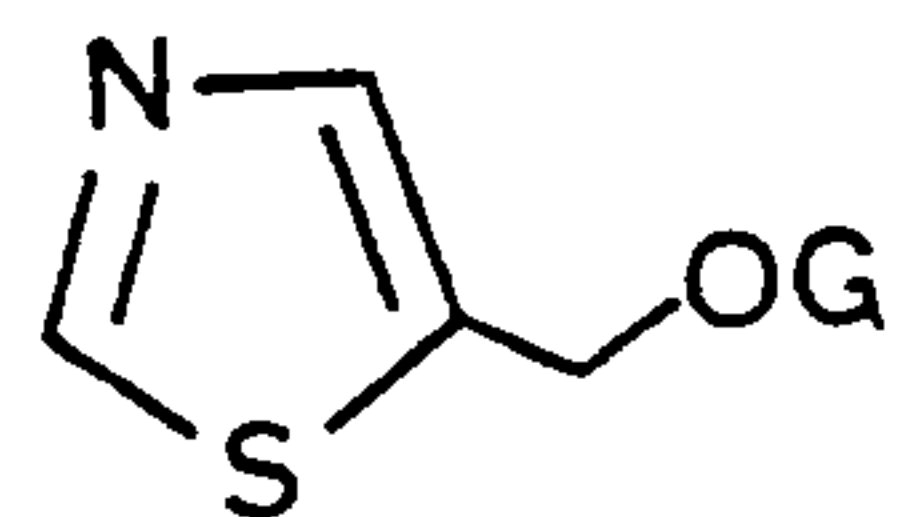
B.4274

 $\underline{0}^6$ -(5-oxazolylmethyl)guanine

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B.4275

 $\underline{0}^6$ -(5-thiazolylmethyl)guanine

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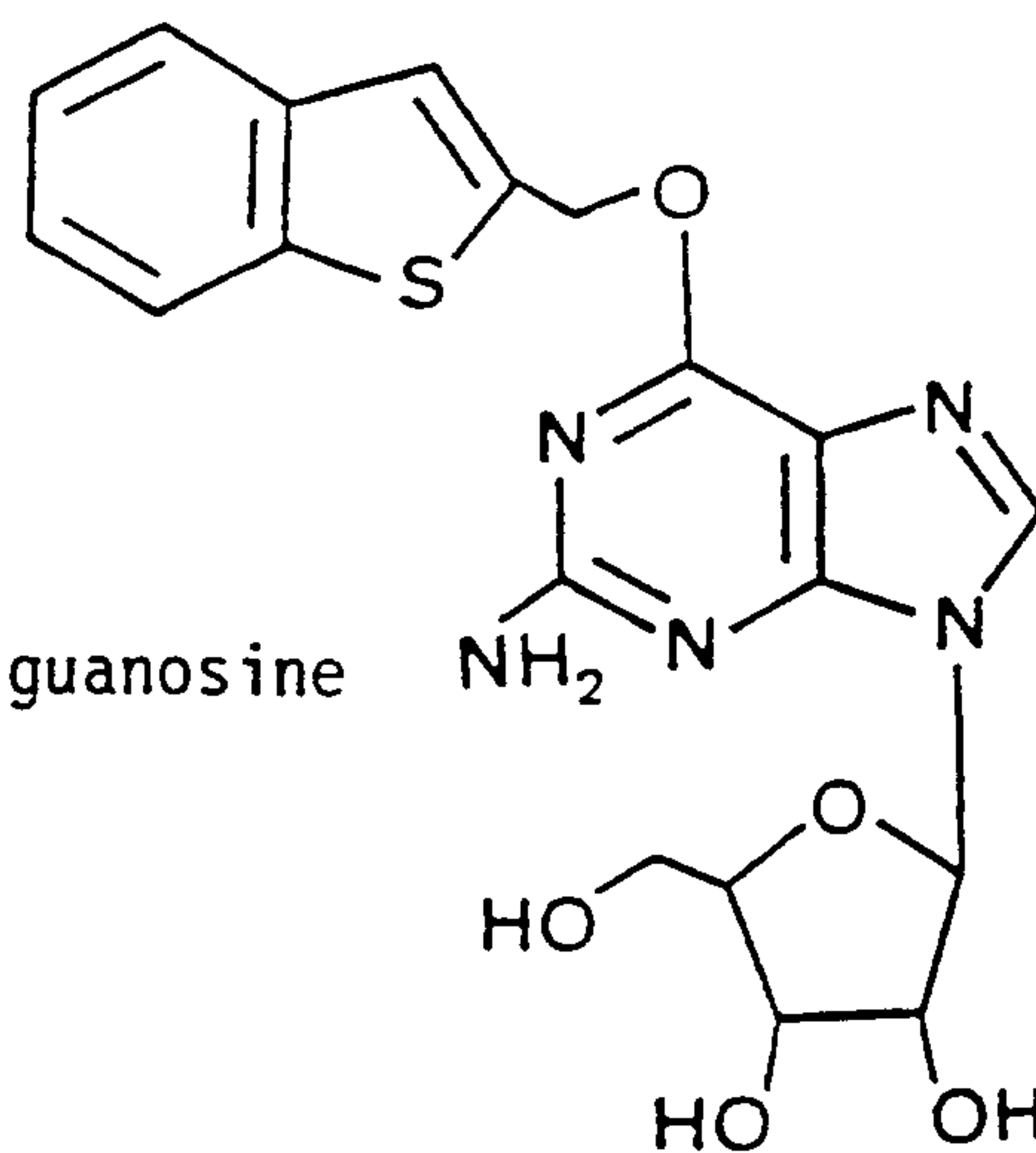
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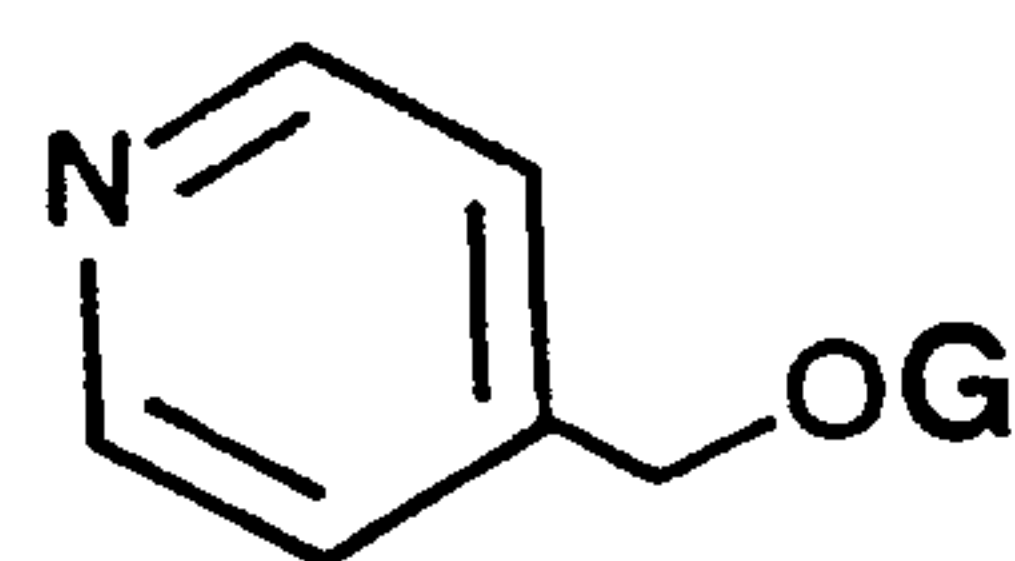
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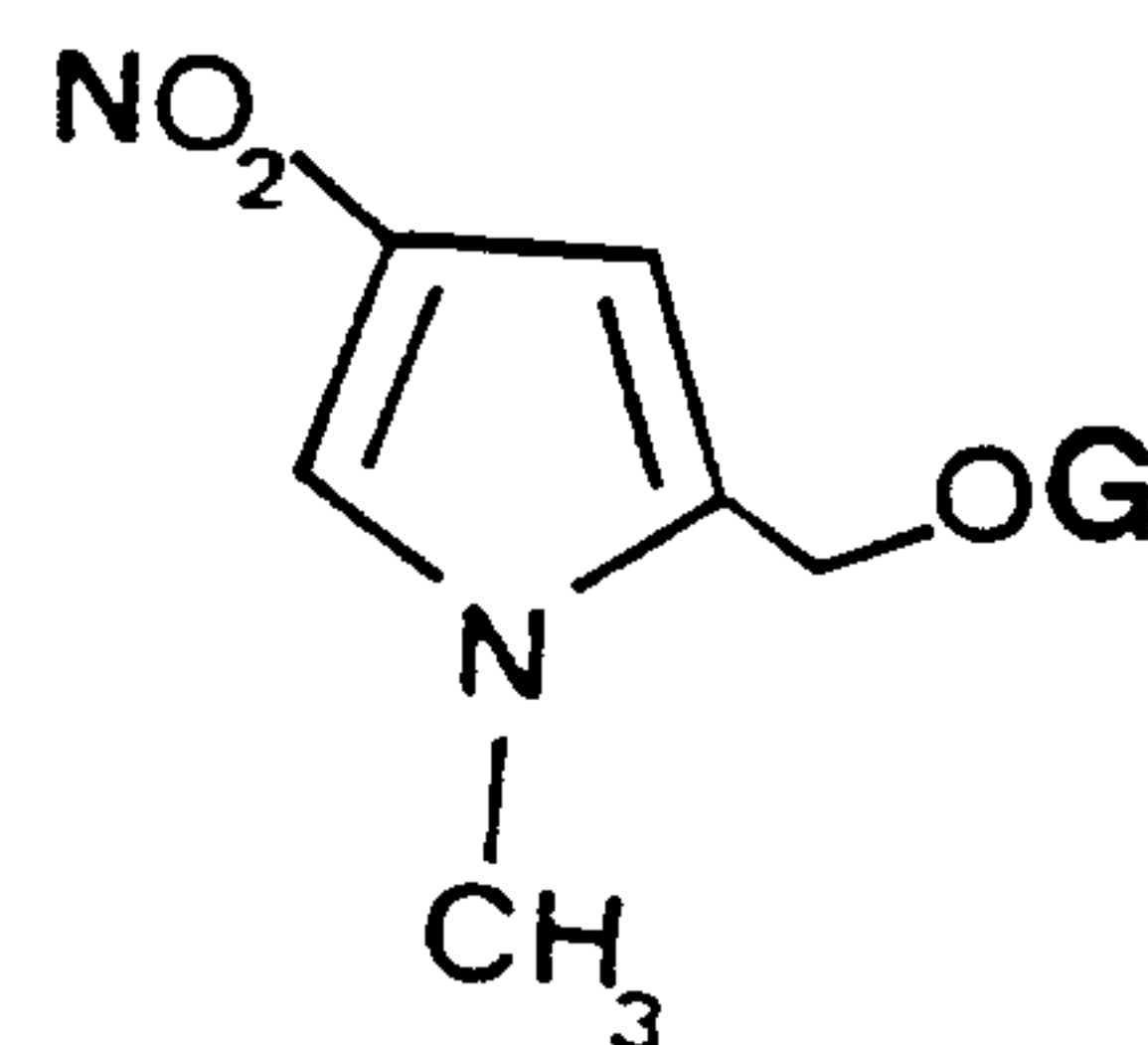
B.4276

 O^6 -(2-benzo[b]thienylmethyl)guanosine

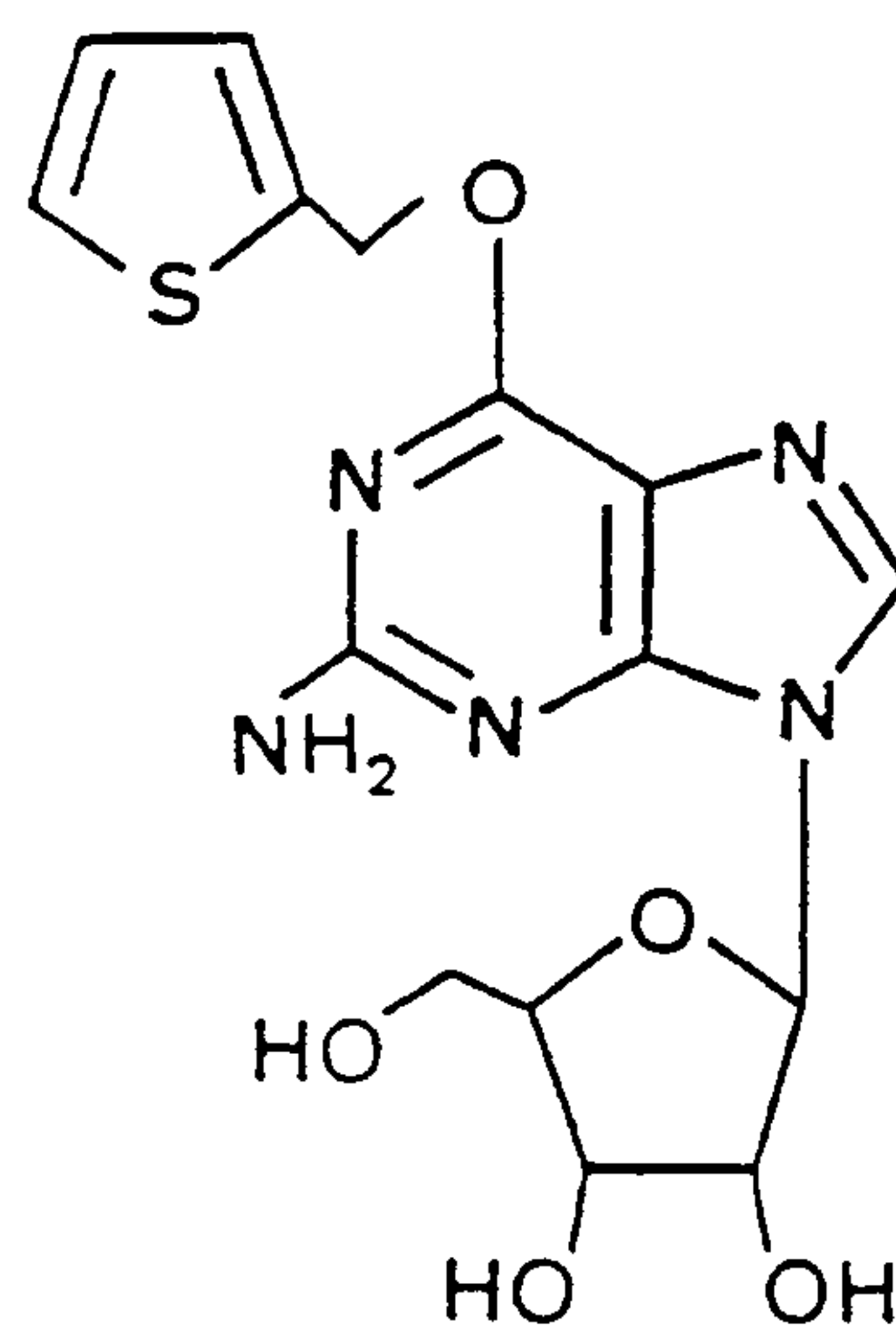
B.4277

 O^6 -(4-picolyl)guanine

B.4278

 O^6 -(1-methyl-4-nitropyrrol-2-ylmethyl)guanine

B.4279

 O^6 -thenylguanosine

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Among the compounds in Table 1 compounds B.4214 and B.4218 are not compounds of the invention. Compound B.4210 (ie the compound of Formula I in which R is 2-pyridyl, R' is H and Y is H) is not a preferred compound of the invention.

5 Particularly preferred compounds of the invention include:

B.4205 $\underline{O}^6$ -thenylguanine

B.4206 $\underline{O}^6$ -(3-thienylmethyl)guanine

B.4212 $\underline{O}^6$ -piperonylguanine

B.4226 $\underline{O}^6$ -(2-benzo[b]thienylmethyl)guanine

10 B.4266 $\underline{O}^6$ -(2-benzofuranylmethyl)guanine

B.4275 $\underline{O}^6$ -(5-thiazolylmethyl)guanine

and compounds substituted in the heterocyclic ring of R by a halo, cyano or ester group, including

B.4229 $\underline{O}^6$ -(5-methoxycarbonylfurfuryl)guanine

15 B.4269 $\underline{O}^6$ -(5-bromophenyl)guanine

B.4273 $\underline{O}^6$ -(5-cyanofurfuryl)guanine.

Other preferred compounds include

B.4209 $\underline{O}^6$ -3-furymethylguanine

B.4276 $\underline{O}^6$ -(2-benzo[b]thienylmethyl)guanosine

20 B.4277 $\underline{O}^6$ (4-picolyl)guanine.

The most preferred compounds of the invention are those that inactivate ATase in vitro and/or in mammalian cells and/or tumour xenografts more effectively than $\underline{O}^6$ -benzylguanine (BeG) and that

25 sensitise mammalian cells and/or tumour xenografts to the killing or growth inhibitory effects of nitrosoureas and or methylating agents more effectively than BeG. Preferred compounds should also have, in comparison to BeG, reduced toxicity to normal tissues and/or to the entire organism when used in combination with such agents. Preferred

30 compounds should not themselves be toxic or show more than minimal toxicity at the doses required to inactivate ATase, neither should any hydrolysis products of a preferred compound that was chemically unstable be toxic. Although the invention is not limited by any theory, preferred compounds may need to be less stable than BeG so

35 that they undergo spontaneous chemical degradation soon after achieving maximal inactivation of ATase: in this way any action of metabolic processes that might act on the agent to generate toxic species would be minimised. Preferred compounds should be less able to sensitise human bone marrow or other normal cell types to the toxic

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effects of alkylating agents so that they would not exacerbate the known toxicity or generate new toxicities of these agents in normal human tissues.

5 Preferred compounds of the invention include those having a relatively low I_{50} value in Table 4 herein (e.g. below $1.0\mu\text{M}$, more particularly below $0.04\mu\text{M}$) and/or having a relatively short half life in Buffer I (representing conditions for an in vitro assay) and/or Phosphate buffered saline (PBS) (representing conditions in
10 physiological medium) in Table 4 herein (e.g. below 20 hours in Buffer I or below 16 hours in PBS).

A relatively short half-life can be regarded as an indicator that a compound of the invention would be less stable than
15 $\underline{O}^6$ -benzylguanine due to the reactivity of $\text{RR}'\text{CH}-$ and would tend to break down by hydrolysis in physiological medium.

The influence of the group $\text{RR}'\text{CH}-$ in the compounds of formula I enabling them to act as ATase inhibitors is determined by electronic,
20 steric and physicochemical factors. Steric factors may be related to the nature of the environment of the cysteine receptor site in ATase. Preferably R' is H. A secondary carbon atom attached to $\underline{O}^6$ (as in the DL compounds B.4214 or B.4217) has been found to reduce greatly the inactivating activity, probably because of the bulk of the
25 substituent.

Preferably the cyclic group in R does not have a methyl group in the vicinal position (as in B.4222) although the influence of vicinal substitution is evidently much less when R is heterocyclic than in the
30 naphthyl isomers B.4213 and B.4265.

Physicochemical factors such as stability, solubility and water-lipid partition are relevant to the selection of compounds for use in vivo, affecting formulation, absorption and transport, for
35 example. Selection of compounds may also be influenced by their differential distribution into different tissues.

One embodiment of the invention provides a pharmaceutical composition containing compounds of formula 1, wherein Y, R and R' are

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as defined above, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient. Optionally the composition may also contain an alkylating agent such as a chloroethylating or methylating agent.

5

In a further embodiment, the present invention provides a method for depleting ATase activity in a host comprising administering to the host an effective amount of a composition containing a compound of formula 1 wherein Y, R and R' are as defined above, or a
10 pharmaceutically acceptable salt thereof, more particularly a pharmaceutical composition as defined above. This method may alternatively be defined as a method of depleting ATase mediated DNA repair activity in a host.

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The invention further provides a method for treating tumour cells in a host comprising administering to the host an effective amount of a composition containing a compound of formula I wherein Y, R and R' are as defined above or a pharmaceutically acceptable salt thereof, more particularly a pharmaceutical composition as defined
20 above and administering to the host an effective amount of a composition containing an alkylating agent. The method may be used for treatment of neoplasms including those which are known to be sensitive to the action of alkylating agents e.g. melanoma and glioma and others whose resistance to treatment with alkylating agents alone
25 may be overcome by the use of an inactivator according to the invention.

The invention also provides a process for preparing compounds of formula I comprising the steps of: reacting sodium hydride with a
30 solution of RR'CHOH (wherein R and R' are as defined above) in an organic solvent, preferably at or below room temperature; adding 2-amino-N,N,N-trimethyl-1H-purin-6-aminium chloride or 2-amino-6-chloropurine riboside; treating the reaction mixture with weak acid and ether; and extracting the desired product.

