PATENT SPECIFICATION (11) 1 594 840

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(21) Application No. 53541/77 (22) Filed 22 Dec. 1977
(31) Convention Application No. 51/154 941
(32) Filed 24 Dec. 1976 in
(33) Japan (JP)
(44) Complete Specification published 5 Aug. 1981
(51) INT CL³ C07D 405/12; A61K 31/505; (C07D 405/12, 239/60, 311/04)
(52) Index at acceptance
C2C 1602 1673 200 213 215 247 250 252 253 25Y 28X 311 31Y 351 352 366 368 387 491 628 678 67X 698 761 764 802 80Y AA TL

(19)



(54) 5-FLUOROURACIL DERIVATIVES AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

(71) We, EISAI CO., LTD., of 6—10, 4-chome, Koishikawa, Bunkyo-ku, Tokyo, Japan, a Japanese company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

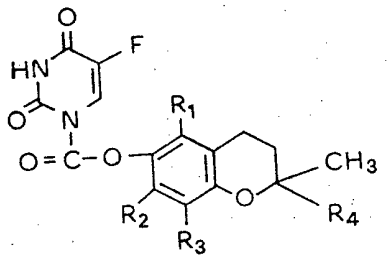
This invention relates to 5-fluorouracil derivatives and to pharmaceutical compositions containing said derivatives.

Heretofore, alkylating agents such as busulfan, chlorambutyl and merphalan, and antimetabolites such as methorexate, 6-mercaptopurine, 6-mercaptopurine ribo- nucleoside and 5-fluorouracil, have been used as chemotherapeutics to various malignant tumours such as gastric cancer, heptoma, bladder cancer, seminoma, leukemia, lymphosarcoma, breast cancer, lung cancer and uterine cancer.

Of these chemotherapeutics, 5-fluorouracil has clinical utility because of the advantages that it is effective against a relatively large number of types of tumours, that its effect is increased by combination therapies with other antitumour drug(s), and that the effect of radiotherapy is enhanced by using 5-fluorouracil. However, careful attention is required because 5-fluorouracil shows relatively severe toxicity at the effective dose. Under such circumstances, many investigations have been made to find more useful 5-fluorouracil derivatives which were less toxic to the host and showed more activity to tumours. As one result of the investigation, N_1-(2'tetrahydrofurfuryl)-5-fluorouracil (FT—207) was found.

FT—207 shows remarkably less toxicity i.e. 1/7—1/5 of that of 5-fluorouracil *per se* and shows less antitumour activity. However, in clinical applications a considerably long period of time is required to obtain remission by administration of FT—207 alone.

According to the present invention we provide a 5-fluorouracil derivative represented by the general formula:



(I)

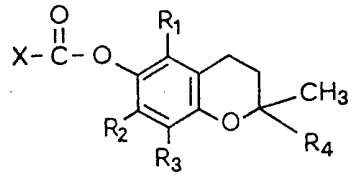
wherein R_1, R_2 and R_3 each represents a hydrogen atom or a methyl group, and R_4 represents a saturated or unsaturated, straight or branched alkyl group.

The present derivatives can be used in methods for treating various malignant tumours such as gastric cancer, hepatoma, bladder cancer, seminoma, leukemia, lymphosarcoma, breast cancer, lung cancer and uterine cancer.

Examples of derivatives I include N_1 - α - tocopheryl carbonyl - 5 - fluorouracil, N_1 - β - tocopheryl carbonyl - 5 - fluorouracil, N_1 - γ - tocopheryl carbonyl - 5-fluorouracil, N_1 - δ - tocopheryl carbonyl - 5 - fluorouracil, N_1 - α - tocotrienyl carbonyl - 5 - fluorouracil, N_1 - (2,2,5,7,8 - pentamethyl chromanyl - 6 - carbonyl) - 5-fluorouracil, N_1 - (2,5,7,8 - tetramethyl - 2 - isohexylchromanyl - 6 - carbonyl) - 5-fluorouracil, N_1 - [2,5,7,8 - tetramethyl - 2 - (4',8' - dimethylnonyl) - chromanyl-6 - carbonyl] - 5 - fluorouracil.

