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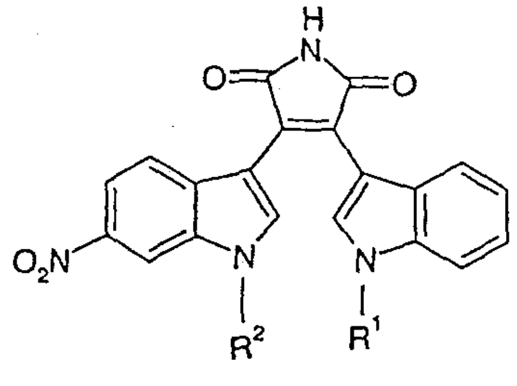


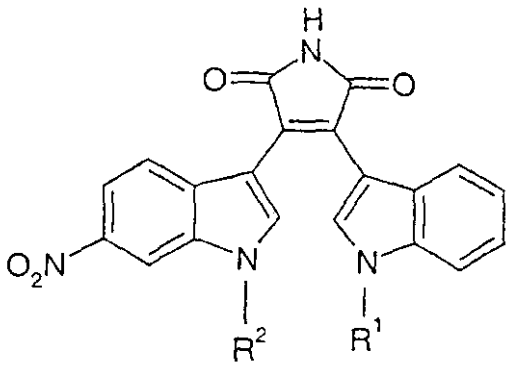
(I)

(57) Abstract

Substituted pyrroles of formula (I) wherein R^1 is hydrogen and R^2 is methyl or R^1 is methyl and R^2 is hydrogen or R^1 is hydroxymethyl and R^2 is methyl as well as pharmaceutically acceptable prodrugs or pharmaceutically acceptable salts thereof are antiproliferative agents useful in the treatment of cancer.

SUBSTITUTED BISINDOLYMALEIMIDES FOR THE INHIBITION OF CELL PROLIFERATION

The invention relates to substituted pyrroles of formula



I

wherein R^1 is hydrogen and R^2 is methyl or

R^1 is methyl and R^2 is hydrogen or

R^1 is hydroxymethyl and R^2 is methyl

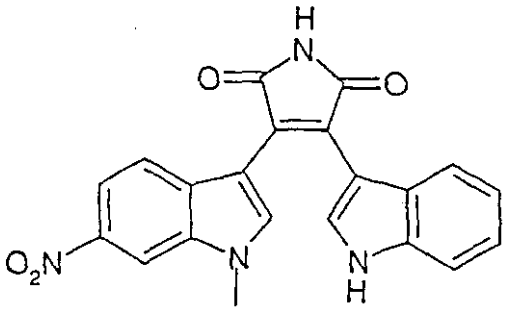
as well as to pharmaceutically acceptable prodrugs or pharmaceutically acceptable salts thereof.

The compounds of formula I have antiproliferative activity, specifically, they inhibit cell division in G2/M phase of the cell cycle and are generally referred to as "G2/M phase cell-cycle" inhibitors.

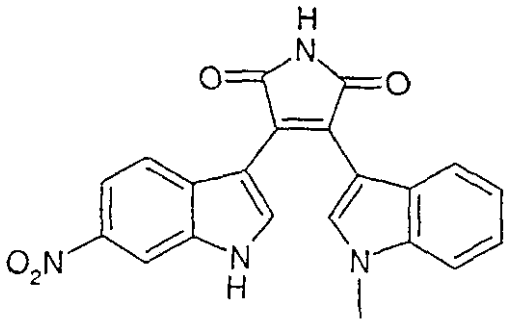
The compounds of formula I are covered by formula I of U.S.P. 5,057,614 without being specifically disclosed as a group or individually. In addition, the above-mentioned activity of the

compounds of the present invention has nowhere been disclosed or made obvious in U.S.P. 5,057,614 and, therefore, is surprising.

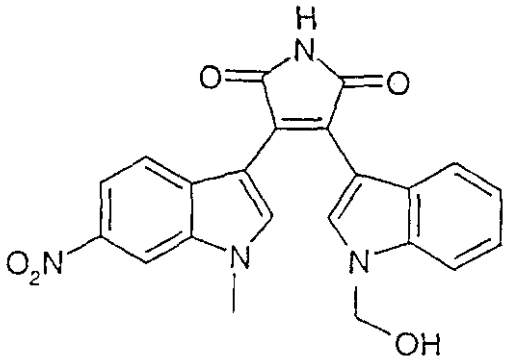
Formula I above comprises the following three compounds:



I-1



I-2



I-3

The term "pharmaceutically acceptable prodrugs" means a compound that may be converted under physiological conditions or by solvolysis to any of the compounds of formula I or to a pharmaceutically acceptable salt of said compounds.

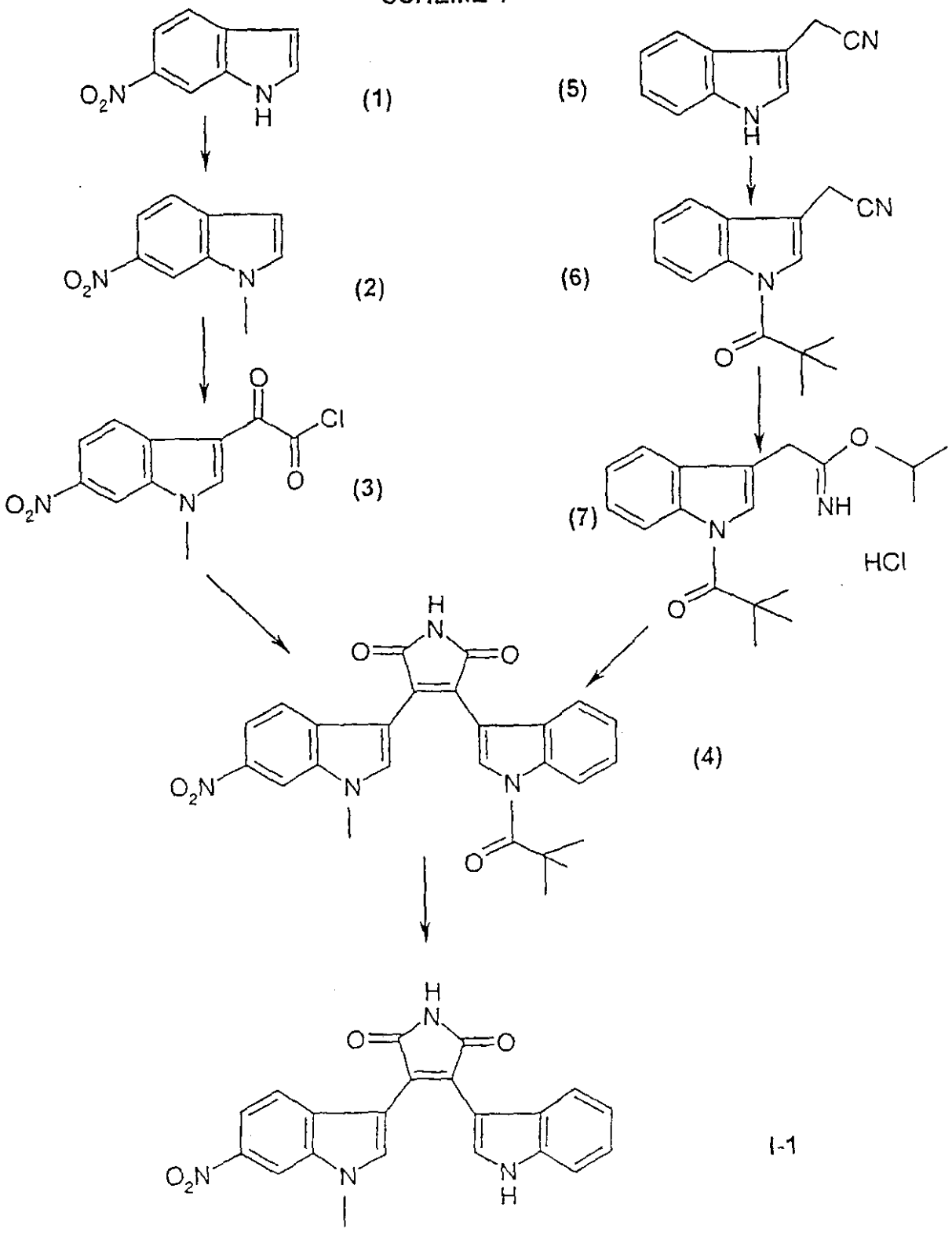
The compounds of formula I, as well as pharmaceutically acceptable salts of said compounds, are prepared by reactions represented in the following Schemes. The synthesis of each of these compounds is also described in Examples 1-3.

Compound I-1 can be prepared by reacting (1-methyl-6-nitro-1H-indol-3-yl)-oxo-acetyl chloride (3) with [1-(2,2-dimethyl-propionyl)-1H-indol-3yl]-3-ethanimidic acid 1-methyl-ethylester hydrochloride (7) and treating the reaction product with a base.

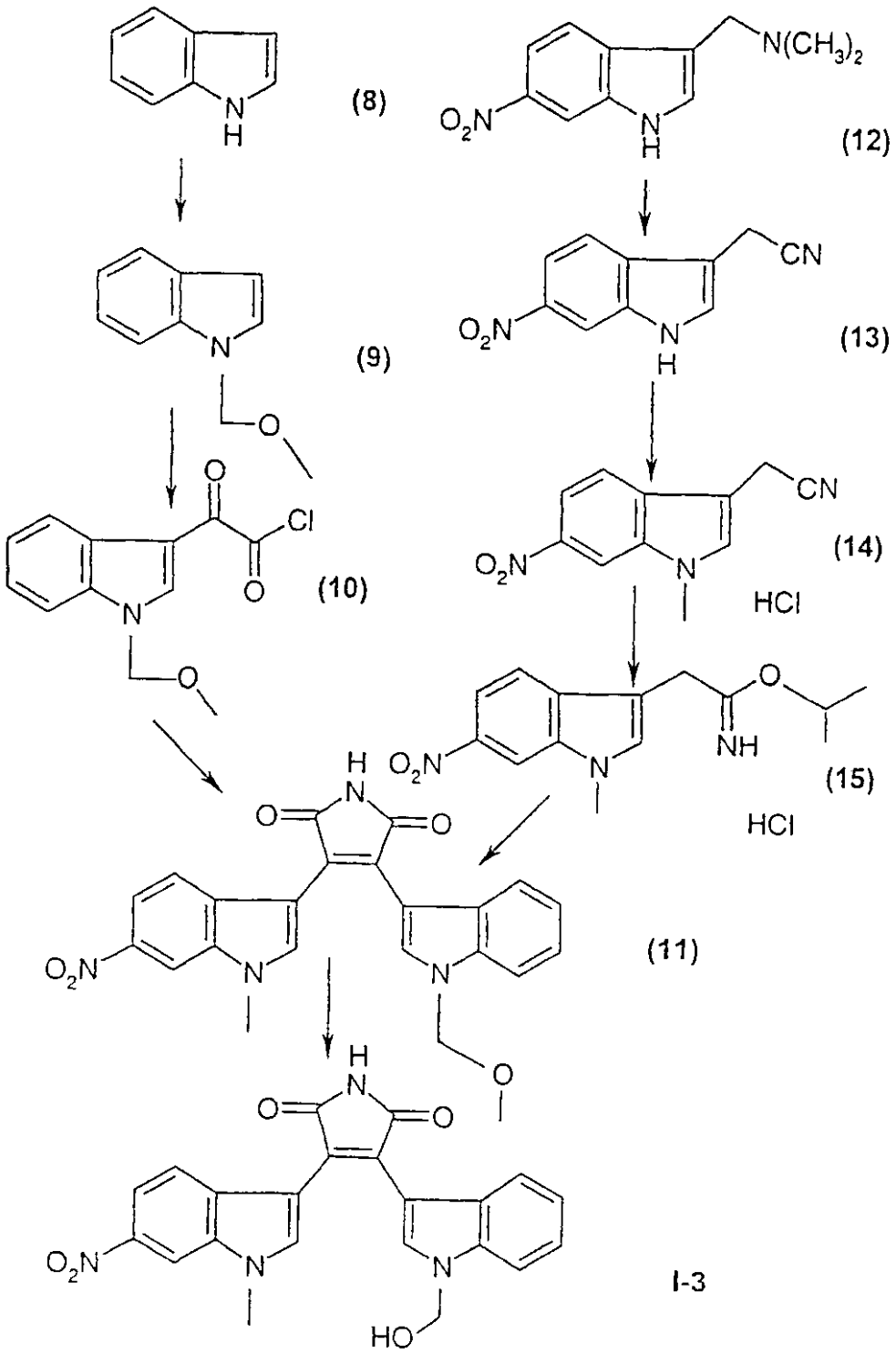
Compound I-2 can be prepared by reacting (1-methyl-1H-indol-3-yl)-oxo-acetyl chloride (17) with [1-(2,2-dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-3-ethanimidic acid 1-methyl-ethylester hydrochloride (19) and treating the reaction product with a base.

