

6,9 BIS(SUBSTITUTED-AMINO)BENZO[g]ISOQUINOLINE-5,10-DI-
ONES

BACKGROUND OF THE INVENTION

Field of the invention

5 This invention is directed to 6,9 bis(substituted-amino)benzo[g]isoquinoline-5,10-diones, and more particularly, to 6,9-substituents which are (aminoalkyl)amino substituents. These compounds have been shown to have antitumor activity in vitro and in vivo.

10 Background

Certain 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones have been reported which show antitumor activity in clinical trials. Of particular interest has been ametantrone, 1,4-bis[[2-(2-hydroxyethylamino)ethyl]amino]anthracene-9,10-dione and mitoxantrone, 5,8-dihydroxy-1,4-bis[[2-(2-hydroxyethylamino)ethyl]amino]anthracene-9,10-dione. (Zea-Cheng et al., J. Med. Chem., (1978), 21, 291-4; Cheng et al., "Progress in Medicinal Chemistry", Ellis, G.P. and West, G.B., eds.; Elsevier: Amsterdam, 1983; pp. 20, 83 and references cited therein). Mitoxantrone is a broad spectrum oncolytic agent, whose activity is similar to that of the anthracycline antibiotic doxorubicin. Clinical trials have demonstrated that mitoxantrone has particularly promising activity in the treatment of advanced breast cancer, acute leukemia and lymphoma (Legha, Drugs of Today, (1984), 20, 629). Although animal studies have demonstrated a diminished cardiotoxicity in comparison to doxorubicin, some clinical cardiotoxicity has been observed also with mitoxantrone, mostly in patients previously treated with doxorubicin (R. Stuart Harris



et al., Lancet, (1984), 219, and references cited therein).

Ametantrone has been reported to be, in animals, about 10-fold less potent and cardiotoxic than mitoxantrone. Because a delayed toxicity is observed only with mitoxantrone after administration of the two drugs by the i.p. route to non-tumor bearing rats at equieffective antitumor dosages, it is suggested that the presence of the 5,8-dihydroxy substitution in mitoxantrone might be implicated in the delayed deaths (Corbett et al., Cancer Chemother. Pharmacol., (1981), 6, 161).

In addition, both mitoxantrone and ametantrone have a remarkable myelodepressive toxicity and both compounds show cross-resistance to cell histotypes developing resistance against doxorubicin mediated by overexpression of glycoprotein P. Such a resistance, which is named multidrug resistance, involves a number of antitumor antibiotics, among which amsacrine and podophyllotoxinic derivatives, and it is one of the main reasons for therapeutical failures in the treatment of solid tumors with said antibiotics.

Therefore, search is required for novel anthracenedione antitumor agents, having a higher therapeutical index than mitoxantrone and being effective both in inhibiting or delaying the growth of those solid tumors which are more refractory to chemotherapeutic treatment (such as lung, breast and colon tumors) and against tumor histotypes developing multidrug resistance.

In the search for safer, active analogues of anthracenediones, compounds bearing hydroxy substituents and/or (aminoalkyl)amino side chains at various posi-



tions on the anthracene-9,10-dione nucleus have been studied (Cheng et al., Drugs of the Future, (1983), 8, 229) without notable improvement.

Aza- and diaza anthracene-9,10-diones such as 6,9-bis(ethoxycarbonylamino)benzo[g]quinoline-5,10-dione (1), 6,9-bis(ethoxycarbonylamino)benzo[g]isoquinoline-5,10-dione (2), 6,9-bis(ethoxycarbonylamino)benzo[g]quinazoline-5,10-dione (3), were disclosed by Potts et al. (Synthesis, 1983, 31). These compounds were reported to be related to the antitumor 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones; however, no antitumor activity data was reported for any of the above compounds. 1-[(aminoalkyl)amino] derivatives (4) of 6,9-dihydroxybenzo[g]isoquinoline-5,10-dione related to mitoxantrone have been disclosed as DNA intercalators but they were completely devoid of any "in vitro" or "in vivo" antitumor activity (Croisy-Delcey et al., Eur. J. Med. Chem., (1988), 23, 101-106).

The 6,9-bis[(aminoalkyl)amino]benzo[g]quinoline-5,10-diones of formulas 5a-d (see Table I) were disclosed in J. Med. Chem. (1985), 28, 1124-26, where they were referred to as 5,8-bis[(aminoalkyl)amino]-1-aza-anthracenediones.

As reported in table II herein below reported the prior art compounds 5 are clearly less active than the corresponding carbocyclic analogues (6).

In fact, as reported in table II, the compounds 5a and 5b are less cytotoxic than the analogues 6a and 6b. Moreover the compound 5a, which is poorly cytotoxic in vitro, is inactive in vivo, and it is both less active and less potent than the carbocyclic analogue 6a.



Even though the introduction of an heteroatom is a common process in the medicinal chemistry, its effect must be evaluated case by case.

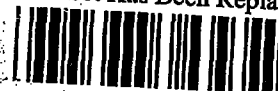
5 In this particular case of aza-analogues of anthracenediones the prior art teachings clearly show that the introduction of a nitrogen atom into the anthracenedione skeleton is detrimental for antitumor activity: In fact the aza-substituted anthracenediones are less cytotoxic than the corresponding anthracene-
10 diones and inactive "in vivo".

Thus a person skilled in the art, would have not considered the introduction of heteroatoms into the anthracenedione skeleton as a possible way to obtain more effective antitumor agents.

15 It is well known that the screening for the discovery of the antitumor agent mitoxantrone (J. Med. Chem., (1978), 21, 291-4) among a number of analogues has been carried out by using the experimental model of murine leukemia P388: this model is then predictive of
20 antitumor activity in humans at least for this class of antitumor antibiotics. Many other clinically active antitumor antibiotics are active on murine leukemias P388 and L1210 such as m-amsacrine and doxorubicin.

Moreover mitoxantrone has shown good activity in
25 other significative experimental tumors such as murine Lewis lung carcinoma and MX1 human mammary carcinoma.

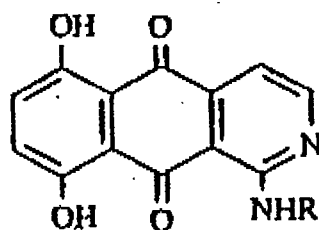
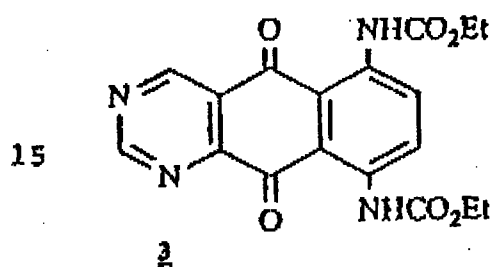
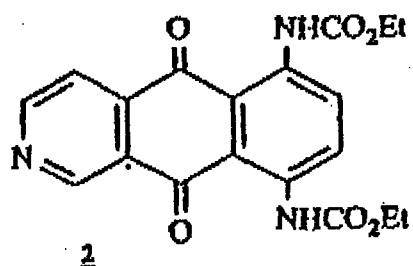
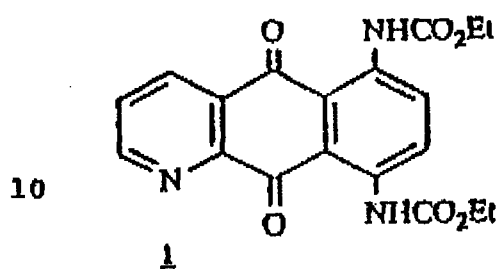
We have now discovered that the compounds of the invention 6,9-bis[(aminoalkyl)amino]benzo[g]isoquinoline-5,10-diones are active as antitumor agents: they are
30 highly effective against murine leukemias L1210 and P388 and against Lewis lung carcinoma and MX1 human



mammary carcinoma.

Table I
Prior art compounds

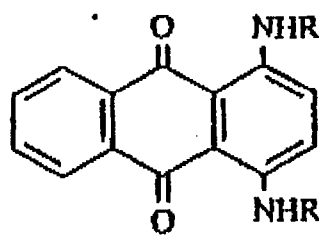
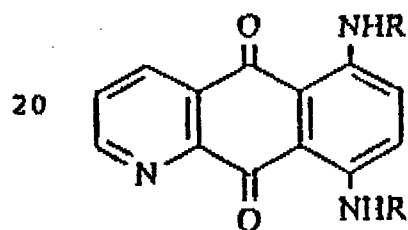
5



4a R = CH₂CH₂N(CH₃)₂

4b R = CH₂CH₂CH₂N(Et)₂

4c R = CH₂CH₂NHCH₂CH₂OH



5a R = CH₂CH₂N(CH₃)₂

6a R = CH₂CH₂N(CH₃)₂

25

5b R = CH₂CH₂N(Et)₂

6b R = CH₂CH₂N(Et)₂

5c R = CH₂CH₂CH₂N(CH₃)₂

5d R = CH₂CH₂NHCOCH₃

30



Table II

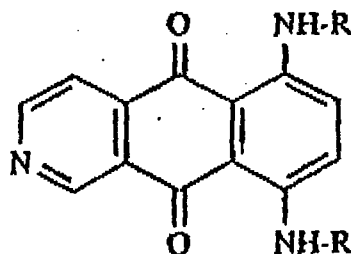
Antitumor activity of prior art compounds 5 and 6 against murine L 1210 leukemia (from J. Med. Chem. (1985), 28, 1124-1126)

5	in vitro		in vivo	
	ID ₅₀ (µg/ml)	Dose (mg/kg)	T/C	
<u>5a</u>	0.16	50	130	
<u>5b</u>	2.4	100	toxic	
<u>5c</u>	1.7			
10 <u>6a</u>	0.08	30	150	
<u>6b</u>	0.30			

BRIEF SUMMARY OF THE INVENTION

The compounds of the invention have the formula

(I)



(I)

wherein R is C₁-C₁₀ alkyl, phenyl or C₇-C₁₀ aralkyl;

C₂-C₁₀ alkyl having one or two substituents selected from the group consisting of OR₁ and -NR₂R₃;

C₂-C₁₀ alkyl interrupted by one or two oxygen atoms or by one member selected from the group consisting of -NR₄-, cis -CH=CH-, trans -CH=CH-, -C≡C-, and optionally substituted by one or two hydroxy (OH) or -NR₂R₃ groups;

R₁ is selected from the group consisting of hydrogen, C₁-C₆ alkyl, phenyl, C₇-C₁₀ aralkyl, -CHO, -COR₅, -COOR₅, -S(O₂)R₅, C₂-C₆ alkyl optionally substituted by



-NR₂R₃;

R₂ and R₃ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₇-C₁₀ aralkyl, phenyl, C₂-C₁₀ alkyl substituted with one or two hydroxy (OH) groups, -CHO, -COR₅, -COOR₅, -S(O₂)R₅, one of R₂ or R₃ is H and the other is -C(=NH)NH₂, or R₂ and R₃ taken together with the nitrogen atom to which they are bound form an ethyleneimine ring or a 5- or 6-membered aromatic or non-aromatic heterocyclic ring which might contain another heteroatom such as sulfur, oxygen or nitrogen;

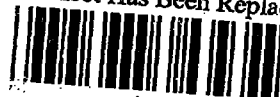
R₄ is selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₂-C₁₀ hydroxyalkyl, C₂-C₁₀ alkyl substituted by -NR₂R₃, C₇-C₁₀ aralkyl, phenyl, -COR₅, -COOR₅ or -S(O₂)R₅;

R₅ is selected from the group consisting of C₁-C₁₀ alkyl, C₇-C₁₀ aralkyl, α-, β-, or γ-naphthyl, phenyl o-, m-, or p-tolyl;

as free bases and their salts with pharmaceutically acceptable acids.

The present invention also concerns the tautomeric forms, the single enantiomers and diastereoisomers of the compounds of formula (I), as well as mixtures thereof.

The present invention also concerns the non-toxic salts of the compounds of formula (I) with acids acceptable for pharmaceutical and veterinary use such as those obtained by addition of inorganic acids like hydrochloric, hydrobromic, sulfuric, phosphoric, pyrophosphoric acid and/or of organic acids such as acetic, propionic, citric, benzoic, lactic, maleic,



fumaric, succinic, tartaric, glutamic, aspartic, gluconic, ascorbic acids and the like.

DETAILED DESCRIPTION OF THE INVENTION

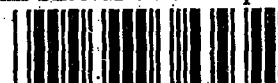
5 In compounds (I) the term "phenyl" means phenyl rings which can optionally contain substituents such as (C₁-C₄)alkyl groups, CF₃, halogen atoms, nitro, amino, acetylamino, formylamino, dimethylamino, diethylamino, hydroxy, methoxy and ethoxy groups.

10 Preferred examples of C₁-C₁₀ alkyl groups are methyl, ethyl, n-propyl, sec-propyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, n-hexyl.

Preferred examples of C₇-C₁₀ aralkyl are benzyl and 4-methoxybenzyl. When in compounds of formula (I) R is a C₂-C₁₀ alkyl interrupted by one or two oxygen atoms or by one member selected from the group consisting of ^{R₄}-N-, cis-CH=CH-, trans-CH=CH-, -CEC- and optionally substituted by one or two hydroxy or -NR₂R₃ groups, at least two carbon atoms are preferably interposed between said oxygen atoms and/or the -NR₄- and -NR₂R₃ groups.

20 When in compounds of formula (I) the -NR₂R₃ substituent is a 5-6 member aromatic or not aromatic heterocyclic ring which may contain another heteroatom such as sulfur, oxygen and nitrogen, preferred examples of said heterocyclic rings are 1-imidazolyl, 1-pyrrolyl, 1-tetrahydropyrrolyl, 1-pyrazolyl, 4-morpholinyl, 1-piperidinyl, 1-piperazinyl, 1-(4-methyl)-piperazinyl, 1-(4-benzyl)-piperazinyl.