There are various processes which can be used for synthesis of the derivatives (I) depending on their chemical structures. One of the processes is illustrated as follows:

5-fluorouracil is reacted with a carbonate of a 6-chromanol derivative of the general formula (II):



(II)

wherein R_1, R_2, R_3 and R_4 have the same meanings as defined above, and X represents a halogen atom or lower alkoxy group, to obtain the desired derivatives (I). In this reaction, it is preferable to use the starting material, 5-fluorouracil, in the form of an alkali metal salt thereof. The reaction can advantageously be carried out by using an organic solvent such as dimethyl formamide or dimethyl acetamide. The reaction proceeds best at an equimolar ratio of reactants and at a temperature ranging from room temperature to about 50°C.

The following shows data for the acute toxicities and antitumour activities of N_1 - d,l - α - tocopheryl carbonyl - 5 - fluorouracil, a compound according to this invention(hereinafter referred to as Compound A of this invention). FT—207, and 5-fluorouracil (hereinafter abbreviated to 5—FU), are selected as standard drugs for comparative purposes.

1. *Acute toxicity*

LD_{50} (mg/Kg) after three weeks observation in CDF_1 mice (female, 8—10 weeks old)

TABLE 1

	Intravenous administration	Intraperitoneal administration	Subcutaneous administration	Oral administration
Compound A	–	>4000	>4000	>4000
FT–207	750	800	830	930
5–FU	135	150	175	120

From Table 1, it can be seen that the Compound A has a lower acute toxicity than FT—207 or 5—FU.

In addition, similar results were also obtained for the acute toxicity (LD_{50}) in ICR/JCL mice.

2. *Antitumour activity against Sarcoma-180 (S—180)*

(1) Inhibitory effect on the growth of S—180 ascites form (Intraperitoneal administration)

Implantation

10^6 cells of S—180 were intraperitoneally implanted in ICR/JCL mice (female, 8—10 weeks old).

Administration

i) To test groups:

The Compound A was administered as a solution in soybean oil and 5—FU was administered as saline solution, at 24 hours after the implantation.

ii) To control groups:

Soybean oil was administered as control for the Compound A.
Saline solution was administered as control for 5—FU.

Observation

Ten days after the implantation of the tumour cells, the total packed cell volume (TPCV) was measured. The measurement of TPCV was carried out in accordance with the method of Hoshi, A. *et al,* Chemical & Pharmaceutical Bulletion *17* (4), 848—850 (1969). The results are shown in Table 2, wherein T/C% means the ratio of the TPCV of test groups to that of the control group.

TABLE 2

Drug administered	Dose (mg/Kg)	Number of animals	Tumour growth (TPCV : T/C%)
Soybean oil	–	16	100
Compound A	4000	8	0
	2000	8	0
	1000	8	0
	500	8	3.3
	250	8	30.4
	125	8	64.7
	62	8	77.5
Saline solution (control)	–	16	100
5—FU	500	8	(died)
	250	8	(died)
	125	8	4.3
	68	8	26.4
	34	8	43.3

It is understood from Tables 1 and 2 that ED_{50} of the Compound A of this invention is about 170 mg/Kg and its safety index (LD_{50}/ED_{50}) is more than 24, while ED_{50} of 5—FU is about 25 mg/Kg and its safety index is about 5. This results show that the Compound A is less toxic when used as an antitumour agent.

(2) *Survival effect*

Implantation

10^6 cells of S—180 were intraperitoneally implanted in LCR/JCL mouse (female, 6—8 weeks old).

Administration

i) To test group:

Compound A was administered as a solution in soybean oil at 24 hours after the implantation.

h) To control group:

Soybean oil was administered as a control for Compound A.

Observation

The number of days survival after implantation was measured. The increase in life-span over control groups (ILS=T/C%—100) was calculated. The results are shown in Table 3.

TABLE 3

Drug administered	Dose (mg/Kg)	Number of animals	Mean survival days	ILS (%) over control
Soybean oil (Control)	—	8	13.8	0
Compound A	4000	8	39.0	182.6
	2000	8	36.0	160.9
	1000	8	43.5	215.2
	500	8	33.0	139.1

It is confirmed from Table 3 that the Compound A exhibits a marked effect by a single administration of 1/8 LD_{50}.