35

Brief Description of Drawings

The invention shall be described in greater detail with reference to the accompanying drawings in which:

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Figure 1 is a graph of percentage residual activity of purified recombinant human ATase following incubation with different concentrations of various inactivators. Each point shows the mean of 3 measurements. The line at 50% residual activity is used for calculating I_{50} values i.e. the concentration of inactivator required to produce a 50% reduction in ATase activity.

Figure 2 is two graphs of percentage cell growth against alkylating agent concentration ($\mu\text{g/ml}$), showing the sensitization effect of O^6 -benzylguanine (BeG) and O^6 -thenylguanine (B.4205) at two different concentrations (0.1 and $1.0\mu\text{M}$) on sensitization of Raji cells to BCNU. The line at 90% growth is used for calculating D_{90} values i.e. the dose of BCNU at which there was 90% growth as compared to untreated controls i.e. 10% growth inhibition.

Figure 3 is four graphs of percentages cell growth, against alkylating agent concentration (μM), showing the effect of BeG and B.4205 at four different concentrations (0.1, 0.5, 1.0 and $5.0\mu\text{M}$) on sensitization of Raji cells to temozolomide.

Figure 4 is a graph extrapolated from Figure 3 of values for D_{50} (μM) (i.e. the dose of temozolomide at which there was 50% growth as compared to untreated controls) against inactivator concentration (μM).

Figure 5 is two graphs of percentage cell growth against inactivator concentration, showing the growth inhibiting effect of O^6 -furfurylguanine (B.4203) and B.4205 on Chinese hamster V79 cells(RJK0) and a subline thereof (+120).

Figure 6 is two graphs of percentage cell growth against inactivator concentration, showing the growth inhibition effect of B.4203 and B.4205 on two Xeroderma pigmentosum subclones.

Figure 7 is four graphs of percentage cell growth against inactivator concentration (μM) showing the effect of degradation products of inactivators B.4203, B.4205, B.4212 (O^6 -piperonylguanine) and B.4226 (O^6 -[2-benzo(b)thienylmethyl]guanine) on Raji cell growth.

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Figure 8 is a diagram of ATase activity (fm/mg) against time (hours) showing the depletion of ATase activity in A375 xenografts in nude mice for untreated controls, corn oil treated controls and BeG (30 mg/kg) and B.4205 (30 mg/kg) treated extracts.

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Figures 9 - 13 are graphs of results of xenograft studies. In each figure the top graph shows percentage tumour volume against time (days) for A375 tumour xenografts in nude mice treated with BCNU alone, with BeG in combination with BCNU, and B.4205 in combination with BCNU. The lower graph in each figure shows the number of surviving mice in each treatment group against time (days) following the treatments illustrated in the top graph.

The particulars for each figure are as follows:-

15	Figure	Mice	BCNU Concentration (mg/kg)	Inactivator (mg/kg)
	9	ASU	Approx. 15	60
20	10	ASU	25	60
	11	OLAC	10	30
25	12	ASU	16	60
	13	ASU	16	60

Figure 14 is 3 graphs of percentage survival against temozolomide concentration (μM), showing the survival of bone marrow cells following treatment with inactivator ($10\mu\text{M}$) or DMSO (control) in combination with increasing doses of temozolomide.

Modes for carrying out the Invention

O^6 -hetarylalkylguanine derivatives having ATase inactivating characteristics may be synthesized by adapting the standard preparation presented below as appropriate.

2-Amino-N,N,N-trimethyl-1H-purin-6-aminium chloride is prepared in accordance with the procedure described by Kiburis et al., J.

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Chem.Soc. (C), 1971, 3942. Details of the conditions for reaction of this quaternary salt with sodium benzyloxide (to give O⁶-benzyl-guanine) not disclosed in MacCoss, Chen and Tolman, Tetrahedron Lett., 1985, 26, 1815, were given in MacCoss, Tolman, Wagner and Hannah, European Patent Application No. 184,473, but these were not suitable for preparation of relatively sensitive analogues, and the standard preparation below was devised by the inventors.

Standard preparation of O⁶-hetarylalkylguanines (Formula I, y = H)

10 Sodium hydride (60% in oil; 0.8 g, 20 mmol) is added to a solution of RR'CHOH (56 mmol, ca. 5 ml) in DMSO (5 ml) and the mixture is stirred at room temperature for 1 hour. For solid or higher molecular weight alcohols, up to 10ml DMSO may be used instead of 5ml. 2-Amino-N,N,N-trimethyl-1H- purin-6-aminium chloride (2.29 g, 10 mmol) is added and stirring is continued for a further 1 hour. The change in UV spectrum is then complete (λ_{max} 312 $\rightarrow$ 284 nm) and the almost clear solution is treated with acetic acid (1.7 ml). After cooling and dilution with ether (300 ml), the mixture is set aside (2 hours) and the solid (A) collected. Trituration with water gives the product. A second fraction can be obtained by evaporation of the ether-DMSO filtrate and trituration of the residue successively with ether and water. Alternatively the product may be extracted from the solid (A) with warm acetonitrile. The recrystallised compounds show a single spot in TLC (C₆H₆-MeOH, 4:1) and are characterised by analysis and their NMR spectra. Frequently, they contain solvent of crystallisation. Melting points and analytical data are given in Table 2, UV and ¹H NMR data in Table 3. NMR spectra were measured on a Bruker WP80 or MSL 300 instruments.

30 This standard preparation procedure (with variations indicated by symbols in Table 2) was used to make the compounds listed in Tables 2a and 3a. In compounds B.4217 and B.4219, R' is methyl; in the remaining compounds R' is H. O⁶-benzylguanine and Compounds B4214, B4218 and B4231 listed in Table 4 below were also made by this standard preparation procedure for comparative testing purposes.

The variations indicated by the symbols in Table 2 are as follows:
a 5 mmol sodium hydride per mmol quaternary salt are used in preparation of this compound. When the standard amount (2 mmol)

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is used, 40% of the quaternary salt can be recovered in the reaction work-up.

b This compound is made by hydrolysis of the methyl ester B.4229 (145 mg, 0.5 mmol) in 2-methoxyethanol (2.5 ml) and water (2.5 ml) by treatment with 2M-NaOH (2.5 ml) for 4 hours at room temperature. Neutralisation with acetic acid (0.32 ml, 5.5 mmol), gentle evaporation, trituration with water (3 ml) and filtration give a solid which on extraction with hot methanol yields the acid B.4234.

c The required figures are based on the monohydrate of a mixture of 4 parts of sodium salt of the acid and 3 parts of the acid, requiring Na, 4.3%. Found: Na, 4.44%.

d 3 mmol alcohol RCH_2OH per mmol quaternary salt are used instead of the standard 5.6 mmol.

e For alcohols which are too sensitive to sodium hydride in DMSO at room temperature, RCH_2ONa is prepared in DMF (2.5 ml at -10°C ; 3 mmol RCH_2OH ; 2 mmol sodium hydride). 1 mmol quaternary salt is added after 15-20 minutes and stirring continued for 2-3 hours at room temperature

f Products are extracted with acetonitrile.

Preparation of Ribosides (formula I, Y= ribosyl)

A solution of alkoxide made as in the Standard Procedure above from sodium hydride (60% in oil; 120 mg, 3mmol) and $\text{RR}'\text{CHOH}$ (4.6 mmol) in dry DMSO (2 ml) during 1 hour is treated with 2-amino-6-chloropurine riboside (302 mg, 1 mmol) and stirred for 5 minutes at room temperature, then 15 minutes at $60-65^\circ\text{C}$. The reaction is then complete as indicated by the change in UV spectrum ($\lambda_{\text{max}}^{311} \rightarrow 284\text{nm}$). Cooling and thorough trituration with ether (100 + 15 ml) and filtration yield a solid which is treated with water (10 ml). The pH is brought from 11 to 8 by passing CO_2 briefly. Filtration removes inorganic material and the dried residue is extracted at room temperature with methanol (4 x 10 ml portions, each containing a drop of pyridine). Evaporation of almost all the

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methanol and addition of a little ether yield the product, almost pure by TLC (C_6H_6 -MeOH 4:1). It is recrystallised from methanol and a trace of pyridine, dissolving and concentrating below 40°C, sometimes with final addition of a little ether.

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This procedure was used to make the compounds listed in Tables 2b and 3b. Traces of impurities are difficult to remove but NMR spectra show that the nucleosides are ca. 90% pure. Yields are of the order of 30-40%.

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TABLE 2a

Test No.	$\overline{O\delta}$ -Substituent RR' ¹ CH-	Yield % (based on solvate)	Solvent for recrystn.	M.p.(decomp) (°C)	Formula	Analysis			
						Found	C	H	N
B.4203	furfuryl	85	MeOH	Indeterminate: from 185	$C_{10}H_9N_5O_2 \cdot 1/2 H_2O$	Req.	50.14 50.01	4.00 4.19	29.24 29.17
B.4205	thenyl	72	EtOH	Indeterminate gradual from 160	$C_{10}H_9N_5OS \cdot 1/3 EtOH$	Found Req.	48.40 48.78	4.61 4.22	26.45 26.63
B.4206	3-thienylmethyl	74	MeOH	205	$C_{10}H_9N_5OS \cdot MeOH$	Found Req.	47.61 47.3	4.88 4.69	25.67 25.07
B.4209	3-furylmethyl	61	EtOH	191	$C_{10}H_9N_5O_2$	Found Req.	52.07 51.93	4.16 3.92	30.25 30.29
B.4210	2-picoly	71	MeOH	220-221	$C_{11}H_{10}N_6O \cdot 1/2 H_2O$	Found Req.	52.85 52.60	4.73 4.41	32.79 33.47
B.4211	3-picoly	53	MeOH	226-228	$C_{11}H_{10}N_6O \cdot 1/2 H_2O$	Found Req.	52.53 52.60	4.78 4.41	32.50 33.47
B.4212	piperonyl	74	EtOH	195-197	$C_{13}H_{11}N_5O_3 \cdot 3/4 H_2O$	Found Req.	52.22 52.25	4.48 4.22	23.64 23.44
B.4213	2-naphthylmethyl	74	EtOH	224-226	$C_{16}H_{13}N_5O$	Found Req.	65.91 65.97	4.58 4.50	23.99 24.05
B.4217	α -methylthienyl	27	MeCN	150 Upwards	$C_{11}H_{11}N_5OS$	Found Req.	50.39 50.57	4.40 4.24	26.96 26.80
B.4219	1-(3-thienyl)ethyl	57	MeCN	150 Upwards	$C_{11}H_{11}N_5OS$	Found	50.59	4.33	27.10

TABLE 2a (continued)

Test No.	O ⁶ -Substituent RR ¹ CH-	Yield % (based on solvate)	Solvent for recrystn.	M.p.(decomp) (°C)	Formula	Analysis		
						C	H	N
B.4220	5-methylthienyl ^f	29	MeCN	140 Upwards	C ₁₁ H ₁₁ N ₅ OS			
B.4221	5-methylfurfuryl ^f	38	MeCN	Indeterminate	C ₁₁ H ₁₁ N ₅ O ₂ ·0.5H ₂ O	Found 52.18 Req. 51.98	4.81 4.76	27.47 27.56
B.4222	3-methylthienyl	29	MeOH	Indeterminate	C ₁₁ H ₁₁ N ₅ OS			
B.4226	2-benzo[b]thienylmethyl	66	MeOH	198-208	C ₁₄ H ₁₁ N ₅ OS·MeOH	Found 53.93 Req. 54.69	4.49 4.59	21.47 21.26
B.4229	5-methoxycarbonyl -furfuryl ^{a,f}	43	MeOH	120-130 (with effervescence)	C ₁₂ N ₁₁ N ₅ O ₄ ·1.5H ₂ O	Found 45.88 Req. 45.57	4.21 4.46	21.86 22.14
B.4234	5-carboxyfurfuryl ^b	67	MeOH	210-260	C ₁₁ H ₉ N ₅ O ₄ ·H ₂ O ₂	Found 42.69 Req. 43.2	3.52 3.4	22.68 22.9
B.4265	1-naphthylmethyl	75	MeOH	210 Upwards	C ₁₆ H ₁₃ N ₅ O·0.5MeOH	Found 64.37 Req. 64.48	4.85 4.92	22.81 22.79
B.4266	2-benzofuranylmethyl	58	MeOH	196-198	C ₁₄ H ₁₁ N ₅ O ₂ ·MeOH	Found 57.31 Req. 57.50	4.78 4.83	22.53 22.35
B.4269	5-bromothienyl ^{d,e,f}	14	EtOH	170-180	C ₁₀ H ₈ BrN ₅ OS	Found 36.81 Req. 36.82	2.52 2.47	20.81 21.47
B.4271	5-azapiperonyl ^d	63	EtOH	230-240	C ₁₂ H ₁₀ N ₆ O ₃ ·0.25EtOH	Found 50.27 Req. 50.40	3.86 3.89	27.94 28.22

TABLE 2a (continued)

Test No.	Q ⁶ -Substituent RR ¹ CH-	Yield % (based on solvate)	Solvent for recrystn.	M.p.(decomp) (°C)	Formula	Analysis			
							C	H	N
B.4273	5-cyanofurfuryl _{d,f}	15	MeOH	91-100 (with effervescence)	C ₁₁ H ₈ N ₆ O ₂ ·H ₂ O	Found Req.	48.25 48.19	3.70 3.67	29.82 30.65
B.4274	5-oxazolymethyl _{d,f}	32	MeOH	180-215	C ₉ H ₈ N ₆ O ₂ ·0.25H ₂ O	Found Req.	45.58 45.66	3.70 3.62	35.50 35.52
B.4275	5-thiazolymethyl _{d,f}	40	MeOH	190-220	C ₉ H ₈ H ₆ OS·0.5H ₂ O	Found Req.	42.14 42.02	3.73 3.53	32.43 32.67
B.4277	4-picoly _d	72	MeOH	230 Upwards	C ₁₁ H ₁₀ N ₆ O	Found Req.	54.82 54.55	4.19 4.16	34.48 34.71
B.4278	1-methyl-4-nitro- pyrrol-2-ylmethyl _{d,e}	22	MeOH	140-210	C ₁₁ H ₁₁ N ₇ O ₃ ·0.5H ₂ O	Found Req.	44.17 44.29	4.17 4.06	32.49 32.87

TABLE 2b

Test No.	$\overline{O^8}$ -Substituent RR ¹ CH-	Yield % (based on solvate)	Solvent for recrystn.	M.p.(decomp) (°C)	Formula	Analysis		
						C	H	N
<u>Ribosides</u>								
B.4268	plperonyl		MeOH	from 150 (with effervescence)	C ₁₈ H ₁₉ N ₅ O ₇ .2H ₂ O	Found 47.08 Req. 47.69	4.58 5.11	15.12 15.45
B.4276	2-benzo[b]thienylmethyl		MeOH- ether	140-155	C ₁₉ H ₁₉ N ₅ O ₅ S.H ₂ O	Found 50.75 Req. 50.98	4.47 4.73	16.83 15.65
B.4279	thenyl		MeOH- ether	from 120 (with effervescence)	C ₁₅ H ₁₇ N ₅ O ₅ S.0.5H ₂ O	Found 46.67 Req. 46.39	5.00 4.67	18.48 18.03

TABLE 3a

Test No.	O ⁶ -Substituent RR ¹ CH- furfuryl	λ _{max} (MeOH) (nm)	δ _H [ppm from TMS;(CD ₃) ₂ SO], <u>Δ</u> (Hz)
B.4203	furfuryl	243,285	5.43(s), 6.32(s), 6.49(dd, <u>Δ</u> 3.1,1.5), 6.66 (d, <u>Δ</u> 3.1), 7.71 (d, <u>Δ</u> 1.5),7.81 (s), 12.42(bs)
B.4205	thenyl	243,284	5.67(s), 6.30(s), 7.04(dd, <u>Δ</u> 5.1, 3.5),7.31(dd, <u>Δ</u> 3.5,1.2), 7.56(dd, <u>Δ</u> 5.1, 1.2), 7.84(s), 12.47(bs)
B.4206	3-thienylmethyl	242,284	5.47(s), 6.27(s), 7.26(dd, <u>Δ</u> 4.8, 1.2),7.54 (dd, <u>Δ</u> 4.8, 2.9), 7.62(bs), 7.80(s), 12.40(bs)
B.4209	3-furylmethyl	245,284	5.34(s), 6.26(s), 6.65(d, <u>Δ</u> 1.4), 7.66 (t, <u>Δ</u> 1.4), 7.82(s), 7.83(bs),12.41(bs)
B.4210	2-picolyl	247,262 269sh, 285	5.58(s), 6.27(s), 7.50(m), 7.84(s), 8.58(dd, <u>Δ</u> 4.8,1.2), 12.47(bs)
B.4211	3-picolyl	245,263sh, 270sh,285	5.58(s), 6.36(s),7.49(dd, <u>Δ</u> 7.9, 4.8),7.87(s), 8.01(dt, <u>Δ</u> 7.9, 1.8), 8.62(dd, <u>Δ</u> 4.8, 1.8), 8.80(bs), 12.48(bs)
B.4212	piperonyl	242,287	5.38(s), 6.02(s), 6.24(s), 6.95(m), 7.79(s), 12.37(bs)
B.4213	2-naphthylmethyl	244,282	5.67(s), 6.28(bs), 7.46-8.04(m), 12.47(bs)
B.4217	α -methylthienyl	243,284	1.74(d, <u>Δ</u> 6.6), 6.24(s), 6.74(q, <u>Δ</u> 6.6), 7.01(m), 7.24(m), 7.49(m), 7.81(s), 12.40(bs)
B.4219	1-(3-thienyl)ethyl	242,283	1.67(d, <u>Δ</u> 6.5), 6.20(s), 6.54(q, <u>Δ</u> 6.5), 7.24(m) 7.50 (m), 7.82(s), 12.37(bs)
B.4220	5-methylthienyl	246,284	2.42 (d, <u>Δ</u> 3.4), 5.57(s), 6.25(s), 6.70(d, <u>Δ</u> 3.4), 7.08(d), 7.81 (s), 12.41(bs)
B.4221	5-methylfurfuryl	240,283	2.28(s), 5.37(s), 6.11(m), 6.27(s), 6.53(d, <u>Δ</u> 3.0), 7.81(s), 12.44(bs)
B.4222	3-methylthienyl	244,283	2.28(s), 5.60(s), 6.25(s), 6.91(d, <u>Δ</u> 5.0), 7.45 (d, <u>Δ</u> 5.0), 7.80(s), 12.44(bs)

TABLE 3a (continued)

Test No.	O ⁶ -Substituent	λ_{max} (MeOH) (nm)	δ_{H} [ppm from TMS;(CD ₃) ₂ SO], $\underline{J}$ (Hz)
B.4226	2-benzo[b]thienylmethyl	240,267,282 (RCH ₂ OH: 241, 262, 289w, 300w)	5.78(s), 6.38(s), 7.38(m), 7.60(s), 7.83(s), 7.85(m), 12.47(bs)
B.4229	5-methoxycarbonyl- furfuryl	251,280sh (RCH ₂ OH: 259)	3.82(s), 5.50(s), 6.39(s), 6.87 (d, $\underline{J}$ 3.4), 7.32(d, $\underline{J}$ 3.4), 7.83(s), 12.45(bs)
B.4234	5-carboxyfurfuryl	250,280 (RCH ₂ OH: 256)	5.43(s), 6.35(s), 6.68(d, $\underline{J}$ 3.3), 6.87 (d, $\underline{J}$ 3.3), 7.85(s), 12.53(bs)
B.4265	1-naphthylmethyl	244,283,293 sh	5.96(s), 6.36(s), 7.58(m), 7.72(d, $\underline{J}$ 6.8) 7.79 (s) 7.89(m), 8.11(m), 12.44(bs)
B.4266	2-benzofuranyl-methyl	246,278,284 (RCH ₂ OH: 246, 275w, 283w)	5.62(s), 6.40(s), 7.13(s), 7.27 (dt, $\underline{J}$ 7.3, 1.1), 7.34(dt, $\underline{J}$ 7.3, 1.3), 7.65(m), 7.68 (m), 7.85 (s), 12.48 (bs)
B.4269	5-bromothenyl	247,284 RCH ₂ OH: 246	5.62(s), 6.36(s), 7.17 (ABq, $\underline{J}$ 3.7) 7.85(s), 12.47(bs)
B.4271	5-azapiperonyl	241,290 (RCH ₂ OH: 234, 294)	5.40(s), 6.18(s), 6.39(s), 7.48 (d, $\underline{J}$ 1.8), 7.83(s), 12.46(bs)

TABLE 3a (continued)