30 Particularly preferred are compounds of formula (I) in which R is a C₂-C₁₀ alkyl selected from the group consisting of:



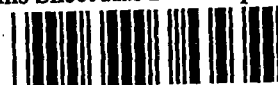
- residue of formula $-(CH_2)_p-NH_2$ wherein p is 2, 3 or 4;
- residue of formula $-(CH_2)_p-NR_2R_3$ wherein p is as above defined and R_2 and R_3 are a C_1-C_6 alkyl or taken together with the nitrogen atom, they form an heterocyclic ring selected from the group consisting of 1-ethyleneimine, 1-pyrrolidine, 4-morpholine, 1-piperazine, 4-methyl-1-piperazine, 4-benzyl-1-piperazine, 1-piperidine,
- 10 - residue of formula $-(CH_2)_p-NR_2R_3$ wherein p is as above defined and R_2 is hydrogen and R_3 is a C_1-C_6 alkyl,
- residue of formula $-(CH_2)_p-NH-\overset{NH}{\underset{||}{C}}-NH_2$ wherein p is as above defined
- 15 - residue of formula $-(CH_2)_p-NH-(CH_2)_q-OH$ wherein p and q are independently an integer selected from the group consisting of 2, 3, 4,
- residue of formula $-(CH_2)_p-OH$ wherein p is as above defined
- 20 - residue of formula $-(CH_2)_p-O-(CH_2)_q-OH$ wherein p and q are as above defined.

Specific examples of the preferred compounds of this invention are as follows:

- 25 6,9-bis[[2-(amino)ethyl]amino]benzo[g]isoquinoline-5,10-dione;
- 6,9-bis[[2-(4'-morpholino)ethyl]amino]benzo[g]isoquinoline-5,10-dione;
- 6,9-bis[[2-(dimethylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione;
- 30 6,9-bis[[2-(diethylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione;

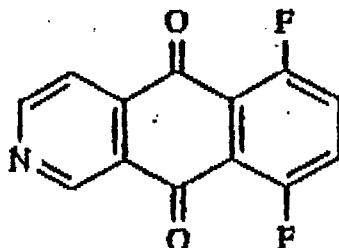


- line-5,10-dione;
6,9-bis[[2-[di(sec-propyl)amino]ethyl]amino]benzo[g]-
isoquinoline-5,10-dione;
6,9-bis[[2-(1'-pyrrolidino)ethyl]amino]benzo[g]isoqui-
5 noline-5,10-dione;
6,9-bis[[2-(1'-aziridino)ethyl]amino]benzo[g]isoquino-
line-5,10-dione;
6,9-bis[[2-(methanesulfonyloxy)ethyl]amino]benzo[g]iso-
quinoline-5,10-dione;
10 6,9-bis[[4-(amino)butyl]amino]benzo[g]isoquinoline-
5,10-dione;
6,9-bis[[3-(amino)propyl]amino]benzo[g]isoquinoline-
5,10-dione;
6,9-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]benzo[g]-
15 isoquinoline-5,10-dione;
6,9-bis[[3-(dimethylamino)propyl]amino]benzo[g]isoqui-
noline-5,10-dione;
6,9-bis[[2-(2-hydroxy)ethyl]amino]benzo[g]isoquinoline-
5,10-dione;
20 6,9-bis[[2-(2-hydroxyethoxy)ethyl]amino]benzo[g]iso-
quinoline-5,10-dione;
6,9-bis[[2-(methylamino)ethyl]amino]benzo[g]isoquino-
line-5,10-dione;
6,9-bis[[2-(ethylamino)ethyl]amino]benzo[g]isoquinoli-
25 ne-5,10-dione;
6,9-bis[[2-(n-propylamino)ethyl]amino]benzo[g]isoqui-
noline-5,10-dione;
6,9-bis[[2-(sec-propylamino)ethyl]amino]benzo[g]isoqui-
noline-5,10-dione;
30 6,9-bis[[2-(guanidino)ethyl]amino]benzo[g]isoquinoline-
5,10-dione;



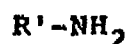
6,9-bis([(2-amino-2,2-dimethyl)ethyl]amino)benzo[g]isoquinoline-5,10-dione.

The compounds of this invention can be prepared by reaction of 6,9-difluorobenzo[g]isoquinoline-5,10-dione
5 of formula (II)



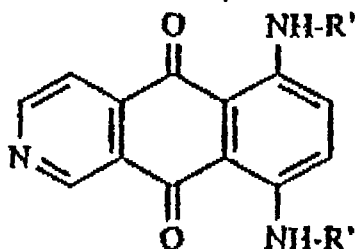
(II)

10 with a compound of formula (III)



(III)

wherein R' has the same meanings as defined in formula
15 (I) for R or it is a group which may be converted to R, to give a compound of formula (Ia):



(Ia)

and then optionally performing one or more of the following steps:

25 a) when R' is other than R, converting R' into R to obtain a compound of formula (I);

b) when R' is one of the groups defined above for R in compounds of formula (I), and in which case the compounds of formula (Ia) are the same as the compounds
30 of formula (I), R' can be optionally converted into another R group to give another compound of formula



(I);

c) optional salification and/or solvation of the obtained compounds of formula (I) or separation of the isomers thereof.

5 The reaction of the compound of formula (II) with a compound of formula (III) is generally carried out in the presence of a stoichiometric amount or a slight molar excess of a compound of formula (III) in a solvent such as methylene chloride, chloroform, 1,1,1-trichloroethane, dimethoxyethane, tetrahydrofuran, dimethylsulfoxide, dimethylformamide, pyridine, picoline, and mixtures thereof, or, if it is desired, using compound (III) itself as the solvent, optionally in the presence of an inorganic base such as an alkaline or alkaline-earth carbonate or hydrogen carbonate or an organic base such as a trialkylamine, at a temperature from 0°C to the reflux temperature of the solvent.

20 Preferably the reaction is carried out in a solvent such as pyridine, chloroform or dimethylsulfoxide, using from 2 to 10 equivalents of compound (III) for 1 equivalent of compound (II) and working at a temperature ranging from room temperature to 50°C.

25 If it is desired the compounds of formula (I) in which R is an hydroxyalkylamino alkyl group of formula $-(CH_2)_p-NH-(CH_2)_q-OH$, wherein p and q are as defined above, can be obtained by reaction of a compound of formula (I) in which R is a group of formula $-(CH_2)_p-OS(O_2)R_5$ with a compound of formula (IV)



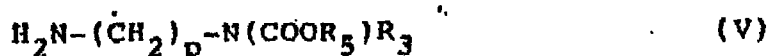
30 wherein E is a hydroxy protective group such as a trialkylsilane, (dialkyl)arylsilane, formyl, acetyl,





which reaction may be optionally followed by removal of the protective group E.

If it is desired the compounds of formula (I) in which R is a group of formula $-(CH_2)_p-NR_2R_3$ wherein one of R_2 or R_3 is hydrogen and the other is hydrogen or a C_1-C_6 alkyl may be obtained by reaction of compound (II) with a monoprotected diamine of formula (V):



wherein R_3 is hydrogen or a C_1-C_6 alkyl and p and R_5 are as defined in formula (I), to give a compound of formula (Ia) in which R' is a group of formula $-(CH_2)_p-N(COOR_5)R_3$, which reaction may be followed by removal of the protective group $COOR_5$.

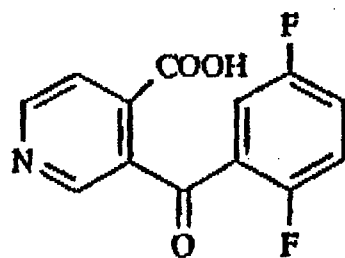
Useful teachings for the removal of the above mentioned protective groups can be found in Green, T.W., Wuts, P.G.M., "Protective Groups in Organic Synthesis", second Edition, John Wiley and sons, (1991).

The compounds of formula (I) in which R is a group of formula $-(CH_2)_p-NH-C(=NH)NH_2$ can be prepared by reaction of a compound of formula (I) in which R is a group of formula $-(CH_2)_p-NH_2$ with the reagent of formula (VI):

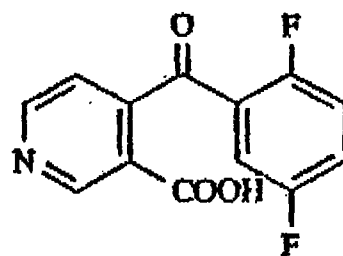


for example following the procedure described in Tetra. Lett. (1988), 29, 3183-3186.

The 6,9-difluorobenzo[g]isoquinoline-5,10-dione of formula (II) may be prepared by a multistep procedure involving the Friedel-Crafts acylation of 1,4-difluorobenzene with pyridine-3,4-dicarboxylic acid anhydride, which results in compounds having the structure according to formulas (VIIa) and (VIIb):



(VIIa)



(VIIb)

Compounds according to formulas (VIIa) and (VIIb) may be then subjected to a cyclization reaction in 30% fuming sulfuric acid at approximately 140°C to give the compound of formula (II).

The compounds of formula (III), (IV) and (V) are known, commercially available or they can be prepared accordingly to known procedures.

For example, the compounds of formula (V) may be prepared according to the procedures described in Synth. Comm., (1990), 20, 2559 and in J. Med. Chem., (1990), 33, 97.

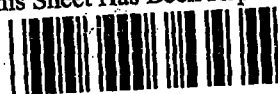
The evaluation of the biological activity for the compounds of this invention was performed "in vitro" and "in vivo" following the protocols developed by the U.S. National Cancer Institute.

The evaluation of the "in vitro" cytotoxic activity of the compounds of the invention was performed using a human colon adenocarcinoma cell line (Lovo) isolated from a metastatic nodule and a subline with acquired resistance to a number of antitumor agents, among which doxorubicin, VP-16 and vincristine. This subline (named Lovo/DX) shows reduced accumulation of doxorubicin and overexpression of a protein (Grandi, M., Geroni, C., Giuliani, F.C., British, J. Cancer, (1986), 54, 515). The compounds were tested according



to the MTT assay (Mosman, T., "Rapid Colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay", J. Immunol. Methods, (1983), 65, 55-63; Green, L.M., "Rapid colorimetric assay for cell viability; application to the quantitation of cytotoxic and growth inhibitory lymphokines", J. Immunol. Methods, (1984), 70, 257-268) in comparison with mitoxantrone, ametantrone and doxorubicin. The data are reported in table VI.

10 In general representative compounds of this invention were equally cytotoxic as ametantrone and mitoxantrone in the Lovo cell line, the highest cytotoxicity (superior to that of mitoxantrone) being shown by the compound 6,9-bis[2-(dimethylamino)ethyl]amino]-
15 benzo[g]isoquinoline-5,10-dione (10a) described in example 4 of the experimental section. When ametantrone or mitoxantrone were tested in the Lovo/DX cell line, a resistance index R.I. (defined as the ratio of the IC_{50} for the resistant cell line to the IC_{50} for the sensible cell line) as high as 101.2 and 29.0 respectively
20 was found, showing that this subline did have an acquired resistance to mitoxantrone and ametantrone. On the other hand, when representative compounds of this invention were tested in the same resistant subline no
25 cross resistance with mitoxantrone and ametantrone occurred, as can be observed for compounds 10a, 12c, 10b, 10e and 10d described in the examples 4, 22, 5, 8 and 7 of the experimental section, respectively. These compounds, in general, showed on this resistant subline the
30 same IC_{50} shown by ametantrone on the sensible Lovo cell line. Compound 10a, in particular, was more active



than mitoxantrone in the Lovo/DX cell line.

These "in vitro" data suggest that representative compounds of this invention may be useful in order to overcome the multidrug resistance mediated mechanism of tumor resistance.

5 The "in vitro" cytotoxic evaluation was also performed on L 1210 murine leukemia cells using the above mentioned cells maintained in suspension cultures (McCoy's 5A medium supplemented with 10% horse serum, glutamine, penicillin and streptomycin) grown in an humidified environment of 10% carbon dioxide and 90% air at 37°C. The compounds were dissolved in dimethylsulfoxide (DMSO) and added to the suspended cells in appropriate concentrations. After 72 hours of continuous exposure, the cell concentration was counted using a Coulter Counter and growth inhibition calculated using the formula:

% growth inhibition = $1 - \left[\frac{\text{cell number treated}}{\text{cell number DMSO alone}} \right] \times 100$.

20 The IC₅₀ was calculated from the growth inhibition data and they are reported in table VII in comparison to the prior art compound 5a (see table I for structure of 5a).

Studies of the biological activity "in vivo" of representative compounds of the invention were performed using the P 388 and L 1210 murine leukemia models.

P 388 murine leukemia cells were intraperitoneally (ip) or intravenously (iv) injected in CD2F1 mice. Treatment was initiated approximately 24 hours after tumor transplantation and dosages of the drug were administered.

nistered ip (P388 ip/ip) or iv (P388 iv/iv) according to preestablished protocols, usually at 3-day (P388 iv/iv) or 4-day (P388 ip/ip) intervals. The studies were done over a 60-day period and the date of death for each animal was recorded. The % T/C was determined using the mean survival time (MST) for each group according to the formula

$$\% \text{ T/C} = [(MST \text{ treated}) / (MST \text{ control})] \times 100$$

For example two representative compounds of this invention, namely 6,9-bis[2-(amino)ethylamino]benzo[g]isoquinoline-5,10-dione 10i as the dimaleate salt (10i maleate) described in example 12 and 6,9-bis[2-(dimethylamino)ethylamino]benzo[g]isoquinoline-5,10-dione 10a described in example 4 showed an activity superior to that of mitoxantrone in the P 388 iv/iv model. Compound 10i of the present invention, both as the hydrochloride (10i.HCl) and the dimaleate salt (10i.maleate) described in example 12, was superior to mitoxantrone also in the P 388 ip/ip model. Moreover the above representative compounds of the invention showed antileukemic activity over a wide range of well tolerated dosages and in particular they were active at dosages which were lower than the maximum tolerated dose, providing indication for a more favourable therapeutic index in comparison to mitoxantrone. The results are shown in table VIII and table IX.

