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<p>(21) International Application Number: PCT/GB98/01522 (22) International Filing Date: 26 May 1998 (26.05.98) (30) Priority Data: 9710698.3 24 May 1997 (24.05.97) GB (71) Applicant (for all designated States except US): VERKAIK, Margaretha, Sophia, Elizabeth [GB/GB]; Culdees, Fortingall, By Aberfeldy, Perthshire PH15 2LG (GB). (71)(72) Applicant and Inventor: ANAND, Chaman, Lal [GB/GB]; 34 Vorlich Gardens, Bearsden, Glasgow G61 4QY (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): STIMSON, William, Howard [GB/GB]; 7 Lawn Park, Fairways, Milngavie, Glasgow G62 6HG (GB). GRAY, Alexander, Irvine [GB/GB]; 48 Lochinver Drive, Cathcart, Glasgow G44 3NL (GB). (74) Agent: MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES</p>		
<p>(57) Abstract</p> <p>The invention provides compositions comprising uscharin and the use of uscharin to combat cell proliferation for example in the treatment of cancer. Administration of uscharin may kill or reduce the growth rate of cancer cells and may also be of application in other medical conditions presenting symptoms of excessive or uncontrolled cell proliferation. The composition may be administered by any convenient route and formulated accordingly. The composition may be administered locally or generally and may be suitably dissolved and/or suspended in a pharmaceutically acceptable liquid carrier medium.</p>		

1 PHARMACEUTICAL COMPOSITION CONTAINING USCHARIDIN OR ITS ANALOGUES

2

3 This invention relates to a composition comprising the
4 cardenolide glycoside uscharin.

5

6 Plants of the family *Asclepidaceae* are known to be
7 extremely poisonous. Such plants have a history of use
8 in folk medicines in those areas where they occur
9 naturally, for example in South East Asia and Africa.

10 Two of the best known representatives of the
11 *Asclepiadaceae* are *Calotropis gigantea* and *Calotropis*
12 *procera*. Extracts from *Calotropis procera* plants have
13 traditionally been used as an abortifacient, for
14 infanticide, for rheumatic pain and to produce a
15 purgative.

16

17 The stems, flowers and leaves of plants from the family
18 *Asclepiadaceae* (including *Calotropis gigantea* and
19 *Calotropis procera*) are known to contain certain
20 compounds known as cardenolides. In several species
21 substantial amounts of cardenolides have been found to
22 be concentrated in the latex (Roeske et al, in
23 *Biochemical Interactions Between Plants and Insects*
24 published in Volume 10 of *Recent Advances in*

1 Phytochemistry, Plenum Press, New York (ed. Wallace),
2 Seiber et al, Phytochemistry 21:2343 (1982), Seiber et
3 al, in Isopentoids in Plants, Academic Press (ed Nes,
4 1984) and Seiber et al, in J. Chem. Ecol. 6:321
5 (1980)). The natural production of cardenolides in
6 *Asclepias curassavia* has been reported by Groeneveld
7 et al in Phytochemistry 29(11):3479-3486 (1990).
8 Examples of cardenolide glycosides found in *C. procera*
9 are voruscharin, uscharin, uscharidin, calotropin,
10 calactin, calotoxin, and calotropagenin. Formula I
11 shows the chemical structure of these cardenolides.
12

1 It has now been found that the cardenolide uscharin is
2 particularly useful for medical purposes. Whilst
3 uscharin has been isolated and its chemical structure
4 determined, no utility for this compound has previously
5 been reported.

6
7 The present invention thus provides a composition
8 comprising uscharin, the analogues and salts thereof as
9 active ingredient together with a pharmaceutically
10 acceptable carrier or excipient.

11
12 Further, the present invention also provides the use of
13 uscharin, the analogues and salts thereof for medical
14 (including veterinary) purposes.

15
16 Previously, certain cardenolide glycosides such as
17 calotropin and uzarigenin have been noted to have
18 cytotoxic activity against primate tumour cells.
19 Certain cardenolide glycosides from the *Asclepiadaceae*
20 family share structural and pharmacological
21 similarities with the *Digitalis* cardiac glycosides.
22 Whilst we do not wish to be bound by theoretical
23 considerations it is believed that the cytotoxicity of
24 some cardenolide glycosides is related to the
25 inhibition of the plasma membrane bound Na^+/K^+ ATPase
26 (ie analogous to the manner in which *Digitalis* cardiac
27 glycosides exert their toxic effects). However, it has
28 also been shown that whilst some cardenolide glycosides
29 are cytotoxic to cell cultures they have no in vivo
30 tumour-inhibiting activity. This is true of calotropin
31 and uzarigenin.

32
33 It has never previously been proposed that uscharin
34 would be useful for medical applications. The
35 inventors' results have shown that at 1mg/ml a primary

1 extract of *Calotropis gigantea* known as CGE-1 does have
2 tumour inhibiting activity in rats (weighing about
3 200g) and does not lead to the death of the test
4 animals.

5
6 Typically, the use of uscharin according to the present
7 invention is to combat cell proliferation for example
8 in the treatment of cancer. Thus administration of
9 uscharin may kill or reduce the growth rate of cancer
10 cells and may also be of application in other medical
11 conditions presenting symptoms of excessive or
12 uncontrolled cell proliferation.

13

14 The word "combat" is used herein to refer to treatment
15 of an existing condition so as to alleviate or reverse
16 the symptoms of the condition in an affected human or
17 animal and to prevent such a condition in a healthy
18 human or animal.

19

20 The composition according to the present invention may
21 be administered by any convenient route and mention may
22 be made of enteral, parenteral, topical administration
23 and the composition will be formulated accordingly.
24 Conveniently, the composition may be administered
25 locally to the affected site, generally by means of
26 injection. Thus the uscharin will be suitably
27 dissolved and/or suspended in a pharmaceutically
28 acceptable liquid carrier medium, which will generally
29 be aqueous-based, for example an isotonic solution.
30 Alternatively, the composition according to the
31 invention may be taken orally.

32

33 Formulations for parenteral administration include
34 aqueous and non-aqueous isotonic sterile injection
35 solutions which may contain anti-oxidants, buffers,

1 bacteriostats and solutes which render the formulation
2 isotonic with the blood of the intended recipient; and
3 aqueous and non-aqueous sterile suspensions which may
4 include suspending agents and thickening agents. The
5 formulations may be presented in unit-dose or multi-
6 dose sealed containers, for example, ampoules and
7 vials, and may be stored in a freeze-dried
8 (lyophilized) condition requiring only the addition of
9 the sterile liquid carrier, for example water for
10 injections, immediately prior to use. Extremoraneous
11 injection solutions and suspensions may be prepared
12 from sterile powders, granules and tablets of the kind
13 previously described.

14
15 The dose will depend on a number of factors known to
16 the skilled physician including the severity of the
17 conditions, the identity of the recipient; and also the
18 efficacy and toxicity of the particular composition
19 which is being administered. Generally doses in the
20 range 0.1-100 mg/kg body weight may be used,
21 particularly 1-10 mg/kg. The frequency of
22 administration will vary depending on the rate of
23 metabolism or excretion of the administered compound,
24 but may be repeated daily, optionally as two or more
25 sub-doses. Unit doses of 20 to 500 mg, preferably 100
26 to 400 mg may be used.

27
28 A single dosage may be given daily or smaller
29 quantities or dosage units may be given at intervals
30 throughout a 24 hour period, for example dosage units
31 given 2, 3 or 4 times throughout the day.