Compound I-3 can be prepared by reacting (1-methoxymethyl-1H-indol-3-yl)-oxo acetyl chloride (10) with (1-methyl-6-nitro-1H-indol-3-yl)-3-ethanimidic acid 1-methylethylester hydrochloride (15) and treating the reaction product with an acid.

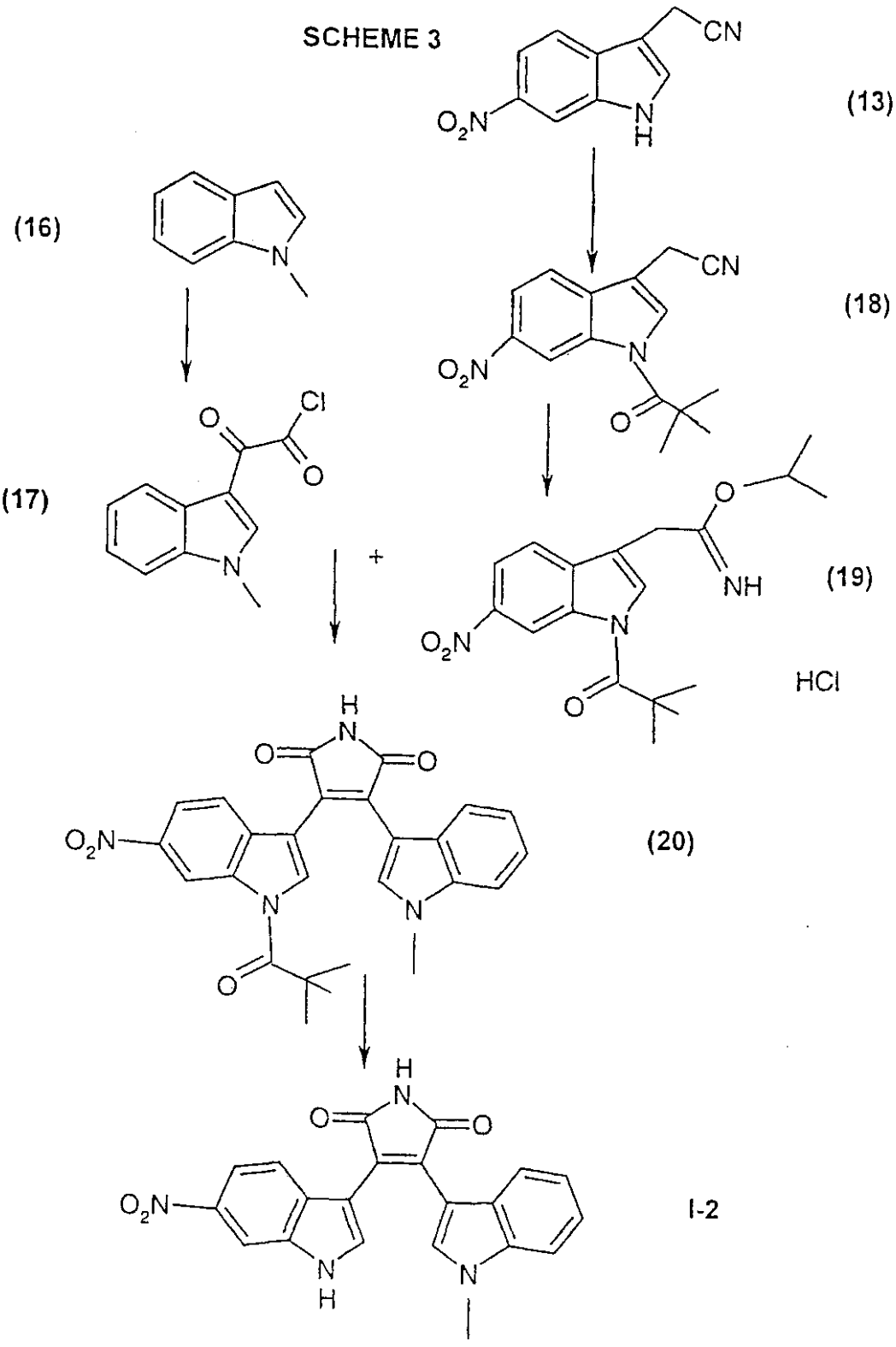
SCHEME 1



SCHEME 2



SCHEME 3



The antiproliferative activity of the compounds of the invention is demonstrated below. These effects indicate that the compounds are useful in treating cancer, in particular solid tumors.

The estrogen receptor negative epithelial breast carcinoma line (MDA-MB-435) was purchased from American Type Cell Culture Collection (ATCC; Rockville, MD) and was grown in the medium recommended by ATCC. For analysis of the effect of the test compounds on growth of these cells, the cells were plated at 2000 cells per well in a 96-well tissue culture plate ("test plate"), and were incubated overnight at 37°C with 5% CO_2. The next day, the test compounds were dissolved in 100% dimethyl sulfoxide (DMSO) to yield a 10 mM stock solution. Each compound was diluted with sterile distilled water to 1 mM and then was added to triplicate wells of a 96-well "master plate" containing medium in a sufficient quantity to yield a final concentration of 40 μM. The compounds were serially diluted in medium in the "master plate." One-fourth final volume of the diluted compounds was transferred to duplicate "test plates." DMSO was added to a row of "control cells" such that the final concentration of DMSO in each well was 0.1%. The "test plates" were returned to the incubator and 3 days post addition of test compound one "test plate" was analyzed as described below. Similarly, 5 days after addition of test compound, the second "test plate" also was analyzed as described below.

3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (thiazolyl blue; MTT) was added to each well to yield a final concentration of 1 mg/ml. The plate was then incubated at 37°C for 3 hours. The MTT-containing medium was then removed and 50 μl 100% ethanol was added to each well to dissolve the resulting formazan metabolite. To ensure complete dissolution, plates were shaken for 15 minutes at room temperature. Absorbencies were read in a microtiter plate reader (Molecular Dynamics) at a wavelength of 570 nm with a 650 nm reference. Percent inhibition was calculated by subtracting the blank from all wells, then subtracting the division of the average absorbance of each test triplicate by the average of the controls from 1.00. Inhibitory concentrations (IC_{50} and IC_{90}) were determined from the linear regression of a plot of the logarithm of the concentration versus the percent inhibition.

The colon adenocarcinoma line SW480 and the colon carcinoma line HCT-116 also were obtained from the ATCC and were tested according to the same protocol provided above with the following modifications. Cell line SW480 was plated at 1000 cells per well and analyzed at 6 days post addition of the test compound. Cell line HCT-116 was plated at 750 cells per well and analyzed at 4 days post addition of test compound. For the MTT analysis, plates were centrifuged at 1000 rpm for 5 minutes prior to aspiration of the MTT-containing medium, and 100 µl 100% ethanol was used to dissolve the formazan.

The results of the foregoing *in vitro* tests are set forth below in Tables I-III.

TABLE I

Antiproliferative Activity In Cell Line MDA-MB-435

Compound	IC_{50} (µM)
Compound I-1	0.03*
Compound I-3	0.05*
Compound I-2	0.6*

* An average of at least three separate experiments.

TABLE II

Antiproliferative Activity In Cell Line HCT-116

Compound	IC_{50} (µM)
Compound I-1	0.17*
Compound I-3	0.23*
Compound I-2	1.66*

* An average of at least three separate experiments.

TABLE III

Antiproliferative Activity In Cell Line SW480

Compound	IC_{50} (μM)
Compound I-1	0.20*
Compound I-3	0.22*
Compound I-2	1.86*

* An average of at least three separate experiments.

For analysis of the effect of the compounds on cell cycle progression, MDA-MB-435 cells (ATCC; Rockville, MD) were plated at 1 x 10^6 cells/10 mls per 10 cm dish in the following growth medium: RPMI 1640 + 10% Heat-Inactivated Fetal Bovine Serum, 2 mM L-glutamine and 50 U/ml pen-strep (all from GIBCO/BRL, Gaithersburg, MD). The cells were incubated overnight at 37° C with 5% CO_2. The next day, 10 μl of each of the compounds to be tested, in a 100% DMSO solution, was added to individual dishes to obtain 1/1000x final concentration of the stock solution. In addition, 10 μl 100% DMSO was added to a control dish. The final concentration of DMSO in all plates, including the control, was 0.1%. The plates were returned to the incubator.

Thereafter, at various periods of time, the medium in each plate was removed to a 50 ml centrifuge tube. The cell layer remaining in the dish was then washed with 5 ml of phosphate buffered saline (PBS; GIBCO/BRL). The PBS was removed and combined with the medium in the appropriate tube. The cells were trypsinized for 5 minutes at 37° C, and the solution was collected and combined with the medium and PBS in the appropriate tubes. The tubes were then centrifuged for 5 minutes at 1200 rpm. The cells were fixed by removing the supernatant, tapping the tube to distribute the pellet, then adding 5 mls of cold 70% ethanol while vortexing gently. The cells were then stored at -20° C for >24 hours.

The cell-containing tubes were taken out of freezer and allowed to sit at room temperature for 20-30 minutes. The tubes were centrifuged at 3000 rpm for 5 minutes. The supernatant was removed, the pellets were washed with 5 ml PBS, and the tubes were centrifuged as above. Subsequently, the supernatant was removed, and the pellet was resuspended in 0.5 ml PBS. Thereafter, 0.5 ml RNAse A (1 mg/ml in PBS) was added to each tube, and the tubes were incubated at 37° C for 15 minutes. 100 µl propidium iodide (Sigma, St. Louis, MO) (1 mg/ml in PBS) was added to each tube, and the tubes were then incubated at room temperature for 2-3 minutes. Each resulting solution was passed through a filter cap tube (Becton Dickinson, San Jose , CA, #2235).

Samples were read in a FACSort machine (Becton-Dickinson) using the manufacturer's CellQUEST program, and analyzed with the manufacturerís ModFIT software. This measurement provides an indication of the percent of cells in each of the following phases: G0/G1, DNA synthesis (S) and G2/M phases.

The results of a cell cycle progression experiment analyzed at day 1 post addition of test compounds I-1, I-2 and I-3 are summarized below in Table IV.