The antitumor activity of representative compounds of this invention was evaluated also in the L 1210 murine leukemia model.

L 1210 leukemia cells were intraperitoneally (ip) injected in CDF1 mice and treatment was initiated ap-



proximately 24 hours after tumor transplantation. Dosages of the drugs were administered ip according to preestablished protocols, usually at 4-day intervals. The studies were done over a 60-day period and the date of death for each animal was recorded. The % T/C was determined using the mean survival time (MST) for each group according to the formula

$$\% \text{ T/C} = [(MST \text{ treated}) / (MST \text{ control})] \times 100$$

The results are shown in table X and XI

From table X the surprisingly superior antileukemic activity of representative compounds of the invention (namely compound 10a of example 4, compound 10i. HCl of example 12 and 6,9-bis[[2-(2-hydroxyethylamino)-ethylamino]benzo[g]isoquinoline-5,10-dione 10l prepared in example 17 (dimaleate salt)) in comparison to the prior art compound 5a is evident.

From table XI a clearly favourable comparison again emerges between the excellent antileukemic activity of compound 10i . HCl of the invention and mitoxantrone.

Activity against solid tumors of the compounds of the invention was demonstrated using the murine Lewis Lung carcinoma and the human mammary carcinoma MX-1 models. These tumor models are enclosed in the panel of eight transplanted tumors used by the U.S.-NCI as screen panel for selection of clinically useful antitumor agents (R.K.Y. Zee Cheng and C.C. Cheng, "Screening and evaluation of anticancer agents", Meth. and Find. Expl. Clin. Pharmacol., (1988), 10(2), 67-101). As an example, table XII shows activity data against these two solid tumors of one representative compound of this



invention, 6,9-bis[[(2-amino)ethyl]amino]benzo[g]iso-quinoline-5,10-dione dimaleate (101, maleate; example 12).

Since representative compounds of this invention
5 show good results against "in vivo" models of murine P 388 and L 1210 leukemias, murine Lewis Lung carcinoma and human mammary carcinoma MX-1, which as noted above are predictive of good results in humans, the compounds disclosed herein are expected to be operative against
10 human leukemias and solid tumors.

The compounds of the present invention may therefore be used as active ingredients of therapeutic compositions to induce regression and/or palliation of cancers in mammals when administered in amounts ranging
15 from about 1 mg to about 0.4 g per kilogram of body weight. A preferred dosage regimen would be from about 1 mg to about 50 mg per kilogram of body weight per day. Unit dosages may be employed so that from about 70 mg to about 3.5 g of the active compound for a subject
20 of about 70 kg of body weight are administered in a 24-hour period. The dosage may be adjusted to be compatible to other treatment regimens, such as radiation therapy.

The pharmaceutical composition may be in the form
25 of tablets, capsules, gel capsules, suppositories, lyophilized powders and solutions for intravenous administration.

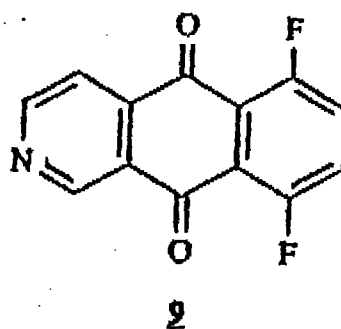
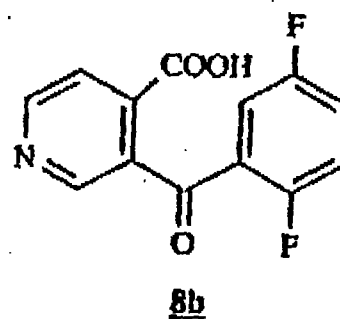
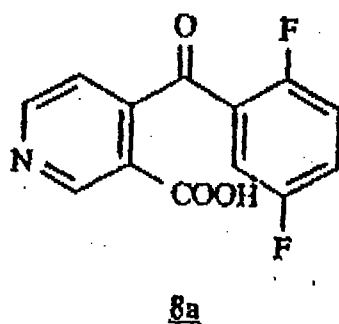
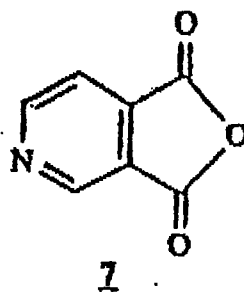
The invention is illustrated by the following non-limiting examples, and variations which are readily apparent to those skilled in the art. The pertinent formulae are illustrated in the tables I, III, IV and V.
30



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REPLACED

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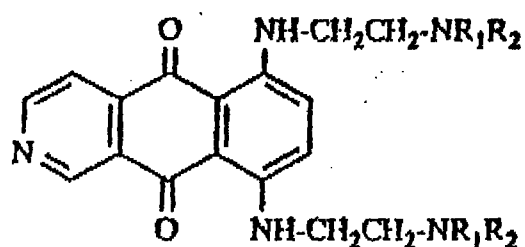
TABLE III



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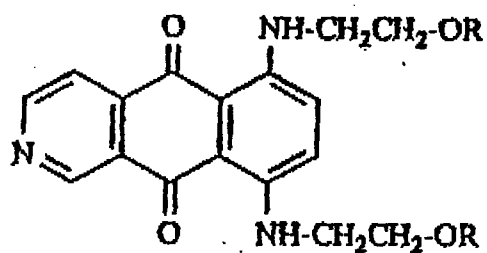
TABLE IV



- 10a, $R = R_1 = \text{CH}_3$
 b, $R = R_1 = \text{CH}_2\text{CH}_3$
 c, $R = R_1 = \text{CH}(\text{CH}_3)_2$
 d, $R = R_1 = -\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$
 e, $R = R_1 = -\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_2-$
 f, $R = R_1 = -\text{CH}_2\text{CH}_2-$
 g, $R = \text{H}, R_1 = \text{CO}_2\text{tBu}$
 h, $R = \text{CH}_3, R_1 = \text{CO}_2\text{tBu}$
 i, $R = R_1 = \text{H}$
 j, $R = \text{H}, R_1 = \text{COCH}_3$
 k, $R = \text{H}, R_1 = \text{CH}_3$
 l, $R = \text{H}, R_1 = \text{CH}_2\text{CH}_2\text{OH}$
 m, $R = \text{H}, R_1 = \text{CH}_2\text{CH}_2\text{OCH}_3$
 n, $R = \text{H}, R_1 = \text{CH}_2\text{CH}_3$
 o, $R = \text{H}, R_1 = \text{CH}_2\text{CH}_2\text{CH}_3$
 p, $R = \text{H}, R_1 = \text{CH}(\text{CH}_3)_2$



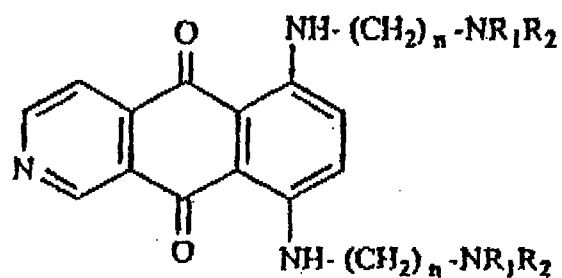
TABLE V



11a, R = H

b, R = SO₂CH₃

c, R = CH₂CH₂OH



12a, n = 3, R = R₁ = H

b, n = 4, R = R₁ = H

c, n = 3, R = R₁ = CH₃



Example 1Pyridine-3,4-dicarboxylic acid anhydride (7)

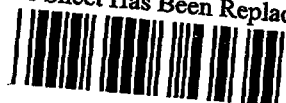
A mixture of pyridine-3,4-dicarboxylic acid (15.0 g, 0.09 mol) and acetic anhydride (30 ml) was refluxed for 2 hours. The excess acetic anhydride was removed by distillation and the anhydride was collected and purified by sublimation (123°C at 3 mm Hg) to yield 7 as a white solid (10.1 g, 76%), m.p. 74-76°C. ¹H NMR (CDCl₃) 9.39 (s, 1H), 9.24 (d, 1H), 7.94 (d, 1H).

Example 24-(2',5'-Difluorobenzoyl)nicotinic acid (8a) and 3-(2'-5'-difluorobenzoyl)isonicotinic acid (8b)

A mixture of 7 (5.0 g, 0.033 mol) and aluminum chloride (17.5 g, 0.131 mol) in 1,4-difluorobenzene was heated in an oil bath at 110°C for 22 hours. The excess of 1,4-difluorobenzene was recovered by distillation. The residue was cooled in an ice bath and quenched with ice water (75 ml) and conc. hydrochloric acid (6.3 ml). The precipitated solid was filtered and dried to yield a white powder (7.7 g, 87%). This material could be crystallized from acetonitrile and water; m.p. 214-217°C; ¹H NMR (DMSO-d₆), 9.15 (s), 8.90 (d), 8.80 (d), 7.90 (d), 7.5 (m), 7.4 (m). Anal. Calcd for C₁₃H₇F₂NO₃: C, 59.32; H, 2.69; N, 5.32. Found: C, 59.03; H, 2.55; N, 5.18.

Example 36,9-Difluorobenzo(g)isoquinoline-5,10-dione (9)

The keto acid mixture of 8a and 8b (3.0 g, 0.011 mol) in fuming sulfuric acid (7.5 ml, 30% SO₃) was heated in an oil bath at 135-140°C for 3 hours. After cooling to room temperature, the mixture was poured into





ice (200 ml) and neutralized with sodium bicarbonate. Extraction with methylene chloride gave 9 as a yellow solid (2.0 g, 72%); m.p. 199-200°C; ^1H NMR (CDCl_3) 9.54 (s, 1H), 9.12 (d, 1H), 8.03 (d, 1H), 7.57 (m, 2H).
5 Anal. Calcd. for $\text{C}_{13}\text{H}_5\text{F}_2\text{NO}_2$: C, 63.67; H, 2.04; N, 5.71. Found: C, 63.86; H, 1.66; N, 5.39.

Example 4

6,9-Bis([2-(dimethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione (10a)

10 A mixture of 6,9-difluorobenzo[g]isoquinoline-5,10-dione, 9 (20 mg, 0.08 mmol) and N,N-dimethylethylenediamine (0.8 mmol, 0.8 ml of a 1 M solution in pyridine) was stirred at room temperature for 48 hours. Most of the pyridine was allowed to evaporate, ether
15 was added, and the product 10a removed by filtration (24 mg, 79%), m.p. 167-168°C; ^1H NMR (CDCl_3) 11.06 (br t, 1H), 10.97 (br t, 1H), 9.63 (s, 1H), 8.92 (d, 1H), 8.13 (d, 1H), 7.32 (m, 2H), 3.54 (q, 4H), 2.7 (t, 4H), 2.38 (s, 12H); mass spectrum m/z (relative intensity)
20 381 (2.0, M^+), 59 (5.4), 59 (100); u.v. λ max (2-methoxyethanol) 580 (sh, 6200), 614 (10,600), 661 (12,700). Anal. Calcd. for $\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_2$: C, 66.12; H, 7.13; N, 18.36. Found: C, 66.22; H, 7.10, N, 18.20.

Example 5

6,9-Bis([2-(diethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione (10b)

25 A solution of N,N-diethylethylenediamine (684 mg, 5.9 mmol) in pyridine (2 ml) was added to 9 (202 mg, 0.82 mmol) in pyridine (2 ml). The purple mixture was
30 stirred at room temperature for 48 hours (TLC analysis at this point showed one blue component: silica gel, 5%

MeOH/CHCl₃). The excess base and pyridine were removed by evaporation with a slow stream of nitrogen gas. The crude material was then placed under vacuum overnight. Upon addition of ice-water to the residue the solid was collected by filtration and dried (330 mg, 84%); m.p. 142-144°C. ¹H NMR (CDCl₃) 11.10 (t, 1H), 11.00 (t, 1H), 9.60 (s, 1H), 8.92 (d, 1H), 8.10 (d, 1H), 7.25 (m, 3H), 3.50 (m, 1H), 2.82 (m, 1H), 2.35 (m, 3H), 1.10 (m, 12H). Anal. Calcd. for C₂₅H₃₅N₅O₂: C, 68.62; H, 16.00; N, 7.31. Found: C, 67.95; H, 16.05; N, 7.28.

Example 6

6,9-Bis[[2-[di(sec-propyl)amino]ethyl]amino]benzo[g]-isoquinoline-5,10-dione (10c)

N,N-diisopropylethylenediamine (588 mg, 4.1 mmol) was added to compound 9 (100 mg, 0.41 mmol) in methanol (1 ml) and water (1 ml). The mixture was allowed to stir at room temperature for 88 h and then poured into ice water. The blue precipitate was recovered by filtration. The solid was purified by column chromatography over silica gel. The initial eluant was chloroform followed by 2% and 20% methanol in chloroform. Concentration of the latter eluants led to 163 mg (81%) of product 10c. mp 134-136°C; ¹H NMR (CDCl₃): 10.95-11.20 (m, 2H), 9.63 (s, 1H), 8.92 (d, 1H), 8.13 (d, 1H), 7.33 (m, 2H), 3.45 (2q, 4H), 3.10 (h, 4H), 2.82 (t, 4H), 1.08 (d, 24H). Anal. Calcd. for C₂₉H₄₃N₅O₂: C, 70.55; H, 8.78; N, 14.19. Found: C, 69.23; H, 8.71; N, 13.43.