32
33 Any type of cancer or condition involving cell
34 proliferation may be treated by the present invention.
35 Uscharin is especially useful for the treatment of

1 cancers such as leukaemia, non-small cell lung cancer,
2 small cell lung cancer, colon cancer, CNS cancer,
3 melanoma, ovarian cancer, renal cancer, prostate
4 cancer, and breast cancer. However the invention is
5 not limited to treatment of these specific conditions
6 since uscharin is believed to be of general effect.

7

8 Cancers where uscharin is particularly efficacious
9 include ovarian cancer and skin cancer.

10

11 Uscharin may be produced by any convenient method, for
12 example by chemical synthesis. Alternatively the
13 uscharin may be conveniently extracted and purified
14 from organisms (for example plants of the family
15 *Asclepiadaceae*) which produce uscharin naturally. It
16 is also envisaged that uscharin may be manufactured
17 using genetically engineered micro-organisms, plants or
18 animals or may be made using cell-culture or other
19 biotechnological techniques.

20

21 Further, the present invention also provides the use of
22 a composition as described above for medical purposes,
23 for example to combat conditions in which cell
24 proliferation is undesirable (eg cancer).

25

26 In another aspect, the present invention provides the
27 use of uscharin in the manufacture of a medicament.
28 Generally such medicament would be of use to combat
29 cancer and other conditions where cell proliferation is
30 undesirable.

31

32 In a further aspect, the present invention provides a
33 method of treatment of a human or non-human animal
34 body, said method comprising administering to said body
35 a composition as described above.

1 The present invention is now further described by means
2 of the following, non-limiting Examples.

3

4 EXAMPLE 1

5

6 PREPARATION OF USCHARIN EXTRACT

7

8 (i) ISOLATION OF CGE-1

9

10 Leaves of *Calotropis gigantea* (500g) were Soxhlet
11 extracted initially with petroleum ether (60-80), then
12 ethyl acetate and finally methanol. The cell culture
13 bioassays showed that the ethyl acetate fraction
14 contained cytotoxic activity. The ethyl acetate
15 extract was subjected to vacuum liquid chromatography
16 (VLC) on silica gel 60H (Merck). Elution was initiated
17 with petroleum ether (60-80) and proceeded with
18 petroleum ether containing progressively greater
19 amounts of ethyl acetate through to ethyl acetate only.
20 Elution was then continued with ethyl acetate
21 containing progressively greater amounts of methanol.

22

23 Samples of the fraction were collected and prepared for
24 cytotoxicity testing by solubilisation in 0.1% Tween.

25

26 The greatest cytotoxic activity ($ED_{50} < 0.10\mu\text{g/ml}$) was
27 found in the 70-80% ethyl acetate in petroleum ether
28 fractions. The cytotoxic compound CGE-1 (72.0 mg)
29 ($ED_{50} < 0.09\mu\text{g/ml}$) was isolated as a white semi-
30 crystalline precipitate from this fraction.

31

32 (ii) ISOLATION OF CGE-2

33

34 Another less cytotoxic compound, CGE-2 (101.0mg) (ED_{50}
35 $< 8.0\mu\text{g/ml}$) was isolated from the 100% ethyl acetate

1 fraction as a semi-crystalline precipitate.

2

3 (iii) PROPERTIES OF CGE-1

4

5 White powder, found 587.2511, $C_{31}H_{41}NO_8S$ requires

6 587,2553. $[\alpha]_D + 10.0^\circ$ (c.0.1, CH_3OH_4) IR

7 $V_{max} \text{ CM}^{-1}$: 3465, 2960, 2920, 2840, 2720, 1735, 1730,

8 1705, 1625, 1540, 1160, 1110, 1060, 1040. EIMS m/z

9 (rel. int.) 587 [M⁺] (4.0), 233 (14.9), 215 (8.6), 187

10 (9.8), 183

11

12 ACTIVITY OF CGE-1

13

14 At a concentration of 1 mg/ml, CGE-1 has a tumor

15 inhibiting activity in rats weighing approximately 200g

16 and does not lead to the death of the rat.

17

18 CGE-1 was found to contain Uscharin.

19

20 EXAMPLE 2

21

22 Isolation of Uscharin from *Calotropis Gigantea* leaves.

23

24 EXTRACTION

25

26 The plant material was minced to a fine powder in a

27 bench grinder. The powder was extracted in a Soxhlet

28 with petroleum ether (60-80) and the ethyl acetate,

29 until exhaustion. The ethyl acetate fraction was

30 concentrated to dryness using a rotary evaporator.

31

32 FRACTIONATION

33

34 Vacuum Liquid Chromatography was used for the initial

35 fractionation of the crude extract Silica gel 60H

1 (Merck) was packed in a scintered funnel under vacuum
2 to give a compact column. The crude extract, adsorbed
3 in silica, was applied to the column. Elution was
4 initiated with petroleum ether and proceeded with
5 petroleum ether containing progressively greater
6 amounts of ethyl acetate than with ethyl acetate
7 through to methanol. The fractions were concentrated
8 using a rotary evaporator. 10 mg of each fraction were
9 prepared for cytotoxicity testing (see MTT assay for
10 method) by solubilisation in DMSO. The fraction
11 containing the greatest cytotoxic activity was
12 subjected to a sephadex column to remove any remaining
13 chlorophyll.
14

15 SEPHADEX COLUMN

16
17 The fraction was dissolved in a minimum volume of
18 chloroform and applied to a column containing
19 lipophilic sephadex LH-20 (Sigma) which had been packed
20 in chloroform. Elution was with chloroform, chloroform
21 with methanol and methanol. As before fraction were
22 dried and tested for activity. The fraction with the
23 greatest activity was further fractionated with a
24 silica gel column.
25

26 SILICA GEL COLUMN

27
28 The fraction was dissolved in a minimum volume of
29 chloroform and applied to a column containing silica
30 gel (packed in chloroform). Elution was with
31 chloroform, chloroform with methanol and methanol.
32 This column yielded a fraction of almost pure uscharin.
33 The pure compound was obtained from this fraction by
34 preparative TLC.
35

1 **PREPARATIVE TLC**

2

3 The fraction was spotted onto glass silica gel plates.
4 The plates were run in ethyl acetate and methanol
5 (97:3). The silica was scratched from the plate and
6 the uscharin eluted with ethyl acetate.

7

8 Once the compound had been isolated, its identity was
9 confirmed by spectroscopic techniques.

10

11 EXAMPLE 3

12

13 CYTOTOXICITY BIOASSAY OF USCHARIN

14

15 Cytotoxicity bioassays were performed. The cell line
16 used was a human ovarian small cell carcinoma SCC Wm
17 1(151) which was grown as a monolayer in Dulbecco's
18 Modified Eagles Medium (Gibco) supplemented with 5%
19 foetal calf serum (v/v), sodium pyruvate (1mM),
20 penicillin (50IU/ml) and streptomycin (50µg/ml).
21 Cultures were maintained in a humidified atmosphere of
22 5% CO₂/95% air at 37°.