TABLE IV

Effect Of Test Compounds On Cell Cycle

Compound	Concentration	% Of Cells In Each Cell Cycle Phase		
		G1	S	G2/M
DMSO	0.1 %	43.93 %	41.08 %	14.99 %
Compound I	0.1 μM	8.27 %	25.21 %	66.52 %
Compound I	0.03 μM	45.30 %	34.67 %	20.03 %
Compound I	0.01 μM	44.95 %	41.04 %	14.00 %
Compound I-3	0.3 μM	1.11 %	24.99 %	73.90 %
Compound I-3	0.1 μM	15.54 %	24.06 %	60.40 %
Compound I-3	0.03 μM	45.45 %	38.06 %	16.50 %
Compound I-2	10 μM	10.41 %	35.25 %	54.34 %
Compound I-2	3 μM	3.26 %	48.75 %	47.99 %
Compound I-2	1 μM	27.21 %	30.19 %	42.60 %

The results summarized in Tables I-IV above demonstrate that compounds I-1, I-2 and I-3 have antiproliferative activity; specifically, they cause an accumulation of cells in the G2/m phase of the cell cycle.

The pyrroles of formula I above and their aforementioned salts can be used as medicaments, for example, in the form of pharmaceutical preparations, which can be administered orally, for example, in the form of tablets, coated tablets, dragees, hard or soft gelatin capsules, solutions, emulsions or suspensions. They can also be administered rectally, for example, in the form of suppositories or parenterally, for example, in the form of injection solutions.

For the manufacture of pharmaceutical preparations these compounds can be formulated with therapeutically inert, inorganic or organic carriers. Lactose, maize starch or derivatives thereof, talc, steric acid or its salts can be used as such carriers for tablets, coated tablets, dragees and hard gelatin capsules. Suitable carriers for soft gelatin capsules are vegetable oils, waxes, fats, semi-solid or liquid polyols. Depending on the nature of the active substance no carriers are, however, generally required in the case of soft gelatin capsules. Suitable carriers for the manufacture of solutions and syrups are, water, polyols, saccharose, invert sugar and glucose. Suitable carriers for injection are water, alcohols, polyols, glycerine, vegetable oils, phospholipids and surfactants, suitable carriers for suppositories are natural or hardened oils, waxes, fats and semi-liquid polyols.

The pharmaceutical preparations can also contain preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifying agents, sweetening agents, coloring agents, flavoring agents, salts for varying the osmotic pressure, buffers, coating agents or antioxidants. They can also contain still other therapeutically valuable substances.

As mentioned above, the pyrroles of formula I and their aforementioned salts can be used in the treatment or control of oncological disorders. The dosage can vary within wide limits and will, of course, be adjusted to the individual requirements in each particular case. In general, in the case of oral or parenteral administration to adult humans weighing about 70 kg, a daily dosage of about 10 mg to about 10,000 mg, preferably from about 200 mg to about 5,000 mg, more preferably to about 1000 mg, should be appropriate, although the upper limit may be exceeded when indicated. The daily dosage can be administered as a single dose or in divided doses, or for parenteral administration, it may be given as continuous infusion.

The following Examples illustrate the present invention.

EXAMPLE 1

Preparation of 3-(1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-1)

A. 1-Methyl-6-nitro-1H-indole (2)

To a slurry of 0.33 g (8.3 mmol) of NaH (60% dispersion in oil) in 30 ml of dried dimethylformamide ("DMF"), was added 0.973 g (6.00 mmol) of commercially available 6-nitro-1H-indole (**1**) at 0-5°C over a period of 10 minutes. After 1 hour stirring at the same temperature, 0.75 ml (12.1 mmol) of methyl iodide was added and the mixture was stirred at the same temperature for 30 minutes, then at room temperature for 1 hour, poured into ice and water and extracted with ethyl acetate. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated to yield 0.814 g (77.5%) of 1-methyl-6-nitro-1H-indole (**2**) as a yellow solid. This material was used without purification.

B. (1-Methyl-6-nitro-1H-indol-3-yl)-oxo-acetyl chloride (3)

To a solution of 1.33 g (7.55 mmol) of 1-methyl-6-nitro-1H-indole (**2**) in 40 ml of ether were added 1.5 ml (17.2 mmol) of oxalyl chloride at 0-5°C under Argon. A precipitate was formed. After 3 hours stirring, the resulting solid was filtered, washed with a small amount of ether and dried to yield 1.9 g (95%) of (1-methyl-6-nitro-1H-indol-3-yl)-oxo-acetyl chloride (**3**) as a yellow solid. This material was used without purification.

C. [1-(2,2-Dimethyl-propionyl)-1H-indol-3-yl]-acetonitrile (6)

Using the procedure of subpart A above, the N-alkylation reaction of 10.2 g (65 mmol) of commercially available (1H-indol-3-yl)-acetonitrile (**5**) with 8.7 ml (71 mmol) of trimethylacetyl chloride and 3.4 g (85 mmol) of NaH (60% dispersion in oil) as a base in 115 ml of DMF yielded 6.6 g (38.7%) of [1-(2,2-dimethyl-propionyl)-1H-indol-3-yl]-acetonitrile (**6**) as a yellow oil after chromatographic purification.

D. [1-(2,2-Dimethyl-propionyl)-1H-indol-3yl]-3-ethanimidic acid 1-methylethylester hydrochloride (7)

To a slurry of 6.6 g (27.5 mmol) of [1-(2,2-dimethyl-propionyl)-1H-indol-3-yl]-acetonitrile (6) from Step C above in 105 ml of 2-propanol, 40 ml (0.563 mol) of acetyl chloride was added dropwise at 0-5°C over a 20 minute period. The reaction mixture was stirred at room temperature overnight, concentrated and the residue was diluted with approximately 75 ml of ethyl acetate, heated for 15 minutes on a steam bath, cooled and placed in a freezer. The precipitate was filtered and dried to yield 6.0 g (65.0%) of [1-(2,2-dimethyl-propionyl)-1H-indol-3-yl]-3-ethanimidic acid 1-methylethylester hydrochloride (7) as a white solid.

E. 3-[1-(2,2-Dimethyl-propionyl)-1H-indol-3-yl]-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (4)

To a solution of 1.25 g (4.69 mmol) of (1-methyl-6-nitro-1H-indol-3-yl)-oxo-acetyl chloride (3) from Step B above and 1.6 g (4.75 mmol) of [1-(2,2-dimethyl-propionyl)-1H-indol-3-yl]-3-ethanimidic acid 1-methyl ethylester hydrochloride (7) from Step D above in 80 ml of methylene chloride was added 2.6 ml (18.65 mmol) of triethylamine at 0°C and under Argon. After stirring at the same temperature for 30 minutes, the reaction mixture was then stirred at room temperature for 3 1/2 hours and diluted with more methylene chloride. The organic phase was washed with water, 0.5N HCl solution, brine, dried over $MgSO_4$ and concentrated to give 3.01 g of a foam. This material was dissolved in 50 ml of toluene and treated with 987.9 mg (5.19 mmol) of p-toluenesulfonic acid at 0°C. After 3 hours stirring at room temperature the reaction mixture was extracted with methylene chloride. The organic phase was washed with a saturated $NaHCO_3$ solution, brine, dried over $MgSO_4$ and concentrated to give 3.9 g of crude material. Chromatographic purification on a silica gel column, yielded 1.7 g (77.%) of 3-[1-(2,2-dimethyl-propionyl)-1H-indol-3-yl]-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (4) as an orange solid. mp >146°C with dec. MS: (M^+), m/z 470.

F. 3-(1H-Indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-1)

1.7 g (3.61 mmol) of 3-[1-(2,2-dimethyl-propionyl)-1H-indol-3-yl]-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (4) from Step E above in 60 ml of methanol was treated with 5.6 ml (8.96 mmol) of a 1.6 molar solution of $NaOCH_3$ in methanol. The reaction was stirred at room temperature for 1 hour, poured in 2N-HCl/ice and extracted with ethyl acetate. The organic extracts were dried on anhydrous $MgSO_4$ and concentrated to yield, after chromatographic purification, 394.7 mg (28%) of 3-(1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-1) as a red solid mp >280°C. MS: (M^+), m/z 386.

EXAMPLE 2

Preparation of 3-(1-hydroxymethyl-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-3)

A. 1-Methoxymethyl-1H-indole (9)

Using the procedure of Example 1, Step A, the N-alkylation reaction of 1.17 g (10 mmol) of commercially available indole (8) with 1 ml (13.1 mmol) of chloromethyl methyl ether and 0.48 g (12 mmol) of NaH (60% dispersion in oil) as a base in 22 ml of DMF yielded 1.4 g (86.9%) of 1-methoxymethyl-1H-indole (9) as a colorless oil, after chromatographic purification.

B. (1-Methoxymethyl-1H-indol-3-yl)-oxo-acetyl chloride (10)

Using the procedure of Example 1, step B, the reaction of 0.23 g (1.43 mmol) of 1-methoxymethyl-1H-indole (9) from Step A above with 0.25 ml (2.86 mmol) of oxalyl chloride in 3.5 ml of ether produced 0.174 g (48.5%) of (1-methoxymethyl-1H-indol-3-yl)-oxo-acetyl chloride (10) as a yellow solid. This material was used without purification.

C. (6-Nitro-1H-indol-3-yl)-acetonitrile (13)

To a stirred solution of 44.27 g (0.204 mol) of 6-nitrogramine (12) [Jackson B. Hester *J. Org. Chem.*, 29: 1158 (1964)] in 450 ml of acetonitrile 44.59 g (0.31 mol) of methyl iodide was added at 0-5°C over a period of an hour. The reaction mixture was stirred at room temperature for three hours, then a solution of 26.6 g (0.543 mol) of sodium cyanide in 225 ml of water added at once. The reaction mixture was heated at 32°C overnight, cooled to room temperature and the product extracted 3 times with a total of 800 ml of ethyl acetate and 300 ml of water. The combined extracts were washed with water, 1N HCl solution, a saturated sodium bicarbonate solution, dried on $MgSO_4$ and the solvent evaporated in vacuo. The orange-brown residue (41.3 g) was dissolved in 200 ml of warm ethyl acetate and passed through a small pad of silica gel to produce 28.9 g (70.4%) of (6-nitro-1H-indolyl-3-yl)-acetonitrile (13) as a yellow solid after evaporation of the solvent.

D. (1-Methyl-6-nitro-1H-indol-3-yl)-acetonitrile (14)

65.5 g (0.474 mol) of powdered potassium carbonate was added to a solution of 28.9 g (0.143 mol) of (6-nitro-1H-indol-3-yl)-acetonitrile (13) from Step C above in 230 ml of dimethylformamide at room temperature. The suspension was stirred for 40 minutes then 25.48 g (0.179 mol) of methyl iodide was added dropwise over 65 minutes. After stirring at room temperature over night the reaction mixture was cooled and poured into a total of 600 ml of water. The precipitate was filtered, washed with a little water and dried on phosphor anhydride until reaching constant weight. The procedure yielded 30.4 g (95.4%) of (1-methyl-6-nitro-1H-indol-3-yl)-acetonitrile (14), which was used without further purification.

E. (1-Methyl-6-nitro-1H-indol-3-yl)-3-ethanimidic acid 1-methylethylester hydrochloride (15)

A stream of HCl gas was bubbled into a stirred suspension of 82 g (0.382 mol) of (1-methyl-6-nitro-1H-indol-3-yl)-acetonitrile (14) from Step D above, in 1000 ml of 2-propanol at 0-10°C. After adding approximately 350 g of HCl, ether was added to the reaction mixture until a precipitate was formed. The solid was collected, washing with ether and dried in vacuo to yield

102 g (85.7%) of (1-methyl-6-nitro-1H-indol-3-yl)-3-ethanimidic acid 1-methylethylester hydrochloride (**15**).