Example 7

6,9-Bis[[2-(1'-pyrrolidino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10d)



A solution of 1-(2-aminoethyl)pyrrolidine (690 mg, 6.0 mmol) in pyridine (2 ml) was added to 9 (202 mg, 0.82 mmol) in pyridine (2 ml). The mixture was stirred at room temperature for 49 hours. The excess base and
5 pyridine were removed using a slow stream of nitrogen. The residue was placed under vacuum overnight. Cold water was added to the residue and a tacky solid separated. The product was extracted with chloroform (3 x 35 ml), the extracts dried over sodium sulfate and the
10 chloroform removed by rotary evaporation to yield the title compound (260 mg, 73%); TLC, silica gel, 5% MeOH/CHCl₃, one blue spot; m.p. 141-142°C. ¹H NMR (CDCl₃) 11.15 (t, 1H), 11.00 (t, 1H), 9.65 (s, 1H), 8.90 (d, 1H), 8.05 (d, 1H), 7.35 (m, 2H), 3.65 (m, 4H),
15 2.90 (m, 4H), 2.70 (m, 8H), 1.85 (m, 8H). Anal. Calcd. for C₂₅H₃₁N₅O₂: C, 69.26; H, 7.21; N, 16.15. Found: C, 69.02; H, 7.25; N, 16.00.

Maleate salt. Under a nitrogen atmosphere a solution of maleic acid (271 mg; 2.34 mmol) in ethanol (5
20 ml) was added to a stirred suspension of compound 10d (450 mg; 1.04 mmol) in ethanol (20 ml). After stirring for two hours diethyl ether (25 ml) was slowly added and the precipitate was filtered and dried under vacuum at 40°C to give the hygroscopic dimaleate salt of 10d
25 (580 mg); m.p. 164-166°C.

Anal. Calcd. for C₃₃H₃₉N₅O₁₀ · H₂O: C, 57.96; H, 6.04; N, 10.24. Found: C, 57.85; H, 5.99; N 10.14.

Example 8

6,9-Bis[2-(4'-morpholino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10e)
30

A solution of 4-(2-aminoethyl)morpholine (220 mg,



1.7 mmol) in pyridine (2 ml) was added to 9 (102 mg, 0.42 mmol) in pyridine (1 ml). The purple mixture was stirred at room temperature for 120 hours. The excess amine and pyridine were evaporated using a slow nitrogen stream and the residue placed under vacuum overnight. The mixture was taken up into chloroform and then filtered. Removal of the chloroform left a blue solid which on TLC (silica gel, 25% MeOH/75% CHCl₃/few drops of aq. ammonium hydroxide) showed one blue spot (140 mg, 72%); ¹H NMR (CDCl₃) 11.015 (m, 1H), 10.90 (m, 1H), 9.62 (d, 1H), 8.90 (d, 1H), 8.10 (d, 1H), 7.25 (m, 2H), 3.80 (m, 8H), 3.55 (m, 4H), 2.75 (t, 4H), 2.50 (m, 8H). Anal. Calcd. for C₂₅H₃₁N₅O₄: C, 64.22; H, 6.68; N, 11.98. Found: C, 63.95; H, 6.78; N, 11.89.

Example 9

6,9-Bis[[2-(1'-aziridino)ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (10f)

The procedure of example 4 was repeated reacting compound 9 with N-(2-aminoethyl)aziridine and the residue obtained after evaporation of pyridine was purified by silica gel column chromatography.

A major blue band eluted with 10% CH₃OH in CHCl₃ was collected. Crystallization from a mixture of methylene chloride and ligroine yielded a dark blue solid, m.p. 100-103°C; ¹H NMR (CDCl₃) 11.23 (br, 1H), 11.15 (br, 1H), 9.73 (s, 1H), 8.91 (d, 1H), 8.12 (d, 1H), 7.40 (d, 1H), 7.38 (d, 1H), 3.69 (q, 4H), 2.60 (t, 4H), 1.85 (m, 4H), 1.24 (m, 4H); mass spectrum m/z (relative intensity) 377 (100, M⁺), 278 (72.7), 56 (12.6). Anal. Calcd. for C₂₁H₂₃N₅O₂: C, 66.82; H, 6.14; N, 18.55. Found: C, 66.50; H, 6.00; N, 18.20.



Example 10

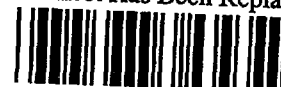
6,9-Bis[[2-(N-(t-butoxycarbonyl)amino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10g)

5 A solution of 9 (0.10 g, 0.41 mmol) and N-(t-butoxycarbonyl)ethylenediamine (Synth. Comm., (1990), 20, 2559; 0.65 g, 4.10 mmol) in pyridine (4.0 ml) was stirred at room temperature for 24 hours. Addition of water (20 ml) led to 10g which was filtered, washed with water and dried (0.17 g, 80%), m.p. 213-216°C. Crystallization from a CHCl₃: CCl₄ mixture gave a dark blue solid m.p. 215-216°C; ¹H NMR (CDCl₃) 11.07 (br, 1H), 10.96 (br, 1H), 9.49 (s, 1H), 8.90 (d, 1H), 7.99 (d, 1H), 7.32 (s, 2H), 5.30 (br, 2H), 3.58 (m, 4H), 3.47 (m, 4H), 1.56 (s, 18H); mass spectrum m/z (relative intensity) 525 (100, M⁺) 339 (51.9), 57 (87.7). Anal. Calcd. for C₂₇H₃₅N₅O₆: C, 61.70; H, 6.73; N, 13.32. Found: C, 61.18; H, 6.29; N, 12.88.

Example 11

20 6,9-Bis[[2-(N-t-butoxycarbonyl-N-methylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10h)

6,9-difluorobenzo[g]isoquinoline-5,10-dione (0.29 g, 1.18 mmol) was added to a stirred solution of N-(t-butoxycarbonyl)-N-methylethylenediamine (0.824 g, 4.73 mmol) (J. Med. Chem. 1990, 33, 97) in dry pyridine (5 ml). The reaction mixture was stirred in a nitrogen atmosphere for 24 hours at room temperature and then for further 8 hours at 50°C. The solvent was removed under reduced pressure and the blue residue obtained was taken up with CH₂Cl₂ (50 ml), washed with a 5% NaHCO₃ solution (2 x 30 ml) and with water (30 ml). The combined organic phases were dried over Na₂SO₄ and the sol-



vent evaporated under reduced pressure.

The blue residue was purified by column chromatography (silica gel 230-400 mesh, 50 g, eluent CH_2Cl_2 : AcOEt: MeOH 93:5:2) yielding 400 mg (61%) of blue crystals, m.p. 161.5 - 162.5°C after recrystallization from CH_2Cl_2 : hexane.

^1H NMR (CDCl_3) 1.49 (s, 18H); 2.94 (s, 6H); 3.45-3.75 (m, 8H); 7.25-7.55 (m, 2H); 8.12 (d, 1H); 8.95 (d, 1H); 9.62 (s, 1H); 10.95-11.23 (m, 2H, exchangeable with D_2O).

Example 12

6,9-Bis[[2-(amino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (101)

From difluoride 9: Under a nitrogen atmosphere 1,2-diaminoethane (8.72 ml; 130.5 mmol) was added to a stirred solution of compound 9 (4.00 g, 16.3 mmol) in pyridine (80 ml). A slightly exothermic reaction ensued that was moderated by cooling in a water bath at 20°C. The reaction mixture was stirred at room temperature for 34 hours, then it was cooled to -5°C for 30 minutes, the solid was separated by suction under a nitrogen flow and dried overnight under vacuum at 30°C. The crude material obtained (6.07 g) was recrystallized by dissolution in methanol (20 ml) at 40°C and reprecipitation with methylene chloride (100 ml) and n-hexane (300 ml) to give 5.12 g of 101 as a blue solid.

^1H NMR (CDCl_3): 11.10-11.30 (2m, 2H), 9.53 (s, 1H), 8.93 (d, 1H), 8.13 (d, 1H), 7.35 (m, 2H), 3.55 (q, 4H), 3.10 (t, 4H).

HPLC Lichrospher 100 RP18, 5 μm ; eluent: sodium heptanesulfonate 2 g/l in H_2O : CH_3CN : dioxane 75:20:5, pH 3



with H_3PO_4 ; $\lambda = 245$ nm; flow 1 ml/min; $R_T = 8.80$ minutes.

5 Maleate salt: Under a nitrogen atmosphere the free base 10i (3.50 g; 10.8 mmol) was dissolved in a mixture of ethanol (170 ml) and methanol (30 ml) by heating at 50°C for 10 minutes, then a solution of maleic acid (2.87 g, 24.7 mmol) in ethanol (30 ml) was added. After heating for five more minutes at 50°C the mixture was allowed to cool to room temperature and then left at 10 4°C for 2 hours. The precipitate was separated by suction under a nitrogen flow and dried at 40°C under vacuum to give 5.75 g of the crude dimaleate.

 To a stirred suspension of this crude material (5.71 g) in water (90 ml) at 50°C ethanol was added 15 dropwise until a complete solution was obtained (about 300 ml), then more ethanol was added (400 ml) and the mixture was allowed to cool to room temperature. After 12 hours the precipitate was separated by suction, washed with ethanol (15 ml) and diethyl ether (10 ml) and 20 then dried under vacuum at 30°C for 5 hours to give 4.22 g of the dimaleate salt of 10i; m.p. 176-178°C shrinking, it does not melt up to 245°C; Anal. Calcd. for $C_{17}H_{19}N_5O_2 \cdot 2C_4H_4O_4 \cdot 1.5 H_2O$: C, 51.37; H, 5.17; N, 11.98. Found: C, 51.31; H 4.99; N 11.93.

25 1H NMR ($DMSO-d_6$) 10.92 (br, 1H), 10.87 (br, 1H), 9.45 (s, 1H), 9.01 (m, 1H), 8.08 (m, 1H), 7.59 (s, 2H), 6.03 (s, 4H), 3.76 (br, 4H), 3.08 (m, 4H). U.V., 1.528 mg in water (50 ml), nm (E 1%): 641 (271), 597 (245), 313 (98), 273 (257), 246 (479).

30 Hydrochloride salt:

a) From amine 10i: Hydrogen chloride gas was bubbled



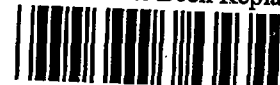
through a solution of the crude amine in chloroform until the solution was no longer blue. The solid was filtered and dried to yield a dark blue hygroscopic solid (0.174 g, 26%), m.p. 209-212°C; ^1H NMR (DMSO- d_6) 10.91 (br, 2H), 9.44 (s, 1H), 9.01 (d, 1H), 8.23 (br, 4H), 8.09 (d, 1H), 7.74 (s, 2H), 3.84 (m, 4H), 3.02 (m, 4H). Anal. Calcd. for $\text{C}_{17}\text{H}_{19}\text{N}_5\text{O}_2 \cdot 2\text{HCl} \cdot 4\text{H}_2\text{O}$; C, 44.94; H, 6.43; N, 15.41. Found: C, 44.52; H, 5.29; N 14.53. The free amine could be regenerated by adding solid potassium carbonate to the NMR sample. A ^1H NMR analysis indicated the presence of the free amine.

b) From deprotection of t-Boc Analogue 10g: Hydrogen chloride gas was bubbled through a solution of 10g (0.160 g, 0.30 mmol) in dry chloroform (10 ml) for 30 minutes. A dark blue solid was filtered and dried (0.115 g, 95%); m.p. 213-215°C whose structure was confirmed as the hydrochloride salt of 10i by ^1H NMR analysis.

Example 13

20 6,9-Bis[[2-(acetylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10j)

The procedure of Example 12 using compound 9 and 1,2-diaminoethane, was repeated and the crude reaction mixture was applied to a silica gel column. Acetic anhydride (30 ml) was added to the column and it was allowed to stand for 15 minutes. A major blue fraction 10j eluted with 1:4 $\text{CH}_3\text{OH}:\text{CHCl}_3$ which was crystallized from a $\text{CHCl}_3:\text{CH}_3\text{OH}$ mixture to yield a blue solid (0.240 g, 35%) m.p. 155-156°C; ^1H NMR (DMSO- d_6) 11.12 (t, 1H), 11.02 (t, 1H), 9.42 (s, 1H), 8.95 (d, 1H), 8.15 (br, 2H), 8.03 (d, 1H), 7.62 (s, 2H), 3.57 (m, 8H), 1.83 (s,





6H); mass spectrum m/z (relative intensity) 409 (2.5, M^+), 337 (6.4), 86 (100). Anal. Calcd. for $C_{12}H_{23}N_5O_4$: C, 61.60; H, 5.67; N, 17.10. Found: C, 61.37; H, 5.42; N, 16.89.

5 Example 14

6,9-Bis[[2-(methylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione trihydrochloride (10k)

Ethanollic HCl (6.7 N, 2.0 ml) was added to a solution of 6,9-bis[[2-(N-t-butoxycarbonyl-N-methylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10h) (440 mg, 0.795 mmol) in $CHCl_3$ (40 ml). The reaction mixture was stirred at room temperature in a nitrogen atmosphere for 24 hours, then the dark green crystals formed were collected by filtration, washed with absolute ethanol and dried at 40°C in vacuo to give 945 mg (94% yield) of pure product, m.p. 188°C dec. (DSC evaluation).

Elemental Analysis Calcd. for $C_{19}H_{26}Cl_3N_5O_2 \cdot 2H_2O$: C, 46.51; H, 6.03; N, 14.14; Cl 23.60. Found: C, 45.75; H 6.06; N, 14.04, Cl 21.32. HPLC (Lichrosper 100 RP18, 5 μ m; Eluent K_2HPO_4 25 mM, n- $C_7H_{15}SO_3Na$ 2 mg/ml in H_2O : CH_3CN : dioxane 80:15:5, pH 2.7 with $HClO_4$; λ 275 nm, flow 1 ml/min., R_T 20.11 minutes.

U.V., 1.07 mg in 20.0 ml of water: nm (E 1%): 641 (290); 597 (267); 313 (106); 273 (278); 247 (486).

1H NMR (D_2O) 2.75 (s, 6H), 3.30-3.45 (m, 4H), 3.80-3.95 (m, 4H), 7.38 (s, 2H); 8.17 (d, 1H), 8.85 (d, 1H), 9.27 (s, 1H).