23

24 Single cell suspensions were obtained by trypsinisation
25 of the monolayer cultures and an equal number of cells
26 (10³-10⁴ depending on the cell line) was inoculated into
27 each 33mm² well of a 96 well plate in 190µl of culture
28 medium. The plates were incubated for 24 hours to
29 allow cells to adhere. At this point 10µl of an
30 appropriate concentration of plant extract or control
31 solvent was added to each well. The cells were exposed
32 to the drug for 3 days after which the medium was
33 removed, the monolayers washed with PBS and fresh
34 medium added. This was repeated 24 hours later.
35 Following a further 24 hours incubation 100µg (50µl of

1 2mg/ml in PBS) MTT (3-(4,5 dimethylthiazol-2-yl)-2, 5-
2 diphenyltetrazolium bromide) was added to each well and
3 the cells were incubated at 37°C for 4 hours. Plates
4 were then processed using a modified version
5 (Carmichael et al, 1987) of the assay first described
6 by Mossman, T.(1983), where DMSO was used in preference
7 to acid isopropanol to solubilise the formazan
8 crystals. The contents of each well were mixed and the
9 plate was read immediately at 540nm on a Flow Titertek
10 Multiscan MCC/340 Mk 11 plate reader. Cells were set
11 up in parallel at two densities, 10^3 and 2×10^3
12 cells/well, and the results from an assay were
13 discarded if the ratio of the OD readings of the two
14 densities was greater than 2.25:1 or less than 1.75:1.

15

16 The results obtained were as shown in Fig. 1

17

18 EXAMPLE 4

19

20 IN VITRO SCREENING OF USCHARIN

21

22 Uscharin was obtained as in Example 2 and was subjected
23 to in vitro cell screening at the National Cancer
24 Institute (NCI), USA in respect of a panel of cancer
25 cell types organised into subpanels representing
26 leukemia, lung cancers, colon cancer, cancer of the
27 central nervous system, melanoma, ovarian cancer, renal
28 cancer, and in some cases prostate cancer and breast
29 cancer also.

30

31 The standard NCI methodology which was employed is
32 described in Michael R Boyd, Principles and Practices
33 of Oncology, Vol. 3, No. 10 (Oct. 1989) and Monks A. et
34 al., Journal of the National Cancer Institute, Vol. 83,
35 No. 11, (5th June, 1991).

1 The results of two separate screening experiments
2 carried out using uscharin are given in Tables 1 and 2.

3

4 The data are derived from Dose-Response Curves and two
5 typical curves for leukemia and colon cancer are given
6 for illustrative purposes in Figures 1 and 2 attached
7 hereto.

8

9 The Dose-Response Curve is created by plotting the
10 Calculated Percent Growth (PG) of each cell line
11 against the $\log_{(10)}$ of the corresponding drug
12 concentration. The cell line curves are grouped by
13 cell type, or subpanel. Mean $\log_{(10)}$ concentrations for
14 all cell lines tested are calculated at three points:
15 where the test compound achieved 50% inhibition of cell
16 growth (GI_{50}), where the test compound achieved 0% cell
17 growth or total growth inhibition (TGI), and where the
18 test compound achieved 50% cell kill or 50% lethal
19 concentration (LC_{50}). Reference lines are shown at the
20 percent growth values of +50 (GI_{50}), 0 (TGI) and -50
21 (LC_{50}).

22

23 Percentage Growth (PG) - of the compound on a cell line
24 is currently calculated according to one of the
25 following expressions:

26

27 If $(\text{Mean OD}(\text{test}) - \text{Mean OD}(\text{tzero})) \geq 0$, then

28

29
$$PG = 100 \times (\text{Mean OD}(\text{test}) - \text{Mean OD}(\text{tzero})) / (\text{mean}$$

30
$$\text{OD}(\text{ctrl}) - \text{Mean OD}(\text{tzero}))$$

31

32 If $(\text{Mean OD}(\text{test}) - \text{Mean OD}(\text{tzero})) < 0$, then $PG = 100 \times$
33 $(\text{Mean OD}(\text{test}) - \text{Mean OD}(\text{tzero})) / \text{Mean OD}(\text{tzero})$

34

35

1 Where:

2

3 Mean OD (tzero) = The average of optical density
4 measurements of SRB-derived colour
5 just before exposure of cells to
6 the test compound.

7

8 Mean OD (test) = The average of optical density
9 measurements of SRB-derived colour
10 after 48 hours with no exposure of
11 cells to the test compound.

12

13 Mean OD (ctrl) = The average of optical density
14 measurements of SRB-derived colour
15 after 48 hours with no exposure of
16 cells to the test compound.

17

18 It is clear from the results given in Tables 1 and 2
19 that uscharin has an inhibitory effect on the growth of
20 a wide variety of cancer cell lines in vitro.

21

22 EXAMPLE 5

23

24 IN VITRO SCREENING OF USCHARIDIN

25

26 Uscharidin was also subjected to in vitro cell
27 screening in the manner described in Example 4.

28 Results are given in Table 3 and Figure 3, and these
29 show that Uscharidin also exerts an inhibitory effect
30 on a variety of cancer cell lines in vitro.

31

1 EXAMPLE 6

2

3 IN VITRO SCREENING OF CALOTOXIN

4

5 Calotoxin was also subjected to in vitro cell screening
6 in the manner described in Example 4. Results are
7 given in Table 4 and Figure 4, which show that
8 calotoxin also exerts an inhibitory effect on a variety
9 of cancer cell lines in vitro.

10

11 EXAMPLE 7

12

13 IN VITRO EXPERIEMENT WITH USCHARIN IN NUDE MICE

14

15 The SCCI cells (human tumour cell line) where grown (1
16 x 10⁵/ml seeding density) in 25 ml RPMI 1640 (10% foetal
17 calf serum, 5% glutamine) in 75 cm² tissue culture
18 flasks. The cells were harvested at log growth phase
19 (5 days approximately) and washed once in saline before
20 injection into the mice.

21

22 The "nude" mice (BALB/c nude) are reared and contained
23 within a sealed isolator. The mice were injected with
24 1 x 10⁷ cells subcut on the back, right hand side near
25 the shoulder blades. After 7 days the mice were split
26 randomly into the study groups (10-15 animals per
27 group). Each was then treated with a different regime,
28 the variable being time between injections and dose of
29 drug at each injection, control groups were also
30 included in the overall pla of the experiement.

31

32 During the trial a daily check was made on the animals
33 and any animal removed if the tumour size became too
34 large (>5-7% total body weight) or if the animal is
35 showing signs of distress. Additional to this the

1 tumour should be assessed every 3-4 days by an
2 independent observer and the result recorded. Once an
3 animal is removed from the study the tumour size,
4 volume and weight was determined and the tumour stored
5 for further cytological study. The reason for the
6 animals removal from the study was also recorded, if
7 this was not due to tumour size. The results are shown
8 in the following tables.

9

Using nude mice injected with 10^7 SCC-1 cells injected
on day 0 and drug treatment started on day 9.