F. 3-(1-Methoxymethyl-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (**11**)

Using the procedure of Example 1, Step E, the condensation reaction of 1.3 g (5.17 mmol) of oxoacetyl chloride (**10**) from Step B above, with 1.7 g (5.45 mmol) of (1-methyl-6-nitro-1H-indole)-3-ethanimidic acid 1-methyl ethylester hydrochloride (**15**) from Step E above in 95 ml of methylene chloride, yielded 1.08 g (48.5%) of 3-(1-methoxymethyl-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (**11**) as an orange solid, mp >250°C with dec. MS: (M^+), m/z 430.

G. 3-(1-Hydroxymethyl-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-3)

A solution of 727.5 mg of 3-[1-(methoxymethyl)-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (**11**) from Step F above in 65 ml of THF was treated with approximately 40 ml of 2N HCl. The reaction mixture was refluxed for 5 hours, cooled and the product was extracted with ethyl acetate. The organic phase was dried on $MgSO_4$ and the solvent evaporated to give an orange solid. Chromatographic purification of this material yielded 123.3 mg of 3-(1-hydroxymethyl-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-3) as a red solid, mp 210-213°C. MS: (M^+), m/z 416.

EXAMPLE 3

Preparation of 3-(1-Methyl-1H-indol-3-yl)-4-(6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-2)

A. (1-Methyl-1H-indol-3-yl)-oxo-acetyl chloride (17)

Using the procedure of Example 1, Step B, the reaction of 6 ml (47 mmol) of commercially available 1-methyl-1H-indole (16) with 8 ml (92 mmol) of oxalyl chloride in 120 ml of ether, produced 7.6 g (73.2%) of (1-methyl-1H-indol-3-yl)-oxo-acetyl chloride (17) as a yellow solid. This material was used without purification.

B. [1-(2,2-Dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-acetonitrile (18)

Using the procedure of Example 1, Step A, the N-alkylation reaction of 346.6 mg (1.72 mmol) of 6-nitro-1H-indolyl-3-acetonitrile (13) from Example 2, Step C with 0.3 ml (2.44 mmol) of trimethylacetyl chloride and 70.8 mg (1.77 mmol) of NaH (60% dispersion in oil) as a base in 8 ml of DMF yielded after chromatographic purification, 287.7 mg (43.2%) of [1-(2,2-dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-acetonitrile (18) as a yellow oil.

C. [1-(2,2-Dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-3-ethanimidic acid 1-methylethyl-ester hydrochloride (19)

A stream of HCl gas was bubbled for 3 minutes into a constantly stirred suspension of 1.45 g (5.08 mmol) of 1[-(2,2-dimethyl-propionyl)-6-nitro-1H-indolyl]-3-acetonitrile (18) from Step B above in 90 ml of 2-propanol at 0-5°C. The reaction mixture was stirred at room temperature for 21 hours. The solvent was evaporated in vacuo to give 1.95 g (100%) of a yellow solid (19). This material was used without further purification.

D. 3-[1-(2,2-Dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione (20)

Using the procedure of Example 1, Step E, 1.1 g (4.96 mmol) of oxoacetyl chloride (17) from Step A above was reacted with 1.95 g (5.08 mmol) of [1-(2,2-dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-3-ethanimidic acid 1- methylethylester hydrochloride (19) from Step C above and 2.1 ml (17.94 mmol) of triethylamine in 120 ml of methylene chloride, the resulting product was treated with 1.1 g (5.78 mmol) of p-toluenesulfonic acid monohydrate in 80 ml of toluene, yielding 1.3 g (62.1%) of 3-[1-(2,2-dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione (20) as an orange solid; mp >245 °C with dec. MS: (M^+), m/z 470.

E. 3-(1-Methyl-1H-indol-3-yl)-4-(6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-2)

Using the procedure of Example 1, Step F, the N-deprotection reaction of 1.3 g (2.76 mmol) of 3-[1-(2,2-dimethyl-propionyl)-6-nitro-1H-indol-3-yl]-4-(1-methyl-1H-indol-3-yl)-pyrrole-2,5-dione. (20) from Step D above with 4.3 ml (6.88 mmol) of a 1.6 molar solution of NaOCH3 in 65 ml of methanol yielded 300.6 mg (28.1%) of 3-(1-methyl-1H-indol-3-yl)-4-(6-nitro-1H-indol-3-yl)-pyrrole-2,5-dione (I-2) as a red solid after crystallization from ethyl acetate and hexane; mp >260 °C. MS: (M^+), m/z 386.

EXAMPLE 4

TABLET FORMULATION

Item	Ingredients	mg/Tablet					
1	Compound A*	5	25	100	250	500	750
2	Anhydrous Lactose	103	83	35	19	38	57
3	Croscarmellose Sodium	6	6	8	16	32	48
4	Povidone K30	5	5	6	12	24	36
5	Magnesium Stearate	1	1	1	3	6	9
	Total Weight	120	120	150	300	600	900

*Compound A represents a compound of the invention.

Manufacturing Procedure:

1. Mix Items 1, 2 and 3 in a suitable mixer for 15 minutes.
2. Granulate the powder mix from Step 1 with 20% Povidone K30 Solution (Item 4).
3. Dry the granulation from Step 2 at 50°C.
4. Pass the granulation from Step 3 through a suitable milling equipment.
5. Add the Item 5 to the milled granulation Step 4 and mix for 3 minutes.
6. Compress the granulation from Step 5 on a suitable press.

EXAMPLE 5

CAPSULE FORMULATION

Item	Ingredients	mg/Tablet				
1	Compound A *	5	25	100	250	500
2	Hydrous Lactose	159	123	148	--	--
3	Corn Starch	25	35	40	35	70
4	Talc	10	15	10	12	24
5	Magnesium Stearate	1	2	2	3	6
	Total Fill Weight	200	200	300	300	600

* Compound A represents a compound of the invention.

Manufacturing Procedure:

1. Mix Items 1, 2 and 3 in a suitable mixer for 15 minutes.
2. Add Items 4 & 5 and mix for 3 minutes.
3. Fill into a suitable capsule.

EXAMPLE 6

INJECTION SOLUTION/EMULSION PREPARATION

Item	Ingredient	mg/ml
1	Compound A *	1 mg
2	PEG 400	10-50 mg
3	Lecithin	20-50 mg
4	Soy Oil	1-5 mg
5	Glycerol	8-12 mg
6	Water q.s.	1 ml

* Compound A represents a compound of the invention.

Manufacturing Procedure:

1. Dissolve item 1 in item 2
2. Add items 3, 4 and 5 to item 6 and mix until dispersed, then homogenize.
3. Add the solution from step 1 to the mixture from step 2 and homogenize until the dispersion is translucent.
4. Sterile filter through a 0.2 µm filter and fill into vials.

EXAMPLE 7

INJECTION SOLUTION/EMULSION PREPARATION

Item	Ingredient	mg/ml
1	Compound A *	1 mg
2	Glycofurol	10-50 mg
3	Lecithin	20-50 mg
4	Soy Oil	1-5 mg
5	Glycerol	8-12 mg
6	Water	q.s. 1 ml

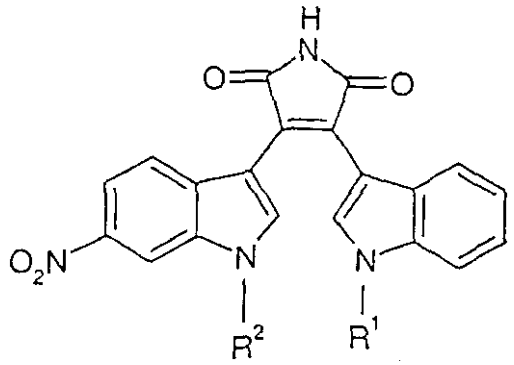
* Compound A represents a compound of the invention.

Manufacturing Procedure:

1. Dissolve item 1 in item 2
2. Add items 3, 4 and 5 to item 6 and mix until dispersed, then homogenize.
3. Add the solution from step 1 to the mixture from step 2 and homogenize until the dispersion is translucent.
4. Sterile filter through a 0.2 µm filter and fill into vials.

CLAIMS

1. A compound of formula



I

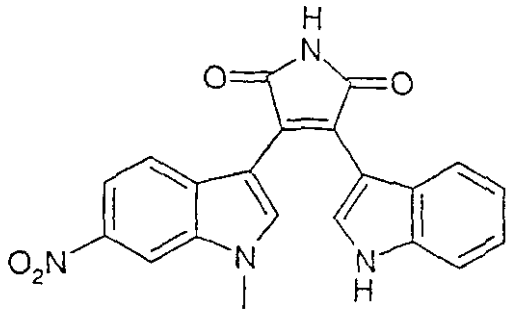
wherein R^1 is hydrogen and R^2 is methyl or

R^1 is methyl and R^2 is hydrogen or

R^1 is hydroxymethyl and R^2 is methyl

as well as pharmaceutically acceptable prodrugs or pharmaceutically acceptable salts thereof.

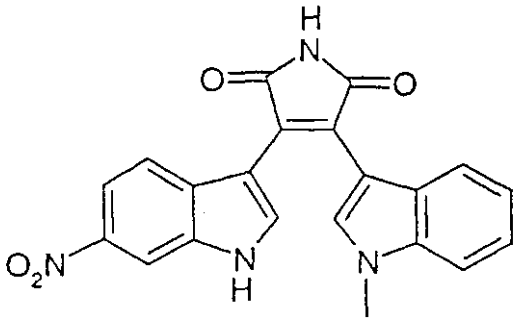
2. A compound of claim 1 of the formula



I-1

and pharmaceutically acceptable prodrugs or pharmaceutically acceptable salts of said compound.

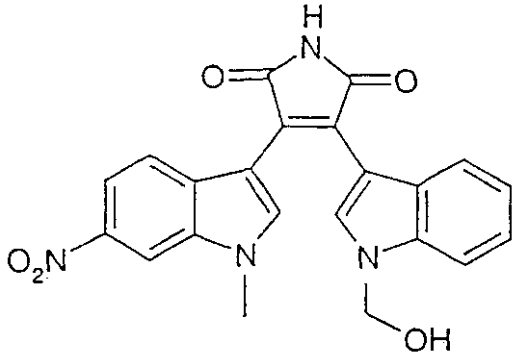
3. A compound of the formula



I-2

and pharmaceutically acceptable prodrugs or pharmaceutically acceptable salts of said compound.

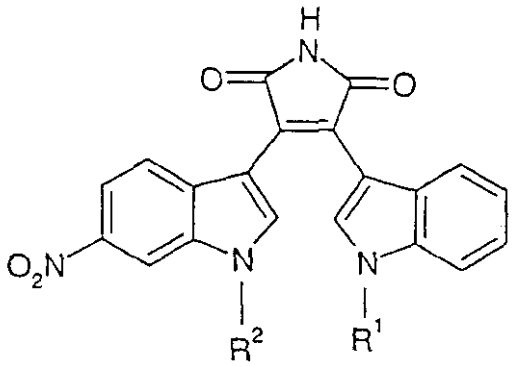
4. A compound of the formula



I-3

and pharmaceutically acceptable prodrugs or pharmaceutically acceptable salts of said compound.

5. A pharmaceutical composition comprising a compound of formula



I

wherein R^1 is hydrogen and R^2 is methyl or
R^1 is methyl and R^2 is hydrogen or
R^1 is hydroxymethyl and R^2 is methyl

or a pharmaceutically acceptable salt or prodrug of said compound and a pharmaceutically acceptable carrier.