(3) *Effect on growth of solid tumour by oral administration*

Implantation

1.8×10^6 cells of S—180 were implanted subcutaneously in ICR/JCL mice (female, 6—8 weeks old).

Administration

i) To test group:

Each of the drugs was orally administered as a solution in safflower oil at three hours after the implantation.

ii) To control group:

Safflower oil was administered as control for each of the drugs.

Observation

Twenty days after administration, the tumour weights were measured and the ratio of the tumour weight to that of the control group (T/C%) was calculated. The results are shown in Table 4.

TABLE 4

Drug administered	Dose (mg/Kg)	Number of animals	Tumour growth (weight : T/C %)
Safflower oil (control)	–	8	100
Compound A	62	8	106
	125	8	87
	250	8	33
	500	8	47
	1000	8	38
	2000	8	36
FT–207	25	8	148
	50	8	269
	100	8	58
	200	8	43
	400	8	34
5–FU	10	8	156
	20	8	55
	40	8	52
	80	8	63

From Table 4, the minimum effective dose of the Compound A is about 250 mg/Kg, that of FT—207 is about 100 mg/Kg, and that of 5—FU is about 20 mg/Kg. As for the ratios of minimum effective dose to toxic dose (LD_{50}) in each of the drugs, the Compound A gives less than 1/16; FT—207 gives about 1/10; and 5—FU gives about 1/6, respectively. Therefore, the bioavailability of the Compound A is higher than those of the other two drugs.

3. *Antitumour activity on L—1210 leukemia*

1) *Survival effect*

Implantation

10^5 cells of L—1210 leukemia were intraperitoneally implanted in CDF_1 mice (female, 8—10 weeks old).

Administration

i) To test group:

Compound A was intraperitoneally administered as a solution in a medium chain triglyceride (MCT) at 24 hours after the implantation.

East of 5—FU and FT—207 was also intraperitoneally administered as a saline solution at 24 hours after the implantation.

(ii) To control group:

MCT was administered as control for Compound A.

Saline solution was administered as controls for 5—FU and FT—207.

Observation

The mean survival time after implantation was determined, and the increase in life-span (ILS) over the control was calculated. The results are shown in Table 5.

TABLE 5

Drug administered	Dose (mg/Kg)	Number of animals	Mean survival days	ILS % over controls
MCT (control)	–	6	7.0	0
Compound A	125	6	7.0	0
	250	6	8.0	14
	500	6	9.8	40
	1000	6	12.1	73
	2000	6	12.5	79
	4000	6	13.5	93
Saline	–	27	7.0	0
5–FU (Saline)	15	6	8.1	15
	30	6	8.8	25
	60	6	9.7	38
FT–207 (Saline)	50	6	7.0	0
	200	6	7.1	1

From Table 5, it does not appear certain that FT—207 is effective at a dose of about 1/4 LD_{50}. On the contrary, it can be confirmed that the Compound A is effective even at a dose of about 1/8 LD_{50}.

Additionally, in comparison of the dose of the Compound A with that of 5—FU, wherein the dose shows the corresponding increase in life-span (ILS), the ratio of said dose to the LD_{50} in the Compound A is smaller than that of 5—FU. Therefore, the availability of the Compound A is higher than that of 5—FU.

2) *Duration of antitumour activity*

Administration

i) To test group:

Compound A was intraperitoneally administered in CDF_1 mice (female, 8—10 weeks old) as a solution in MCT, before the implantation as shown in Table 6.

Each of FT—207 and 5—FU was intraperitoneally administered as a saline solution, before the implantation as shown in Table 6.

ii) To control groups:

MCT was administered as control for Compound A.

Saline solution was administered as controls for FT—207 and 5—FU.

Implantation

10^5 cells of L—1210 leukemia were intraperitoneally implanted in the mice.

Observation

The number of days survival after the implantation were counted. The results were shown in Table 6.