Example 15

30 6,9-Bis[(2-hydroxyethyl)amino]benzo[g]isoquinoline-5,10-dione (11a)



Difluoride 9 (1.0 g, 4.1 mmol) and 2-aminoethanol (2.5 g, 40.8 mmol) in pyridine (7 ml) were stirred at room temperature for 18 hours. The mixture was poured into water (50 ml) and the product 11a was filtered and dried (1.26 g, 94%), m.p. 236-239°C. Recrystallization from methanol yielded dark blue needles m.p. 237-239°C; ¹H NMR (DMSO-d₆) 11.28 (t, 1H), 11.19 (t, 1H), 9.43 (s, 1H), 8.94 (d, 1H), 8.03 (d, 1H), 7.56 (s, 2H), 5.01 (t, 2H), 3.69 (m, 4H), 3.54 (m, 4H). Anal. Calcd. for C₁₇H₁₇N₃O₄: C, 62.38; H, 5.25; N, 12.83. Found: C, 62.21; H, 5.12; N, 12.50.

Example 16

6,9-Bis[(2-methanesulfonyloxyethyl)amino]benzo[g]isoquinoline-5,10-dione (11b)

A solution of 11a (0.30 g, 0.92 mmol) in dry pyridine (4 ml) was stirred at room temperature under nitrogen for 10 minutes. Methanesulfonyl chloride (0.24 g, 2.07 mmol) was added and the mixture stirred for 20 minutes. The mixture was quenched into ice-water (20 ml) and the solid was filtered. Crystallization from a chloroform-ligroine mixture gave a blue solid (0.294 g, 66%); m.p. 116-118°C; ¹H NMR (CDCl₃) 10.98 (br, 2H), 9.59 (s, 1H), 8.97 (d, 1H), 8.09 (d, 1H), 7.38 (d, 1H), 7.34 (d, 1H), 4.50 (t, 4H), 3.83 (q, 4H), 3.09 (s, 6H). Anal. Calcd. for C₁₉H₂₁N₃O₈S₂: C, 47.20; H, 4.39; N, 8.69. Found: C, 46.80; H, 4.31; N, 8.48.

Example 17

6,9-Bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]benzo[g]isoquinoline-5,10-dione (101)

a) From mesylate (11b)

A solution of (11b) (0.40 g, 0.83 mmol) and 2-



(trimethylsilyloxy)ethylamine (2.21 g, 16.5 mmol) in pyridine (5.0 ml) was stirred at room temperature under a nitrogen atmosphere for 48 hours. The pyridine was removed under a nitrogen flow and the residue was taken up in methylene chloride. This solution was washed with saturated sodium bicarbonate and dried over sodium sulfate. Removal of the solvent left a blue oil which was dried under vacuum overnight. Chromatography on a silica gel column resulted in a cleavage of the Si-O bond to yield 101. This product was eluted with a 1:5:5 Et₃N:CH₃OH:CHCl₃ mixture. Concentration gave 101 as an oil: ¹H NMR (CDCl₃) 11.19 (br, 1H), 11.06 (br, 1H), 9.46 (s, 1H), 8.83 (d, 1H), 7.98 (d, 1H), 6.98 (s, 2H), 3.82 (m, 4H), 3.52 (m, 4H), 3.07 (m, 4H), 2.96 (m, 4H); U.V. (2-methoxyethanol): 570 (sh, ε = 7200), 612 (ε = 13,200), 658 (ε = 14,000).

b) From difluoride (9)

Under a nitrogen atmosphere 2-[(2-hydroxyethyl)-amino]ethylamine, (6.19 ml; 61.2 mmol) was added to a stirred suspension of compound 9 (1.00 g; 4.08 mmol) in pyridine (30 ml) at 0°C.

After one hour the reaction mixture was allowed to reach room temperature and left at this temperature for three hours. The reaction mixture was applied to a silica gel (100 g) column chromatography and eluted first with CHCl₃:CH₃OH 90:10, then with CHCl₃:CH₃OH 85:15 and finally with CHCl₃:CH₃OH:NH₄OH 85:15:1. The fractions containing the product were pooled, the solvents were removed and the residue was subjected to a second purification by silica gel (95 g) column chromatography, eluting with CHCl₃:CH₃OH:NH₄OH conc. from

95:5:0 to 80:20:2. A final purification over grade I basic alumina (25 g) eluting with CH_2Cl_2 : EtOH 96:4 gave a residue of 500 mg that was crystallized from a mixture of ethanol (0.5 ml) and methylene chloride (10 ml) to give 101 (142 mg).

Maleate salt: The free amine 101 (138 mg; 0.334 mmol) was dissolved in ethanol (6 ml) under a nitrogen atmosphere and a solution of maleic acid (87 mg; 0.75 mmol) in ethanol (3 ml) was added at room temperature. After cooling for 3 hours at 4°C the blue crystals obtained were separated by suction under a nitrogen flow, washed with ethanol (1 ml) and diethyl ether (2 ml) and dried under vacuum at 40°C to give the dimaleate salt of 101 (160 mg), m.p. 132-133°C; ^1H NMR ($\text{DMSO}-d_6$) 10.94 (br, 1H), 10.88 (br, 1H), 9.47 (s, 1H), 9.02 (d, 1H), 8.60 (br, 2H), 8.09 (d, 1H), 7.63 (s, 2H), 6.01 (s, 4H), 5.32 (br, 2H), 3.89 (m, 4H), 3.71 (m, 4H), 3.26 (m, 4H), 3.12 (m, 4H). U.V. 1.355 mg in H_2O (25 ml): nm (E 1%) : 641 (220), 598 (208), 312 (85), 272 (221), 246 (406).

Anal. Calcd. for $\text{C}_{21}\text{H}_{27}\text{N}_5\text{O}_4 \cdot 2\text{C}_4\text{H}_4\text{O}_4 \cdot \text{H}_2\text{O}$: C, 52.52; H, 5.57; N, 10.64. Found: C, 52.49; H, 5.56; N, 10.61.

Example 18

6,9-Bis[[2-(2-methoxyethylamino)ethyl]amino]benzo[g]-isoquinoline-5,10-dione (10m)

A solution of 11b (0.15 g, 0.21 mmol) and 2-methoxyethylamine (0.47 g, 6.20 mmol) in pyridine (2.0 ml) was stirred at room temperature under a nitrogen blanket for 48 hours. The pyridine and excess amine were removed under vacuum. The residue was dissolved in methylene chloride, washed with a saturated sodium bi-



carbonate solution and dried over sodium sulfate. Upon removal of the solvent the residue was purified by column chromatography on silica gel by eluting with 1:1 $\text{CH}_3\text{OH}:\text{CHCl}_3$. Crystallization from a ethanol and pentane mixture led to 10m (0.091 g, 66%); m.p. 174-175°C; ^1H NMR (CDCl_3) 11.08 (br, 1H), 10.97 (br, 1H), 9.56 (s, 1H), 8.90 (d, 1H), 8.05 (d, 1H), 7.31 (s, 2H), 3.62 (m, 8H), 3.43 (s, 6H), 3.08 (t, 4H), 2.94 (t, 4H), 2.66 (br s, 2H); mass spectrum m/z (relative intensity) 441 (100, M^+), 354 (57.4), 266 (50.5). Anal. Calcd. for $\text{C}_{23}\text{H}_{31}\text{N}_5\text{O}_4$: C, 62.57; H, 7.09; N, 15.86. Found: C, 62.51; H, 6.92; N, 15.47.

Example 19

6,9-Bis[[2-(2-hydroxyethoxy)ethyl]amino]benzo[g]isoquinoline-5,10-dione (11c)

A solution of 2-(2-aminoethoxy)ethanol (446 mg, 4.25 mmol) in pyridine (1 ml) was added to compound 9 (100 mg, 0.41 mmol) in pyridine (1 ml). The mixture was stirred at room temperature for 45 h. The excess amine and pyridine were removed via a slow stream of nitrogen gas and the residual solid was placed under vacuum overnight. A cold saturated NaCl solution was added to the solid and the product was extracted with chloroform (6 x 25 ml). The extracts were dried over magnesium sulfate and concentrated on the rotary evaporator to yield 140 mg (82%) of product 11c; mp 97-99°C. ^1H NMR (CDCl_3): 11.15-11.35 (2m, 2H), 9.60 (s, 1H), 8.85 (d, 1H), 8.08 (d, 1H), 7.30 (m, 2H), 3.85 (m, 8H), 3.57-3.75 (2m, 9H), 2.75-3.25 (br s, 1H).

Example 20

6,9-Bis[[3-(amino)propyl]amino]benzo[g]isoquinoline-



5,10-dione (12a)

1) Free Amine: A solution of 1,3-diaminopropane (870 mg, 11.76 mmol) in chloroform (3 ml) was added to 9 (300 mg, 1.22 mmol) in chloroform (5 ml). The purple mixture was allowed to stir for 166 hours at room temperature. The mixture was filtered and the salts washed with chloroform. The solvent was removed by rotary evaporation and the residue was placed under vacuum overnight to yield 290 mg (93%) of 12a; TLC (silica gel, 25% MeOH/75% chloroform/few drops of aq. ammonium hydroxide) indicated one blue spot; m.p. 105°C (softens) 112-113°C. ^1H NMR (DMSO- d_6) 11.15 (m, 1H), 11.05 (m, 1H), 9.45 (s, 1H), 8.90 (d, 1H), 8.0 (d, 1H), 7.55 (br s, 2H), 3.55 (br m, 4H), 4.65 (t, 4H), 3.75 (t, 4H), 1.75 (t, 4H). Anal. Calcd. for $\text{C}_{19}\text{H}_{23}\text{N}_5\text{O}_2$: C, 64.57; H, 6.56; N, 19.81. Found: C, 65.00; H, 6.50; N, 19.78.

2) Maleate Salt: A solution of maleic acid (11 mg, 0.86 mmol) in methanol was added to 12a (100 mg, 0.28 mmol) dissolved in methanol (2 ml) and ethyl acetate (2 ml). The addition of more ethyl acetate gave a blue precipitate which was filtered and dried under vacuum (120 mg, 79%), ^1H NMR (DMSO- d_6) 11.10 (t, 1H), 11.00 (t, 1H), 9.45 (s, 1H), 8.95 (d, 1H), 8.05 (d, 1H), 7.75 (br, 4H), 7.60 (s, 2H), 6.0 (s, 4H), 3.60 (m, 4H), 2.95 (t, 4H), 1.95 (m, 4H). Anal. Calcd. for $\text{C}_{23}\text{H}_{27}\text{N}_5\text{O}_6$: C, 58.84; H, 5.80; N, 14.92. Found: C, 58.75; H, 5.70; N, 14.85.

Example 21

30 6,9-Bis[[4-(amino)butyl]amino]benzo[g]isoquinoline-5,10-dione (12b)





- 1) Free Amine: A solution of 1,4-diaminobutane (960 mg, 10.9 mmol) in chloroform (3 ml) was added to 9 (319 mg, 1.3 mmol) in chloroform (6 ml). The purple reaction mixture was stirred at room temperature for 217 hours (9 days). The precipitate salts were removed by filtration and the filtrate concentrated under a slow nitrogen stream to yield the free amine 12b (400 mg, 80%); TLC (Silica gel, 25% MeOH/75% CHCl₃/few drops of aq. ammonium hydroxide); m.p. 80°C, (softens) 90-92°C. ¹H NMR (DMSO-d₆) 11.15 (m, 1H), 11.05 (m, 1H), 9.42 (s, 1H), 8.90 (d, 1H), 8.0 (d, 1H), 7.5 (br s, 2H), 3.45 (m, 4H), 2.60 (m, 4H), 1.70 (m, 4H), 1.45 (m, 4H). Anal. Calcd. for C₂₁H₂₇N₅O₂: C, 66.12; H, 7.13; N, 18.36. Found: C, 64.26; H, 6.95; N, 17.42.
- 2) Maleate Salt: A solution of maleic acid (116 mg, 1 mmol) in ethyl acetate (4 ml) was added to 12b (124 mg, 0.40 mmol) in a methanol (6 ml) and ethyl acetate (8 ml) solution. A blue oil separated and the mixture was placed in the refrigerator overnight. The solvents were removed by decantation and the remaining solid washed thoroughly with ethyl acetate to yield a blue, very hygroscopic solid, 125 mg (63%). ¹H NMR (DMSO-d₆) 11.20 (m, 1H), 11.10 (m, 1H), 9.45 (s, 1H), 8.95 (d, 1H), 8.05 (d, 1H), 7.70 (br s, 4H), 6.00 (s, 4H), 3.55 (m, 4H), 2.85 (m, 4H), 1.70 (m, 8H). Anal. Calcd. for C₂₉H₃₅N₅O₁₀: C, 56.76; H, 5.75; N, 11.41. Found: C, 53.65; H, 6.10; N, 10.05.

Example 22

- 6,9-Bis([3-(dimethylamino)propyl]amino)benzo[g]isoquinoline-5,10-dione (12c)

A solution of 3-dimethylaminopropylamine (700 mg,

6.9 mmol) in pyridine (2 ml) was added to compound 9 (100 mg, 0.41 mmol) in pyridine (2 ml). The mixture was stirred at room temperature for 120 h. The excess diamine and pyridine were removed under a slow stream of nitrogen gas and the residue placed under vacuum overnight. The residue was added to cold water and extracted with chloroform (4 x 20 ml). The extract was dried over Na_2SO_4 and concentrated by rotary evaporation. The blue solid was purified by column chromatography over silica gel. The initial eluant was 10% methanol/90% chloroform followed by 25% methanol/75% chloroform. The product was eluted by addition of small amounts of ammonium hydroxide to 25% methanol/75% chloroform. Concentration of these eluants led to 92 mg (55%) of the desired product 12c mp 107-109°C. ^1H NMR (CDCl_3): 11.00-11.20 (2m, 2H), 9.63 (s, 1H), 8.93 (d, 1H), 8.12 (d, 1H), 7.37 (s, 2H), 3.55 (q, 4H), 2.55 (m, 4H), 2.35 (s, 12H), 2.00 (m, 4H).