GROUP NO. 1

0.1 mg CGE-1/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm ³)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	27	4356.4	1.7492	64.8	22.41	1
B	55	-	NONE	-	-	5
C	30	4141.3	2.5658	85.5	45.28	1
D	30	299.8	1.8196	60.7	52.24	1
E	37	2752.8	1.5783	42.7	33.37	1
F	55	-	NONE	-	-	5
G	55	-	NONE	-	-	5
H	55	-	NONE	-	-	5
I	33	3414.9	1.8805	57.0	28.69	1
J	55	-	NONE	-	-	5
K	37	828.9	0.6773	18.3	8.19	2
L	27	2223.8	1.6854	62.4	48.92	1
M	27	1556.2	0.7728	28.6	5.45	1
N	27	3457.9	1.9394	71.8	52.94	1
O	55	-	NONE	-	-	5
MEAN		2559.11	1.6298	54.64	33.05	
S.D.		1437.34	0.5844	21.20	18.29	

GROUP NO. 2

0.1 mg CGE-1/ Animal/ 10 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm ³)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	27	2993.1	2.0570	76.2	49.92	1
B	55	-	NONE	-	-	5
C	55	-	NONE	-	-	5
D	55	-	NONE	-	-	5
E	55	664.8	0.4333	7.9	17.91	5
F	55	3148.8	2.0378	37.1	16.96	5
G	55	134.4	0.1285	2.3	8.17	5
H	55	-	NONE	-	-	5
I	55	-	NONE	-	-	5
J	55	-	NONE	-	-	5
K	55	-	NONE	-	-	5
L	55	-	NONE	-	-	5
M	26	2025.9	1.3238	50.9	6.90	3
N	30	1548.8	1.2677	42.3	10.79	1
O	30	544.1	0.3827	12.8	25.29	4
MEAN		1579.99	1.0901	32.79	19.42	
S.D.		1201.27	0.7933	26.68	14.9	

GROUP NO. 3

0.5 mg CGE-1/ Animal/ 5 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm ³)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE	-	-	5
B	55	219.6	0.2082	3.8	18.18	5
C	55	-	NONE	-	-	5
D	19	1494.7	1.1889	62.6	2.33	3
	19	203.2	0.0948	5.0	-	
E	19	-	NONE	-	-	3
F	23	3912.0	2.5341	110.2	13.13	1
G	28	4463.2	2.5717	91.8	23.42	1
H	37	-	NONE	-	-	2
I	28	1666.5	1.0930	39.0	12.96	1
J	19	23.7	0.0038	0.2	-	3
K	33	1457.9	1.2546	38.0	19.22	1
L	29	1532.5	0.8926	30.8	12.49	1
M	29	2972.3	1.6348	56.4	17.79	1
N	37	537.9	0.4997	13.5	9.70	2
O	37	-	NONE	-	-	2
MEAN		1848.36	1.1976	45.12	14.36	
S.D.		1504.32	0.8738	36.61	6.18	

GROUP NO. 4

0.5 mg CGE-1/ Animal/ 10 days

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm ³)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	28	1482.1	1.1211	40.0	28.48	1
B	27	3499.1	2.5087	92.9	32.54	1
C	42	1930.3	1.4088	33.5	13.58	1
D	42	2177.3	1.5067	35.9	17.14	1
E	55	-	NONE	-	-	5
F	27	6882.3	3.1626	117.1	42.37	1
G	33	760.9	0.7467	22.6	50.31	1
H	55	-	NONE	-	-	5
I	55	-	NONE	-	-	5
J	55	-	NONE	-	-	5
K	55	64.5	0.1127	2.0	17.78	5
L	29	-	NONE	-	-	2
M	55	-	NONE	-	-	5
N	23	4929.6	2.6126	113.6	37.52	1
O	55	-	NONE	-	-	5
MEAN		2715.76	1.6475	57.2	29.97	
S.D.		2272.64	1.0344	44.08	13.18	

GROUP NO. 5

CONTROL (0.1 ml Saline/ Animal/ 5 days)

MOUSE	TUMOUR					
	DAY REMOVED	VOL. (mm ³)	WEIGHT (g)	RATE (mg/D)	NECROTIC (%)	REASON
A	55	-	NONE	-	-	5
B	55	-	NONE	-	-	5
C	55	-	NONE	-	-	5
D	55	-	NONE	-	-	5
E	23	4570.9	2.4227	105.3	35.2	1
F	50	3138.3	1.9475	39.0	4.43	1
G	55	-	NONE	-	-	5
H	55	-	NONE	-	-	5
I	3	-	NONE	-	-	3
J	23	5493.0	3.1602	137.4	59.07	1
K	28	2500.7	1.8958	67.7	6.68	1
L	28	3246.9	1.9716	70.4	31.86	1
M	55	-	NONE	-	-	5
N	28	4120.3	2.2965	82.0	46.07	1
O	55	-	NONE	-	-	5
MEAN		3845.02	2.2707	83.63	30.55	
S.D.		1093.88	0.4797	34.01	21.59	

1 NOTES:-

2 REASONS:

3

4 (1) Removed due to tumour size.

5 (2) Removed due to another illness.

6 (3) Found dead in cage.

7 (4) Removed because the tumour was about to rupture.

8 (5) Removed at end of the experiment.

9

TABLE 5

Table 5 gives a summary of the results.

	Tumour Growth (mg/day)	% Necrosis*	% Mortality at 40 days
Group 1 (0.1mg/5 days)	54.6 ± 21.1	33.1 ± 18.3	84
Group 2 (0.1mg/10 days)	32.8 ± 26.7	19.4 ± 14.9	55
Group 3 (0.5mg/5 days)	45.1 ± 36.6	14.4 ± 6.2	90
Group 4 (0.5mg/10 days)	57.2 ± 44.1	30.0 ± 13.2	62
Control	83.6 ± 34.0	30.6 ± 21.6	100

* from histological examination

Values are means ±SD, n=15

From these results it can be seen that a reduction in percentage mortality due to the cancer cells of up to 45% can be achieved by administration of the compound of the invention (Uscharin).

1 CLAIMS

2

3 1. A composition comprising uscharin or analogues or
4 salts thereof as active ingredient together with a
5 pharmaceutically acceptable carrier or excipient.

6

7 2. The use of uscharin, analogues or salts thereof
8 for medical (including veterinary) purposes.

9

10 3. The use of uscharin as claimed in the preparation
11 of a medicament.

12

13 4. A composition as claimed in Claim 1 or 2 wherein
14 the uscharin is suspended or dissolved in an
15 acceptable liquid carrier medium.

16

17 5. A composition as claimed in Claim 4 wherein the
18 carrier medium is aqueous based.

19

20 6. A use as claimed in Claims 2 or 3 wherein 0.1-100
21 uscharin per kg body weight is used.

22

23 7. A method of treatment of a human or non-human
24 animal body, said method comprising administering
25 to said body a composition comprising uscharin.

26

27 8. A method as claimed in Claim 7 wherein a unit dose
28 of composition comprises between 20 and 500 mg
29 uscharin.

30

31

National Cancer Institute Developmental Therapeutics Program

In-Vitro Testing Results

(PCT/GB 9 8 / 0 1 5 2 2)

NSC: D- 654033 -O/1	Experiment ID: 9207SC89	Test Type: 8	Units: Molar
Report Date: September 8, 1992	Test Date: July 20, 1992	QNS:	MC:
COMI: Uscharin	Stain Reagent: Dual-Pass	SSPL: OCXW	