Test No.	Q ⁶ -Substituent	$\lambda_{\text{max}}(\text{MeOH})$ (nm)	δ_{H} [ppm from TMS;(CD ₃) ₂ SO], $\underline{J}$ (Hz)
B.4273	5-cyanofurfuryl	248,286 (RCH ₂ OH: 248)	5.53(s), 6.42(s), 6.99(d, $\underline{J}$ 3.5), 7.67(d, $\underline{J}$ 3.5) 7.87(s), 12.52(bs)
B.4274	5-oxazolylmethyl	243,286	5.56(s), 6.38(s), 7.44(s), 7.85(s), 8.45(s), 12.48(bs)
B.4275	5-thiazolylmethyl	244,286	5.76(s), 6.42(s), 7.85(s), 8.14(s), 9.13(s), 12.49(bs)
B.4277	4-picolyl	244,265sh,286	5.58(s), 6.34(s), 7.47(d, $\underline{J}$ 5.7), 7.88(s), 8.60 (d, $\underline{J}$ 5.7), 12.51(bs)
B.4278	1-methyl-4-nitro- pyrrol-2-ylmethyl	244,285,320sh (RCH ₂ OH: 280, 320)	3.78(s), 5.46(s), 6.40(s), 6.98(s), 7.84(s), 8.08(s), 12.49(bs)

TABLE 3b

Test No.	Q ⁶ -Substituent	$\lambda_{\text{max}}(\text{MeOH})$ (nm)	δ_{H} [ppm from TMS; (CD ₃) ₂ SO], $\underline{J}$ (Hz)
<u>Ribosides</u>			
B.4268	piperonyl	246,287 (RCH ₂ OH 240,288)	3.53 and 3.62(2 x dd, $\underline{J}$ 11.9, 3.75), 3.89 (q, $\underline{J}$ 3.5), 4.10(dd, $\underline{J}$ 4.9, 3.5), 4.45 (t, $\underline{J}$ 5.5), 5.1 (bs), 5.38(s), 5.78 (d, $\underline{J}$ 6.0), 6.02(s), 6.51(s), 6.93(d, $\underline{J}$ 7.7), 7.00 (dd, $\underline{J}$ 7.7, 1.5), 7.10(d, $\underline{J}$ 1.5), 8.10(s)
B.4276	2-benzo[b]thienyl/methyl	241,252, 269,287 (RCH ₂ OH 241,262 289w, 300w)	3.57 and 3.67(2 x m), 3.91 (q, $\underline{J}$ 3.6), 4.12 (q, $\underline{J}$ 5.2), 5.16 (m), 5.45(d, $\underline{J}$ 6.2), 5.83 (s + m), 6.61(s), 7.40(m), 7.63(s), 7.88(m), 7.97(m), 8.16(s)
B.4279	thenyl	246,285	3.57 and 3.64 (2 x m), 3.92(q, $\underline{J}$ 3.5), 4.13(m), 4.49 (q, $\underline{J}$ 5.2), 5.16(m), 5.45(m), 5.70(s), 5.81 (d, $\underline{J}$ 6.0), 6.57(s), 7.07(dd, $\underline{J}$ 5.1, 3.5), 7.34 (d, $\underline{J}$ 0.9), 7.60(d, $\underline{J}$ 1.3), 8.14(s), 12.40(bs)

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The starting alcohols $RR'CHOH$ were usually made by reduction of the corresponding aldehydes, often commercially available, by sodium borohydride. A different approach was used for the precursors of the ester B.4229 and the nitrile B.4273. Sucrose was converted ¹ via 5-chloromethylfurfural into 5-hydroxymethylfurfural. Oxidation ² of this aldehyde gave the carboxylic acid which was esterfied by the method of Bocchi et al. ³ to methyl 5-(hydroxymethyl)furoate, ⁴ required for B.4229. The oxime ⁵ of the aldehyde was dehydrated by the method of Carotti et al. ⁶; the crude reaction product was treated with conc. aqueous ammonia in methanol before extraction into dichloromethane. Distillation afforded 5-cyanofurfuryl alcohol, ⁷ required for B.4273.

For B.4266, Vilsmeier reaction ⁸ of benzofuran yielded the 2-aldehyde, reduced to the required alcohol, ⁹ while for B.4226 lithiation and treatment with dimethylformamide gave the 2-aldehyde and in turn the alcohol. ¹⁰

For B.4274, dimethyl tartrate was oxidised ¹¹ to methyl glyoxylate which reacted ¹² with tosylmethyl isocyanide to give methyl oxazole-5-carboxylate. ¹³ This was reduced by lithium aluminium hydride by the method of Fallab ¹⁴ to the alcohol. ¹⁵

For B.4275, bromomalondehyde ¹⁶ and thiourea yielded 2-aminothiazole-5-carboxaldehyde. ¹⁷ Deamination by amyl nitrite ¹⁷ followed by sodium borohydride reduction gave 5-hydroxymethylthiazole ¹⁴.

For B.4271, 5-azapiperonyl alcohol (m.p. 82-84°C; found, C, 54.60; H, 4.58; N, 9.09; $C_7H_7NO_3$ requires C, 54.90; H, 4.61; N, 9.15%) was prepared from the corresponding aldehyde. ¹⁸

For B.4278, 1-methylpyrrole-2-carboxaldehyde was nitrated ¹⁹ and reduced to the alcohol ²⁰ by sodium borohydride.

By way of specific example, the preparation of 0⁶-thenylguanine (B.4205) will now be described:

Preparation of 0⁶-thenylguanine

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A solution of thenyl alcohol (3.18 ml, 33.6 mmol) in DMSO (3 ml) was treated with sodium hydride (60% in oil; 0.48g, 12 mmol), stirring cautiously at first. After 1 hour 2-amino-N, N, N-trimethyl-1H-purin-6-aminium chloride (1.37g, 6 mmol) was added and stirring continued 1 hour more. Acetic acid (1.0 ml) was added, cooling briefly, and the mixture diluted with ether (180 ml). The solid (2.09g) was collected after 1-2h. Evaporation of ether from the filtrate and distillation of DMSO and excess thenyl alcohol (b.p. 48-57°C/0.2 mm) left a residue which on trituration with ether yielded a second solid fraction (0.36g). The combined solids were rubbed (trituration) with water (6 ml), yielding product (1.335g, 90%) showing a strong spot in TLC (C₆H₆-MeOH, 4:1) with only traces of impurity. Dissolution in hot ethanol (30 ml), clarification by filtration through Celite, and concentration (to 10 ml) using a rotary evaporator yielded B.4205 (1.125g, 71% of material containing 1/3 EtOH per mole as solvate).

Compounds of formula I in which Y is R''XCHR''' (seco-nucleosides) may be prepared by an analogous preparation to the reaction of O⁶-benzylguanine with α-chloro-ethers (MacCoss et al., Tetrahedron Lett.; European Patent Application No. 184,473., loc. cit.) or with alkyl bromides (e.g. Kjellberg, Liljenberg and Johansson, Tetrahedron Lett., 1986, 27, 877; Moschel, McDougall, Dolan, Stine, and Pegg, J. Med. Chem., 1992, 35, 4486).

Typical "sugar" components corresponding to R''XCHR''', leading to seco-nucleosides, are made by methods described in e.g. McCormick and McElhinney, J. Chem. Soc., Perkin Trans. 1, 1985, 93; Lucey, McCormick and McElhinney, J. Chem. Soc., Perkin Trans. 1, 1990, 795.

Compounds of formula I in which Y is ribosyl or deoxyribosyl (nucleosides) may be prepared by methods analogous to the syntheses of O⁶-benzylguanine riboside and 2-deoxyriboside (Moschel et al. 1992; cf. Gao, Fathi, Gaffney et al., J. Org. Chem., 1992, 57, 6954; Moschel, Hudgins and Dipple, J. Amer. Chem. Soc., 1981, 103, 5489) (see preparation of Ribosides above).

Industrial Applicability

The amount of the compound of the present invention to be used

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varies according to the effective amount required for treating tumour cells. A suitable dosage is that which will result in a concentration of the compound of the invention in the tumor cells to be treated which results in the depletion of ATase activity, e.g. about 1 - 2000
5 mg/kg, and preferably 1 - 800 mg/kg, particularly 1-120 mg/kg, prior to chemotherapy with an alkylating agent.

The pharmaceutical composition of the invention may be formulated in conventional forms with conventional excipients, as described for
10 example in WO 91/13898.

The composition may contain the inactivator according to the invention together with an alkylating agent; or the composition may comprise two parts, one containing the inactivator and the other containing the alkylating agent. The method of administering the
15 compounds of the invention to a host may also be a conventional method, as described in WO 91/13898 for example. For administration of an inactivator according to the invention to patients, the pharmaceutical composition may suitably contain the inactivator in a suitable vehicle such as 40% polyethyleneglycol 400 in saline
20 solution, or in saline or 3% ethanol (in saline), for intravenous injection, or in a powder form in suitable capsules for oral administration.

Alkylating agents may be administered in accordance with known
25 techniques and in conventional forms of administration, as described in WO 91/13898 for example or preferably as a single dose immediately after or up to 24 hours after but preferably around 2 hours after administration of the ATase inactivating agents and also at doses lower than those used in standard treatment regimen. A reduction in
30 dose may be necessary because the inactivators would generally be anticipated to increase the toxicity of the alkylating agents. Examples of chloroethylating agents include 1,3 bis (2-chloroethyl) -1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), fotemustine, mitozolomide and clomesone and those described in
35 McCormick, McElhinney, McMurphy and Maxwell J. Chem. Soc. Perkin Trans. I, 1991, 877 and Bibby, Double, McCormick, McElhinney, Radacic, Pratesi and Dumont Anti-Cancer Drug Design, 1993, 8, 115. Examples of methylating agents include temozolomide (British Patent GB 2 104 522) and dacarbazine, procarbazine, and streptozocin.

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METHODS

Purification of Recombinant ATases

The cDNA cloning and overexpression of the human ATase has been reported previously²³. Purification of the recombinant proteins was achieved either by affinity chromatography through a DNA-cellulose column as described by Wilkinson *et al.*,^{25, 26} or by DEAE-cellulose ion-exchange chromatography. For the latter, the ATase protein was partially purified by ammonium sulphate precipitation (30 - 60%) and dialysed against 10 mM Tris-HCl pH 7.5, 1 mM DTT, 2 mM EDTA, 10% glycerol, before loading on a DEAE-cellulose column. The ATase was then eluted with a 0-0.1 M NaCl gradient. The purified human ATase protein retained activity for more than one year when stored at high concentration at -20°C in buffer I [50 mM-Tris/HCl (pH 8.3)/3 mM-dithiothreitol/1 mM-EDTA] and could be thawed and refrozen several times without substantial loss of activity.

Incubation with Inactivators and ATase Assay

Compounds to be tested were dissolved in DMSO to a final concentration of 10 mM and diluted just before use in buffer I containing 1 mg/ml bovine serum albumin (IBSA). Recombinant ATase was diluted in IBSA and titrated in order that the reaction be conducted under ATase, and not substrate, limiting conditions. In each assay, fixed amounts of ATase (60-75 fmol) were incubated with varying amounts of O⁶-benzylguanine, or test compound in a total volume of 200 µl of IBSA containing 10 µg of calf thymus DNA at 37°C for 1 hour. The [³H]-methylated-DNA substrate (100 µl containing 6.7 µg of DNA and 100 fmol of O⁶-methylguanine) was added and incubation continued at 37°C for 1 hour, until the reaction was completed. Following acid hydrolysis of the DNA as previously described²¹ the [³H]-methylated protein was recovered and quantitated by liquid scintillation counting. Samples were typically assayed in duplicate and experiments repeated several times. I₅₀ is the concentration of inactivator required to produce a 50% reduction in ATase activity.

Cell Culture and preparation of extracts

Mammalian cells were cultured under standard conditions. For example, Raji (a human lymphoblastoid cell line from a Burkitt's lymphoma) cells were grown in suspension culture in RPMI medium supplemented

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with 10% horse serum. A375M cells are human melanoma cells from which the xenografts described below were established following subcutaneous injection into nude mice: WiDr cells are a human colon carcinoma cell line: Hamster+120 cells are a mitozolomide-selected
5 subline of a Chinese hamster lung fibroblast V79 cell line called RJK0: Yoshida cells are a rat carcinosarcoma cell line and YR_{bus} is a busulphan resistant subline thereof: XP cells are an SV40-transformed fibroblast cell line originally from the skin of a Xeroderma pigmentosum patient, pHMGhAT2b cells are a clone of these
10 cells that have been transfected with a mammalian cell expression vector containing the human ATase cDNA and pHMGla cells are a clone that have been transfected with the expression vector only (i.e. one not containing the human ATase cDNA). Cell pellets were resuspended in cold (4°C) buffer I containing 2 µg/ml leupeptin and sonicated
15 for 10 seconds at 12 µ peak to peak distance. After cooling in ice, the cells were sonicated for a further 10 seconds at 18 µ. Immediately after sonication, 0.01 volumes of 8.7 mg/ml phenylmethanesulphonylfluoride in ethanol was added and the sonicates centrifuged at 15 000 g for 10 minutes at 4°C to pellet cell
20 debris. The supernatant was kept for determination of ATase activity (see below).

Stability of Inactivators at 37°C.

Inactivators (10mM in DMSO) were diluted to 0.1mM in prewarmed
25 degassed buffer I (1mM EDTA, 50mM Tris pH 8.3) or PBS (pH 7-7.2). PBS (Phosphate buffered saline) is 0.8% NaCl, 0.02% KCl, 0.15% Na₂H₂PO₄, 0.02% KH₂PO₄, pH 7.2. Samples were immediately transferred to a CARY13 spectrophotometer (cuvette block held at 37°C) and scanned at an appropriate wavelength (according to the
30 spectral properties of the compound) at 3-10 minute intervals for up to 80 hours. The results were expressed as percentage absorbance change versus time and T1/2 values (half life) extrapolated from this.

Inactivation of ATase activity in mammalian cells.

35 Cells were diluted to 10⁶/ml in culture medium containing either the appropriate concentration of inactivator or an equivalent volume of vehicle (DMSO). Following incubation at 37°C for 2 hours the cells were harvested by centrifugation, washed twice with PBS and the resulting cell pellets (between 1-2 x 10⁷ per pellet) stored at -20°C.

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ATase activity was determined as described above, in duplicate sonicated cell extracts and expressed as the percentage activity remaining based on that present in the untreated controls (for example 350-450 fm/mg in Raji cells). I_{50} (i.e. concentration of inactivator required to reduce ATase activity by 50%) values were extrapolated from this data.

Sensitization of Raji and A375M cells to BCNU and temozolomide.

Sensitization of Raji cells to the cytotoxic effects of BCNU and temozolomide following a 2 hour pretreatment with inactivator was analysed using an XTT assay²². Briefly, cells were plated at 1000 cells/well in 96 well plates and incubated at 37°C for 30 minutes prior to the addition of medium containing either the appropriate concentration of inactivator or an equivalent volume of vehicle. Following a 2 hour incubation at 37°C, medium containing either increasing doses of BCNU, temozolomide or equivalent vehicle was added and the cells allowed to grow for 6 days. At this time XTT solution was added and the cells incubated for a further 4 hours at 37°C. The resulting red/orange formazan reaction product was quantified by measuring absorption at 450nm on a microtitre platereader.

Sensitization of A375M cells to the cytotoxic effects of BCNU was analysed by MTT assay²⁴ differing from the XTT assay described above as follows. A375M cells (1000 per well) were allowed to grow for 24 hours then treated with the inactivator and 2 hours later with BCNU. After 6 days MTT solution (4mg/ml) was added and cells incubated for 3 hours at 37°C. Medium was aspirated and the resulting purple formazan crystals were solubilised in DMSO (120µl) and cell viability was quantified by measuring absorption at 540nm using a microtitre plate reader.

From this data the percentage growth of cells relative to that in control wells was determined for a range of BCNU or temozolomide doses in both the presence and absence of inactivator. Raji sensitization to BCNU (D_{90}^C/D_{90}^I) was determined by dividing the D_{90} (i.e. dose at which there was 90% growth versus untreated controls i.e. 10% growth inhibition) calculated for data on use of BCNU alone (D_{90}^C) by that for BCNU plus inactivator (D_{90}^I). A value of one (1) thus indicates no sensitization by the inactivator. Raji

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sensitization to temozolomide and A375M sensitization to BCNU were determined using the corresponding D_{50} values (i.e. the doses at which there was 50% growth inhibition).

5 **Sensitivity of mammalian cells to the inactivators or their hydrolysis products**

In order to assess the effects on cell growth alone, Xeroderma pigmentosum cells and Chinese Hamster V79 cells were exposed to increasing concentrations (up to 600 μ M) of selected inactivators for 10 2h at 37⁰. In some cases, the inactivators were allowed to undergo hydrolysis at 37⁰C for 20 hours and then added to Raji cells in order to assess the extent to which the decomposition products of the agents might inhibit cell growth. After 6 days the extent of cell growth was determined as described above.

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Sensitization of bone marrow cells to temozolomide (GM-CFC assay)

For the granulocyte/macrophage colony-forming cell (GM-CFC) assay primary human bone marrow samples were obtained from patients undergoing cardiothoracic surgery. Following removal of erythrocytes 20 from the samples, cells were plated out at 1-2 x 10⁵/ml in 300mOsm Iscoves medium containing 10 μ M inactivator or equivalent volume of DMSO, 20% foetal calf serum, 10% 5637 conditioned medium as a source of growth factors and 0.3% agar noble, in 1ml petri dishes containing appropriate dose of temozolomide and incubated at 37⁰C in an 25 atmosphere of 5% CO₂ and 95% air. After 9 days, colonies comprising of more than 50 cells were counted. The colonies represented precursor cells of the granulocyte/macrophage lineage (huGM-CFC). Survival was expressed as a % of the number of colonies at zero dose temozolomide.

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Xenograft studies

Animals

BALB-C derived athymic male mice (nu/nu athymic) weighing between 25-35g were obtained from the in-house breeding colony of the Paterson 35 Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 9BX, England (ASU mice). The animals were housed in a sterile environment until required for experiments. In one

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experiment mice were obtained from Harlan-Olac, Harlan UK Limited, Shaw's Farm, Blackthorn, Bicester, Oxon. OX6 OPT, England (OLAC mice). These mice weighed between 17 - 23g and were subsequently found to be more sensitive to the toxic effects of the combination (inactivator and BCNU) treatment. For this reason lower doses were administered.

Cells

A375M (human melanoma) cells were grown in DMEM containing 10% fetal bovine serum. The cells were prepared for in vivo inoculation by incubation with 0.01% trypsin.

Tumours

Cells (10^6) in 100 μ l of PBS were injected subcutaneously into the right-hand flank of 8-10 week old nu/nu athymic mice. These cells were allowed to develop into a tumour for 3-4 weeks for passaging into experimental animals. Tumour blocks measuring 2mm x 2mm x 2mm were implanted subcutaneously on the right hand flank. These animals were ready for use in inactivator experiments in 7-10 days.

Drug Treatment

Nu/Nu mice were treated with either 30 or 60 mg/kg O^6 -benzylguanine, or B.4205 and the appropriate vehicle control (i.p), 60 - 90 minutes prior to 10 - 25 mg/kg BCNU (i.p.). O^6 -benzylguanine and B.4205 were prepared as a 5 mg/kg solution in corn oil and BCNU (2mg/ml) in PBS + 3% ethanol.

Tumour Measurements

Xenograft tumour measurements were taken every 1-2 days by workers using digital calipers. Tumour volume was calculated using the formula $(h \times w \times l) \pi / 6$. The experimental animals were also weighed every 1-2 days. Measurements continued until tumours in the control animals reached the maximum allowable volume (i.e. 1cm x 1cm x 1cm).

RESULTS

Tables 4, 5 and 6 shows the physical, biochemical and in vivo data for each of the inactivators. The tests listed are explained in the Methods section.