TABLE 6

Drug administered	Administration time of drug (number of days before tumour Implantation)	Number of animals	Mean survival days
No administration	–	7	9.1
MCT (control)	0	4	9.0
	1	8	10.3
	2	7	10.1
	2	7	10.1
	3	7	10.1
	4	7	9.9
	6	5	9.0
Compound A (Dose : 1000 mg/Kg)	0	8	11.6
	1	6	13.0
	2	8	16.4
	3	8	11.8
	4	7	11.2
	6	4	13.5
Saline	1	5	8.8
	2	5	9.0
	4	5	9.0
FT–207 (Saline) (Dose : 200 mg/Kg)	1	5	8.8
	2	5	9.0
	4	5	9.0
5–FU (Saline) (Dose : 50 mg/Kg)	1	5	9.0
	2	5	9.0
	4	5	8.8

In Table 6, it is shown that the Compound A exhibits a survival effect after administration either on the same day of the implantation of the tumour cells, or on 1—6 days prior to the implantation, and that the Compound A shows antitumour effect for 6 days.

As described above, derivatives of this invention have very lower toxicity and a broader range between toxic dose and effective dose than 5—FU and FT—207. The life in a patient of derivatives of this invention is more effective than 5—FU and FT—207. In view of these points, the derivatives of this invention appear to be excellent antitumour agents.

The typical dose of derivatives of this invention as antitumour agents amounts to 1—100 mg/Kg per day, preferably 5—100 mg/Kg for an adult.

The derivatives of this invention may be administered either orally or parenterally as pharmaceutical compositions containing the derivative and pharmaceutically acceptable excipient. Possible administration forms include powders, granules, tablets, capsules, liquids for injection, suppositories and ointments. The formulations can be prepared using a conventional excipient by means of a conventional process.

The following examples are illustrative of this invention, but are not to be construed as limiting the invention. Parts and percentages are by weight unless otherwise specified.

Example 1.

Synthesis of N_1 - d,l - α - tocopheryl carbonyl - 5 - fluorouracil

A suspension was prepared by adding 10.3 g (0.13 mols) of 55% oily sodium hydride to 100 ml of dimethyl formamide. The suspension was cooled to below 5°C. 150 ml of dimethyl formamide solution containing 16.9 g (0.13 mols) of 5-fluorouracil were added slowly dropwise to the suspension while stirring for 30 minutes, and the mixed solution was further kept for one hour at the same condition. To the solution 100 ml of dimethyl formamide solution containing 64.1 g (0.13 mols) of d,l-α-tocopheryl chloroformate were added slowly dropwise for 30 minutes. The solution was heated to 40°C and stirred for additional one hour. After the stirring, the reaction solution was allowed to cool to room temperature, and subjected to filtration. The filtrate was concentrated under a reduced pressure. The resulting residue in an amount of 76 g was extracted twice with 300 ml of benzene. The benzene extracts were combined, and concentrated under reduced pressure. The resulting residue in an amount of 71 g was recrystallized from ethanol to obtain the desired compound as a white crystalline powder having a melting point of 119—120°C.

Yield: 64.7 g (85%).

Elementary analysis of the compound for $C_{34}H_{51}FN_2O_5$:

	C	H	N
Calculated (%)	69.59	8.76	4.77
Found (%)	69.44	8.70	4.79

Infrared absorption spectrum: (KBr method)

Three absorption bands due to carbonyl group are observed in the range 1700—1770 cm^{-1}.

Nuclear magnetic resonance spectrum: ($CDCl_3$)

δ 8.22 (d:1, J_{5-6}=8.0H_z, H_6)
2.60 (t:2 J=7H_z)
2.09—2.04 (s: 9 Phenylmethyl group)
1.80 (t:2, J=7H_z)
1.60—1.18 (m: 18)
0.9 —0.8 (d :15)

Mass spectrum: M^+=586

Example 2.

Synthesis of N_1 -(2,2,5,7,8 - pentamethyl chromanyl - 6 - carbonyl)-5 - fluorouracil

2.47 g (0.019 mols) of 5-fluorouracil and 5.13 g (0.019 mols) of 2,2,5,7,8-pentamethylchromanyl-6-chloroformate were subjected to the reaction and the subsequent treatment according to the procedure of Example 1. The desired material was thus obtained in the form of a white crystalline powder having a melting point of 208—210°C.