Example 23

6,9-bis([2-(ethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione (10n)

A solution of N-ethylethylenediamine (400 mg, 4.5 mmol) in pyridine (1 ml) was added to compound 9 (98 mg, 0.40 mmol) in pyridine (1 ml). The mixture was stirred at room temperature for 66 h. The pyridine and excess diamine were removed under a slow nitrogen stream and the residual material was placed under vacuum overnight. Chloroform was then added and the chloroform washed twice with cold water. The chloroform extract was dried over MgSO_4 and the solvent removed by rotary evaporation. The resultant blue solid was





purified by column chromatography over silica gel using 5% methanol/95% chloroform as the initial eluant. The eluant was gradually changed to 5%, 10% and then 50% methanol in chloroform. The desired product was eluted using 50% methanol/48% chloroform/2% ammonium hydroxide. Removal of the solvent led to 32 mg (21%) of product (10n). mp 101-102°C.

¹H NMR (CDCl₃) 11.10 (m, 1H), 11.00 (m, 1H), 9.6 (s, 1H), 8.9 (d, 1H), 8.05 (d, 1H), 7.3 (m, 2H), 3.55 (m, 4H), 3.0 (t, 4H), 2.8 (q, 4H), 1.15 (t, 6H).

Example 24

6,9-bis[[2-(propylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10o)

A solution of N-propylethylenediamine (403 mg, 4.0 mmol) in pyridine (1 ml) was added to compound 9 (98 mg, 0.40 mmol) in pyridine (1 ml). The reaction mixture was stirred at room temperature for 24 h and concentrated under a slow stream of nitrogen. The residual material was placed under vacuum overnight. Ice water was added to the residual and the aqueous layer was extracted with chloroform (5 x 40 ml). The extracts were dried over magnesium sulfate and the chloroform removed by rotary evaporation. The blue solid was purified by column chromatography over silica gel. The initial eluant was 5% methanol/95% chloroform followed by increasing the methanol amounts gradually to 10%, 20%, 30%, 40% and 50%. The desired compound could be eluted using 60% methanol/40% chloroform containing some ammonium hydroxide. Removal of the eluant led to 50 mg (30%) of the product (10o). mp 105-106°C.

¹H NMR (CDCl₃) 11.15 (m, 1H), 11.05 (m, 1H), 9.6 (s,

1H), 8.9 (d, 1H), 8.10 (d, 1H), 7.3 (m, 2H), 3.6 (m, m, 4H), 3.0 (t, 4H), 2.75 (t, 4H), 1.6 (m, 4H, H₂O peak superimposed), 0.95 (t, 6H).

Example 25

5 6,9-bis[[2-(isopropylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10p)

10 A solution of N-isopropylethylenediamine (450 mg, 4.5 mmol) in pyridine (2 ml) was added to compound 9 (110 mg, 0.45 mmol) in pyridine (1 ml). An instantaneous permanganate-like coloration resulted. The mixture was stirred at room temperature for 40 h. The pyridine and excess diamine were removed under a slow nitrogen stream and the residue was placed under vacuum overnight. Chloroform (20 ml) was added followed by ice water. Since the blue coloration appeared in the aqueous layer, this was extracted with chloroform (4 x 25 ml). The combined extracts were dried over MgSO₄ and the chloroform removed by rotary evaporation. The resultant blue solid was purified by column chromatography over silica gel using chloroform as the initial eluant followed by gradual changes to 2%, 5%, 10%, 20%, 40% and 50% methanol in chloroform. The desired product was eluted from the column with 50% methanol/49% chloroform/1% ammonium hydroxide. Removal of the eluants led to 56 mg (30%) of the desired product (10p). mp 136-137°C.

15 ¹H NMR (CDCl₃) 11.1 (t, 1H), 11.0 (t, 1H), 9.6 (s, 1H), 8.9 (d, 1H), 8.1 (d, 1H), 7.30 (m, 2H), 3.6 (m, 4H), 3.0 (t, 4H), 2.9 (m, 2H), 1.05 (d, 6H).

30 Example 26

In vitro biological evaluation: MTT assay on human co-



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lon adenocarcinoma Lovo cells.

The MTT assay was performed according to Mosmann, T., J. Immunol. Methods, (1983), 65, 55-63 and Green, L. M., J. Immunol. Methods, (1984), 70, 257-268.

5 Immediately before use, compounds and reference standards are dissolved in proper solvent and subsequently further diluted in complete culture medium. Human colon adenocarcinoma cells Lovo and the subline resistant to doxorubicin (Lovo/DX) (2.5×10^4 cells/ml)
10 are plated in 96-well plates and preincubated for 24 hours in culture medium. After this time, the cells are exposed to drugs for 144 hours, then the thiazolyl blue tetrazolium bromide solution (MTT solution) is added and the cells are reincubated for 4 hours. The superna-
15 tant is removed and 150 μ l of dimethylsulfoxide are added to solubilize formazan crystals. The plate is read at 570 nm with microplate reader and the inhibiting concentration of 50% cellular growth (IC_{50} ; μ g/ml) is calculated.

20 The results are reported in Table VI

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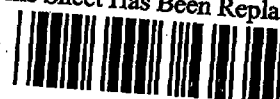
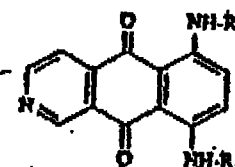




TABLE VI

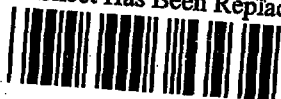
IN VITRO CYTOTOXIC ACTIVITY OF REPRESENTATIVE COMPOUNDS OF
THE INVENTION ON LOVO AND LOVO/DX CELLS BY MTT ASSAY;



COMPOUND	EXAMPLE	R =	IC50 µg/ml ± S.D. a)		
			LoVo	LoVo/Dx	R.I. b)
<u>10a</u>	4		0.045	0.085	1.9
<u>12c</u>	22		0.15 ± 0.07	0.36 ± 0.04	2.4
<u>10b</u>	5		0.29 ± 0.05	0.69 ± 0.1	2.4
<u>10e</u>	8		5.32 ± 1.55	2.29 ± 0.67	0.4
<u>10d</u>	7		0.20 ± 0.03	0.52 ± 0.15	2.6
<u>10i</u> (maleate)	12		0.21 ± 0.08	6.70 ± 1.8	31.8
<u>12a</u>	20		1.64 ± 0.02	20.9 ± 0.72	12.7
Mitoxantrone			0.01 ± 0.009	0.29 ± 0.1	29.0
Anetantrone			0.33 ± 0.1	33.4 ± 13.9	101.2
Doxorubicin			0.03 ± 0.009	4.28 ± 1.8	142.7

a) MTT Assay, 144 hrs drug exposure.

b) R.I. = (IC50 LoVo/Dx)/(IC50 LoVo).



Example 27

In vitro biological evaluation: L1210 murine leukemia

L1210 Murine leukemia cells were routinely maintained in suspension cultures in McCoy's 5A medium supplemented with 10% horse serum glutamine, penicillin and streptomycin and grown in a humidified environment of 10% carbon dioxide and 90% air at 37°C.

To assess the in vitro toxicity, each compound was dissolved in dimethylsulfoxide and added to 1 ml of L1210 cells (10^5 cells/tube) to attain final concentrations of 0.01, 0.1 and 1 μ g of drug/ml of culture. After 72 hours of continuous exposure to the drug, the cell concentration was determined with a Coulter Counter. Growth inhibition was calculated for each drug using the following formula:

$$\% \text{ growth inhibition} = 1 - [\text{cell number treated} / \text{cell number DMSO alone}] \times 100.$$

The growth inhibition data was then used to calculate the IC_{50} value (the calculated drug concentration required to inhibit cell growth by 50% of control).

The results are reported in Table VII.



TABLE VII IN VITRO CYTOTOXIC ACTIVITIES OF REPRESENTATIVE COMPOUNDS OF THE INVENTION AGAINST L1210 LEUKEMIA IN COMPARISON TO THE PRIOR ART COMPOUND 5a

5	Compound	Example	IC ₅₀ (μg/ml)
	<u>10a</u>	4	0.006
	<u>10f</u>	9	0.002
	<u>10i</u> (a)	12	0.05
	<u>10i</u> (b)	17	0.06
10	<u>5a</u> (c)	/	0.155

(a) Hydrochloride Salt: 10i.HCl

(b) Dimaleate Salt: 10i.maleate

(c) Prior art compound, 6,9-bis[2-(dimethylamino)-ethyl]amino]benzo[g]quinoline-5,10-dione: A.P. Krapcho et al., J. Med. Chem. (1985), 28, 1124-1126.

Example 28

In vivo biological studies: P388 Murine Leukemia (iv/iv, d 1, 4, 7)

P388 Murine Leukemia cells were maintained in vivo by serial intraperitoneal (i.p.) injections of 10⁶ cells in DBA2 mice. For test purposes, CDF1 mice were inoculated intravenously (i.v.) with 10⁶ P388 cells and treatment was initiated 24 hr later. The i.v. dose of drug was administered on days 1, 4 and 7. Mice were observed daily for signs of toxicity and survival. The date of death was recorded for each animal that died or was sacrificed during the 60 day study. The median survival time (MST) for each treatment group was calculated and the % T/C was determined using the following formula:

$$\% \text{ T/C} = \{(\text{MST treated})/(\text{MST control})\} \times 100$$

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The results of the antileukemic activity of 6,9-bis[[2-(dimethylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (10a) of example 4 and 6,9-bis[[2-(amino)-ethyl]amino]benzo[g]isoquinoline-5,10-dione dimaleate
5 (10i maleate) of example 12 in comparison to mitoxantrone are reported in table VIII

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Table VIII ANTI-TUMOR ACTIVITY OF COMPOUNDS 10a, 10i, maleate AND MITOXANTRONE
(MITOX) AGAINST P388 MURINE LEUKEMIA (iv/iv 1,4,7)¹⁾

COMPOUND ²⁾	DOSE (mg/kg/day)	T/Cx (range) ³⁾	TOX ⁴⁾	LTS ⁵⁾
Controls	-	100	0/73	0/73
<u>10i</u> , maleate	18 27	200, 188 203(181-227)	0/16 2/32	0/16 0/32
<u>10a</u>	18 27 40	172, 156 188, 167 183, 156	0/14 3/17 1/16	0/14 0/17 0/16
MITOX	2 3 4	162(154-167) 185(167-217) 118(89-133)	0/42 4/78 20/24	0/42 0/78 0/24

6

- 1) CDF1 male mice were transplanted iv with 10 cells/mouse. Treatment was given iv on days 1,4,7 after tumor transplantation (day 0).
- 2) 10i, maleate and MITOX were dissolved in sterile water; 10a in citric acid 1%.
- 3) Median survival time of treated mice/median survival time of controls x 100:
- 4) Number of toxic deaths/total number of mice.
- 5) Long term survivors: animals tumor free at the end of the experiment (60 days).

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Example 29

In vivo biological studies: P388 Murine Leukemia
(ip/ip, d 1, 5, 9)

P388 Murine Leukemia cells were maintained in vivo
5 by serial intraperitoneal (ip) injections of 10^6 cells
in DBA2 mice. For test purposes, CDF1 mice were inocu-
lated i.p. with 10^6 P388 cells and treatment was ini-
tiated 24 hours later. The i.p. dose of drug was admi-
nistered on days 1, 5 and 9. Mice were observed daily
10 for signs of toxicity and survival. The date of death
was recorded for each animal that died or was sacrific-
ed during the 60-day study. The median survival time
(MST) for each treatment group was calculated and the %
T/C was determined using the following formula:

15
$$\% \text{ T/C} = [(MST \text{ treated}) / (MST \text{ control})] \times 100$$

The results of the antileukemic activity of 6,9-
bis[[2-(amino)ethyl]amino]benzo[g]isoquinoline-5,10-
dione (101) as the dihydrochloride (101.HCl) and dima-
leate (101.maleate) salts (example 12) in comparison to
20 mitoxantrone are reported in table IX



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Table IX. ANTITUMOR ACTIVITY OF 101.HCl, 101.maleate AND MITOXANTHONE (MITOX) AGAINST
ASCITIC P388 MURINE LEUKEMIA (ip/ip 1,5,9)

2) COMPOUNDS	DOSE (mg/kg/day)	3) % T/C (range)	4) TOX	5) LTS (day 60)
<u>101.HCl</u>	3.1	144	0/8	0/8
	6.25	196	1/8	0/8
	12.5	239	0/8	0/8
	25	367	0/8	2/8
<u>101.maleate</u>	25	185	0/8	0/8
	37.5	260	1/8	1/8
MITOX	1.5	186(160-225)	6/26	1/26
	3	173(142-205)	21/42	1/42

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- 1) CDF1 mice were injected ip with 10 cells/mouse; treatment was given ip on days 1,5,9 after tumor transplantation (day 0)
- 2) 101.HCl and 101.maleate were dissolved in sterile water and MITOX in saline.
- 3) Median survival time of treated mice/median survival time of controls x 100
- 4) No. of toxic deaths/ total No. of mice
- 5) Long term survivors at the end of the experiment (60 days).