TABLE 4

INACTIVATOR

INACTIVATOR	M.Wt	I ₅₀ (μM)	T1/2(h) in Buffer I	Raji I ₅₀ (μM)	T1/2(h) in PBS	Raji sensitization (D ₉₀ -C/D ₉₀ -I)		
						0.1μM	0.5μM	1.0μM
<u>O</u> ⁶ -benzylguanine	241	0.04	> 64	0.10	> 16	*1.9 ± 0.7	*2.0 ± 0.4	*3.8 ± 1.8
<u>O</u> ⁶ -allylguanine	191	22	> 64					
B.4203 <u>O</u> ⁶ -furfurylguanine.1/2H ₂ O	240	0.08	0.3	0.04	0.17	2.6		5.7
B.4205 <u>O</u> ⁶ -thenylguanine.1/3EtOH	262	0.018	1.2	0.02	0.6	8.4		8.0
B.4206 <u>O</u> ⁶ -3-thienylmethylguanine.1MeOH	279	0.03	> 80	0.06	> 16	1.6		6.0
B.4209 <u>O</u> ⁶ -3-furylmethylguanine	231	0.15		0.15	> 16	1.5	1.4	2.8
B.4210 <u>O</u> ⁶ -2-picolylguanine.1/2H ₂ O	251	35		> 1.0	> 16			36
B.4211 <u>O</u> ⁶ -3-picolylguanine.1/2H ₂ O	251	0.43			> 16			
B.4212 <u>O</u> ⁶ -piperonylguanine.3/4H ₂ O	299	0.02	1.3	0.05	0.72	2.7		10.7
B.4213 <u>O</u> ⁶ -(2-naphthylmethyl)guanine	291	0.15		0.03	> 16	1.2	2.1	2.9

*Mean of 8 individual experiments

TABLE 4(continued)

INACTIVATOR	M.Wt	I ₅₀ (μM)	T1/2(h) In Buffer I	Raji I ₅₀ (μM)	T1/2(h) In PBS	Raji sensitization (D ₉₀ . C/D ₉₀ . I) O. 1μM 0.5μM 1.0μM
B.4214 DL-Q ⁶ -(α-methylbenzyl)guanine	255	> 60				
B.4217 DL-Q ⁶ -(α-methylthienyl)guanine	261	> 500				
B.4218 Q ⁶ -(2-methylbenzyl)guanine	255	58				
B.4219 DL-Q ⁶ -[1-(3-thienyl)ethyl]guanine	261	85		> 1.0	0.12	1.0 1.0 1.0
B.4220 Q ⁶ -(5-methylthienyl)guanine	261	9		> 1.0	> 16	1.0 1.0 1.0
B.4221 Q ⁶ -(5-methylfurfuryl)guanine. 1/2H ₂ O	254	10		> 1.0	> 16	1.0 1.0 1.0
B.4222 Q ⁶ -(3-methylthienyl)guanine	260	0.65		0.47	> 16	1.9 2.1
B.4226 Q ⁶ -(2-benzo[b]thienylmethyl) guanine. 1 MeOH	329	0.03	9.7	0.06	6.7	1.6 10.0
B.4229 Q ⁶ -(5-methoxycarbonylfurfuryl) guanine. 1.5H ₂ O	316	0.09		0.25	> 16	2.1 2.5

TABLE 4(continued)

INACTIVATOR	M.Wt	I ₅₀ (μM)	T1/2(h) in Buffer I	Raji I ₅₀ (μM)	T1/2(h) in PBS	Raji sensitization (D ₉₀ . C/D ₉₀ .h) 0.1μM 0.5μM 1.0μM
B.4231 O ₆ -(2-methoxybenzyl)guanine.1/2H ₂ O	280	60				
B.4234 O ₆ -(5-carboxyfurfuryl)guanine	306	60		> 1.0	> 16	1.0
B.4265 O ₆ -1-(naphthylmethyl)guanine .1/2MeOH	307	95	60			
B.4266 O ₆ -(2-benzofuranylmethyl)guanine .1MeOH	313	0.035			> 16	
B.4268 O ₆ -piperonylguanosine.2H ₂ O	453	0.75			8	
B.4269 O ₆ -(5-bromothienyl)guanine	326	0.0045		0.006		
B.4271 O ₆ -(-5-azapiperonyl)guanine .1/4EtOH	298	0.23			> 16	
B.4273 O ₆ -(5-cyanofurfuryl)guanine.H ₂ O	256	0.006				
B.4274 O ₆ -(5-oxazolymethyl)guanine.1/4 H ₂ O	274	0.34				

TABLE 4(continued)

INACTIVATOR	M.Wt	I ₅₀ (μM)	T _{1/2} (h) in Buffer I	Raji I ₅₀ (μM)	T _{1/2} (h) in PBS	Raji sensitization (D ₉₀ -C/D ₉₀ -I) 0.1μM 0.5μM 1.0μM
B.4275 O ⁶ -(5-thiazolylmethyl)guanine.1/2 H ₂ O	257	0.033				
B.4276 O ⁶ -(2-benzo[b]thienylmethyl) guanosine.H ₂ O	464	0.35				
B.4277 O ⁶ -4-picolylguanine	242	0.13				
B.4278 O ⁶ -(1-methyl-4-nitro- pyrrol-2-ylmethyl)guanine	298	0.55				
B.4279 O ⁶ -thenylguanosine.1/2H ₂ O	388	0.9				

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Table 5

Inactivation of ATase in Mammalian Cells 150(μM)					
INACTIVATOR	CELL LINE				
	Hamster + 120	Rat Yoshida	Human XP	Human Raji	Human WiDr
5					
<u>0</u> ⁶ -benzylguanine	0.20	0.14	0.07	0.10	>0.09
B.4203	0.12	0.06	0.06	0.04	0.08
10 B.4205	0.03	0.02	0.03	0.02	0.02

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Table 6

		A375M sensitization ($D_{50.C}/D_{50.I}$)		
INACTIVATOR		0.5uM	1.0uM	5.0uM
	<u>0</u> ⁶ -benzylguanine	3.1	2.3	4.2
5	<u>0</u> ⁶ -allylguanine	1.3		1.9
	B.4203	2.0		2.5
	B.4205	2.8	2.3	3.5
	B.4206	3.3		4.6
10	B.4209		2.5	
	B.4210		1.5	
	B.4212	2.3		2.5
	B.4213		2.0	
15	B.4220		1.3	
	B.4221		1.0	
	B.4222		1.4	
20	B.4226	3.8		4.5
	B.4229		1.8	
	B.4234		0.8	
	B.4266	6.3		6.3

25 Figure 1 shows the result of the in vitro ATase inactivation assay using 4 of the compounds. B.4206 was somewhat more effective than BeG but B.4212 and B.4205 were considerably better under the assay conditions used. B.4203 was not as effective as BeG.

30 Figure 2 shows that at 0.1uM inactivator, B.4205 was more effective than BeG in sensitizing Raji cells to the growth inhibitory effects of BCNU: at 1.0uM inactivator, B.4205 and BeG were equally effective in this respect.

35 Figure 3 shows that at 0.1uM inactivator, B.4205 was more effective than BeG in sensitising Raji cells to the growth inhibitory effects of temozolomide but that as the doses of the inactivators were increased, sensitization by BeG became more effective whilst that by

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B.4205 remained the same. This lack of dose response with B.4205 but clear dose response with BeG is shown more clearly in Figure 4.

Figure 5 shows that whilst some growth inhibition of B79 cells was produced by B.4203 at doses in excess of 100uM (i.e. at least 100x higher than the I_{50} dose for this compound), no such effects were seen with B.4205 up to the maximum concentration used.

Figure 6 shows that XP cells were as susceptible to the growth inhibitory effects of B.4203 but that these cells were more sensitive than V79 cells to the effects of B.4205. However, the doses required for growth inhibition were at least 100 times that required for ATase inactivation. It can be concluded that the inherent growth inhibitory effects of these inactivators would not contribute detectably to the sensitization of cells to the growth inhibitory effects of the alkylating agents.

Figure 7 shows that no substantial growth inhibitory effects were produced in Raji cells when the given compounds were allowed to undergo hydrolysis (see Methods) before being added to the cells without further addition of alkylating agents. Under these experimental conditions therefore, the decomposition products of the agents would not be expected to contribute to the growth inhibitions seen using combinations of these agents and alkylating agents.

Figure 8 - 13 shows the results of the xenograft study in greater detail. The depletion and recovery of ATase activity following exposure to O^6 -benzylguanine or B.4205 was measured in A375 tumour xenograft (Fig.8) extracts prepared from tissues of animals sacrificed 2 or 24 hours after administration of inactivator. Figures 9-13 indicate the sensitization of A375 tumour xenografts in nude mice to either O^6 -benzylguanine or B.4205 in combination with BCNU as described in the Methods section. In each Figure the top graph shows the percentage increase in tumour volume over the time course of the experiment. The lower graph shows the number of animals in each treatment group and the number surviving following treatment. The graphs show that B.4205 is comparable to O^6 -benzylguanine (BeG) in reducing tumour volume and is substantially less toxic in

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combination with BCNU than BeG in combination with BCNU. In human patients, cutaneous malignant melanoma is treated with BCNU, particularly in the USA (Balch, C.M., Houghton, A. & Peters, L. (1989) "Cutaneous Melanoma" in Cancer: Principles and Practise of Oncology,
5 De Vita, V.T., Helman, S. & Rosenberg, S.A. (eds), pp1499-1542, Lipincott: Philadelphia) so that the human melanoma xenograft grown in nude mice is an animal model system that is clinically highly relevant.

Figure 14 shows the results of the tests on sensitization of
10 human bone marrow cells to temozolomide as described in the Methods section above. The bone marrow cells were obtained from three patients identified as C, D and E respectively. The survival curves shown relate to the cytotoxic effect of combined
inactivator/temozolomide treatment. It is desirable that this effect
15 should be reduced. The results for patients C & E show that B.4205 had a smaller sensitization effect than $\underline{O}^6$ -benzylguanine ($\underline{O}^6$ BeG); for patient D there was no differential but this result is regarded as a reasonable variation in scientific testing with human material.

20 Table 7 shows the toxic effect of inactivator alone on bone marrow cells obtained from 5 patients A - E, including the same patients C - E as in Figure 17. The results for B.4205 are comparable to those for $\underline{O}^6$ -benzylguanine. Note that the cells of patient B were almost twice as sensitive to both BeG and B.4205 in comparison to
25 the other samples.

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Table 7

INACTIVATOR (10uM) TOXICITY IN BONE MARROW CELLS

Patient		Date of Experiment	No. of colonies as % of control (ie no inactivator)	
			<u>O</u> ⁶ -benzylguanine	B.4205
5	A	05/02/93	95	91
	B	25/03/93	46	46
	C	17/05/93	88	97
10	D	05/05/93	88	118
	E	05/05/93	89	89

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Summary of Findings

- 1) All of the compounds have been tested for their ability to inactivate recombinant human ATase under standard conditions in an in vitro assay. Under these conditions two of the compounds (B.4214 and B.4217) did not inactivate ATase up to the highest concentration used but these were compounds in which R' is methyl. The remainder of the compounds inactivated ATase with I_{50} values ranging from 0.0045 to 95 μ molar. Eight compounds (B.4205, B.4206, B.4212, B.4226, B.4266, B.4269, B.4273, B.4275) had I_{50} values lower than that of BeG (Table 4).
- 2) The inactivators underwent hydrolysis in aqueous solution at different rates (half lives 0.17 to >80 hours) but this was not related to their efficiency as ATase inactivators. (Table 4)
- 3) Compounds that were efficient in the inactivation of ATase in vitro ($I_{50} < 1.0 \mu$ M) also inactivated ATase in human cells with I_{50} values that were generally only slightly (mean approx. 1.5 times) higher than those found using recombinant protein in the in vitro assay. (Table 4)
- 4) B.4205 inactivated ATase in human, rat and Chinese hamster cells with similar effectiveness (I_{50} values 0.02-0.03). For B.4203 the range was 0.04-0.12. In the cell lines used B.4205 and B.4203 were up to 7 times more effective than BeG. (Table 5)
- 5) B.4203 and B.4205 were toxic to the XP cells studied and B.4203 was toxic to the V79 cells studied but only at concentrations that were at least 100 times higher than those at which sensitization to BCNU was observed. (Figs 5 & 6)
- 6) Compounds that were efficient in the inactivation of ATase in vitro ($I_{50} < 1.0 \mu$ M) and in Raji cells ($I_{50} < 1.0 \mu$ M) also sensitized Raji and A375M cells to the growth inhibitory effects of BCNU where this was tested. (Table 4)
- 7) At inactivator concentrations of 0.1 μ M, B.4205 was more effective than BeG in sensitising Raji cells to the growth inhibitory effects of temozolomide. (Fig 3)

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- 8) The in vitro assay can be used to predict which compounds are most likely to be effective sensitisers of mammalian cells to the growth inhibitory effects of BCNU and related cytotoxic agents.
- 5 9) B.4205 was similar or slightly more effective than BeG in sensitising human melanoma xenografts grown in nude mice to the growth inhibitory effects of BCNU.(Figs. 9-13)
- 10 10) B.4205 was as effective as BeG in inactivating ATase in human melanoma xenografts grown in nude mice.(Fig. 8)
- 15 11) The in vitro assay and/or the xenograft ATase depletion assay may be used to predict which compounds are most likely to be effective sensitisers of melanoma xenografts to the growth inhibitory effects of BCNU and related cytotoxic agents.
- 20 12) In contrast to BeG, which caused death in up to 70% of the treated animals, B.4205 had very little effect on the sensitivity of nude mice bearing human melanoma xenografts to the acute toxic effects of BCNU under the conditions used. (Figs. 9-13)
- 25 13) BeG sensitised the GM-CFCs in the three human bone marrow samples tested to the toxic effects of temozolomide but in two of these samples, little or no sensitization was produced by B.4205. This assay may therefore be used to predict the possible myelosuppressive effects of ATase inactivators when used in the clinic in combination with BCNU and related agents. (Fig. 14)

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References

1. W.N. Haworth and W.G.M. Jones, J.Chem.Soc., 1944, 667
2. T. Reichstein, Helv.Chim.Acta, 9, 1926, 1066.
- 5 3. V. Bocchi, G. Casnati, A. Dossena and R. Marchelli, Synthesis, 1979, 961.
4. P.A. Finan, J.Chem.Soc., 1963, 3917; J.A. Moore and J.E. Kelly, J. Polym.Sci., Polym.Chem.Ed., 22, 1984, 863.
- 10 5. J. Kiermayer, Chemiker-Zeitung, 19, 1895, 1004.
6. A. Carotti, F. Campagna and R. Ballini, Synthesis, 1979, 56.
7. A.J. Floyd, R.G. Kinsman, Y. Roshan-Ali and D.W. Brown, Tetrahedron, 39, 1983, 3881
8. V.T. Suu, N.P. Buu-Hoi and N.D. Xuong, Bull.Soc.Chim.France, 15 1962, 1875.
9. T. Reichstein and L. Reichstein Helv.Chim.Acta, 13, 1930, 1275.
10. H. Takeshita, H. Mametsuka and H. Motomura. J. Heterocycl.Chim., 23, 1986, 1211.
11. F.J. Wolf and J. Weijlard, Org.Synth., Coll.Vol.4, 1963, 124.
- 20 12. A.M. van Leusen, B.E. Hoogenboom and H. Siderius, Tetrahedron Lett., 1972, 2369.
13. A. Maquestiau, R. Flammang and F.B. Abdelouahab, Heterocycles, 29, 1989, 103.
14. S. Fallab, Helv.Chim.Acta, 35, 1952, 215
- 25 15. A.S. Kende, K. Kawamura and R.J. DeVita, J.Amer.Chem.Soc., 112, 1990, 4070.
16. S. Trofimenko, J.Org.Chem., 28, 1963, 3243.
17. I. Sawhney and J.R.H. Wilson (Shell Internationale), EP. 395,174, 1989 (Chem.Abs., 114, 143410s).
- 30 18. F. Dallacker, P. Fechter and V. Mues, Z. Naturforsch., 34b, 1979, 1729.
19. P. Fournari, Bull.Soc.Chim.France, 1963, 488.
20. L. Grehn, Chem.Scr., 16, 1980, 72.
21. Morten, J.E.N. & Margison, G.P. (1988) Carcinogenesis 9, 45-49
- 35 22. Scudiero, D.A., Shoemaker R.J., Paull K.D., Monks A., Tierney S., Nofziger T.H., Currens M.J., Seniff D, & Boyd M.R. (1988), Cancer Research 48, 4827-4833

- 48 -

23. Fan, C.-Y., Potter, P.M., Rafferty, J.A., Watson, A.J., Cawkwell, L., Searle, P.F., O'Connor, P.J. and Margison, G.P. (1991) Nucleic Acids Res. 18, 5723-5727
- 5 24. Carmichael J., DeGraff W.G., Gazdar A.F., Minna J.D., Mitchell J.B. (1987). Evaluation of a tetrazolium based semiautomated colorimetric assay : assessment of chemosensitivity testing. Cancer Res. 47 : 936-942.
- 10 25. Wilkinson, M.C., Potter, P.M., Cawkwell, L., Georgiadis, P., Patel, D., Swann, P.F. and Margison, G.P. (1989) Nucleic Acids Res. 17, 8475-8484.
- 26 Wilkinson, M.C., Cooper, D.P., Southan, C., Potter, P.M. & Margison, G.P. (1990) Nucleic Acids Res., 18, 13-16.

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In the specification the abbreviations "1h" or "2h" etc. mean "1 hour", "2 hours", etc.

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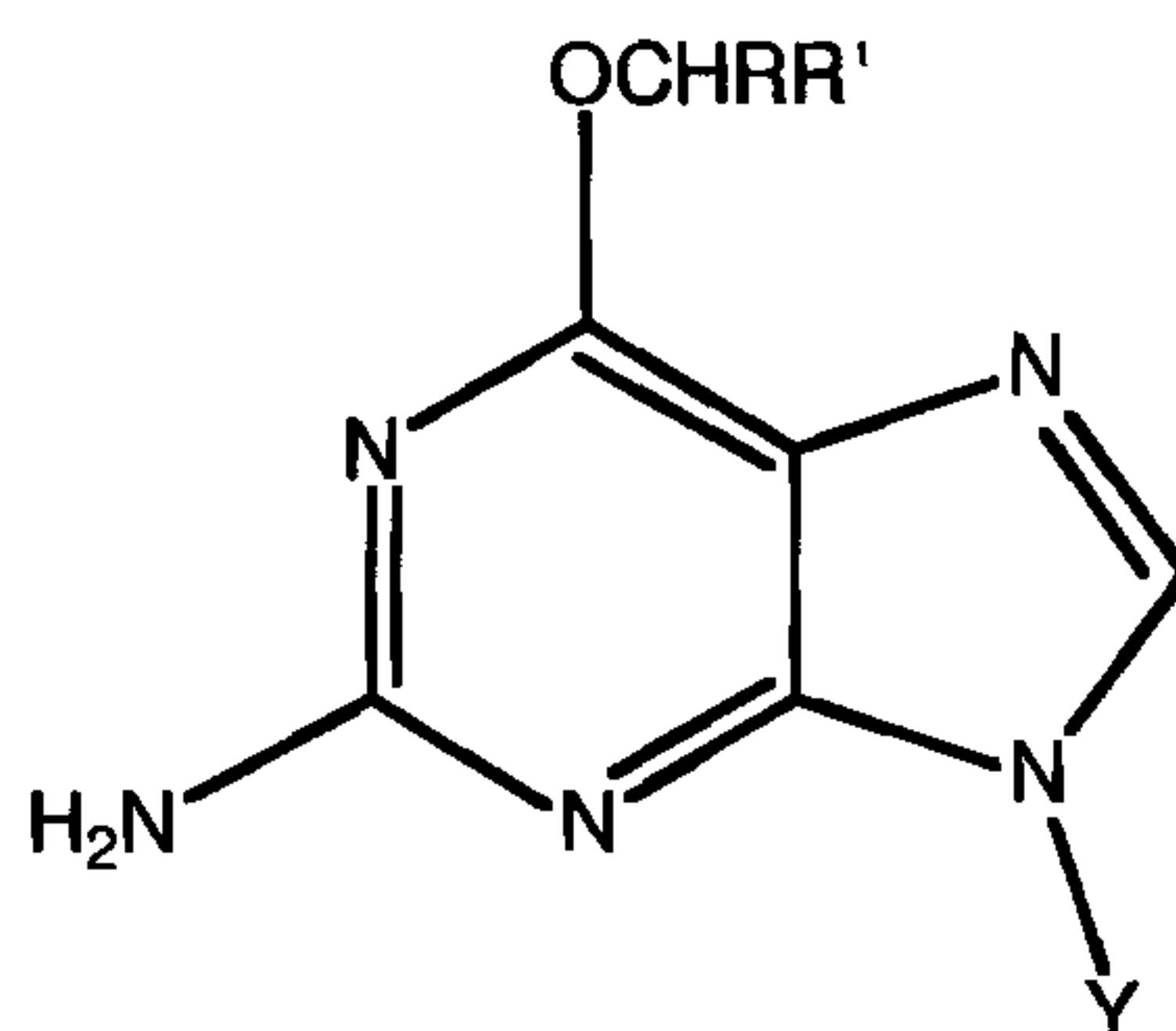
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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. $\underline{Q}^6$ -alkylguanine derivatives of the formula I:



wherein

Y is H, ribosyl, deoxyribosyl, or R''XCHR''', wherein X is O or S, R'' and R''' are C₁-C₂₀alkyl, or substituted derivatives thereof,

R' is H, or C₁-C₂₀alkyl or hydroxyC₁-C₂₀alkyl

R is

(i) a cyclic group having at least one 5 or 6 membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring fused thereto, the or each heterocyclic ring having at least one hetero atom chosen from O, N, or S, or a substituted derivative thereof; or

(ii) naphthyl or a substituted derivative thereof

and pharmaceutically acceptable salts thereof,

provided that when Y or R is a substituted derivative, the compound has the ability to deplete $\underline{Q}^6$ -alkylguanine-DNA alkyltransferase activity.