Yield: 4.9 g (68.5%)

Elementary analysis of the compound for $C_{19}H_{21}FN_2O_5$:

	C	H	N
Calculated (%)	60.63	5.60	7.40
Found (%)	60.75	5.58	7.41

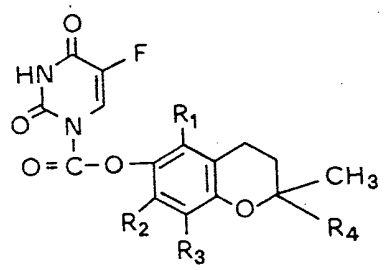
Infrared absorption spectrum: (KBr method)

Three absorption bands due to the carbonyl group were observed in the range 1700—1700 cm^{-1}.

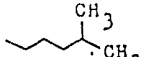
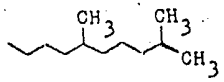
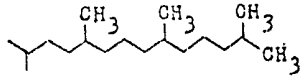
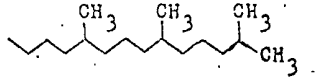
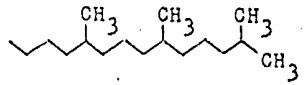
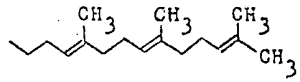
Nuclear magnetic resonance spectrum: (deutero pyridine)

8.24 (d=1 $J_{5-6}=8.0H_z$, H_6)
2.62 (t=2 $J=7H_z$)
2.08—2.04 (s=9 phenylmethyl group)
1.82 (t=2, $J=7H_z$)
1.32 (s=6 methyl group)

Other compounds according to this invention were prepared in a similar manner and are shown as Examples 3 to 8 in the following table.



(I)

Example No.	R_1	R_2	R_3	R_4	Molecular formula / Melting point (°C)	Elementary analysis (%) Calculated / Found C	H	N	F	Remarks
3	CH_3	CH_3	CH_3		$C_{24}H_{31}FN_2O_5$ 198 – 200	64.55 64.71	6.70 6.72	6.28 6.24	4.26 4.18	d, l – form
4	CH_3	CH_3	CH_3		$C_{29}H_{41}FN_2O_5$ 177 – 179	67.41 67.29	8.00 8.05	5.42 5.37	3.68 3.49	d, l – form
5	H	CH_3	CH_3		$C_{33}H_{49}FN_2O_5$ 102 – 103	69.20 69.35	8.62 8.58	4.89 4.94	3.32 3.20	d – form
6	H	H	CH_3		$C_{32}H_{47}FN_2O_5$ 89 – 90	68.79 68.87	8.48 8.51	5.02 5.10	3.40 3.28	d – form
7	CH_3	CH_3	CH_3		$C_{34}H_{51}FN_2O_5$ 132 – 133	69.59 69.71	8.76 8.71	4.77 4.82	3.24 3.34	d – form
8	CH_3	CH_3	CH_3		$C_{34}H_{51}FN_2O_5$ 147 – 149	70.32 70.35	7.81 7.78	4.83 4.90	3.27 3.22	d, l – form

Example 9.

Capsule

N_1-d,1-α-tocopheryl carbonyl-5-fluorouracil	80 g
Micro-crystalline cellulose	40 g
Corn starch	15 g
Lactose	12 g
Polyvinyl pyrolidone	3 g
Total	150 g

In accordance with the above formulation, N_1 - d,1 - α - tocopheryl carbonyl - 5-fluorouracil, micro-crystalline cellulose, corn starch and lactose were mixed together. Aqueous polyvinyl pyrolidone solution was added as a binder to this mixture, and the whole was granulated by a conventional process. These granules were filled in to hard gelatinous capsules to form capsules.

Example 10.

Tablet

N_1-d,1-α-tocopheryl carbonyl-5-fluorouracil	100 g
Micro-crystalline cellulose	40 g
Corn starch	14 g
Lactose	20 g
Calcium carboxymethyl cellulose	10 g
Polyvinyl pyrolidone	6 g
Talc	10 g
Total	200 g

According to the above formulation, N_1 - d,1 - α - tocopheryl carbonyl - 5-fluorouracil, micro-crystalline cellulose, corn starch, lactose and calcium carboxymethyl cellulose were mixed together. Aqueous polyvinyl pyrolidone solution was added as a binder to said mixture, and the whole was granulated by a conventional process. The granules were admixed with the talc to form tablets of 200 mg per tablet.