6. A compound according to any one of claims 1-4 for use as an antitumor drug.

7. The use of a compound claimed in any one of claim 1-4 for the treatment of solid tumors or the preparation of pharmaceutical compositions.

8. The compounds, compositions, processes and uses as hereinbefore described.

*

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/EP 99/01534

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D403/14 A61K31/40

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 C07D 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.
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Y	WO 98 04551 A (F. HOFFMANN-LA ROCHE AG) 5 February 1998 * page 15-16: table; page 2, line 7-10 *	1,5-7
Y	WO 98 04552 A (F. HOFFMANN-LA ROCHE AG) 5 February 1998 * page 2, line 7-10; claim 1 *	1,5-7
A	WO 98 04553 A (F. HOFFMANN-LA ROCHE AG) 5 February 1998 see page 1	1,5-7

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 July 1999	09/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Bijlen, H

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ial Application No
PCT/EP 99/01534

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		EP	0915871 A	19-05-1999
		HR	970417 A	31-08-1998

[19]中华人民共和国国家知识产权局

[51]Int. Cl[7]
C07D403/14
A61K 31/40

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[71]申请人 弗·哈夫曼-拉罗切有限公司
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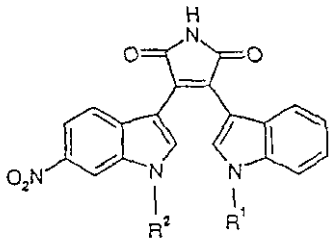
J·柯 G·F·威伯
[74]专利代理机构 中国国际贸易促进委员会专利商标事务所
代理人 郭建新

权利要求书3页 说明书18页 附图页数0页

[54]发明名称 抑制细胞增殖的取代双吲哚基马来酰亚胺

[57]摘要

式(Ⅰ)(其中,R^1是氢且R^2是甲基,或者R^1是甲基且R^2是氢,或者R^1是羟甲基且R^2是甲基)的取代吡咯及其药物上可接受的前体药物或药物上可接受的盐是适用于治疗癌的抗增殖剂。



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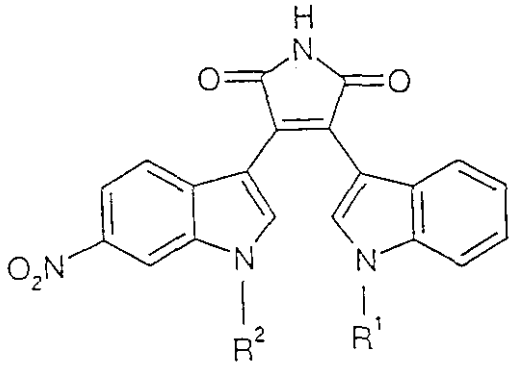
ISSN1008-4274

权 利 要 求 书

1. 下式的化合物



I

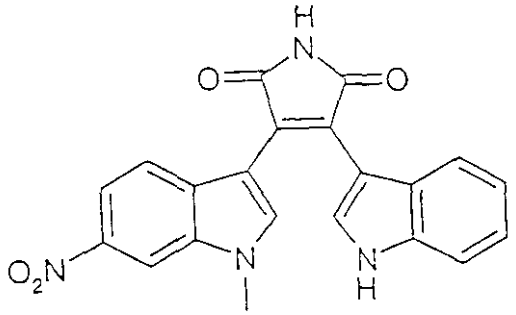
其中，R^1是氢且R^2是甲基，或者

R^1是甲基且R^2是氢，或者

R^1是羟甲基且R^2是甲基

及其药物上可接受的前体药物或药物上可接受的盐。

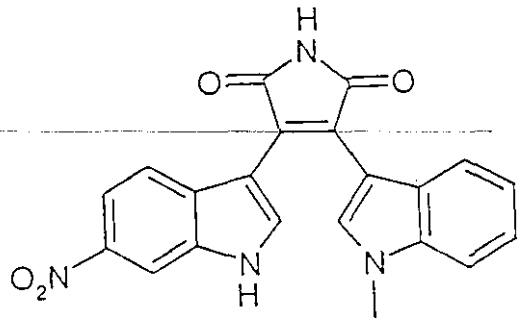
2.下式的权利要求 1 的化合物



I-1

和所述化合物的药物上可接受的前体药物或药物上可接受的盐。

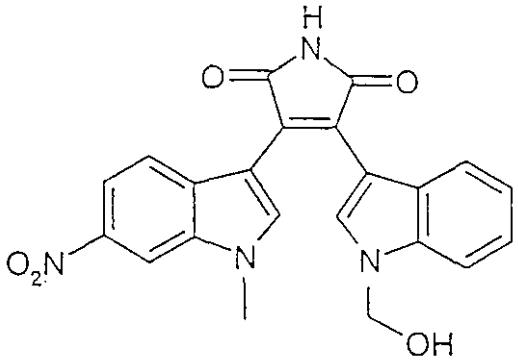
3. 下式的化合物



I-2

和所述化合物的药物上可接受的前体药物或药物上可接受的盐。

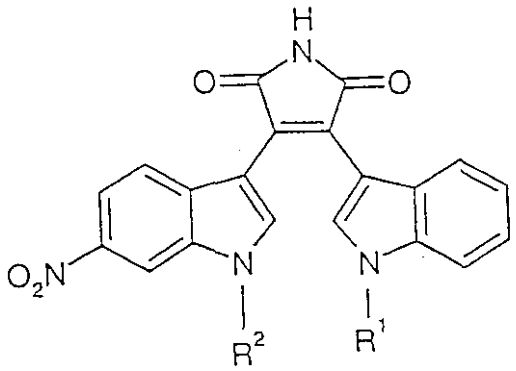
4. 下式的化合物



I-3

和所述化合物的药物上可接受的前体药物或药物上可接受的盐。

5. 一种药物组合物，它包含下式的化合物:



I

其中，R^1是氢且R^2是甲基，或者

R^1是甲基且R^2是氢，或者

R^1是羟甲基且R^2是甲基

或者所述化合物的药物上可接受的盐或前体药物以及药物上可接受的载体。

6. 用作抗肿瘤药物的权利要求 1～4 任一项的化合物。

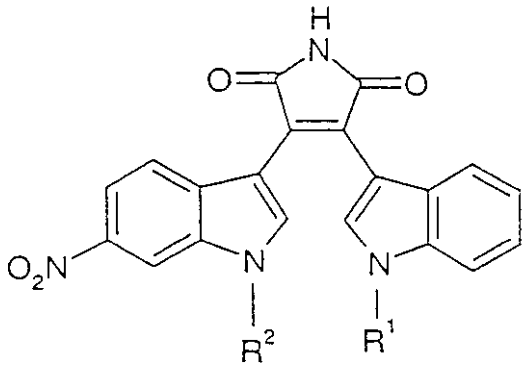
7. 权利要求 1～4 任一项的化合物在治疗实体瘤方面或在制备药物组合物方面的应用。

8. 本文上述化合物、组合物、方法和应用。

说　　明　　书

抑制细胞增殖的
取代双吲哚基马来酰亚胺

本发明涉及下式的取代吡咯



I

其中，R^1是氢且R^2是甲基，或者

R^1是甲基且R^2是氢，或者

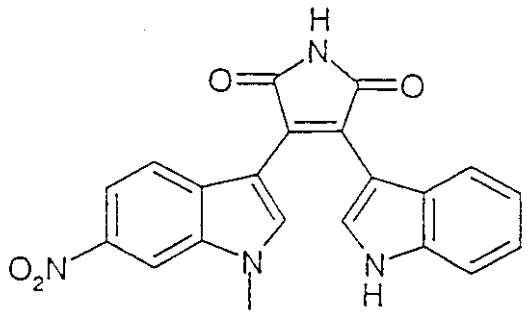
R^1是羟甲基且R^2是甲基

还涉及其药物上可接受的前体药物或药物上可接受的盐。

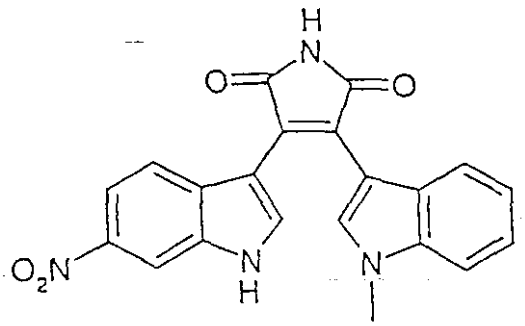
式 I 的化合物具有抗增殖活性，具体地说，它们抑制细胞周期的 G2/M 期中的细胞分裂，所以，通常被称为“G2/M 期细胞周期”抑制剂。

式 I 的化合物被 U.S.P. 5,057,614 的式 I 覆盖（但未作为一类或单独地具体公开）。此外，U.S.P. 5, 057,614 中未公开或表明本发明化合物的上述活性，所以，本发明化合物的上述活性令人感到意外。

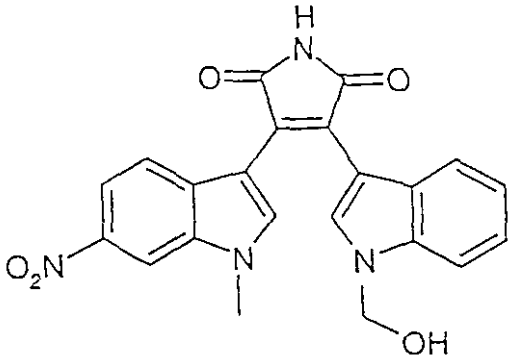
上式 I 包括如下三种化合物:



I-1



I-2



I-3

术语“药物上可接受的前体药物”表示这样的化合物：在生理条件下或通过溶剂解可将它转化为式I的任意化合物或转化为所述化合物的药物上可接受的盐。

式 I 的化合物以及所述化合物的药物上可接受的盐是通过下列示意图所表示的反应制备的。这些化合物中每一种的合成还被描述于实施例 1～3 中。

化合物 I－1 可这样制备：将（1－甲基－6－硝基－1H－吲哚－3－基）－氧代－乙酰氯（3）与[1－(2, 2-二甲基-丙酰)－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（7）反应，再用碱处理反应产物。

化合物 I－2 可这样制备：将（1－甲基－1H－吲哚－3－基）－氧代－乙酰氯（17）与[1－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（19）反应，再用碱处理反应产物。

化合物 I－3 可这样制备：将（1－甲氧基甲基－1H－吲哚－3－基）－氧代－乙酰氯（10）与（1－甲基－6－硝基－1H－吲哚－3－基）－3－乙亚氨基酸 1－甲基乙酯盐酸化物（15）反应，再用酸处理反应产物。