2. A compound according to claim 1 wherein:

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Y is H, ribosyl, deoxyribosyl or R"XCHR"', wherein X is O or S, R" and R"' are C₁-C₂₀alkyl, or substituted derivatives thereof having substitution by one or more of hydroxy, amino, C₁-C₂₀alkylamino, amido or ureido;

R' is as defined in claim 1;

R is

(i) a cyclic group having at least one 5- or 6-membered heterocyclic ring, optionally with a carbocyclic or heterocyclic ring used thereto, the or each heterocyclic ring having at least one heteroatom chosen from O, N or S, or a substituted derivative thereof having substitution in the heterocyclic rings(s) and/or carbocyclic ring(s) by one or more of C₁-C₂₀alkyl, C₁-C₂₀alkenyl, C₁-C₂₀alkynyl, halo, haloC₁-C₂₀alkyl, nitro, cyano, hydroxyC₁-C₂₀alkyl, SO_nR"', where R"' is C₁-C₂₀alkyl and n is 0, 1 or 2; or

(ii) naphthyl or a substituted derivative thereof having substitution by one or more of C₁-C₂₀alkyl, C₁-C₂₀alkenyl, C₁-C₂₀alkynyl, halo, haloC₁-C₂₀alkyl, nitro, cyano, hydroxyC₁-C₂₀alkyl, SO_nR"', where R"' is C₁-C₂₀alkyl and n is 0, 1 or 2;

and pharmaceutically acceptable salts thereof.

3. A compound according to claim 1 wherein R is:
 - (a) a 5- or 6-membered heterocyclic ring, or
 - (b) a benzo derivative thereof, the $\underline{Q}^6$ -alkylguanine moiety being attached to R at either the heterocyclic or the benzene ring.
4. A compound according to claim 1 or 2 wherein R is a heterocyclic ring, having at least one S atom therein.
5. A compound according to claim 1 or 2 wherein R is a heterocyclic ring, having at least one O atom therein.

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6. A compound according to any one of claims 1 to 5 wherein a heterocyclic ring in R is substituted by halo, cyano or $-\text{COOR}^5$ wherein R^5 is $\text{C}_1\text{-C}_{20}$ alkyl.
7. A compound according to any of claims 1 to 6 wherein R is a five membered ring, with or without a second ring fused thereto.
8. A compound according to claim 1 or 2 wherein R is a thiophene ring or a substituted derivative thereof.
9. A compound according to claim 1 or 2 wherein R is a furan ring or a substituted derivative thereof.
10. A compound according to claim 1 wherein at least one ring in R selected from heterocyclic and carbocyclic rings is substituted by halo, haloalkyl, cyano, $\text{SO}_n\text{R}'''$ where R''' is alkyl and $n = 0, 1$ or 2 , or $-\text{COOR}^5$ wherein R^5 is alkyl.
11. A compound according to any one of claims 1 to 10 wherein R' is H.
12. A compound according to claim 1, 2, 3 or 4 which is $\underline{0}^6$ -thenylguanine or a substituted derivative thereof having substitution in the thiophene ring by a halo, cyano or ester group.
13. A compound according to claim 12 which is $\underline{0}^6$ -thenylguanine having substitution in the thiophene ring by a halo group.
14. A compound according to claim 13 which is $\underline{0}^6$ -thenylguanine having bromo-substitution in the thiophene ring.
15. A compound according to claim 1 which is $\underline{0}^6$ -thenylguanine.

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16. A compound according to claim 1 which is $\underline{0}^6$ -(3-thienylmethyl)-guanine.
17. A compound according to claim 1 which is $\underline{0}^6$ -piperonylguanine.
18. A compound according to claim 1 which is $\underline{0}^6$ -furfurylguanine.
19. A compound according to claim 1 which is $\underline{0}^6$ -(3-furylmethyl)-guanine.
20. A compound according to claim 1 which is $\underline{0}^6$ -(2-benzo[b]thienylmethyl)guanine.
21. A compound according to claim 1 which is $\underline{0}^6$ -(2-benzofuranylmethyl)guanine.
22. A compound according to claim 1 which is $\underline{0}^6$ -(5-thiazolylmethyl)guanine.
23. A compound according to claim 1 which is $\underline{0}^6$ -(5-methoxycarbonylfurfuryl)guanine.
24. A compound according to claim 1 which is $\underline{0}^6$ -(5-bromothienyl)guanine.
25. A compound according to claim 1 which is $\underline{0}^6$ -(4-bromothienyl)guanine.
26. A compound according to claim 1 which is $\underline{0}^6$ -(5-cyanofurfuryl)guanine.
27. A compound according to claim 1 which is $\underline{0}^6$ -(2-benzo[b]thienylmethyl)guanosine.

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28. A compound according to claim 1 which is O^6 -(4-picolyl)guanine.
29. A compound according to claim 1 which is O^6 -(2-naphthylmethyl)guanine.
30. A pharmaceutical composition comprising a compound according to any one of claims 1-29 and a pharmaceutically acceptable excipient.
31. A pharmaceutical composition according to claim 30 further comprising an alkylating agent.
32. A composition according to claim 31 wherein the alkylating agent is 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU).
33. A composition according to claim 31 wherein the alkylating agent is temozolomide.
34. Use of a compound according to any one of claims 1 to 29 in a method for depleting O^6 -alkylguanine-DNA alkyltransferase activity in a host.
35. Use of a compound according to any one of claims 1 to 29 and an alkylating agent in a method for depleting O^6 -alkylguanine-DNA alkyltransferase activity in a host.
36. Use of a compound according to any one of claims 1 to 29 and an alkylating agent for treating tumour cells in a host.
37. Use of a compound according to any one of claims 1 to 29, in the manufacture of a medicament for depleting O^6 -alkylguanine-DNA alkyltransferase activity in tumour cells.

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38. Use of a compound according to any one of claims 1 to 29 and an alkylating agent in the manufacture of a one- or two-part pharmaceutical composition for treating tumour cells.

39. Use according to any one of claims 34-38 wherein the compound according to any one of claims 1 to 29 is $\underline{Q}^6$ -thenylguanine or a substituted derivative thereof having substitution in the thiophene ring by a halo group.

40. Use according to any one of claims 35, 36 and 38 wherein the alkylating agent is selected from 1,3 bis (2-chloroethyl)-1-nitrosourea (BCNU) and temozolomide.

41. A process for preparing a compound of the formula I as claimed in claim 1 which comprises reacting sodium hydride with a solution of $RR'CHOH$ (wherein R and R' are as defined in claim 1) in an organic solvent, adding 2-amino-N,N,N-trimethyl-1H-purin-6-aminium chloride or 2-amino-6-chloropurine riboside, treatment with weak acid and ether, and extracting the desired product.

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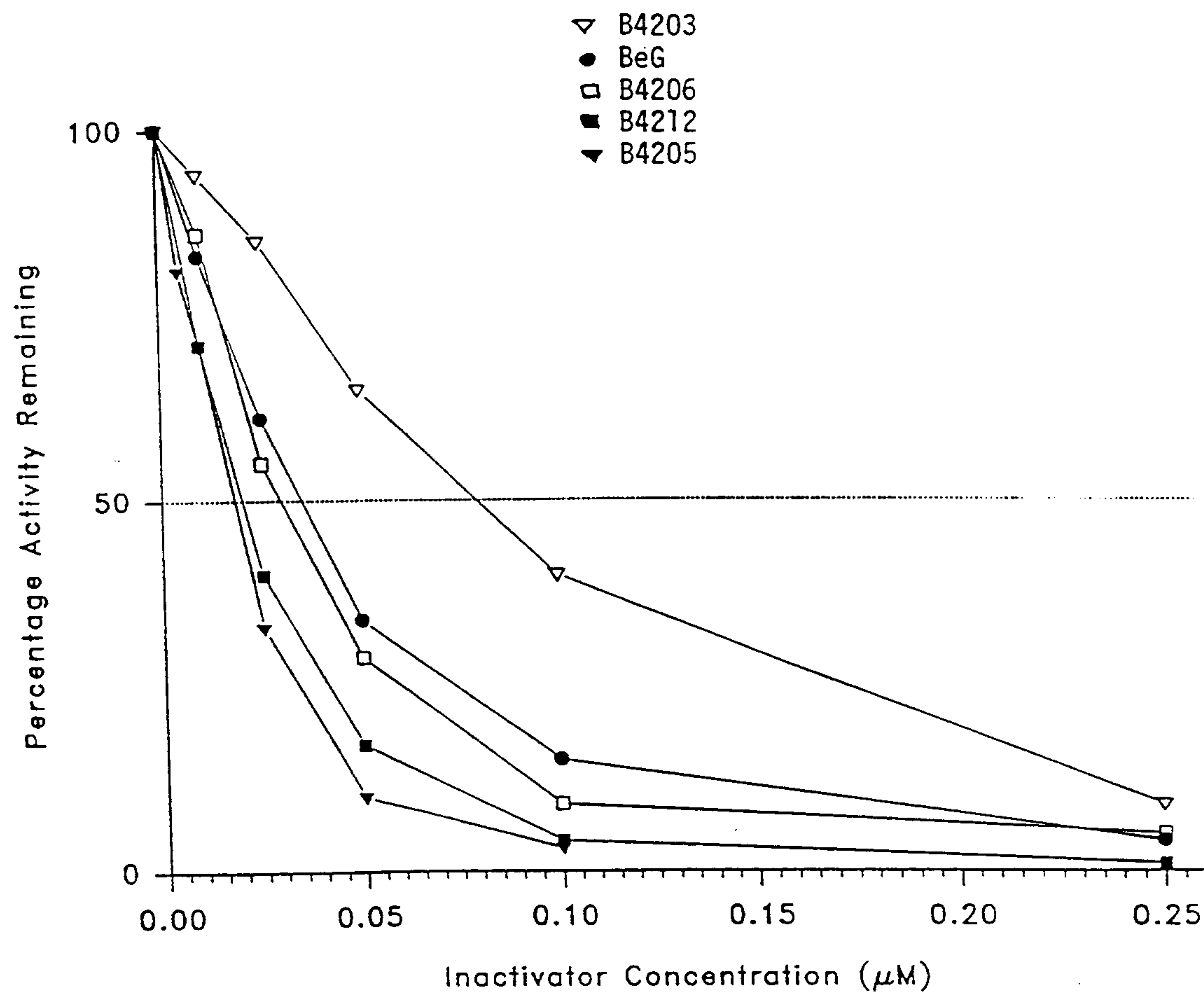


Fig. 1

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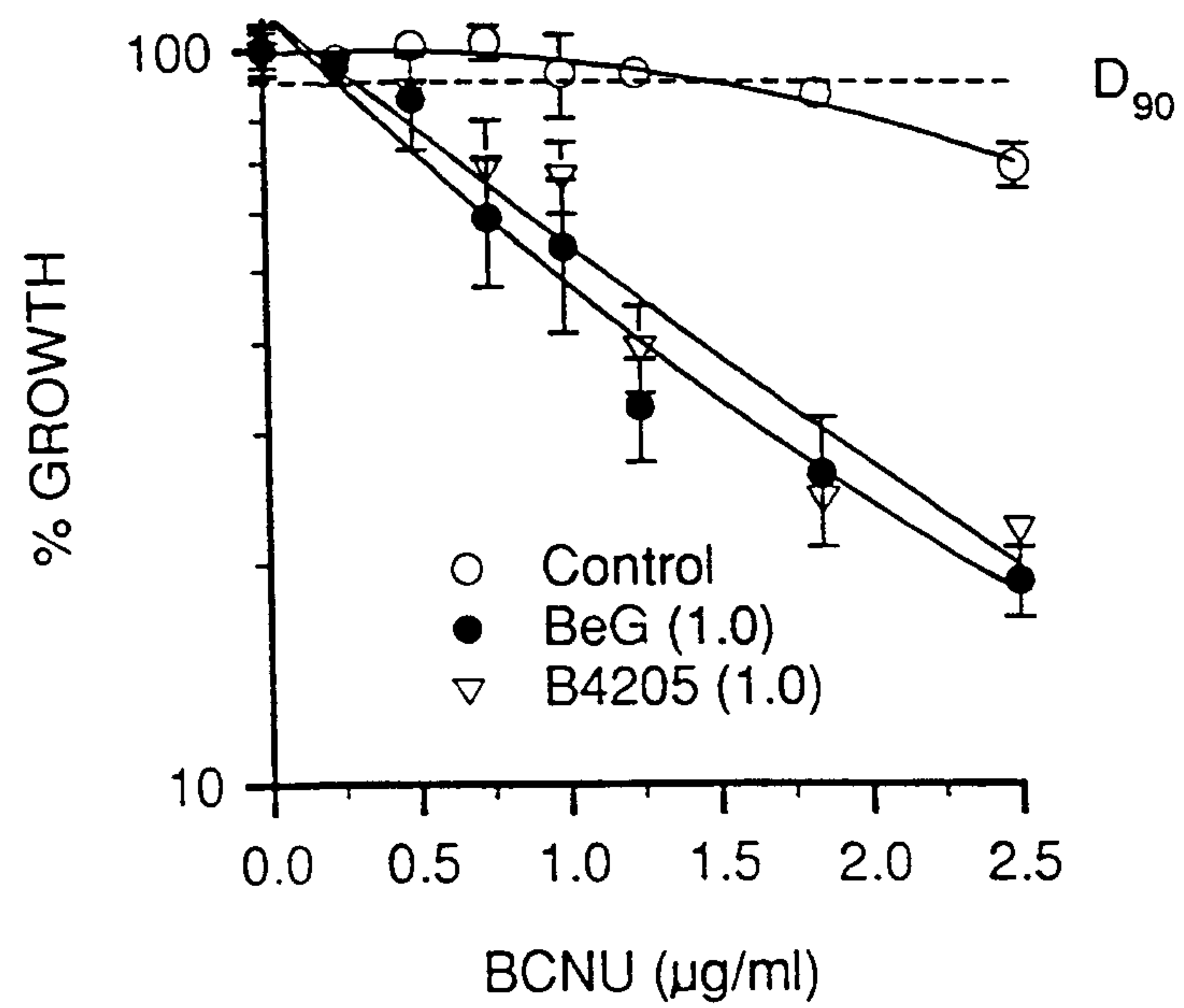
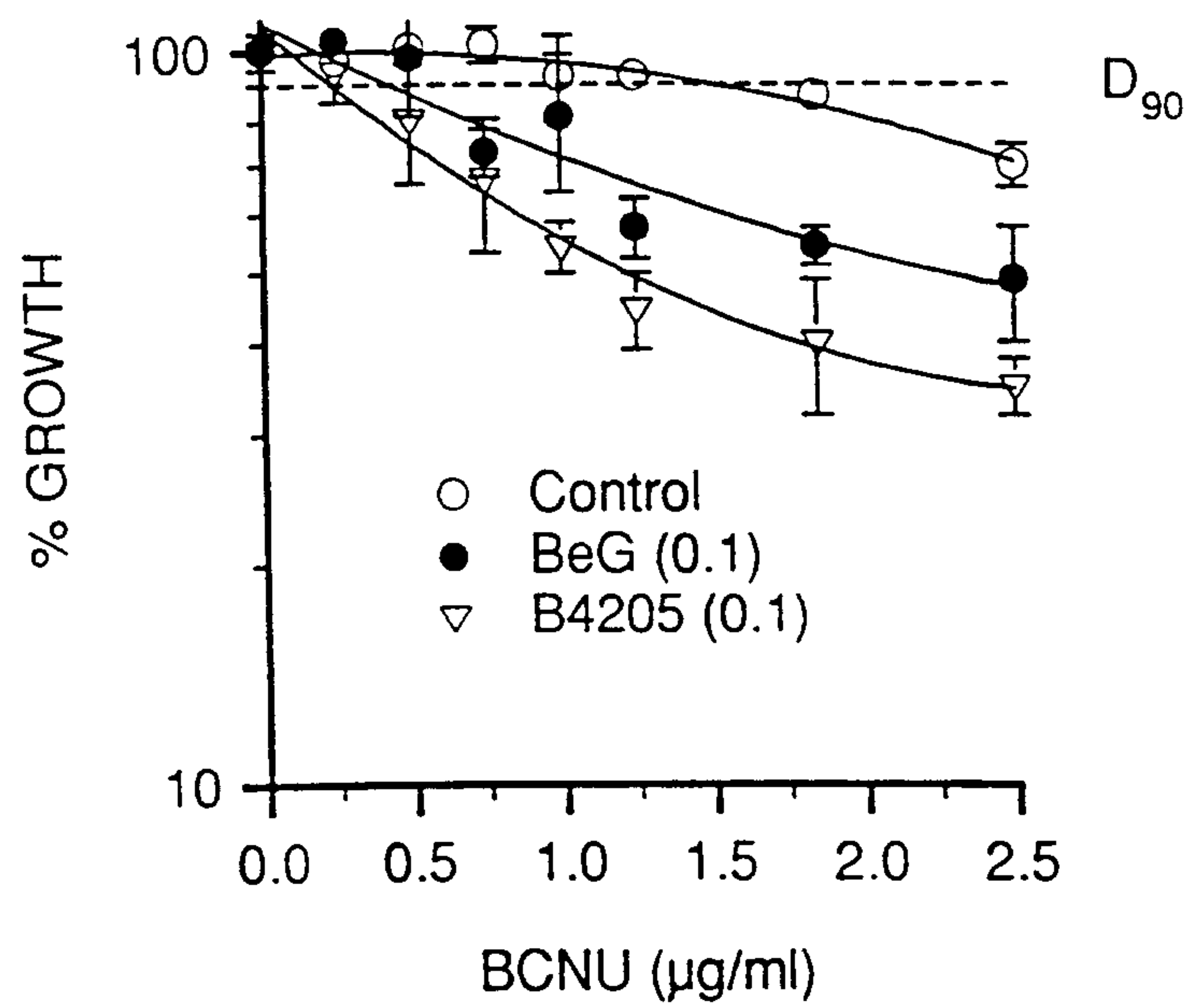


Fig. 2

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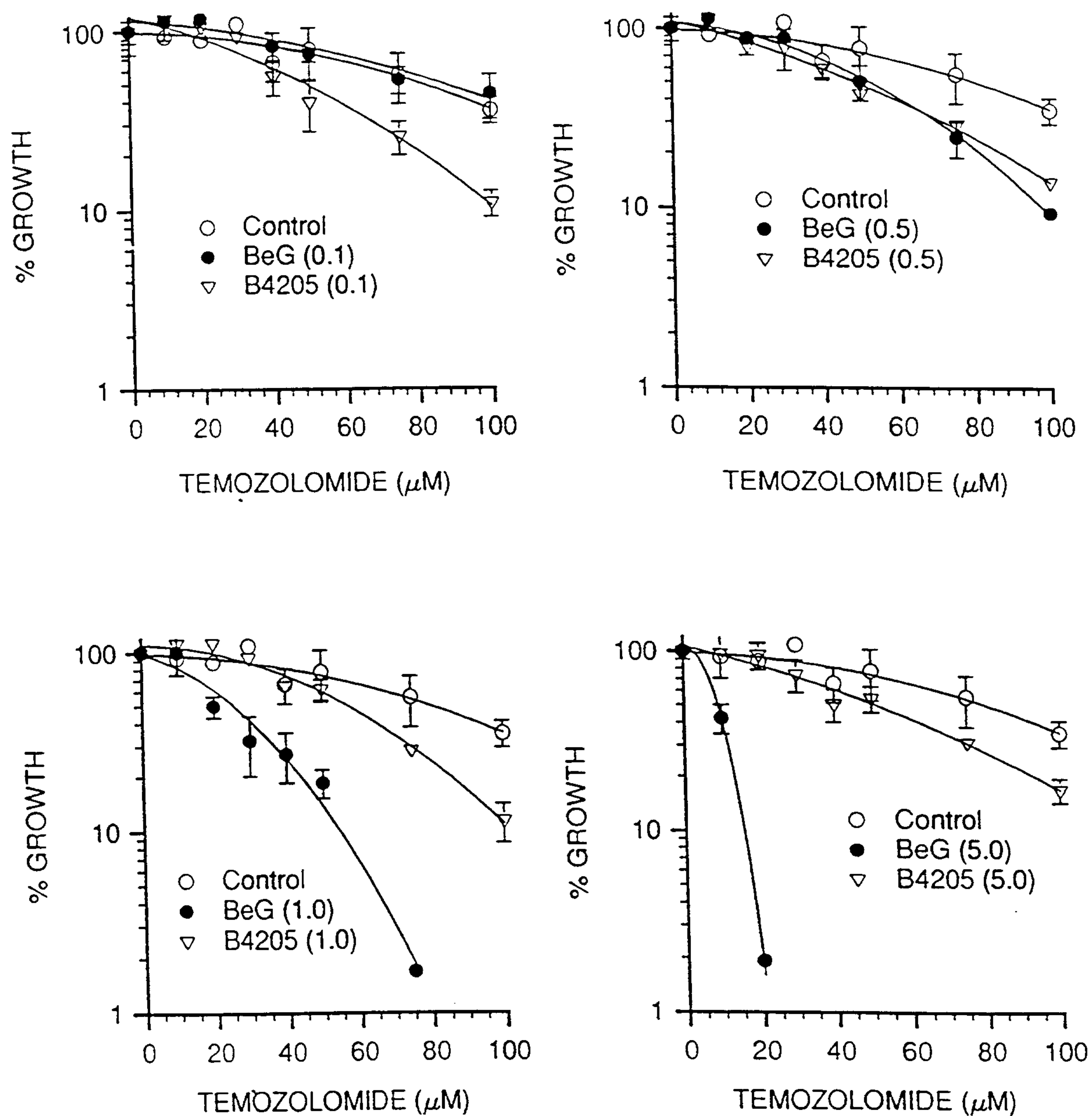