Panel/Cell Line	Time	Ctrl	Mean Optical Densities				Percent Growth				GI50	TCI	LC50		
			-6.0	-7.0	-6.0	-3.0	-4.0	-6.0	-7.0	-6.0				-3.0	-4.0
Leukemia															
CCRF-CEM	0.279	0.993	0.912	0.166	0.134	0.134	0.124	89	-41	-52	-52	-56	1.99E-08	4.66E-08	6.73E-07
HL-60 (TB)	0.357	1.228	1.324	0.102	0.104	0.100	0.102	111	-71	-71	-72	-71	2.16E-08	4.08E-08	7.43E-08
N-362	0.120	0.825	0.904	0.132	0.085	0.104	0.111	111	4	-30	-13	-7	3.74E-08	1.33E-07	>1.00E-04
MOLT-4	0.490	1.377	1.463	0.194	0.163	0.131	0.137	90	-60	-67	-69	-31	1.83E-08	3.93E-08	3.93E-08
AFM1-8224	0.343	1.374	1.330	0.114	0.284	0.316	0.274	97	-24	-46	-42	-49	2.43E-08	4.34E-08	>1.00E-04
SP	0.348	1.430	1.379	0.138	0.227	0.094	0.130	84	-60	-64	-73	-57	1.73E-08	3.83E-08	8.48E-08
Non-Small Cell Lung Cancer															
A549/ATCC	0.381	1.457	1.393	0.133	0.074	0.109	0.094	95	-65	-60	-71	-74	1.91E-08	3.92E-08	8.04E-08
ERXV	1.134	1.728	1.790	0.417	0.244	0.332	0.162	111	-47	-79	-70	-66	2.43E-08	3.06E-08	1.28E-07
HOP-18															
HOP-62	0.864	1.702	1.699	0.208	0.035	0.018	0.014	100	-76	-96	-96	-98	1.92E-08	3.70E-08	7.12E-08
HOP-92	0.636	0.957	0.970	0.354	0.269	0.214	0.173	104	-13	-58	-66	-73	2.69E-08	7.75E-08	6.75E-07
MCI-H226	0.919	1.321	1.347	0.372	0.199	0.220	0.093	110	-38	-78	-76	-90	2.46E-08	3.37E-08	2.01E-07
MCI-H223	0.316	1.407	1.301	0.047	0.080	0.137	0.230	77	63	-84	-70	-52	1.47E-08	3.02E-08	6.21E-08
MCI-H222H	0.364	1.480	1.519	0.620	0.461	0.349	0.273	104	6	-18	-38	-52	3.37E-08	1.79E-07	7.60E-08
MCI-H460	0.177	1.224	1.181	0.030	0.013	-0.007	0.018	96	-83	-93	-100	-90	1.80E-08	3.43E-08	6.32E-08
MCI-H222	0.478	0.783	0.729	0.130	0.044	0.068	0.098	88	-73	-91	-86	-80	1.73E-08	3.33E-08	7.23E-08
LSPL 829	0.456	1.483	1.493	0.054	0.019	0.012	0.013	101	-88	-96	-97	-97	1.88E-08	3.41E-08	6.27E-08
Small Cell Lung Cancer															
DM8 114	0.440	1.308	0.710	0.204	0.100	0.158	0.116	31	-	-77	-64	-74	<1.00E-08	3.74E-08	3.12E-07
DM8 273	0.256	1.331	1.342	-0.001	-0.012	0.013	0.016	101	-100	-100	-93	-94	1.79E-08	3.18E-08	3.64E-08
Colon Cancer															
COLO 203	0.277	1.284	1.213	0.300	0.087	0.188	0.091	93	2	-69	-32	-67	2.98E-08	1.08E-07	3.68E-08
DLD-1	0.133	0.866	0.844	0.035	0.026	0.012	0.030	87	-77	-82	-82	-80	1.86E-08	3.98E-08	6.96E-08
HCC-2998	0.306	0.817	0.901	0.316	0.022	0.004	0.010	118	4	-93	-99	-97	4.03E-08	1.13E-07	3.68E-07
HCT-116	0.231	1.376	1.263	0.094	0.016	0.021	0.069	90	-60	-93	-87	-71	1.83E-08	3.98E-08	6.33E-08
HCT-15	0.318	1.790	1.881	0.073	0.072	0.037	0.060	104	-77	-77	-96	-81	2.03E-08	3.81E-08	7.16E-08
HCT9	0.248	1.271	1.312	0.221	0.031	0.046	0.038	107	-11	-60	-61	-64	3.03E-08	8.05E-08	3.70E-07
HML2															
HML202	0.264	1.047	1.054	0.152	0.012	0.008	0.007	101	-43	-93	-97	-97	2.27E-08	5.05E-08	1.38E-07
SW-620	0.329	1.324	1.299	0.179	0.077	0.134	0.133	98	-22	-66	-41	-42	2.31E-08	6.97E-08	3.61E-07
CNS Cancer															
SF-268	0.496	1.240	1.209	0.338	0.049	0.045	0.093	82	-32	-90	-90	-81	1.92E-08	3.27E-08	2.06E-07
SF-295	0.703	1.321	1.336	0.409	0.301	0.151	0.078	102	-42	-87	-76	-89	2.30E-08	5.12E-08	3.39E-07
SF-329	0.846	1.793	1.702	0.304	0.061	0.093	0.113	90	-64	-93	-89	-87	1.83E-08	3.83E-08	8.11E-08
SNB-19	0.856	1.894	1.910	1.028	0.434	0.358	0.337	102	-16	-47	-30	-41	4.03E-08	1.81E-07	4.43E-08
SNB-75	0.384	0.864	0.811	0.372	0.303	0.414	0.380	82	3	-10	-27	-33	2.33E-08	1.60E-07	>1.00E-04
SNB-78	0.937	1.083	1.118	0.474	0.424	0.403	0.363	103	-13	-24	-27	-33	2.86E-08	7.50E-08	>1.00E-04
U251	0.269	1.179	1.224	0.062	0.016	0.006	0.018	103	-77	-94	-96	-93	2.00E-08	3.78E-08	7.11E-08
XF 498	0.469	0.713	0.710	0.162	0.042	0.014	0.013	99	-66	-91	-97	-97	1.98E-08	3.99E-08	8.04E-08
Melanoma															
LOX IMVT	0.256	1.363	1.346	0.016	-0.001	0.017	0.014	91	-94	-100	-94	-94	1.78E-08	3.25E-08	3.91E-08
MALME-3M	0.644	1.259	1.233	0.235	0.124	0.064	0.069	99	-64	-	-90	-89	2.01E-08	4.08E-08	8.26E-08
M14	0.333	1.147	1.160	0.240	0.038	0.033	0.004	99	-28	-49	-90	-99	2.44E-08	6.03E-08	2.31E-07
M19-MEL	0.284	1.126	1.134	0.368	0.094	0.144	0.144	100	12	-67	-49	-49	3.69E-08	1.42E-07	3.69E-08
SK-MEL-2	0.370	1.337	1.322	0.280	0.091	0.078	0.075	96	-51	-84	-86	-87	2.05E-08	4.30E-08	9.98E-08
SK-MEL-28	0.234	0.542	0.608	0.278	0.198	0.170	0.093	113	8	-22	-35	-64	4.03E-08	1.60E-07	3.56E-08
SK-MEL-5	0.483	1.805	1.896	0.249	0.300	0.179	0.114	99	-49	-59	-43	-72	2.11E-08	4.69E-08	1.38E-07
UACC-237	0.734	2.040	2.117	0.872	0.483	0.463	0.344	104	11	-34	-37	-53	3.86E-08	1.72E-07	6.40E-08
UACC-62	0.314	1.714	1.649	0.463	0.103	0.163	0.093	93	-10	-80	-68	-82	2.67E-08	8.04E-08	3.73E-07
Ovarian Cancer															
IGROV1	0.444	1.377	1.422	0.510	0.237	0.302	0.320	103	7	-42	-32	-28	3.63E-08	1.39E-07	>1.00E-04
OVCA9-3	0.634	1.189	1.228	0.280	0.324	0.296	0.237	109	-57	-51	-55	-61	2.27E-08	4.53E-08	9.03E-08
OVCA9-4	0.417	1.051	0.974	0.048	0.011	0.003	0.004	88	-88	-97	-99	-97	1.64E-08	3.13E-08	6.03E-08
OVCA9-5	0.346	0.848	0.832	0.043	0.017	-0.009	0.012	101	-88	-95	-100	-97	1.86E-08	3.42E-08	6.31E-08
OVCA9-6	0.413	1.794	1.799	0.330	0.092	0.063	0.206	101	-14	-63	-90	-67	2.79E-08	7.40E-08	3.22E-07
SK-OV-3	0.483	1.165	1.097	0.480	0.172	0.251	0.322	90	-1	-64	-48	-	2.75E-08	9.72E-08	3.73E-07
Renal Cancer															
786-0	0.274	1.093	1.062	0.027	0.011	0.008	0.027	96	-90	-96	-97	-90	1.77E-08	3.28E-08	4.09E-08
A498	0.418	1.360	1.348	0.872	0.718	0.344	0.341	98	34	13	-12	-45	5.66E-08	3.38E-08	>1.00E-04
ACHN	0.412	1.349	1.204	0.130	0.024	0.020	0.049	83	-44	-94	-89	-83	1.68E-08	3.27E-08	7.38E-08
CARI-1															
RXF-393	0.856	1.266	1.203	0.413	0.391	0.499	0.866	84	-28	-34	-42	-22	2.02E-08	5.81E-08	3.22E-07
RXF-631															
SN12C	0.239	1.333	1.331	0.022	0.034	0.070	0.040	100	-91	-85	-92	-83	1.83E-08	3.34E-08	8.11E-08
TA-10	0.430	1.037	1.064	0.227	0.042	0.040	0.088	102	-	-94	-91	-86	3.38E-08	1.10E-07	3.58E-07
VO-31	0.789	1.347	1.424	0.713	0.482	0.484	0.807	114	-9	-41	-41	-23	3.30E-08	8.40E-08	>1.00E-04