Example 11.

Liquid for injection

N_1-d-γ-tocopheryl carbonyl-5-fluorouracil	10 g
Nikko HCO—60 (Trade Mark, Nikko Chemical Company, Japan)	40 g
Propylene glycol	80 g
Sorbitol	20 g
Distilled water sufficient to make up total	1 litre

According to the above formulation, a liquid for injection was prepared by a conventional method.

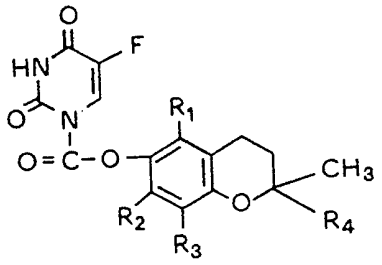
Example 12.

Suppository

Forty grams of N_1 - d,1 - α - tocopheryl carbonyl - 5 - fluorouracil were dissolved in one litre of O.D.O. (medium chain triglyceride produced and sold by Nisshin Oil Co., Ltd., Japan). This solution was made into a film on soft capsules of gelatine to obtain a suppository.

WHAT WE CLAIM IS:—

1. 5-fluorouracil derivative represented by the general formula:



(I)

wherein R_1, R_2 and R_3 each represents a hydrogen atom or a methyl group, and R_4 represents a saturated or unsaturated, straight or branched alkyl group.

2. N_1-α-tocopheryl carbonyl-5-fluorouracil.

3. N_1-β-tocopheryl carbonyl-5-fluorouracil.

4. N_1-γ-tocopheryl carbonyl-5-fluorouracil.

5. N_1-δ-tocopheryl carbonyl-5-fluorouracil.

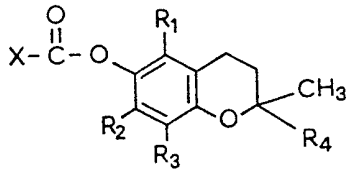
6. N_1-α-tocotrienyl carbonyl-5-fluorouracil.

7. N_1-(2,2,5,7,8-pentamethyl chromanyl-6-carbonyl)-5-fluorouracil.

8. N_1-(2,5,7,8-tetramethyl-2-isohexylchromanyl-6-carbonyl)-5-fluorouracil.

9. N_1 - [2,5,7,8 - tetramethyl - 2 - (4′,8′ - dimethylnonyl) - chromanyl - 6-carbonyl] - 5 - fluorouracil.

10. A method of preparing a 5-fluorouracil derivative of general formula I as defined in Claim 1 which comprises reacting 5-fluorouracil with a carbonate of a 6-chromanol derivative of the general formula

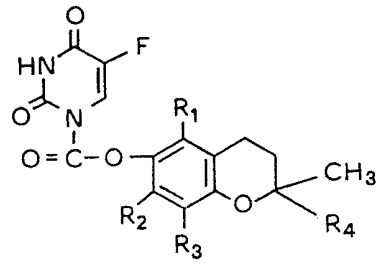


(II)

where R_1, R_2, R_3 and R_4 are as defined in Claim 1 and X represents a halogen atom or lower alkoxy group.

11. A method of preparing a 5-fluorouracil derivative of general formula I as defined in Claim 1, substantially as hereinbefore described in Example 1.

12. A pharmaceutical composition containing a 5-fluorouracil derivative represented by the general formula:



(I)

wherein R_1, R_2 and R_3 each represent hydrogen atom or methyl group, and R_4 represents a saturated or unsaturated, straight or branched alkyl group, and a pharmaceutically acceptable excipient.

13. A pharmaceutical composition according to Claim 12 wherein the 5-fluorouracil derivative of general formula I is as defined in any one of Claims 2 to 9.

14. A pharmaceutical composition according to Claim 12 substantially as hereinbefore described in any one of Examples 9 to 12.

MARKS & CLERK,
Chartered Patent Agents,
57-60 Lincoln's Inn Fields,
London, WC2A 3LS.
Agents for the Applicants.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1981.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.