Fig. 3

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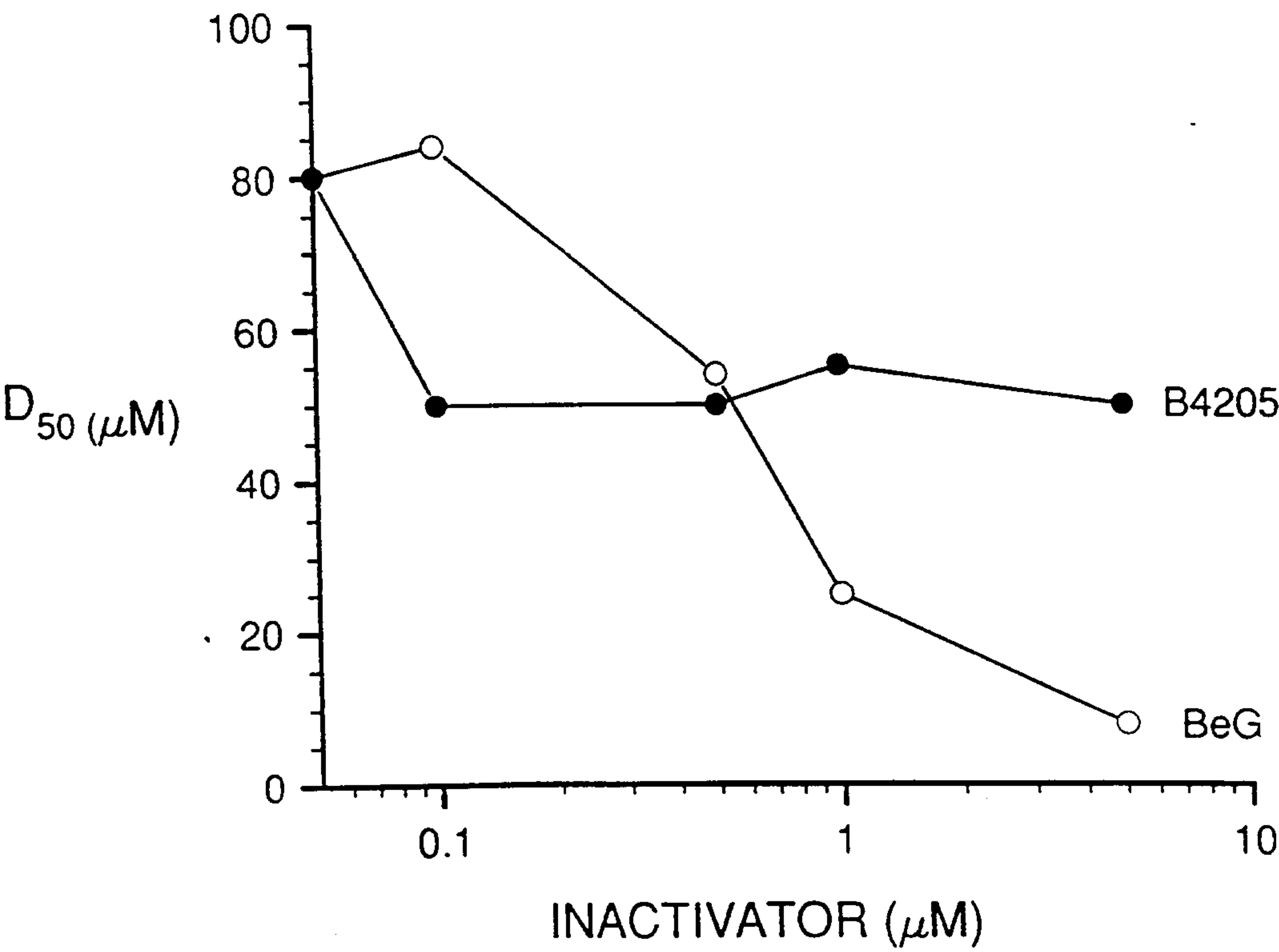


Fig. 4

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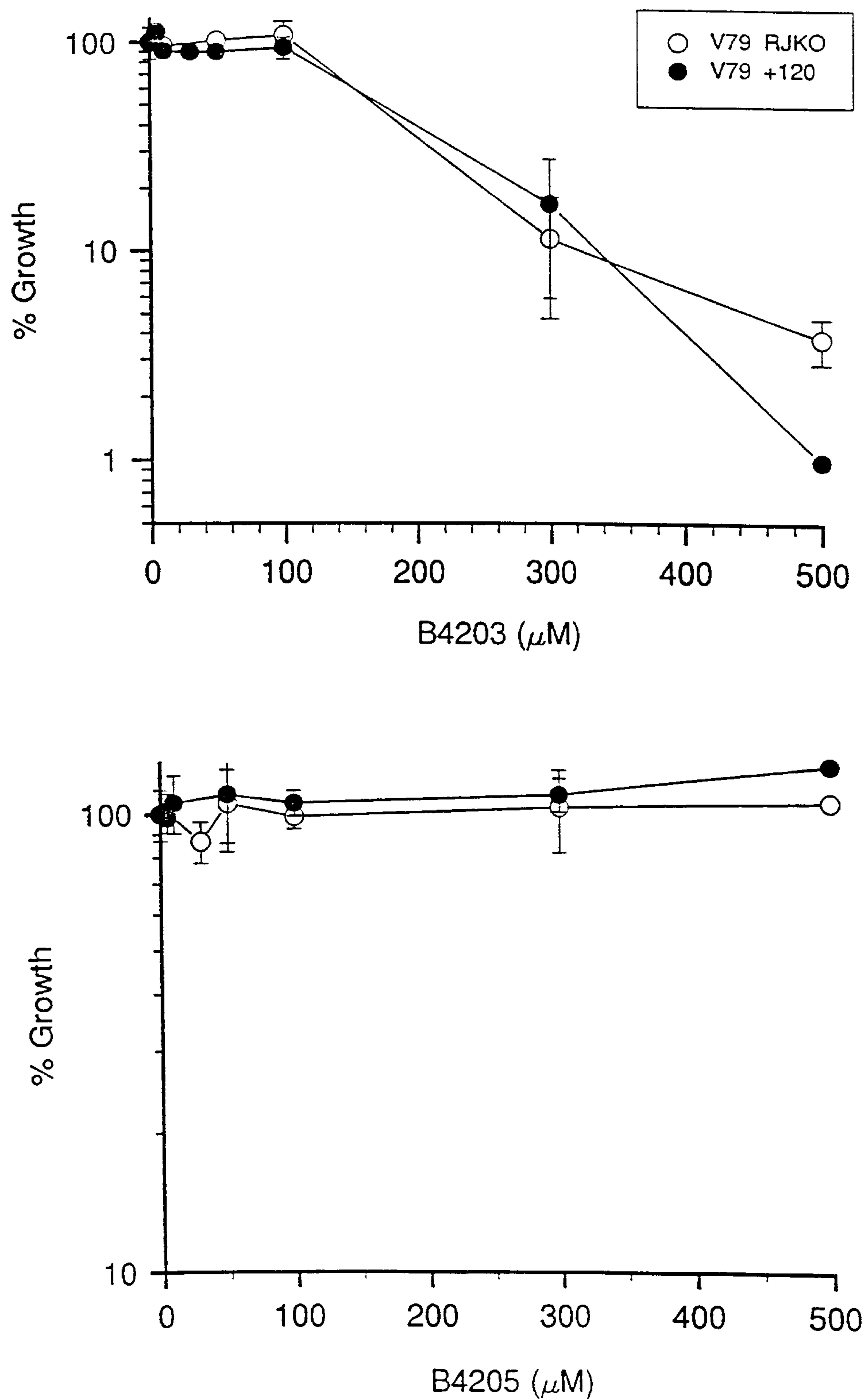


Fig. 5

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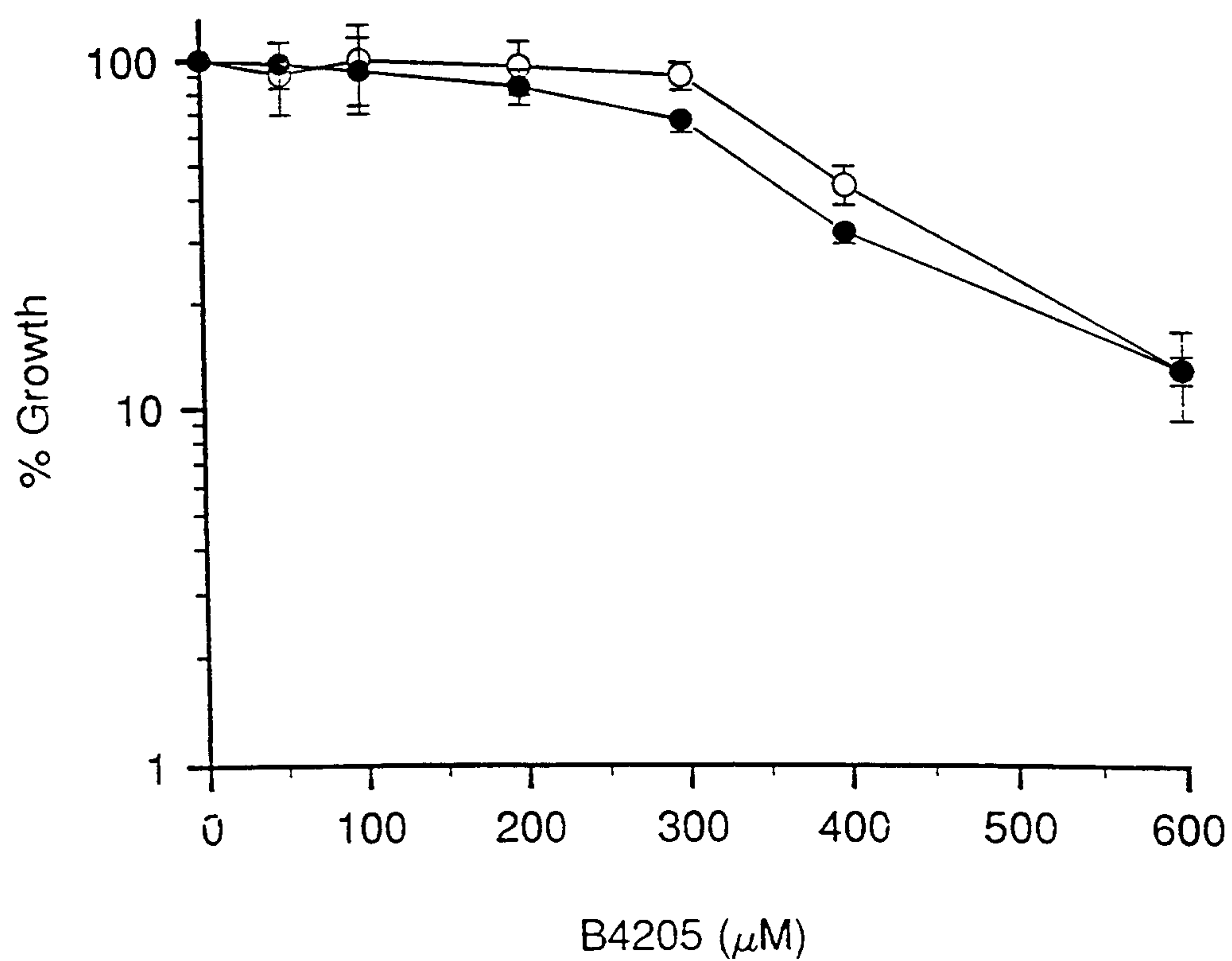
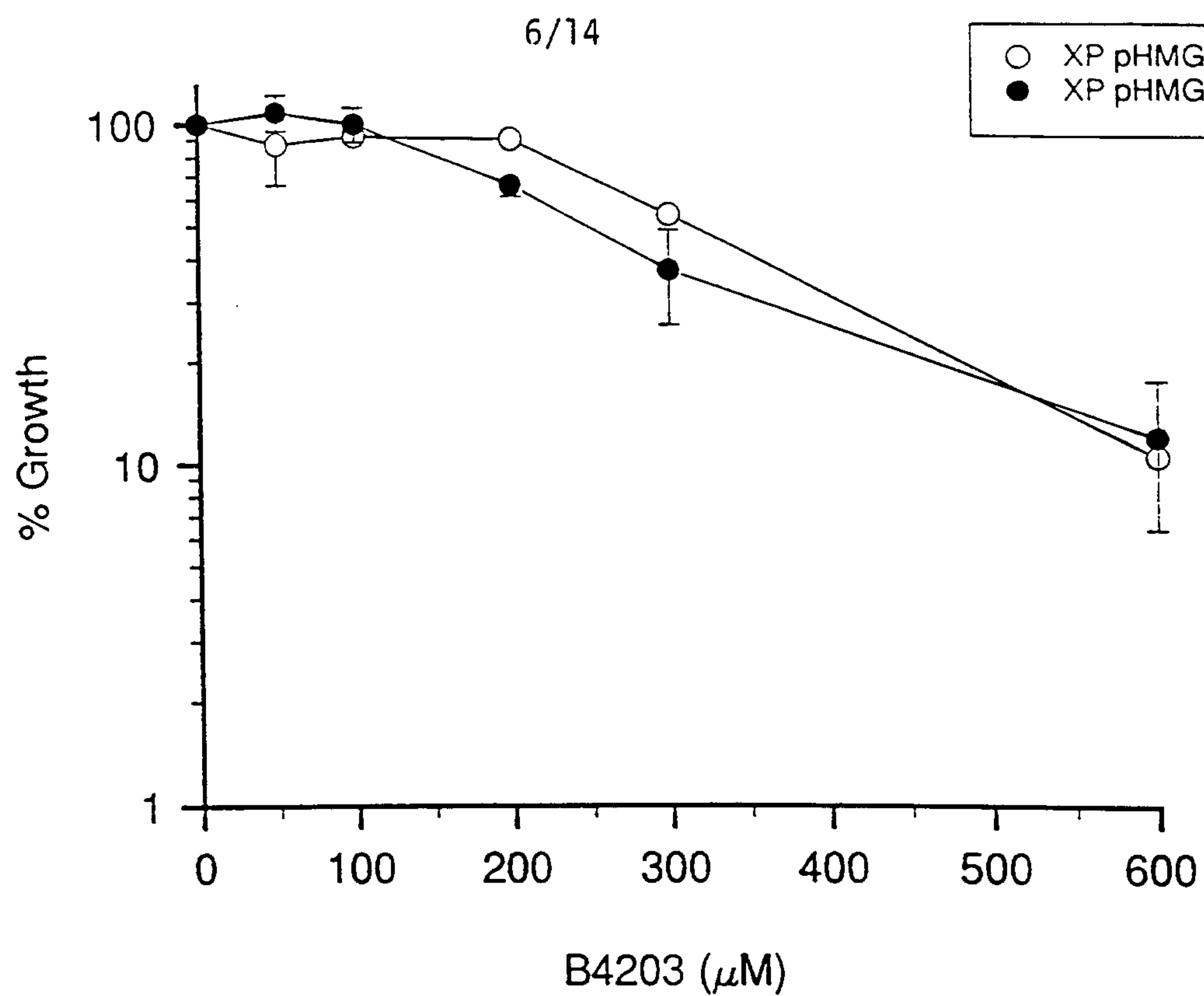


Fig. 6

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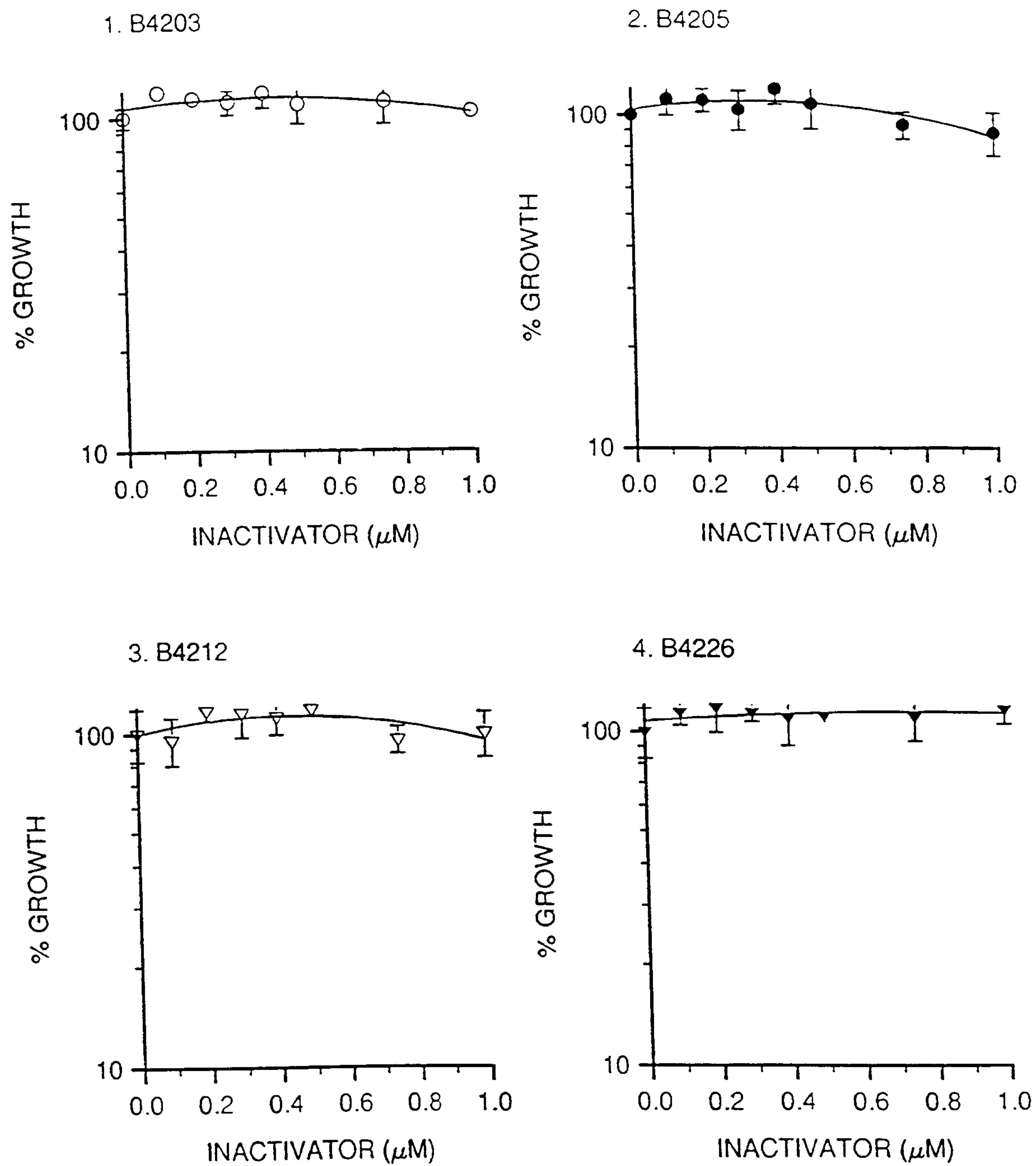