TABLE 1A

ISSPL: UCXW Exp. ID: 9207SC8

Units: Molar

Report Date: September 8, 1992

Test Date: July 20, 1992

Report Date: September 8, 1992

Mean Graphs

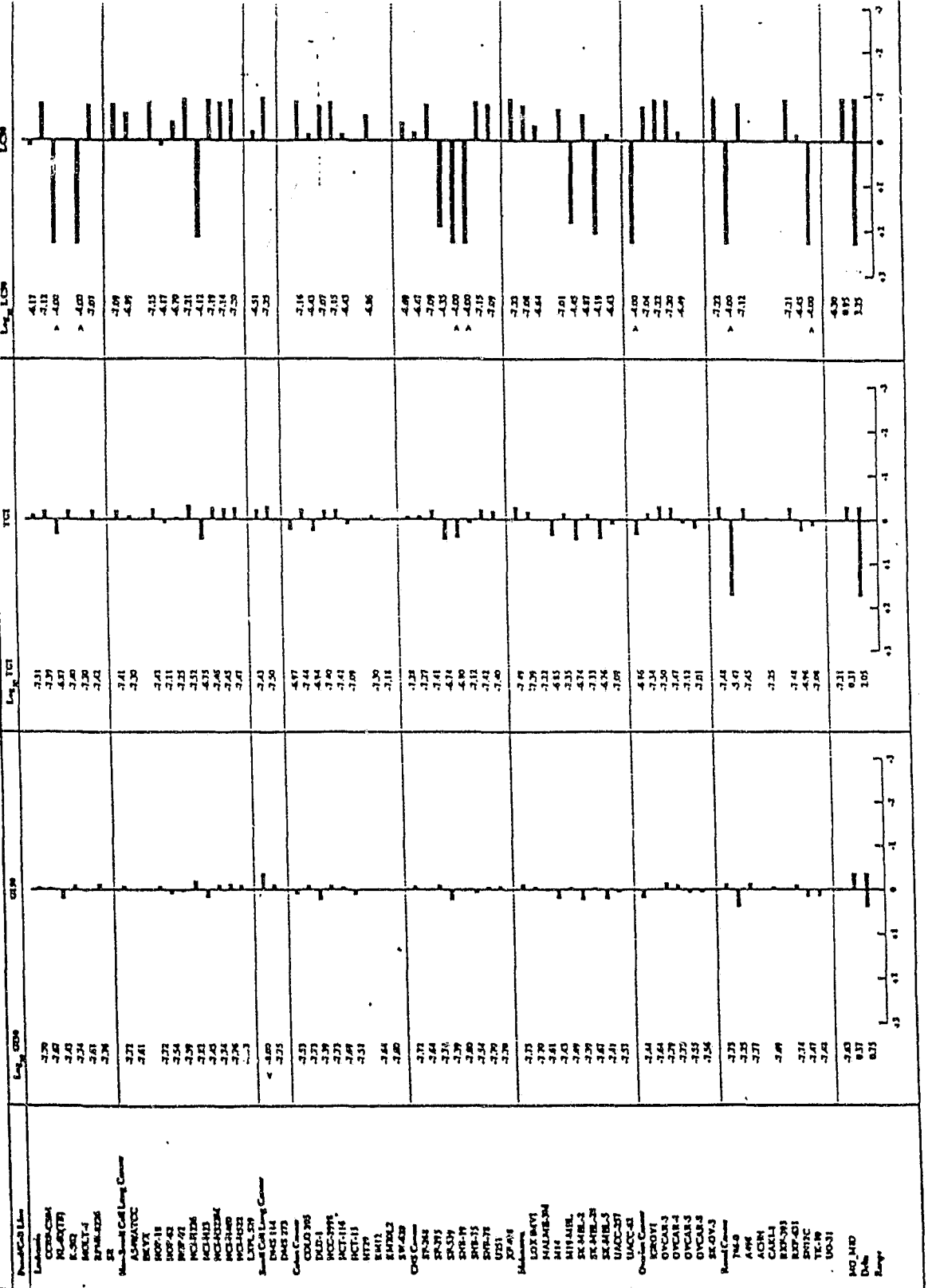
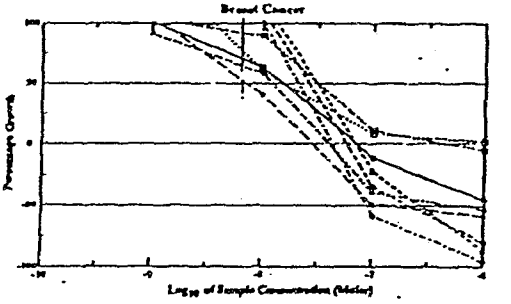
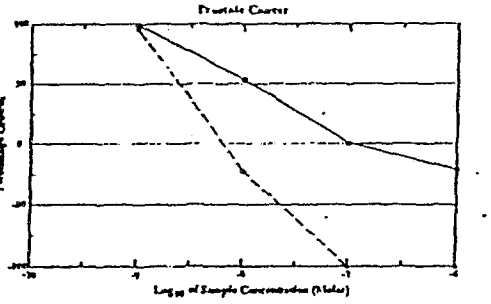
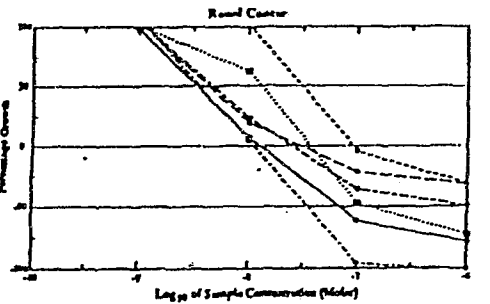
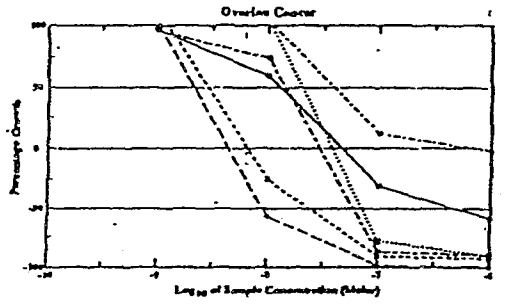
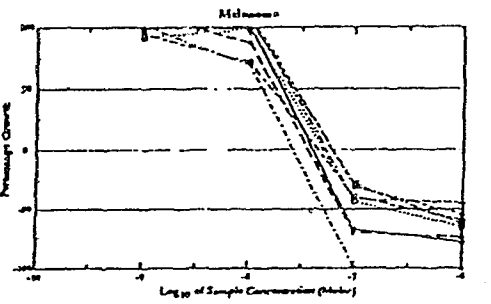
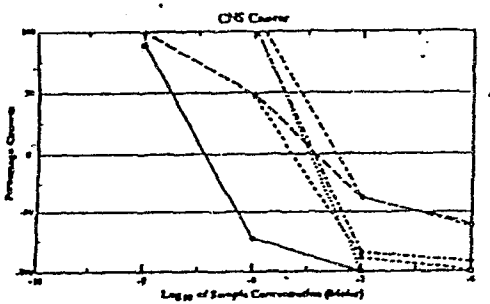
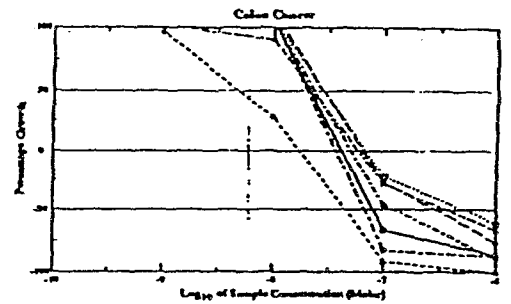
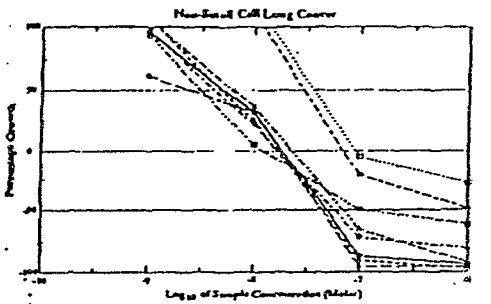
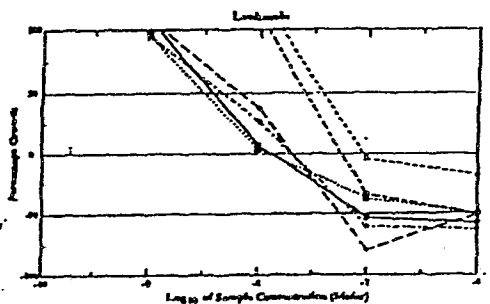