示意图 1

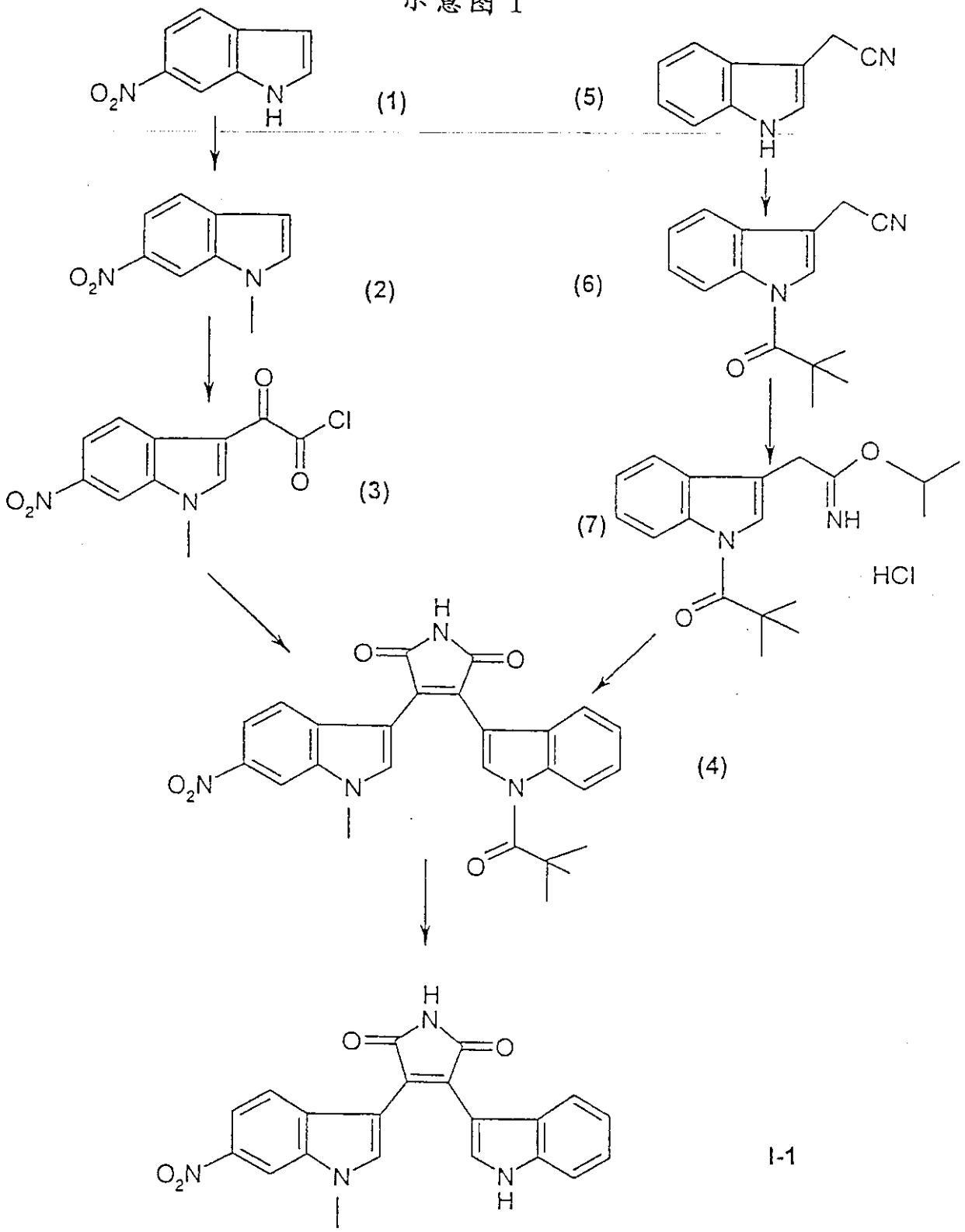


示意图 2

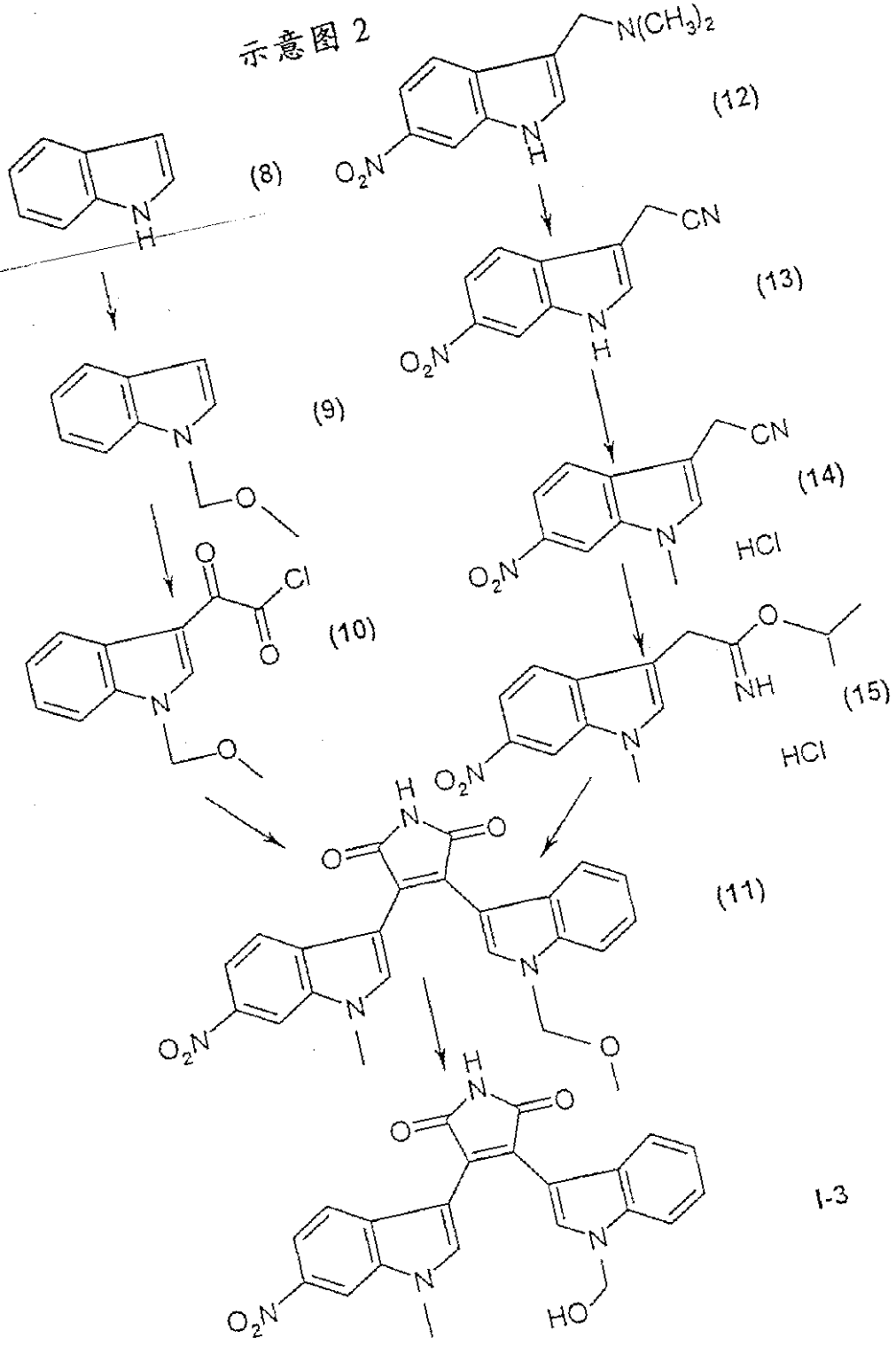
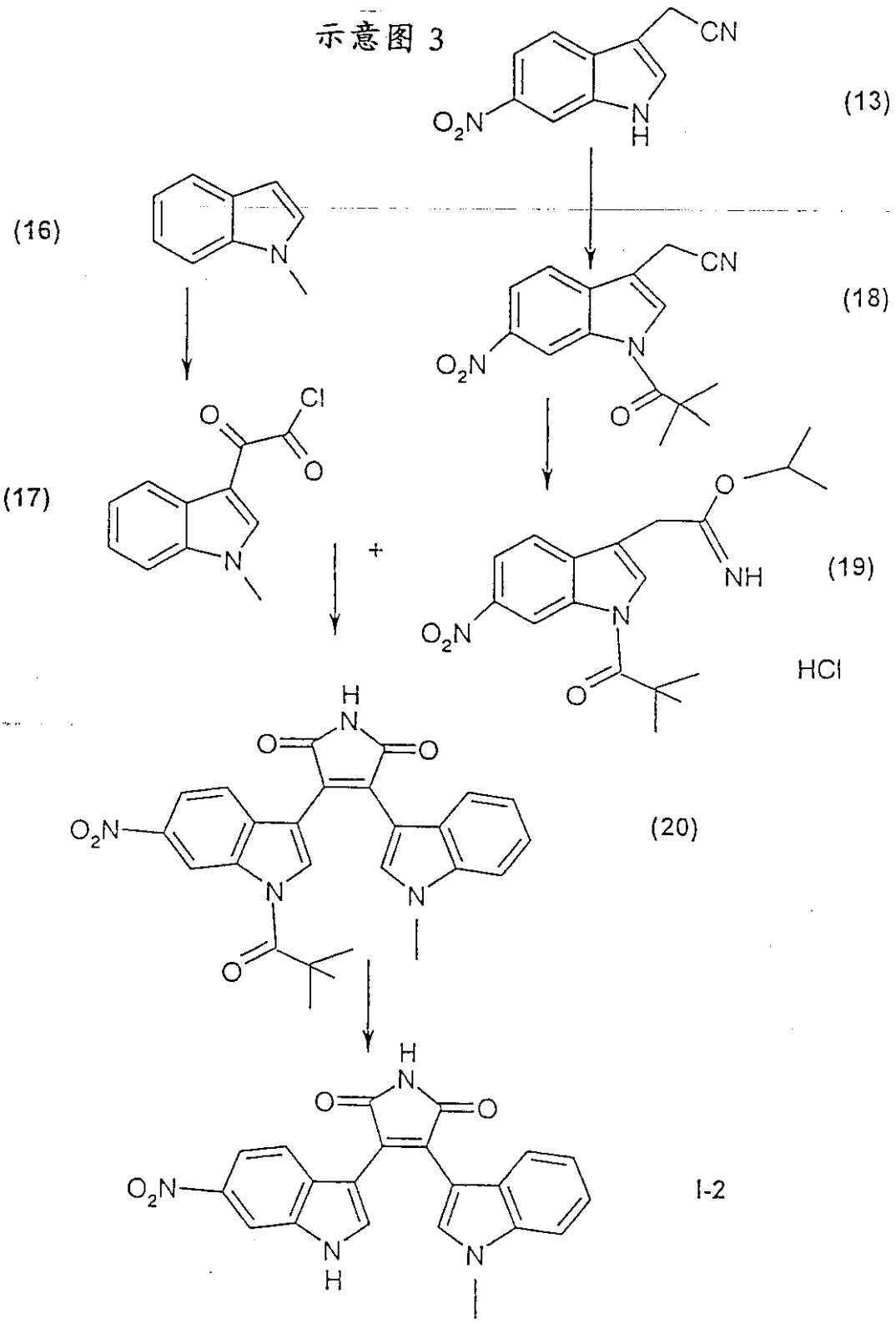


示意图 3



下面阐述本发明化合物的抗增殖活性。这些效果启示：所述化合物适用于治疗癌（尤其实体瘤）。

雌激素受体阴性上皮乳腺癌系（MDA－MB－435）购自美国典型细胞培养物保藏中心（ATCC; Rockville, MD）并在ATCC推荐的培养基中生长。要分析试验化合物对这些细胞生长的影响，将细胞以每孔2000个细胞铺板于96孔组织培养板（“试验板”），并在37℃和5% CO_2存在下保温一夜。次日，将试验化合物溶于100%二甲亚砜（DMSO）而得10mM储备溶液。用无菌蒸馏水稀释每种化合物至1mM，然后加到96孔“主平板”的三份孔中（孔内盛有形成40μM的最终浓度的足量培养基）。用培养基逐个稀释所述“主平板”中的化合物。将四分之一终体积的已稀释化合物转到两份“试验板”上。将DMSO加到一排“对比细胞”中，使每孔的最终DMSO浓度为0.1%。将“试验板”放回培养箱，在添加试验化合物3天后按下述方法分析一块“试验板”。类似地，在添加试验化合物5天后，按下述方法分板第二块“试验板”。