Fig. 7

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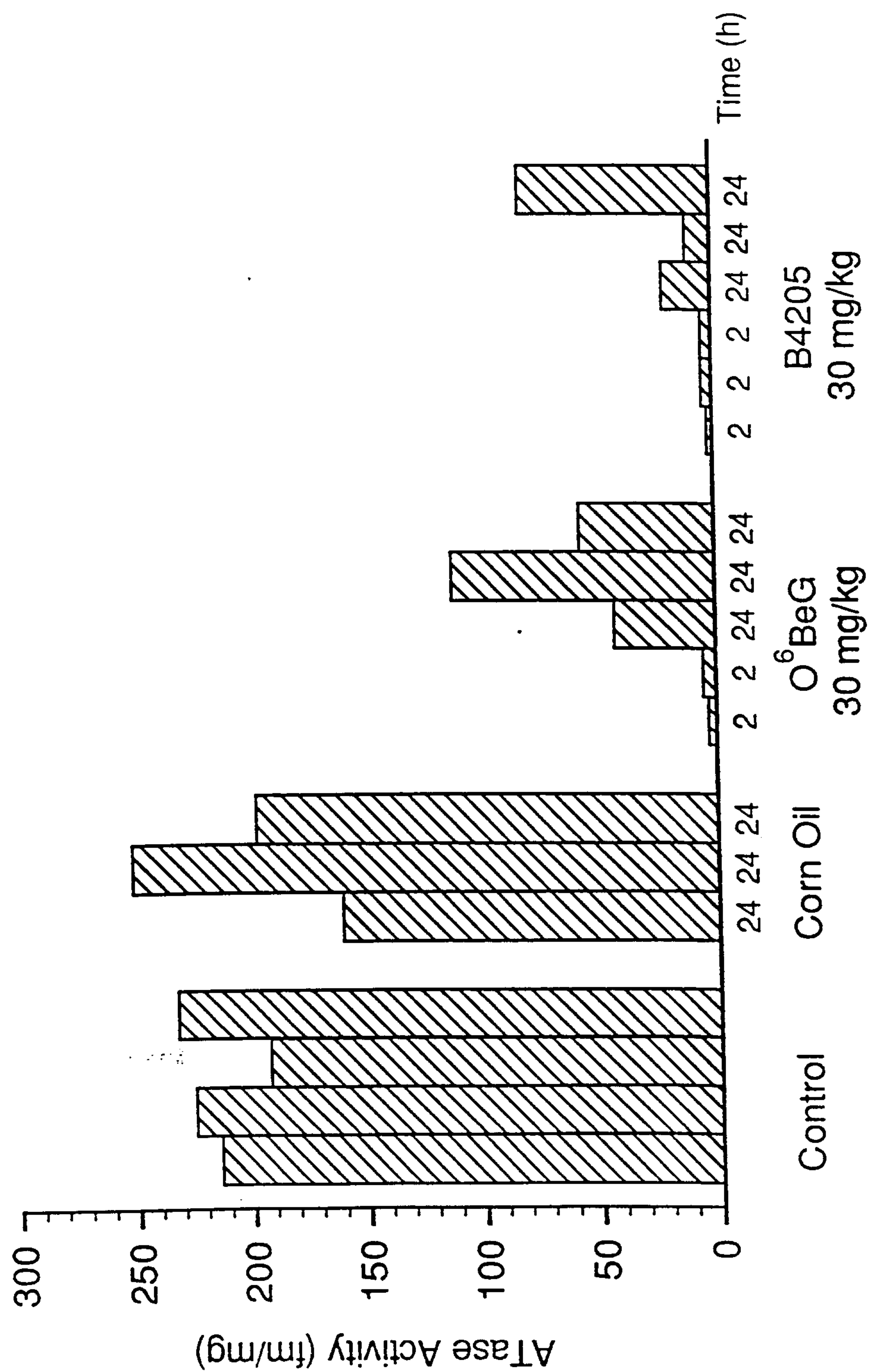


Fig. 8

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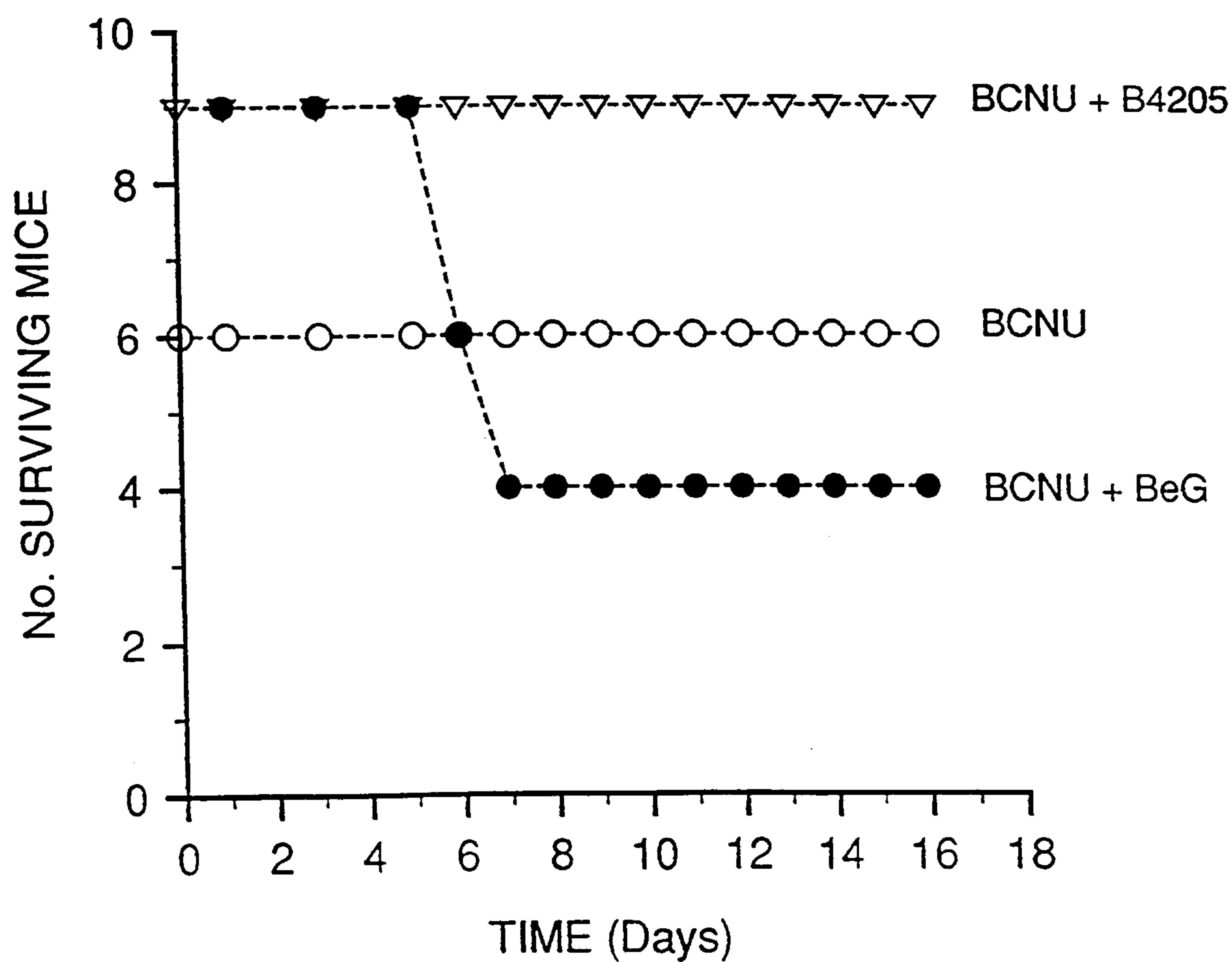
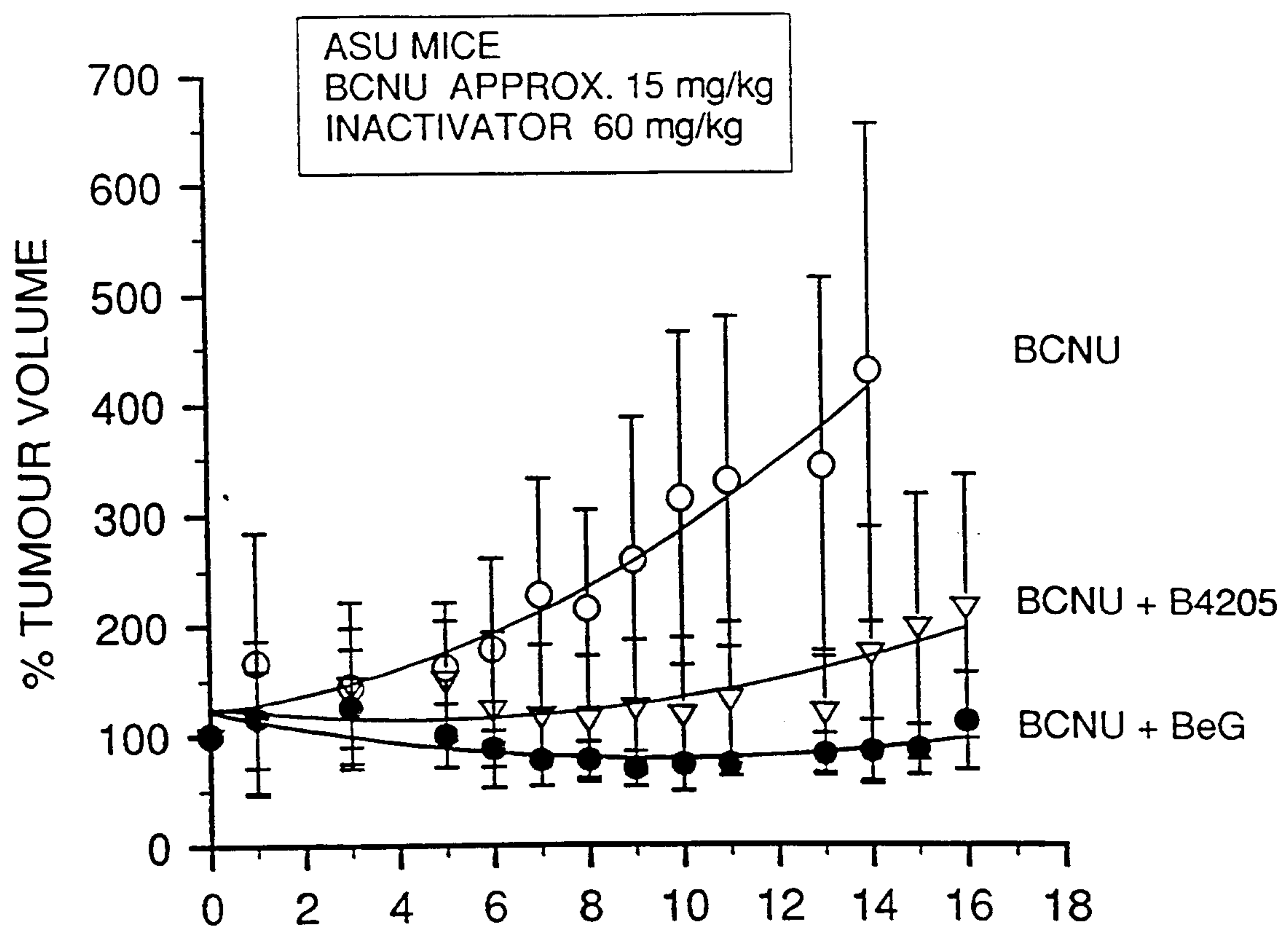


Fig. 9

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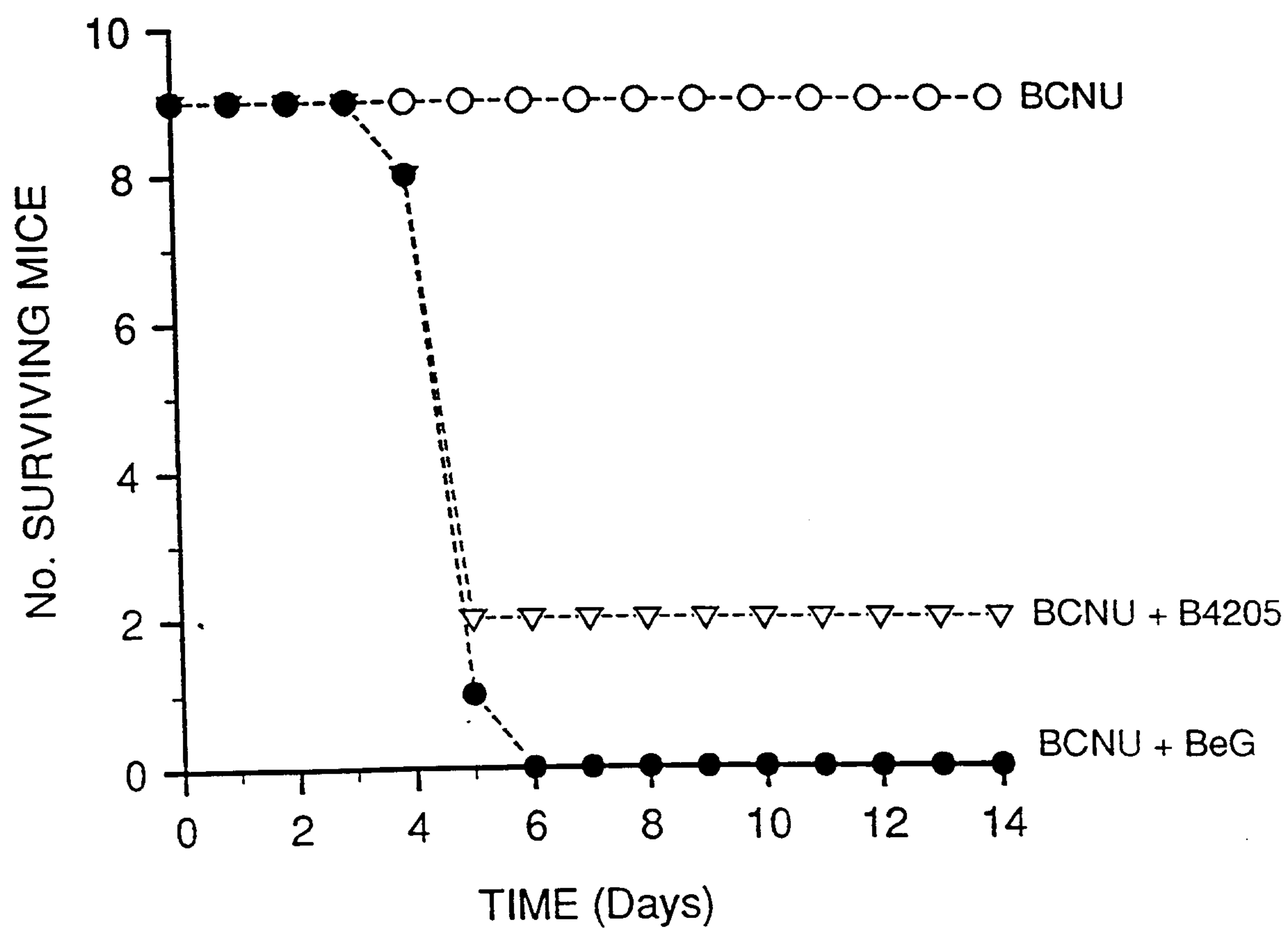
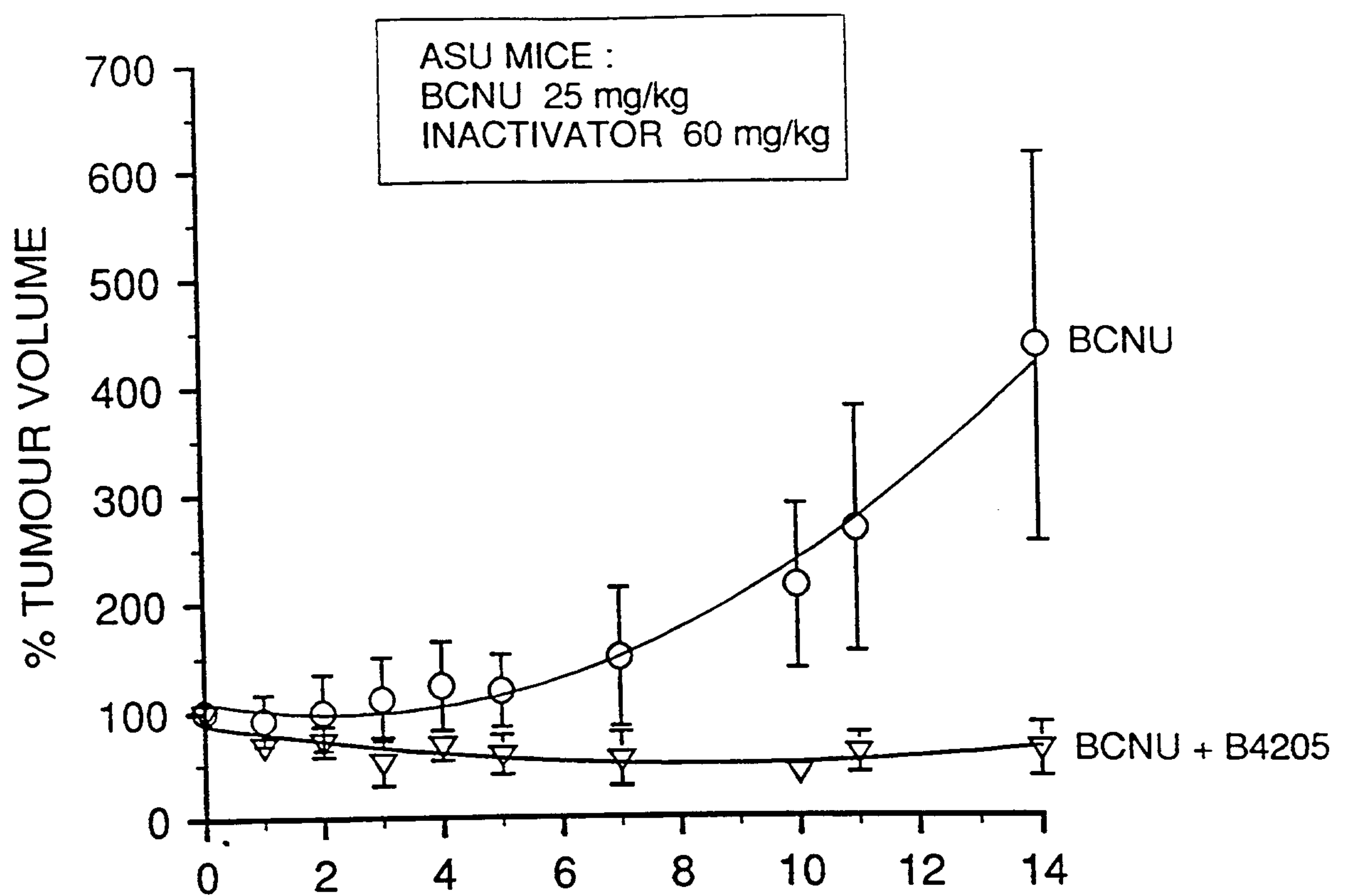


Fig. 10

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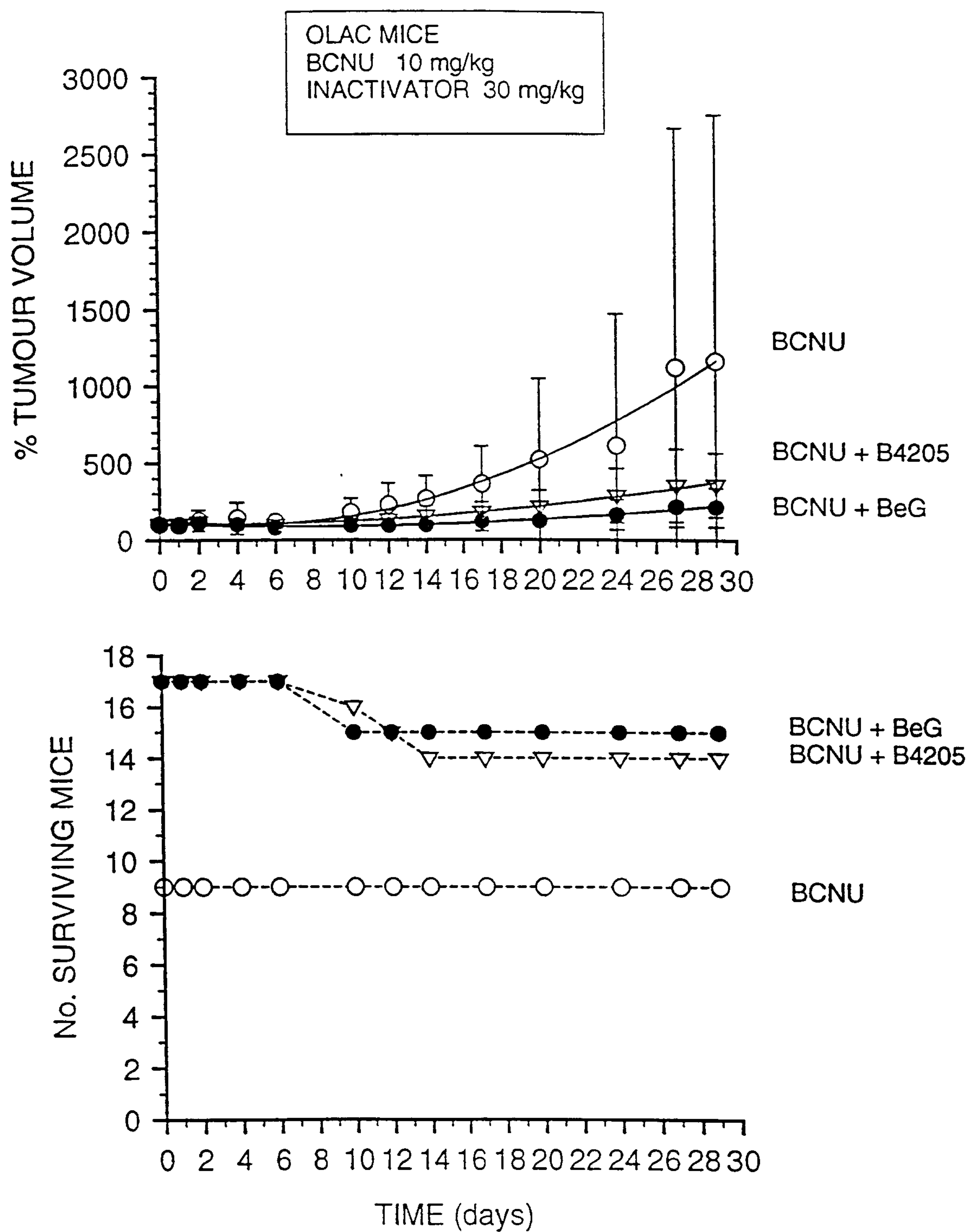