TABLE 18

FIGURE 2



National Cancer Institute Developmental Therapeutics Program		NSC: D-659472-Z/0-1/ (Units: Molar)		SSPL:	Exp. ID: 9302MD33
Mean Graphs - CALOTOXIN		Report Date: March 8, 1993	Report Date: February 23, 1993		
Panel Cell Line	Log ₁₀ GI50	Log ₁₀ TGI	Log ₁₀ LC50	Log ₁₀ LC75	Log ₁₀ LC90
Leukemia					
COF-GEM	< -8.00	-7.51	> -4.00	> -4.00	
HLA(HTB)	-7.97	-7.28	> -4.00	> -4.00	
K-562	-7.08	> -4.00	> -4.00	> -4.00	
MOLT-4	< -8.00	< -7.22	> -4.00	> -4.00	
MDA-MB-231	-7.57				
SK	< -8.00				
Non-Small Cell Lung Cancer					
A549	< -8.00	-7.67	-7.29	-7.29	
A549ATOC	-7.97	-7.60	-7.22	-7.22	
EAH1	-7.29	-7.22	-7.22	-7.22	
EAH2	-7.29	-7.07	> -4.00	> -4.00	
HOP-87	-7.29	-4.57			
HOP-97	-7.29	-7.78			
HOP-107	-7.29	-4.45			
HOP-117	-7.29	-4.45			
HOP-127	-7.29	-4.45			
HOP-137	-7.29	-4.45			
HOP-147	-7.29	-4.45			
HOP-157	-7.29	-4.45			
HOP-167	-7.29	-4.45			
HOP-177	-7.29	-4.45			
HOP-187	-7.29	-4.45			
HOP-197	-7.29	-4.45			
HOP-207	-7.29	-4.45			
HOP-217	-7.29	-4.45			
HOP-227	-7.29	-4.45			
HOP-237	-7.29	-4.45			
HOP-247	-7.29	-4.45			
HOP-257	-7.29	-4.45			
HOP-267	-7.29	-4.45			
HOP-277	-7.29	-4.45			
HOP-287	-7.29	-4.45			
HOP-297	-7.29	-4.45			
HOP-307	-7.29	-4.45			
HOP-317	-7.29	-4.45			
HOP-327	-7.29	-4.45			
HOP-337	-7.29	-4.45			
HOP-347	-7.29	-4.45			
HOP-357	-7.29	-4.45			
HOP-367	-7.29	-4.45			
HOP-377	-7.29	-4.45			
HOP-387	-7.29	-4.45			
HOP-397	-7.29	-4.45			
HOP-407	-7.29	-4.45			
HOP-417	-7.29	-4.45			
HOP-427	-7.29	-4.45			
HOP-437	-7.29	-4.45			
HOP-447	-7.29	-4.45			
HOP-457	-7.29	-4.45			
HOP-467	-7.29	-4.45			
HOP-477	-7.29	-4.45			
HOP-487	-7.29	-4.45			
HOP-497	-7.29	-4.45			
HOP-507	-7.29	-4.45			
HOP-517	-7.29	-4.45			
HOP-527	-7.29	-4.45			
HOP-537	-7.29	-4.45			
HOP-547	-7.29	-4.45			
HOP-557	-7.29	-4.45			
HOP-567	-7.29	-4.45			
HOP-577	-7.29	-4.45			
HOP-587	-7.29	-4.45			
HOP-597	-7.29	-4.45			
HOP-607	-7.29	-4.45			
HOP-617	-7.29	-4.45			
HOP-627	-7.29	-4.45			
HOP-637	-7.29	-4.45			
HOP-647	-7.29	-4.45			
HOP-657	-7.29	-4.45			
HOP-667	-7.29	-4.45			
HOP-677	-7.29	-4.45			
HOP-687	-7.29	-4.45			
HOP-697	-7.29	-4.45			
HOP-707	-7.29	-4.45			
HOP-717	-7.29	-4.45			
HOP-727	-7.29	-4.45			
HOP-737	-7.29	-4.45			
HOP-747	-7.29	-4.45			
HOP-757	-7.29	-4.45			
HOP-767	-7.29	-4.45			
HOP-777	-7.29	-4.45			
HOP-787	-7.29	-4.45			
HOP-797	-7.29	-4.45			
HOP-807	-7.29	-4.45			
HOP-817	-7.29	-4.45			
HOP-827	-7.29	-4.45			
HOP-837	-7.29	-4.45			
HOP-847	-7.29	-4.45			
HOP-857	-7.29	-4.45			
HOP-867	-7.29	-4.45			
HOP-877	-7.29	-4.45			
HOP-887	-7.29	-4.45			
HOP-897	-7.29	-4.45			
HOP-907	-7.29	-4.45			
HOP-917	-7.29	-4.45			
HOP-927	-7.29	-4.45			
HOP-937	-7.29	-4.45			
HOP-947	-7.29	-4.45			
HOP-957	-7.29	-4.45			
HOP-967	-7.29	-4.45			
HOP-977	-7.29	-4.45			
HOP-987	-7.29	-4.45			
HOP-997	-7.29	-4.45			
HOP-1007	-7.29	-4.45			
HOP-1017	-7.29	-4.45			
HOP-1027	-7.29	-4.45			
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Dose Response Curves - USCHARIDIN