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Example 30

In vivo biological studies: L1210 Murine Leukemia
(ip/ip, d 1, 5, 9)

a) L1210 murine leukemia cells were maintained in vivo by weekly intraperitoneal (ip) injections of 10^6 cells in BDF₁ mice. For test purposes, mice were inoculated i.p. with 10^6 L1210 cells and treatment was initiated 24 hours later. The desired dose of drug was administered on days 1, 5 and 9. Mice were observed daily for signs of toxicity and survival. The date of death was recorded for each animal that died or was sacrificed during the 60-day study. The mean survival time (MST) for each treatment group was calculated and the % T/C was determined using the following formula:

$$\% \text{ T/C} = [(MST \text{ treated}) / (MST \text{ control})] \times 100$$

The results are reported in table X

b) L1210 murine leukemia cells were maintained in vivo by weekly intraperitoneal (ip) injections of 10^5 cells in DBA2 mice. For test purposes, CDF1 mice were inoculated i.p. with 10^5 L1210 cells and treatment was initiated 24 hours later. The desired dose of drug was administered on days 1, 5 and 9. Mice were observed daily for signs of toxicity and survival. The date of death was recorded for each animal that died or was sacrificed during the 60-day study. The median survival time (MST) for each treatment group was calculated and the % T/C was determined using the following formula:

$$\% \text{ T/C} = [(MST \text{ treated}) / (MST \text{ control})] \times 100$$

The results are reported in table XI



TABLE X IN VIVO ANTITUMOR ACTIVITIES OF COMPOUNDS 10a,
- 10i . HCl AND 10l AGAINST L1210 LEUKEMIA (ip/ip, d 1,
5, 9) IN COMPARISON TO THE PRIOR ART COMPOUND 5a.

5

	Compound	Example	Dose ^a	%T/C	LTS ^f
10	<u>10a</u> (b)	4	50	151	-
	<u>10i</u> .HCl (c)	12	25	265	5/6
			12.5	265	4/6
	<u>10l</u> .maleate (d)	17	25	415	1/6
			12.5	313	2/6
15	<u>5a</u> (e), (b)	/	50	129	-
	(a) mg/kg, days 1, 5 and 9				
	(b) Compound <u>10a</u> and <u>5a</u> were dissolved in DMSO (<35%) and water				
	(c) Hydrochloride salt dissolved in water				
20	(d) Dimaleate salt dissolved in water				
	(e) Prior art compound, 6,9-bis[[2-(dimethylamino)- ethyl]amino]benzo[g]quinoline-5,10-dione: A.P. Krapcho et al., J. Med. Chem., (1985), <u>28</u> , 1124-1126.				
25	(f) Long Term Survivors: animals tumor-free at the end of the experiment (60 days)				



Table XI. ANTITUMOR ACTIVITY OF COMPOUND 101.HCl AND MITOXANTHRONE (MITOX) AGAINST
ASCITIC MURINE LEUKEMIA L1210 (ip/ip d.1,5,9)
1)

COMPOUNDS	2) DOSE (mg/kg/day)	3) ± T/C	4) TOX	5) LTS
<u>101</u> .HCl	6.25	156	0/8	0/8
	12.5	175	0/8	1/8
	25	250*	1/7	4/7
MITOX	1.5	188	0/8	1/8
	3	194	1/8	1/8

- 5
- 1) CDF1 mice were injected ip with 10 cells/mouse; treatment was given ip on days 1,5,9 after tumor transplantation (day 0).
 - 2) 101.HCl was dissolved in sterile water and MITOX in saline.
 - 3) Median survival time of treated mice/median survival time of controls x 100
 - 4) No. of toxic deaths/ total No. of mice at the end of the experiment (60 days).
 - 5) Long term survivors: animals tumor free at the end of the experiment (60 days).
- * This value was calculated on dead mice.



Example 31In vivo biological studies: antitumor activity against murine Lewis Lung carcinoma and human MX-1 mammary carcinoma

5 a) Murine Lewis Lung carcinoma

C57bl/6 female mice were trasplantated im (intra-limbs) with 10^5 cells. Treatment was given iv (intravenously) on days 1, 7, 15, after tumor transplantation (day 0). The mean tumor weight for each treatment group was calculated according to Geran, R.I. et al., Cancer Chemother. Rep., (1972), 3, 51-61 and the TWI% was calculated 7 days after the last drug treatment using the formula:

10 TWI% = $100 - [(\text{Mean tumor weight of treated mice}) / (\text{mean tumor weight of controls})] \times 100$.

The results are reported in table XII for the representative compound of the invention 6,9-bis[[2-(amino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (101) as the dimaleate salt (101 maleate) of the example 12.

20 b) Human MX-1 mammary carcinoma

CD1 nu/nu female mice were transplanted sc (subcutaneously) with tumor fragments (about 1 mm x 1 mm x 1 mm). Treatment was given iv once a week for three weeks, when tumor weight reached an average of 150 mg.

25 For all individual tumors the weight change (relative tumor weight) for the start of treatment (V_0) was expressed as V_t/V_0 at each day of measurement (V_t). TWI% was calculated 7 days after the last drug treatment using the formula:

30 TWI% = $100 - [(\text{mean relative tumor weight of treated mice}) / (\text{mean relative tumor weight of controls})] \times 100$.

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The results are reported in table XII for the representative compound of the invention 6,9-bis[[2-(amino)ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (10i) as the dimaleate salt (10i maleate) of the example 12.

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TABLE XII

ACTIVITY ON SOLID TUMORS OF 10i MALEATE

TUMOR MODEL AND TREATMENT SCHEDULE	COMPOUND	OPTIMAL DOSE (mg/Kg/day)	T/C % a)	TWI % b)	TOX c)
LEWIS LUNG im/iv 1,8,15 murine lung carcinoma	<u>10i</u> maleate	40	158	80	0/10
		60	182	95	1/10
MX-1 sc/iv q 7d x 3 human mammary carcinoma	<u>10i</u> maleate	17	-	65	0/7
		26.6	-	58	0/7
		40	-	64	1/5

a) Median survival time of treated mice/median survival time of controls x 100.

b) Percent of tumor Weight Inhibition.

c) Number of toxic deaths/total number of mice.

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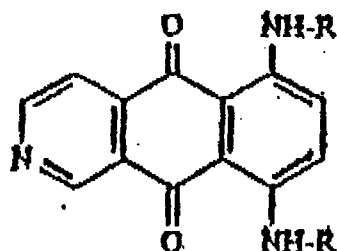
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IN THE CLAIMS:

1. A compound according to formula (I):



(I)

wherein R is

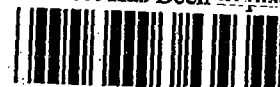
C₁-C₁₀ alkyl; phenyl or C₇-C₁₀ aralkyl;

C₂-C₁₀ alkyl substituted with one or two substituents selected from the group consisting of OR₁ and -NR₂R₃;

C₂-C₁₀ alkyl interrupted by one or two oxygen atoms or by a member selected from the group consisting of -NR₄-, cis -CH=CH-, trans -CH=CH- and -C≡C-, and optionally substituted with one or two hydroxy (OH) or -NR₂R₃ groups; and wherein

R₁ is selected from the group consisting of hydrogen, C₁-C₆ alkyl, phenyl, C₇-C₁₀ aralkyl, -CHO, -COR₅-, -COOR₅, -S(O₂)R₅ and C₂-C₆ alkyl optionally substituted with -NR₂R₃;

R₂ and R₃ are the same or different and are selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₇-C₁₀ aralkyl, phenyl, C₂-C₁₀ alkyl substituted with one or two hydroxy (OH) groups, -CHO, -COR₅, -COOR₅, and -S(O₂)R₅. R₂ and R₃ taken together with the nitrogen atom to which they are bound form an ethyleneimine ring or a 5- or 6-membered aromatic or non-aromatic heterocyclic ring optionally containing another heteroatom selected from the group consisting of sulfur, oxygen and nitrogen, R₂ is H and R₃ is -C(=NH)NH₂ or R₂ is -C(=NH)NH₂ and R₃ is H;





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R_4 is selected from the group consisting of hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} hydroxyalkyl, C_2 - C_{10} alkyl substituted with $-NR_2R_3$, C_7 - C_{10} aralkyl, phenyl, $-COR_5$, $-COOR_5$ and $-S(O_2)R_5$;

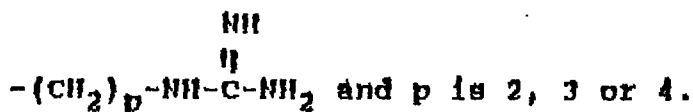
R_5 is selected from the group consisting of C_1 - C_{10} alkyl, C_7 - C_{10} aralkyl, α -, β -, or γ -naphthyl, phenyl, o-, m-, or p-tolyl as free bases and their salts with pharmaceutically acceptable acids.

2. A compound according to claim 1, wherein R is $-(CH_2)_p-NH_2$ and p is 2, 3 or 4.

3. A compound according to claim 1, wherein R is $-(CH_2)_p-NR_2R_3$ and p is 2, 3 or 4 and wherein R_2 and R_3 are a C_1 - C_6 alkyl or taken together with the nitrogen atom form a heterocyclic ring selected from the group consisting of 1-ethylenimine, 1-pyrrolidine, 4-morpholine, 1-piperazine, 4-methyl-1-piperazine, 4-benzyl-1-piperazine and 1-piperidine.

4. A compound according to claim 1, wherein R is $-(CH_2)_p-NR_2R_3$ and p is 2, 3 or 4 and wherein R_2 is hydrogen and R_3 is C_1 - C_6 alkyl.

5. A compound according to claim 1, wherein R is



6. A compound according to claim 1, wherein R is $-(CH_2)_p-NH-(CH_2)_qOH$ and p and q are independently 2, 3 or 4.

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7. A compound according to claim 1, wherein R is $-(CH_2)_p-OH$ and p is 2, 3 or 4.

8. A compound according to claim 1, wherein R is $-(CH_2)_p-O-(CH_2)_q-OH$ and p and q are independently 2, 3 or 4.

9. A compound according to claim 1 selected from the group consisting of:

6,9-bis([2-(amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(4'-morpholino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(dimethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(diethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-[di(sec-propyl)amino]ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(1'-pyrrolidino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(1'-aziridino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(methanesulfonyloxy)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([4-(amino)butyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([3-(amino)propyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-[(2-hydroxyethyl)amino]ethyl]amino)benzo[g]isoquinoline-5,10-dione;

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6,9-bis([3-(dimethylamino)propylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(2-hydroxy)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(2-hydroxyethoxy)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(methylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(ethylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(n-propylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(sec-propylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(guanidino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(2-amino-2,2-dimethyl)ethylamino)benzo[g]isoquinoline-5,10-dione.

10. A compound according to claim 1 selected from the group consisting of:

6,9-bis([2-(amino)ethylamino)benzo[g]isoquinoline-5,10-dione,

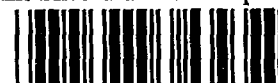
6,9-bis([2-(1'-pyrrolidino)ethylamino)benzo[g]isoquinoline-5,10-dione,

6,9-bis([4-(amino)butylamino)benzo[g]isoquinoline-5,10-dione,

6,9-bis([3-(amino)propylamino)benzo[g]isoquinoline-5,10-dione,

6,9-bis([2-[(2-hydroxyethyl)amino]ethylamino)benzo[g]isoquinoline-5,10-dione, and a salt of any one of said compounds.

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11. A compound according to claim 1 which is 6,9-bis[2-(amino)ethylamino]benzo[g]isoquinoline-5,10-dione or a salt thereof.

12. The compound according to claim 11, wherein said salt is a maleate salt.

13. The compound according to claim 11, wherein said salt is a hydrochloride salt.

14. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically acceptable diluent or excipient.

15. A pharmaceutical composition comprising a compound according to claim 2 and a pharmaceutically acceptable diluent or excipient.

16. A pharmaceutical composition comprising a compound according to claim 3 and a pharmaceutically acceptable diluent or excipient.

17. A pharmaceutical composition comprising a compound according to claim 4 and a pharmaceutically acceptable diluent or excipient.

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18. A pharmaceutical composition comprising a compound according to claim 5 and a pharmaceutically acceptable diluent or excipient.

19. A pharmaceutical composition comprising a compound according to claim 6 and a pharmaceutically acceptable diluent or excipient.

20. A pharmaceutical composition comprising a compound according to claim 7 and a pharmaceutically acceptable diluent or excipient.

21. A pharmaceutical composition comprising a compound according to claim 8 and a pharmaceutically acceptable diluent or excipient.

22. A pharmaceutical composition comprising a compound selected from the group consisting of:

6,9-bis([2-(amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(4'-morpholino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(dimethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(diethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(di(sec-propyl)amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

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6,9-bis([2-(1'-pyrrolidino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(1'-aziridino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(methanesulfonyloxy)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([4-(amino)butylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([3-(amino)propylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-[(2-hydroxyethyl)amino]ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([3-(dimethylamino)propylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(2-hydroxy)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(2-hydroxyethoxy)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(methylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(ethylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(n-propylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(sec-propylamino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(guanidino)ethylamino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(2-amino-2,2-dimethyl)ethylamino)benzo[g]isoquinoline-5,10-dione; and

a pharmaceutically acceptable diluent or excipient.

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23. A pharmaceutical composition comprising a compound selected from the group consisting of:

6,9-bis([2-(amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione,
6,9-bis([2-(1'-pyrrolidino)ethyl]amino)benzo[g]isoquinoline-5,10-dione,
6,9-bis([4-(amino)butyl]amino)benzo[g]isoquinoline-5,10-dione,
6,9-bis([3-(amino)propyl]amino)benzo[g]isoquinoline-5,10-dione,
6,9-bis([2-[(2-hydroxyethyl)amino]ethyl]amino)benzo[g]isoquinoline-5,10-dione, and a salt of any one of said compounds; and a pharmaceutically acceptable diluent or excipient.

24. A pharmaceutical composition comprising 6,9-bis([2-(amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione or a salt thereof and a pharmaceutically acceptable diluent or excipient.

25. The pharmaceutical composition according to claim 24, wherein said salt is a maleate salt.

26. The pharmaceutical composition according to claim 24, wherein said salt is a hydrochloride salt.

27. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 1.

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28. The method according to claim 27, wherein said compound is administered daily in a dosage range of about 1 mg to about 50 mg per kilogram of body weight.

29. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 2.

30. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 3.

31. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 4.

32. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 5.

33. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 6.

34. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 7.

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35. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound according to claim 8.

36. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound selected from the group consisting of:

6,9-bis([2-(amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(4'-morpholino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(dimethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(diethylamino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(di(sec-propyl)amino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(1'-pyrrolidino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(1'-aziridino)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(methanesulfonyloxy)ethyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([4-(amino)butyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([3-(amino)propyl]amino)benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-[(2-hydroxyethyl)amino]ethyl]amino)benzo[g]isoquinoline-5,10-dione;

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6,9-bis([3-(dimethylamino)propylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([(2-hydroxy)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([(2-(2-hydroxyethoxy)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(methylamino)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(ethylamino)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(n-propylamino)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(sec-propylamino)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([2-(guanidino)ethylamino]benzo[g]isoquinoline-5,10-dione;

6,9-bis([(2-amino-2,2-dimethyl)ethylamino]benzo[g]isoquinoline-5,10-dione.

37. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of a compound selected from the group consisting of:

6,9-bis([2-(amino)ethylamino]benzo[g]isoquinoline-5,10-dione,

6,9-bis([2-(1'-pyrrolidino)ethylamino]benzo[g]isoquinoline-5,10-dione,

6,9-bis([4-(amino)butylamino]benzo[g]isoquinoline-5,10-dione,

6,9-bis([3-(amino)propylamino]benzo[g]isoquinoline-5,10-dione,

6,9-bis([2-[(2-hydroxyethyl)amino]ethylamino]benzo[g]isoquinoline-5,10-dione, and a salt of any one of said compounds.

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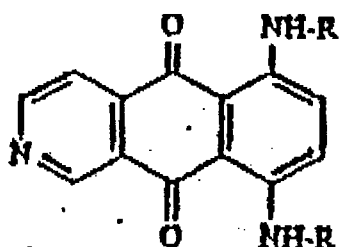
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38. A method of treatment of tumors in a mammal requiring such treatment comprising administration of an effective anti-tumor amount of 6,9-bis((2-(amino)ethyl)amino)benzo[g]isoquinoline-5,10-dione or a salt thereof.

39. The method according to claim 38, wherein said salt is a maleate salt.

40. The method according to claim 38, wherein said salt is a hydrochloride salt.

41. A process for the synthesis of a compound according to Formula I:



(I)

wherein R is

C₁-C₁₀ alkyl; phenyl or C₇-C₁₀ aralkyl;

C₂-C₁₀ alkyl substituted with one or two substituents selected from the group consisting of OR₁ and -NR₂R₃;

C₂-C₁₀ alkyl interrupted by one or two oxygen atoms or by a member selected from the group consisting of -NR₄-, cis -CH=CH-, trans -CH=CH- and -C≡C-, and optionally substituted with one or two hydroxy (OH) or -NR₂R₃ groups; and wherein

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R_1 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, phenyl, C_7-C_{10} aralkyl, $-CHO$, $-COR_5$, $-COOR_5$, $-S(O_2)R_5$ and C_2-C_6 alkyl optionally substituted with $-NR_2R_3$;

R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_7-C_{10} aralkyl, phenyl, C_2-C_{10} alkyl substituted with one or two hydroxy (OH) groups, $-CHO$, $-COR_5$, $-COOR_5$, and $-S(O_2)R_5$, R_2 and R_3 taken together with the nitrogen atom to which they are bound form an ethyleneimine ring or a 5- or 6-membered aromatic or non-aromatic heterocyclic ring optionally containing another heteroatom selected from the group consisting of sulfur, oxygen and nitrogen, R_2 is H and R_3 is $-C(=NH)NH_2$ or R_2 is $-C(=NH)NH_2$ and R_3 is H;

R_4 is selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} hydroxyalkyl, C_2-C_{10} alkyl substituted with $-NR_2R_3$, C_7-C_{10} aralkyl, phenyl, $-COR_5$, $-COOR_5$ and $-S(O_2)R_5$;

R_5 is selected from the group consisting of C_1-C_{10} alkyl, C_7-C_{10} aralkyl, α -, β -, or γ -naphthyl, phenyl, o-, m-, or p-tolyl as free bases and their salts with pharmaceutically acceptable acids, comprising the steps of:

- a) reacting pyridine -3,4-dicarboxylic anhydride with 1,4-difluorobenzene;
- b) cyclizing the product of step a) in fuming sulfuric acid at elevated temperature; and
- c) displacement of fluoride by addition of amine of formula R^1-NH_2 , wherein R^1 is the same as that defined by R.

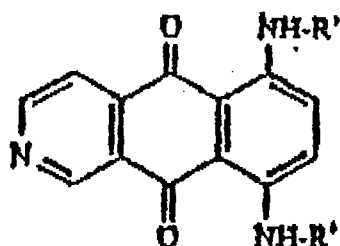




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42. The process according to claim 41, wherein a compound obtained in step (c) has the formula (Ia):



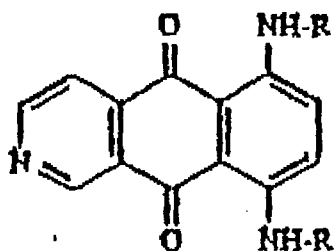
(Ia)

and wherein R' has a meaning other than that defined by R and further comprising the step of converting R' to R.

43. The process according to claim 41, further comprising the step of converting R' in the compound obtained in step (c) to another compound defined by R.

44. The process according to claim 41, further comprising the step of subjecting the compound obtained in step (c) to salification and/or solvation or separating isomers thereof.

45. A process for the synthesis of a compound according to formula I:



(I)

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wherein R is

C_1 - C_{10} alkyl; phenyl or C_7 - C_{10} aralkyl;

C_2 - C_{10} alkyl substituted with one or two substituents selected from the group consisting of OR_1 and $-NR_2R_3$;

C_2 - C_{10} alkyl interrupted by one or two oxygen atoms or by a member selected from the group consisting of $-NR_4-$, cis $-CH=CH-$, trans $-CH=CH-$ and $-C\equiv C-$, and optionally substituted with one or two hydroxy (OH) or $-NR_2R_3$ groups; and wherein

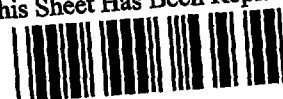
R_1 is selected from the group consisting of hydrogen, C_1 - C_6 alkyl, phenyl, C_7 - C_{10} aralkyl, $-CHO$, $-COR_5$, $-COOR_5$, $-S(O_2)R_5$ and C_2 - C_6 alkyl optionally substituted with $-NR_2R_3$;

R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen, C_1 - C_{10} alkyl, C_7 - C_{10} aralkyl, phenyl, C_2 - C_{10} alkyl substituted with one or two hydroxy (OH) groups, $-CHO$, $-COR_5$, $-COOR_5$, and $-S(O_2)R_5$, R_2 and R_3 taken together with the nitrogen atom to which they are bound form an ethylenimine ring or a 5- or 6-membered aromatic or non-aromatic heterocyclic ring optionally containing another heteroatom selected from the group consisting of sulfur, oxygen and nitrogen, R_2 is H and R_3 is $-C(=NH)NH_2$ or R_2 is $-C(=NH)NH_2$ and R_3 is H;

R_4 is selected from the group consisting of hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} hydroxyalkyl, C_2 - C_{10} alkyl substituted with $-NR_2R_3$, C_7 - C_{10} aralkyl, phenyl, $-COR_5$, $-COOR_5$ and $-S(O_2)R_5$;

R_5 is selected from the group consisting of C_1 - C_{10} alkyl, C_7 - C_{10} aralkyl, α -, β -, or γ -naphthyl, phenyl, o-, m-, or p-tolyl as free bases and their salts with pharmaceutically acceptable acids, comprising the step of:

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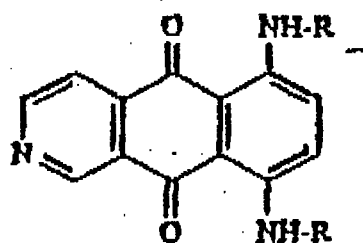
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reacting a compound of formula (I), wherein R is $-(CH_2)_p-OS(O_2)R_5$ and wherein R_5 is as previously defined and p is 2, 3 or 4, with a compound having the formula $H_2N-(CH_2)_q-O-E$, wherein E is selected from the group consisting of trialkylsilane, (dialkyl)arylsilane, formyl and acetyl, and q is 2, 3 or 4.

46. The process according to claim 45, further comprising the step of removing the E group.

47. A process for the synthesis of a compound according to formula I:



(I)

wherein R is

C_1-C_{10} alkyl; phenyl or C_7-C_{10} aralkyl;
 C_2-C_{10} alkyl substituted with one or two substituents selected from the group consisting of OR_1 and $-NR_2R_3$;
 C_2-C_{10} alkyl interrupted by one or two oxygen atoms or by a member selected from the group consisting of $-NR_4-$, cis $-CH=CH-$, trans $-CH=CH-$ and $-C\equiv C-$, and optionally substituted with one or two hydroxy (OH) or $-NR_2R_3$ groups; and wherein

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R_1 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, phenyl, C_7-C_{10} aralkyl, $-CHO$, $-COR_5$, $-COOR_5$, $-S(O_2)R_5$ and C_2-C_6 alkyl optionally substituted with $-NR_2R_3$;

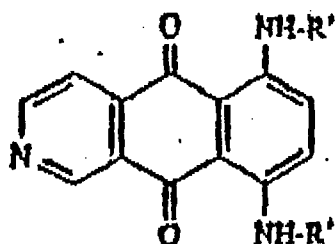
R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_7-C_{10} aralkyl, phenyl, C_2-C_{10} alkyl substituted with one or two hydroxy (OH) groups, $-CHO$, $-COR_5$, $-COOR_5$, and $-S(O_2)R_5$, R_2 and R_3 taken together with the nitrogen atom to which they are bound form an ethyleneimine ring or a 5- or 6-membered aromatic or non-aromatic heterocyclic ring optionally containing another heteroatom selected from the group consisting of sulfur, oxygen and nitrogen, R_2 is H and R_3 is $-C(=NH)NH_2$ or R_2 is $-C(=NH)NH_2$ and R_3 is H;

R_4 is selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_2-C_{10} hydroxyalkyl, C_3-C_{10} alkyl substituted with $-NR_2R_3$, C_7-C_{10} aralkyl, phenyl, $-COR_5$, $-COOR_5$ and $-S(O_2)R_5$;

R_5 is selected from the group consisting of C_1-C_{10} alkyl, C_7-C_{10} aralkyl, α -, β -, or γ -naphthyl, phenyl, o-, m-, or p-tolyl as free bases and their salts with pharmaceutically acceptable acids, comprising the steps of:

a) reacting 6,9-difluorobenzo[g]isoquinoline-5,10-dione with a compound having the formula $H_2N-(CH_2)_p-N(COOR_5)R_3$,

wherein R_3 is hydrogen or a C_1-C_6 alkyl, R_5 is as previously defined and p is 2, 3 or 4 to obtain a compound having the formula:



(Ia)





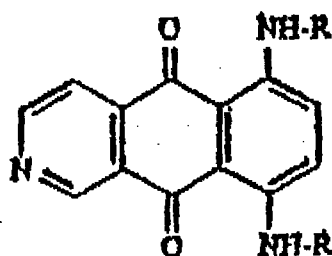
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wherein R' is $-(CH_2)_p-N(COOR_5)R_3$;

b) removing the group $COOR_5$.

48. A process for the synthesis of a compound according to formula I:



wherein R is

C_1 - C_{10} alkyl; phenyl or C_7 - C_{10} aralkyl;

C_2 - C_{10} alkyl substituted with one or two substituents selected from the group consisting of OR_1 and $-NR_2R_3$;

C_2 - C_{10} alkyl interrupted by one or two oxygen atoms or by a member selected from the group consisting of $-NR_4-$, cis $-CH=CH-$, trans $-CH=CH-$ and $-C\equiv C-$, and optionally substituted with one or two hydroxy (OH) or $-NR_2R_3$ groups; and wherein

R_1 is selected from the group consisting of hydrogen, C_1 - C_6 alkyl, phenyl, C_7 - C_{10} aralkyl, $-CHO$, $-COR_5$, $-COOR_5$, $-S(O_2)R_5$ and C_2 - C_6 alkyl optionally substituted with $-NR_2R_3$;

R_2 and R_3 are the same or different and are selected from the group consisting of hydrogen, C_1 - C_{10} alkyl, C_7 - C_{10} aralkyl, phenyl, C_2 - C_{10} alkyl substituted with one or two hydroxy (OH) groups, $-CHO$, $-COR_5$, $-COOR_5$, and $-S(O_2)R_5$; R_2 and R_3 taken together with the nitrogen atom to which they are bound form an ethyleneimine ring or a 5- or 6-membered aromatic or non-aromatic heterocyclic ring

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optionally containing another heteroatom selected from the group consisting of sulfur, oxygen and nitrogen, R_2 is H and R_3 is $-C(=NH)NH_2$ or R_2 is $-C(=NH)NH_2$ and R_3 is H;

R_4 is selected from the group consisting of hydrogen, C_1 - C_{10} alkyl, C_2 - C_{10} hydroxyalkyl, C_2 - C_{10} alkyl substituted with $-NR_2R_3$, C_7 - C_{10} aralkyl, phenyl, $-COR_5$, $-COOR_5$ and $-S(O_2)R_5$;

R_5 is selected from the group consisting of C_1 - C_{10} alkyl, C_7 - C_{10} aralkyl, α -, β -, or γ -naphthyl, phenyl, o -, m -, or p -tolyl as free bases and their salts with pharmaceutically acceptable acids, comprising the step of:

reacting a compound of formula (I), wherein R is $(CH_2)_p-NH_2$ and p is 2, 3 or 4 with a compound having the formula $H_2N-C(=NH)SO_3H$.

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