将3－（4, 5－二甲基噻唑－2－基）－2, 5－二苯基－2H－溴化四唑鎓（噻唑基蓝(thiazolyl blue); MTT）加到每孔中至1mg/ml的最终浓度。再在37℃下将平板保温3小时。然后，除去含MTT的培养基，往每孔添加50μl 100%乙醇而溶解生成的甲臜代谢物。为保证完全溶解，在室温下将平板振荡15分钟。在微量滴定板读数器（Molecular Dynamics）中读取570nm波长处的吸光度（以650nm为参比）。通过从所有的孔扣除空白，再从1.00减去每次试验三份的平均吸光度除以对比物的平均值所得的商而计算抑制百分数。从浓度的对数与抑制百分数的曲线图的线性回归确定抑制浓度（IC_{50}和IC_{90}）。

结肠腺癌系SW480和结肠癌系HCT－116也得自ATCC并按前面提供的同样方案（作如下修改）试验。将细胞系SW480以每孔1000个细胞铺板并在添加试验化合物6天后分析。将细胞系HCT－116以每孔750个细胞铺板并在添加试验化合物4天后分析。至于MTT分析，在吸出含MTT的培养基之前，将平板在1000rpm下离心5分钟，应用100μl 100%乙醇溶解甲臜。

将前面体外试验的结果列于表I－III中。

表 I

在细胞系 MDA－MB－435 中的抗增殖活性

化合物	$IC_{50}(\mu M)$
化合物 I－1	0.03*
化合物 I－3	0.05*
化合物 I－2	0.6*

*至少三个独立试验的平均值

表 II

在细胞系 HCT－116 中的抗增殖活性

化合物	$IC_{50}(\mu M)$
化合物 I－1	0.17*
化合物 I－3	0.23*
化合物 I－2	1.66*

*至少三个独立试验的平均值

表 III

在细胞系 SW480 中的抗增殖活性

化合物	$IC_{50}(\mu M)$
化合物 I－1	0.20*
化合物 I－3	0.22*
化合物 I－2	1.86*

*至少三个独立试验的平均值

要分析所述化合物对细胞周期进展的影响，将 MDA－MB－435 细胞（ATCC; Rockville, MD）以每 10cm 皿 1 $\times 10^6$ 个细胞/10ml 铺板于如下生长培养基中：RPMI 1640＋10％热灭活的胎牛血清，2mM L－谷氨酰胺和 5U/ml pen-strep（都得自 GIBCO/BRL, Gaithersburg, MD）。在 37℃和 5％ CO_2 存在下将细胞保温一夜。次日，将 10μl 每种待测化合

物于 100％DMSO 中的溶液加到各个皿中而得 1/1000x 最终浓度的储备溶液。此外，将 10μl 100％DMSO 加到对比皿中。包括对比板在内的所有平板中 DMSO 的最终浓度是 0.1%。将这些平板放回培养箱中。

然后，在各时间段，将每块平板中的培养基转移到 50ml 离心管中。再用 5ml 磷酸盐缓冲盐水（PBS; GIBCO/BRL）洗涤残留在皿中的细胞层。移出 PBS 并与相应管中的培养基合并。在 37℃下将细胞用胰蛋白酶消化 5 分钟，收集溶液并与相应管中的培养基和 PBS 合并。然后，在 1200rpm 下将这些管离心 5 分钟。这样固定细胞：移出上清液，轻敲离心管使粒状沉淀物分散开，再在缓缓旋转的同时添加 5ml 冷的 70％乙醇。然后，在－20℃下将细胞贮存 24 小时以上。

从冷冻机中取出所述盛有细胞的管，在室温下放置 20～30 分钟。在 3000rpm 下将这些管离心 5 分钟。移出上清液，用 5ml PBS 洗涤粒状沉淀物，象上述那样将管离心。接着，移出上清液，将粒状沉淀物重悬浮于 0.5ml PBS 中。此后，往每支管中添加 0.5ml RNA 酶 A（RNAse A）（1mg/ml 于 PBS 中），将这些管在 37℃下保温 15 分钟。往每支管中添加 100μl 碘化丙锭（Sigma, St. Louis, MO）（1mg/ml 于 PBS 中），再在室温下将管保温 2～3 分钟。将每份形成的溶液滤过滤器帽管（filter cap tube）（Becton Dickinson, San Jose, CA, # 2235）。

在 FACSort 仪（Becton-Dickinson）中应用厂商的 CellQUEST 程序读取样品，并利用厂商的 ModFIT 软件分析。该检测提供了下列每个期内细胞的百分数指示：G0/G1 期、DNA 合成（S）期和 G2/M 期。

在添加试验化合物 I－1、I－2 和 I－3 一天后分析细胞周期进展实验的结果并总结于下表 IV 中。

表 IV

试验化合物对细胞周期的影响

化合物	浓度	每个细胞周期期中的细胞%		
		G1	S	G2/M
DMSO	0.1%	43.93%	41.08%	14.99%
化合物 I	0.1μM	8.27%	25.21%	66.52%
化合物 I	0.03μM	45.30%	34.67%	20.03%
化合物 I	0.01μM	44.95%	41.04%	14.00%
化合物 I－3	0.3μM	1.11%	24.99%	73.90%
化合物 I－3	0. 1μM	15.54%	24.06%	60.40%
化合物 I－3	0.03μM	45.45%	38.06%	16.50%
化合物 I－2	10μM	10.41%	35.25%	54.34%
化合物 I－2	3μM	3.26%	48.75%	47.99%
化合物 I－2	1μM	27.21%	30.19%	42.60%

前述表 I～IV 中总结的结果表明：化合物 I－1、I－2 和 I－3 具有抗增殖活性；确切地说，它们引起细胞周期的 G2/m 期中细胞的积聚。

前述式 I 的吡咯及其前述盐可被用作药物（例如，呈药物制剂的形式），它们可被经口施药，例如，呈片剂、包衣片、锭剂、硬或软明胶胶囊、溶液、乳液或悬浮液的形式。它们还可被经直肠施药，例如，呈栓剂的形式；或者肠胃外施药，例如，呈注射液的形式。

要生产药物制剂，可将这些化合物与治疗上惰性的无机或有机载体配制。乳糖、玉米淀粉或其衍生物、滑石粉、硬脂酸（steric acid）或其盐可作为这样的载体用于片剂、包衣片、锭剂和硬明胶胶囊。用于软明胶胶囊的合适的载体有：植物油、蜡、脂肪、半固态或液态多元醇。不过，根据所述活性物质的性质，就软明胶胶囊来说通常不需要载体。生产溶液和糖浆剂的合适的载体有：水、多元醇、蔗糖、转化糖和葡萄糖。用于注射液的合适的载体有：水、醇、多元醇、甘油、植物油、磷脂和表面活性剂，用于栓剂的合适的载体有：天然油或硬化油、蜡、脂肪和半液态多元醇。

药物制剂还可以含防腐剂、增溶剂、稳定剂、润湿剂、乳化剂、增甜剂、着色剂、调味剂、改变渗透压的盐、缓冲剂、包衣剂或抗氧化剂。它们甚至还可以含其它治疗上有用的物质。

如前所述，式 I 的吡咯及其前述盐可被用于治疗或控制肿瘤病。剂量可在宽范围内变动，当然，应被调节到各具体情况下个体的需要。通常，就给体重约为 70kg 的成人经口或肠胃外施药来说，约 10mg~约 10,000mg、优选约 200mg～约 5,000mg、更优选约 200mg～约 1000mg 的日剂量应是合适的，不过，必要的话可以超出该上限。该日剂量可作为单剂量或均分剂量施药，或者用于肠胃外施药时，可作为连续输注给药。

如下实施例阐述了本发明。

实施例 1

3-（1H-吲哚-3-基）-4-（1-甲基-6-硝基-1H-吲哚-3-基）-吡咯-2，5-二酮（I-1）的制备

A. 1-甲基-6-硝基-1H-吲哚（2）

在 0～5℃下 10 分钟期间，往 0.33g（8.3mmol）NaH（60％油中分散液）于 30ml 干二甲基甲酰胺（“DMF”）的浆液中添加 0.973g（6.00mmol）可商购的 6-硝基-1H-吲哚（1）。在同样温度下搅拌 1 小时后，添加 0.75ml（12.1mmol）碘代甲烷，在相同温度下将混合物搅拌 30 分钟，再在室温下搅拌 1 小时，倾入冰水中，用乙酸乙酯萃取。用盐水洗涤有机相，在 $MgSO_4$ 上干燥，浓缩而得 0.814g（77.5%）1-甲基-6-硝基-1H-吲哚（2）黄色固体。未经纯化而直接应用该物质。

B.（1-甲基-6-硝基-1H-吲哚-3-基）-氧代-乙酰氯（3）

在 0～5℃的氩气氛中，往 1.33g（7.55mmol）1-甲基-6-硝基-1H-吲哚（2）的 40ml 乙醚溶液中添加 1.5ml（17.2mmol）草酰氯。有沉淀形成。搅拌 3 小时后，过滤形成的固体，用少量乙醚洗涤，干燥后得 1.9g（95％）（1-甲基-6-硝基-1H-吲哚-3-基）-氧代-乙酰氯（3）黄色固体。未经纯化而直接应用该物质。

C.[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－乙腈（6）

应用上述子部分 A 的操作方法，用 8.7ml（71mmol）三甲基乙酰氯和 3.4g（85mmol）用作碱的 NaH（油中的 60％分散液）在 115ml DMF 中对 10.2g（65mmol）可商购的（1H－吲哚－3－基）－乙腈（5）进行 N－烷基化反应，用色谱法纯化后得 6.6g（38.7%）[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－乙腈（6）黄色油。

D.[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（7）

在 0～5℃下 20 分钟期间，往 6.6g（27.5mmol）得自如上步骤 C 的[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－乙腈（6）于 105ml 2－丙醇中的浆液中滴加 40ml（0.563mol）乙酰氯。在室温下将反应混合物搅拌一夜，浓缩，用大约 75ml 乙酸乙酯稀释残余物，在蒸汽浴上加热 15 分钟，冷却后放入冷冻机中。过滤沉淀，干燥后得 6.0g（65.0%）[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（7）白色固体。

E. 3－[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－4－(1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（4）

在 0℃的氩气氛中，往 1.25g（4.69mmol）得自如上步骤 B 的（1－甲基－6－硝基－1H－吲哚－3－基）－氧代－乙酰氯（3）和 1.6g（4.75mmol）得自如上步骤 D 的[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（7）的 80ml 二氯甲烷溶液中添加 2.6ml（18.65mmol）三乙胺。在相同温度下搅拌 30 分钟后，再在室温下将反应混合物搅拌 3 1/2 小时，用更多二氯甲烷稀释。用水、0.5N HCl 溶液、盐水洗涤有机相，在 $MgSO_4$ 上干燥，浓缩而得 3.01g 泡沫体。将该物质溶于 50ml 甲苯，在 0℃下用 987.9mg（5.19mmol）对－甲苯磺酸处理。在室温下搅拌 3 小时后用二氯甲烷萃取反应混合物。用饱和 $NaHCO_3$ 溶液、盐水洗涤有机相，在 $MgSO_4$ 上干燥，浓缩得 3.9g 粗物质。在硅胶柱上用色谱法纯化，得 1.7g（77％）3－[1－(2, 2－二甲基－丙酰)－1H－吲哚－3－基]－4－(1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（4）橙色固体。mp>146℃（分解）。MS:

（M^+），m/z 470。

F. 3－（1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－1）

用 5.6ml（8.96mmol）的 1.6 摩尔 $NaOCH_3$ 甲醇溶液处理 1.7g（3.61mmol）得自如上步骤 E 的 3－[1－(2，2－二甲基－丙酰)－1H－吲哚－3－基]－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（4）的 60ml 甲醇溶液。在室温下搅拌反应 1 小时，倾到 2N HCl/冰中，用乙酸乙酯萃取。在无水 $MgSO_4$ 上干燥有机萃取物，浓缩，用色谱法纯化后得 394.7mg（28％）3－（1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－1）红色固体，mp>280℃。MS：（M^+）m/z 386。

实施例 2

3－（1－羟甲基－1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－3）的制备

A. 1－甲氧基甲基－1H－吲哚（9）

应用实施例 1 中步骤 A 的操作方法，用 1ml（13.1mmol）氯甲基甲基醚和 0.48g（12mmol）作为碱的 NaH（油中 60％分散液）在 22ml DMF 中对 1.17g（10mmol）可商购的吲哚（8）进行 N－烷基化反应，用色谱法纯化后得 1.4g（86.9％）1－甲氧基甲基－1H－吲哚（9）无色油。

B.（1－甲氧基甲基－1H－吲哚－3－基）－氧代－乙酰氯（10）

应用实施例 1 中步骤 B 的操作方法，将得自如上步骤 A 的 0.23g（1.43mmol）1－甲氧基甲基－1H－吲哚（9）与 0.25ml（2.86mmol）草酰氯在 3.5ml 乙醚中反应而生成 0.174g（48.5％）（1－甲氧基甲基－1H－吲哚－3－基）－氧代－乙酰氯（10）黄色固体。未经纯化而直接应用该物质。

C.（6－硝基－1H－吲哚－3－基）－乙腈（13）

在 0－5℃下一小时期间，边搅拌边往 44.27g（0.204mol）6－硝基

芦竹碱（12）[Jackson B. Hester, 有机化学杂志(J. Org. Chem.) 29: 1158(1964)]的 450ml 乙腈溶液中添加 44.59g（0.31mol）碘代甲烷。在室温下搅拌反应混合物达三小时，然后，一次性添加 26.6g（0.543mol）氯化钠的 225ml 水溶液。在 32℃下将反应混合物加热一夜，冷却到室温，用总量为 800ml 的乙酸乙酯和 300ml 水将产物萃取 3 次。用水、1N HCl 溶液、饱和碳酸氢钠溶液洗涤合并的萃取液，在 $MgSO_4$ 上干燥，真空蒸发溶剂。将橙棕色残余物（41.3g）溶于 200ml 温热的乙酸乙酯，通过小硅胶垫片，蒸发溶剂后产生 28.9g(70.4%)（6－硝基－1H－吲哚－3－基）－乙腈（13）黄色固体。

D. （1－甲基－6－硝基－1H－吲哚－3－基）－乙腈（14）

在室温下，将 65.5g（0.474mol）粉状碳酸钾加到 28.9g（0.143mol）得自如上步骤 C 的（6－硝基－1H－吲哚－3－基）－乙腈（13）于 230ml 二甲基甲酰胺的溶液中。搅拌该悬浮液达 40 分钟，然后在 65 分钟内滴加 25.48g（0.179mol）碘代甲烷。在室温下搅拌一夜后，冷却反应混合物，倾入总量为 600ml 的水中。过滤沉淀，用少量水洗涤，在磷酐（phosphor anhydride）上干燥直至恒重。该操作产生 30.4g（95.4%）（1－甲基－6－硝基－1H－吲哚－3－基）－乙腈（14），未进一步纯化而直接应用它。

E. （1－甲基－6－硝基－1H－吲哚－3－基）－3－乙亚氨基酸 1－甲基乙酯盐酸化物（15）

在 0～10℃下，将 HCl 气流鼓泡通入搅拌下 82g（0.382mol）得自如上步骤 D 的（1－甲基－6－硝基－1H－吲哚－3－基）－乙腈（14）于 1000ml 2－丙醇的悬浮液中。添加约 350g HCl 后，往反应混合物中添加乙醚直至形成沉淀。收集固体，用乙醚洗涤并真空干燥而得 102g（85.7%）（1－甲基－6－硝基－1H－吲哚－3－基）－3－乙亚氨基酸 1－甲基乙酯盐酸化物（15）。

F. 3－（1－甲氧基甲基－1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（11）

应用实施例 1 中步骤 E 的操作方法，将 1.3g（5.17mmol）得自如上步骤 B 的氧代乙酰氯（10）与 1.7g（5.45mmol）得自如上步骤 E 的

（1－甲基－6－硝基－1H－吲哚）－3－乙亚氨基酸 1－甲基乙酯盐酸化物（15）在 95ml 二氯甲烷中进行缩合反应，生成 1.08g（48.5%）3－（1－甲氧基甲基－1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（11）橙色固体，mp>250℃(分解)。MS: （M^+），m/z 430。

G. 3－（1－羟甲基－1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－3）

用大约 40ml 2N HCl 处理 727.5mg 得自如上步骤 F 的 3－[1－(甲氧基甲基)－1H－吲哚－3－基]－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（11）于 65ml THF 中的溶液。将反应混合物回流 5 小时，冷却，用乙酸乙酯萃取产物。在 $MgSO_4$ 上干燥有机相，蒸发溶剂而得橙色固体。用色谱法纯化该物质而得 123.3mg 3－（1－羟甲基－1H－吲哚－3－基）－4－（1－甲基－6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－3）红色固体，mp 210~213℃。MS: （M^+），m/z 416。

实施例 3

3－（1－甲基－1H－吲哚－3－基）－4－（6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－2）的制备

A. （1－甲基－1H－吲哚－3－基）－氧代－乙酰氯（17）

应用实施例 1 中步骤 B 的操作方法，用 6ml（47mmol）可商购的 1－甲基－1H－吲哚（16）与 8ml（92mmol）草酰氯在 120ml 乙醚中反应，生成 7.6g（73.2%）（1－甲基－1H－吲哚－3－基）－氧代－乙酰氯（17）黄色固体。未经纯化而直接应用该物质。

B.[1－(2,2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－乙腈（18）

应用实施例 1 中步骤 A 的操作方法，用 0.3ml（2.44mmol）三甲基乙酰氯和 70.8mg（1.77mmol）作为碱的 NaH（油中 60% 分散液）在 8ml DMF 中对 346.6mg（1.72mmol）得自实施例 2 步骤 C 的 6－硝基－1H－吲哚基－3－乙腈（13）进行 N－烷基化反应，用色谱法纯化后得

287.7mg（43.2%）[1－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－乙腈（18）黄色油。

C. [1－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（19）

在 0～5℃下，将 HCl 气流鼓泡通入不断搅拌下的 1.45g（5.08mmol）得自如上步骤 B 的 1 [－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚基]－3－乙腈（18）于 90ml 2－丙醇的悬浮液中。在室温下将反应混合物搅拌 21 小时。真空蒸发溶剂而得 1.95g（100%）黄色固体（19）。未进一步纯化而直接应用该物质。

D. 3－[1－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－4－(1－甲基－1H－吲哚－3－基）－吡咯－2，5－二酮（20）

应用实施例 1 中步骤 E 的操作方法，将 1.1g（4.96mmol）得自如上步骤 A 的氧代乙酰氯（17）与 1.95g（5.08mmol）得自如上步骤 C 的 [1－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－3－乙亚氨基酸 1－甲基乙酯盐酸化物（19）和 2.1ml（17.94mmol）三乙胺在 120ml 二氯甲烷中反应，用 1.1g（5.78mmol）对甲苯磺酸一水合物的 80ml 甲苯溶液处理生成的产物，得 1.3g（62.1%）3－[1－(2, 2－二甲基－丙酰)－6－硝基－1H－吲哚－3－基]－4－(1－甲基－1H－吲哚－3－基）－吡咯－2，5－二酮（20）橙色固体；mp>245℃（分解）。MS: （M^+），m/z 470。

E. 3－（1－甲基－1H－吲哚－3－基）－4－（6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－2）

应用实施例 1 中步骤 F 的操作方法，用 4.3ml（6.88mmol）的 1.6 摩尔 $NaOCH_3$ 于 65ml 甲醇中的溶液对 1.3g（2.76mmol）得自如上步骤 D 的 3－[1－（2，2－二甲基－丙酰）－6－硝基－1H－吲哚－3－基]－4－（1－甲基－1H－吲哚－3－基）－吡咯－2，5－二酮（20）进行 N－去保护反应，从乙酸乙酯和己烷中结晶后得 300.6mg（28.1%）3－（1－甲基－1H－吲哚－3－基）－4－（6－硝基－1H－吲哚－3－基）－吡咯－2，5－二酮（I－2）红色固体；mp>260℃。MS: （M^+），m/z 386。

实施例 4

片剂配方

项目	组分	mg/片					
1	化合物 A*	5	25	100	250	500	750
2	无水乳糖	103	83	35	19	38	57
3	交联羧甲纤维素钠 (Croscarmellose Sodium)	6	6	8	16	32	48
4	聚维酮 K30(Povidone K30)	5	5	6	12	24	36
5	硬脂酸镁	1	1	1	3	6	9
	总重量	120	120	150	300	600	900

*化合物 A 代表本发明的化合物。

制备操作:

1. 将第 1、2 和 3 项在合适的混合器中混合 15 分钟。
2. 将步骤 1 的粉状混合物与 20% 聚维酮 K30 溶液（第 4 项）一起造粒。
3. 在 50℃下干燥步骤 2 的粒化物。
4. 使步骤 3 的粒化物通过合适的研磨设备。
5. 将第 5 项加到步骤 4 的研磨后的粒化物中并混合 3 分钟。
6. 在合适的压制机上压制步骤 5 的粒化物。

实施例 5

胶囊配方

项目	组分	mg/片				
1	化合物 A*	5	25	100	250	500
2	含水乳糖	159	123	148	--	--
3	玉米淀粉	25	35	40	35	70
4	滑石粉	10	15	10	12	24
5	硬脂酸镁	1	2	2	3	6
	总填充重量	200	200	300	300	600

*化合物 A 代表本发明的化合物。

制备操作:

1. 将第 1、2 和 3 项在合适的混合器中混合 15 分钟。
2. 添加第 4 & 5 项并混合 3 分钟。
3. 填入合适的胶囊。

实施例 6

注射溶液/乳液制剂

项目	组分	mg/ml
1	化合物 A*	1mg
2	PEG 400	10-50mg
3	卵磷脂	20-50mg
4	豆油	1-5mg
5	甘油	8-12mg
6	水适量	1ml

*化合物 A 代表本发明的化合物。

制备操作:

1. 将第 1 项溶于第 2 项中。
2. 将第 3、4 和 5 项加到第 6 项中并混合直至分散，然后均化。
3. 将步骤 1 的溶液加到步骤 2 的混合物中，均化直至分散液变成半透明。
4. 通过 0.2μm 滤器进行无菌过滤并注入小瓶。

实施例 7

注射溶液/乳液制剂

项目	组分	mg/ml
1	化合物 A*	1mg
2	Glycofurol	10-50mg
3	卵磷脂	20-50mg
4	豆油	1-5mg
5	甘油	8-12mg
6	水	q.s. 1ml

*化合物 A 代表本发明的化合物。

制备操作:

1. 将第 1 项溶于第 2 项中。

2. 将第 3、4 和 5 项加到第 6 项中并混合直至分散，然后均化。

3. 将步骤 1 的溶液加到步骤 2 的混合物中，均化直至分散液变成半透明。

4. 通过 0.2μm 滤器进行无菌过滤并注入小瓶。