NSC: D-659471-Y/0-1/15

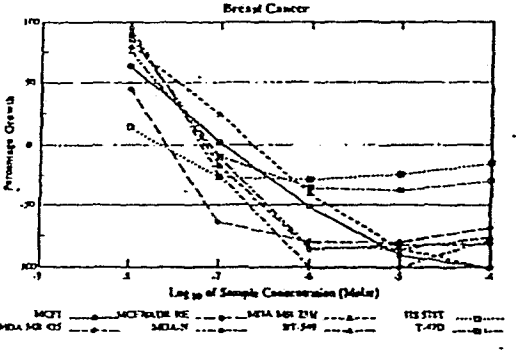
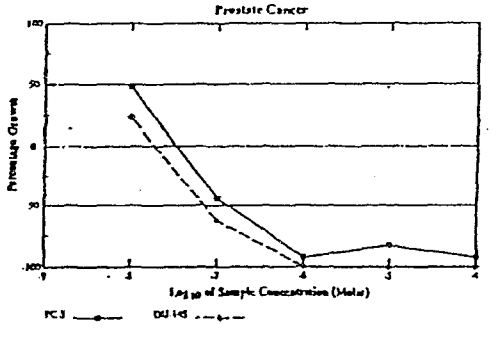
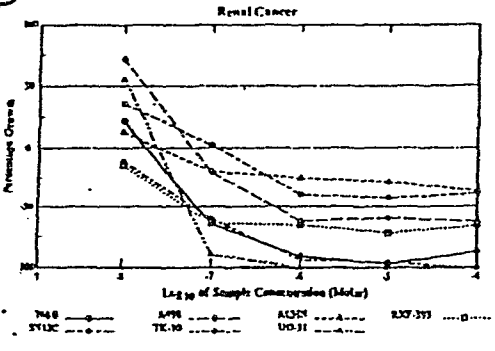
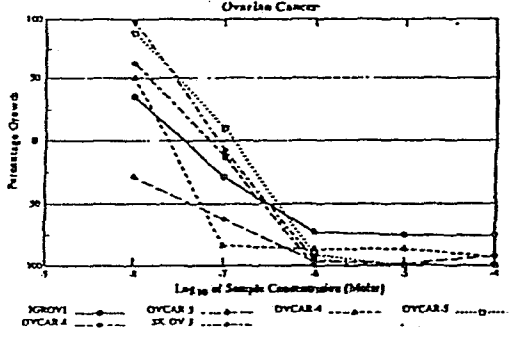
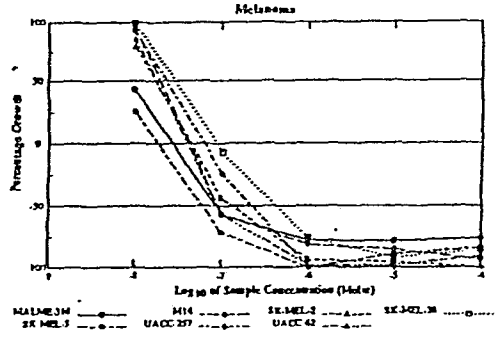
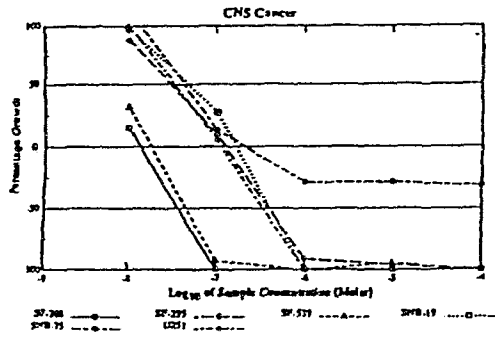
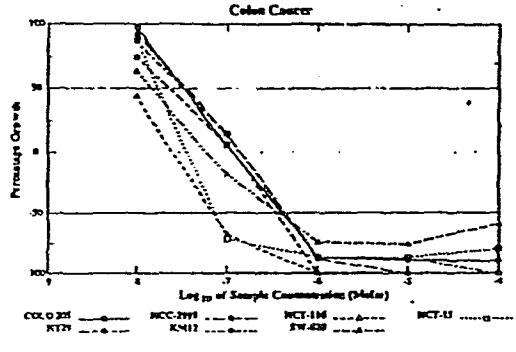
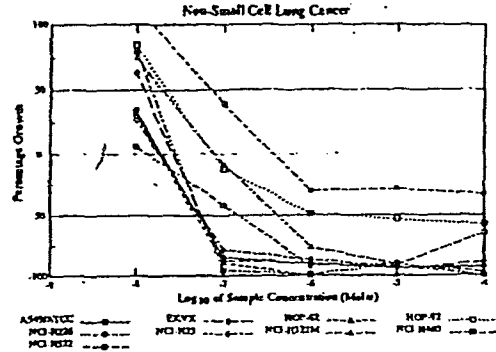
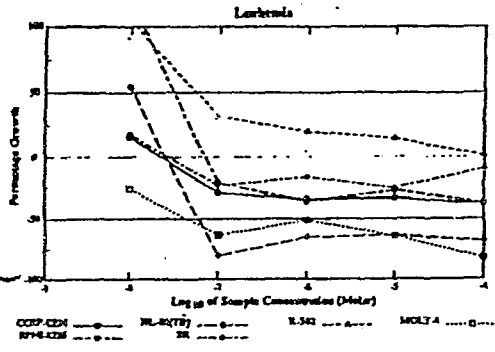
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Exp. ID: 9302MD33

Report Date: March 8, 1993

Test Date: February 23, 1993

723110 / 0-1-93



9 / 9
FIGURE

W/O 98/52562

PCT/GB98/01522

INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/GB 98/01522

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/365

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	J.A. PARSONS: "Cat assay for the emetic action of digitalis and related glycosides (digitoxin, digoxin, lanatoside C ouabain and calactin)" BR. J. PHARMACOL., vol. 42, no. 1, 1971, pages 143-152, XP002078318 see page 145	1-8
P, X	F. KIUCHI ET AL.: "Cytotoxic principles of a Bangladesh crude drug, akond mul (roots of Calotropis gigantea L.)" CHEM. PHARM. BULL., vol. 46, no. 3, 1998, pages 528-530, XP002078319 see the whole document	1-6
A	WO 92 09295 A (MRAK, M.,) 11 June 1992 -/--	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "&" document member of the same patent family

Date of the actual completion of the international search

22 September 1998

Date of mailing of the international search report

02/10/1998

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Klaver, T

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 98/01522

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>A.E.MUTLIB ET AL.: "In vivo and in vitro metabolism of gomphoside, a cardiotonic steroid with doubly-linked sugar." J. STEROID BIOCHEM., vol. 28, no. 1, 1987, pages 65-76, XP002078320</p> <p style="text-align: center;">-----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/01522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9209295 A	11-06-1992	CH 679012 A	13-12-1991
		AU 657283 B	09-03-1995
		AU 8902891 A	25-06-1992
		EP 0514508 A	25-11-1992