Fig. 11

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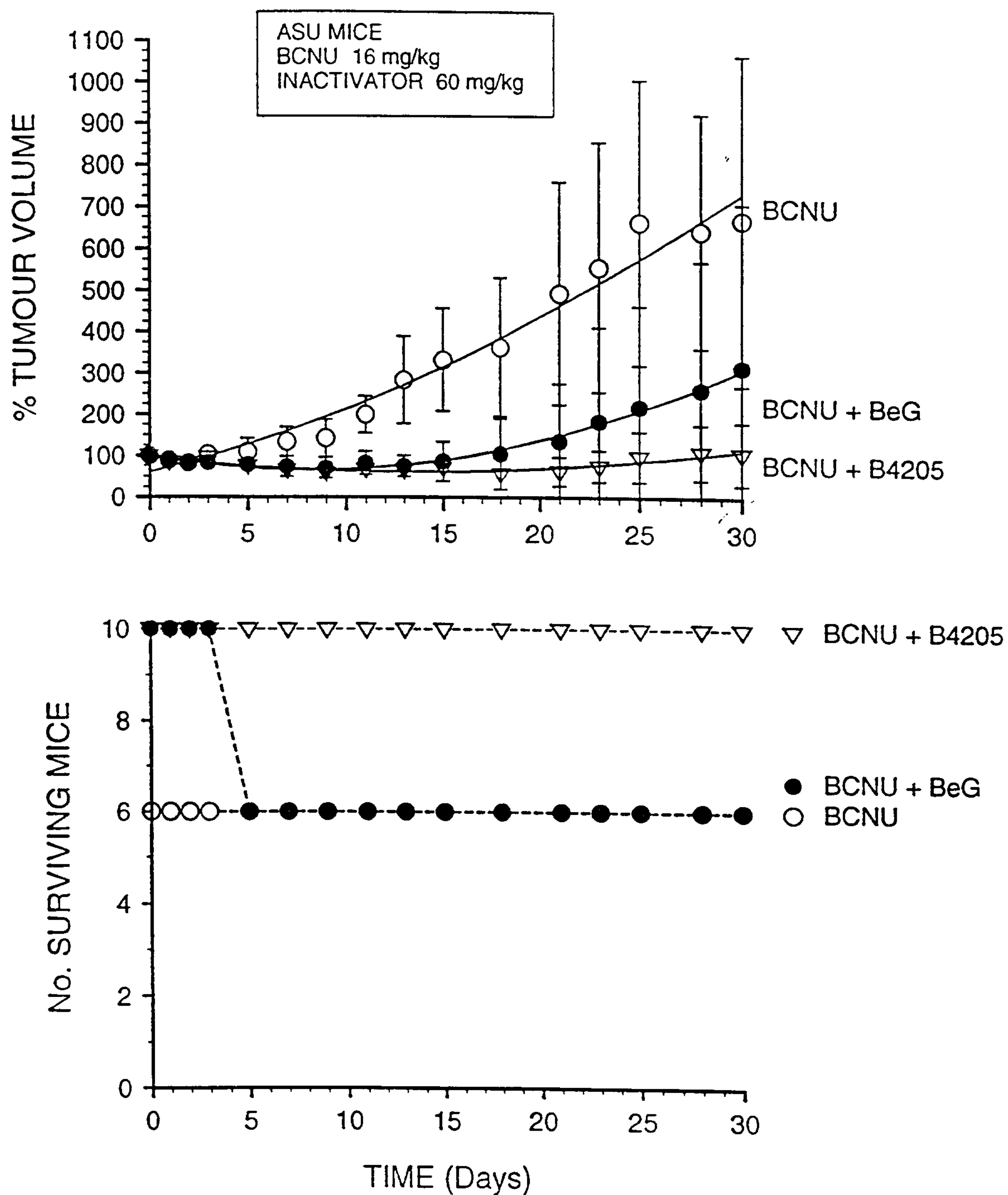


Fig. 12

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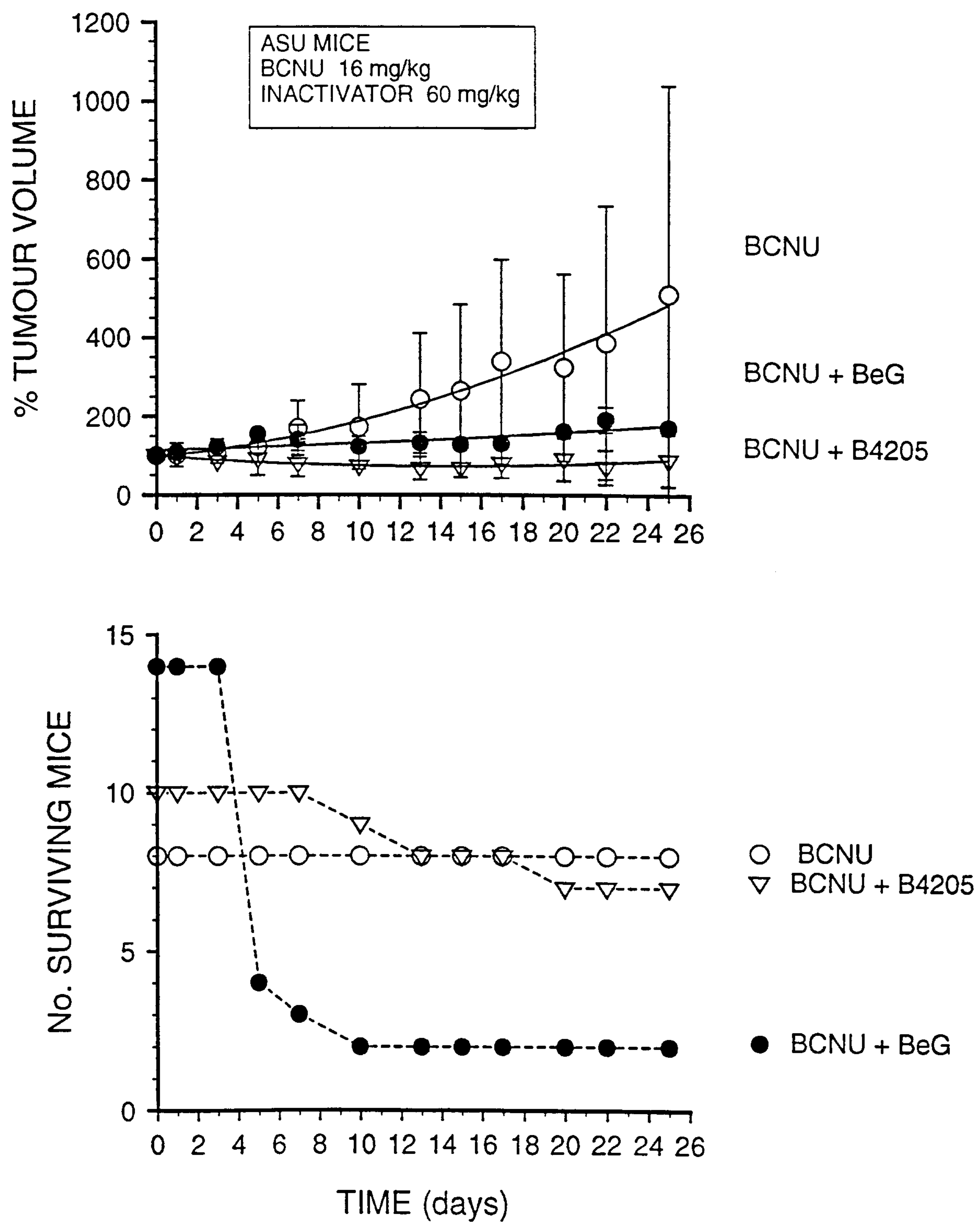
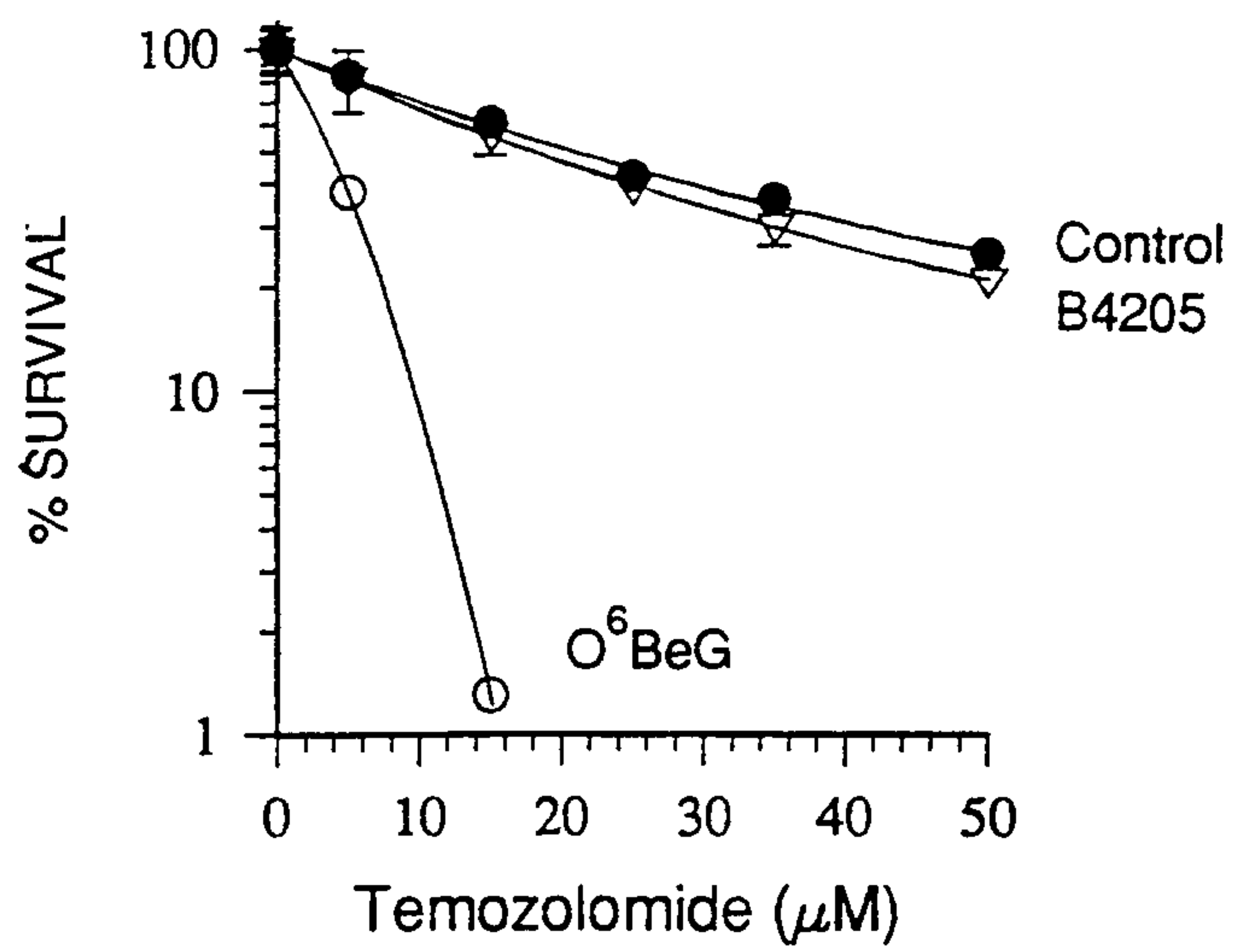
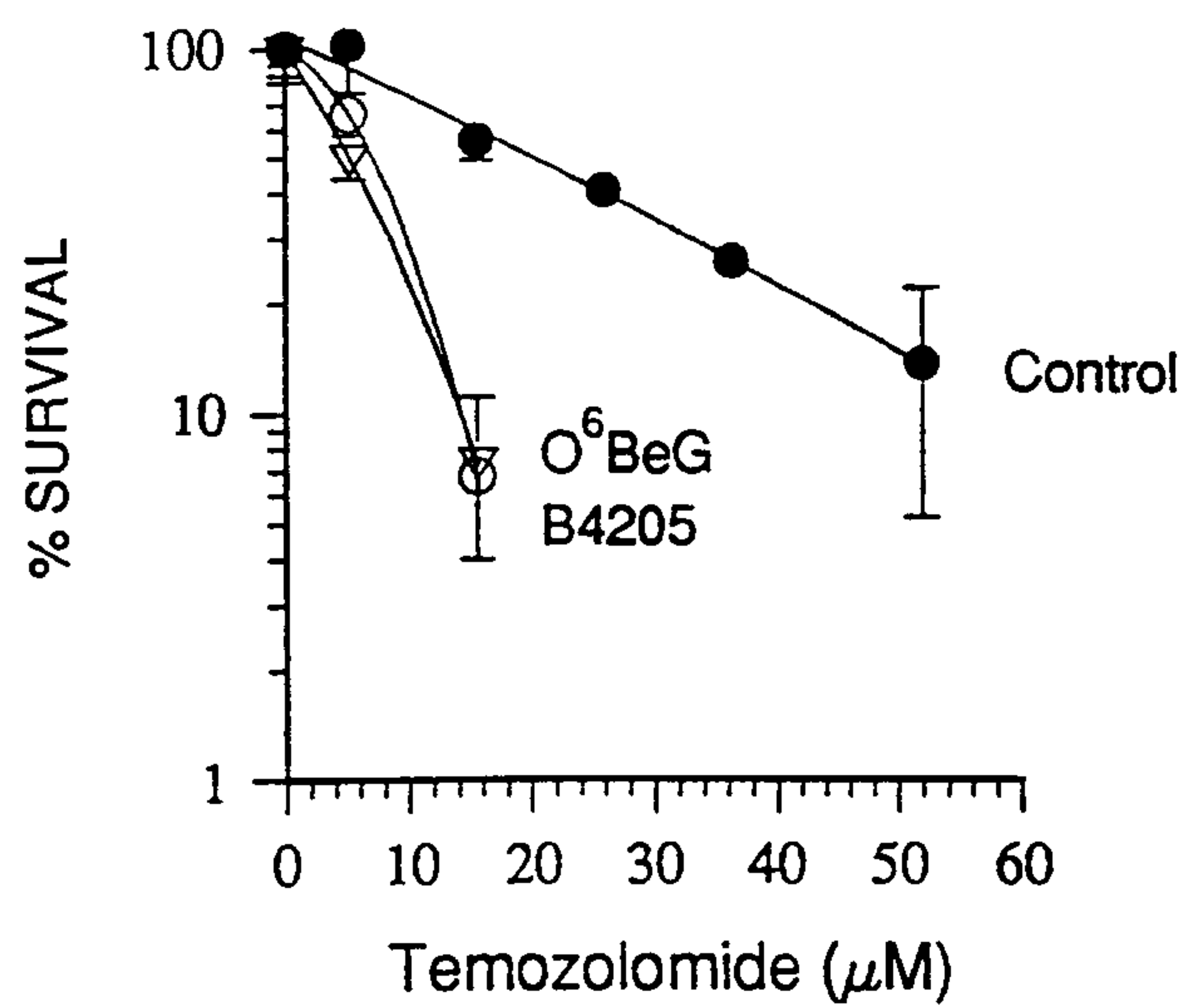


Fig. 13

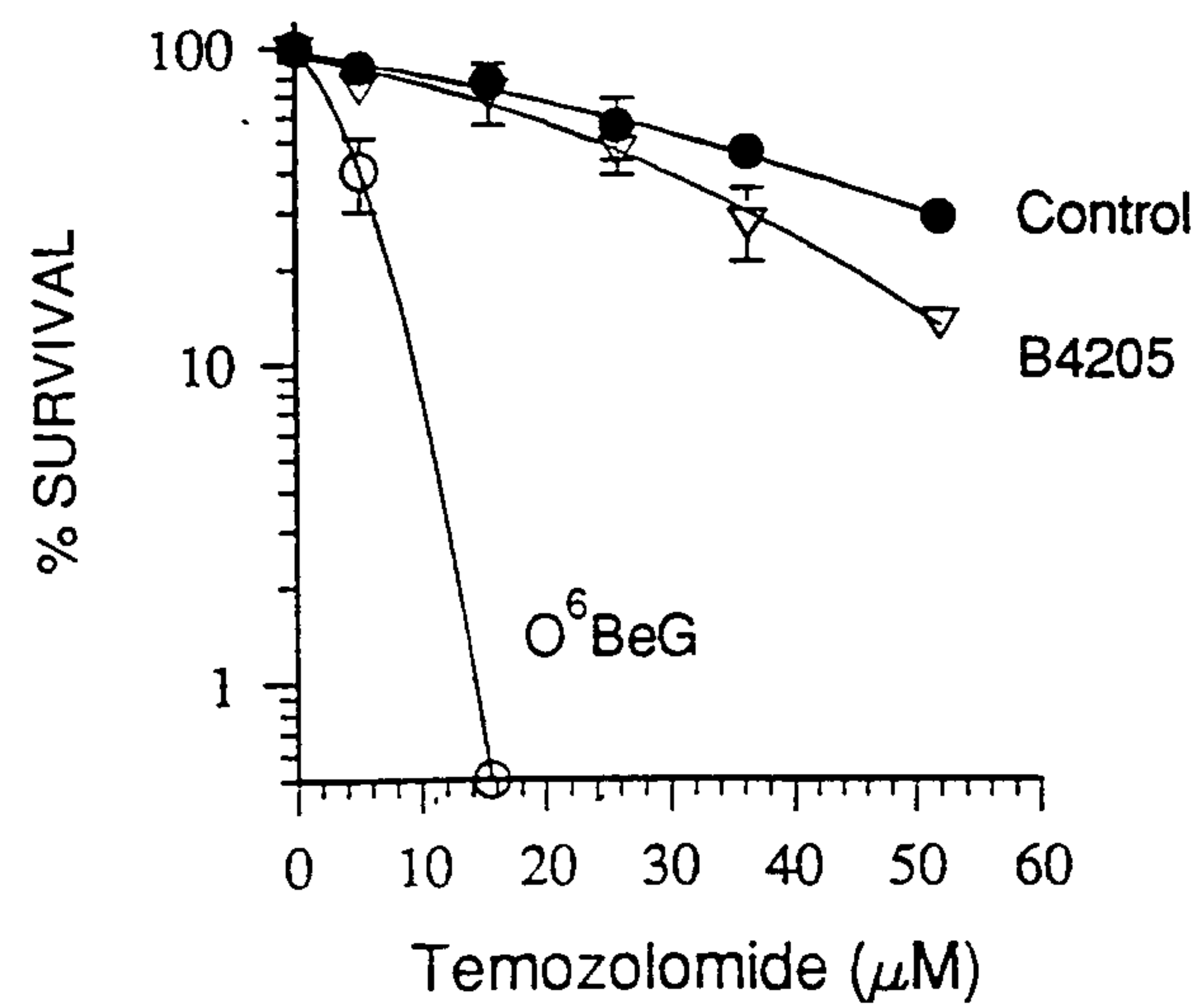
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C



D



E

Fig. 14

