CAMPTOTHECIN DERIVATIVES WITH ANTITUMOR ACTIVITY

The present invention relates to novel camptothecin derivatives having antitumor activity, the processes for the preparation thereof, the use thereof as antitumor drugs and pharmaceutical compositions containing them.

BACKGROUND OF THE INVENTION

Camptothecin is an alkaloid extracted from *Camptotheca acuminata* (Nyssaceae), first described by Wall and Wani in 1966 (J. Am. Chem. Soc. 1966, 88, 3888-3890). Camptothecin, albeit endowed with wide spectrum antitumor activity, especially against colon tumor and other solid tumors and leukemias, is not used in therapy due to its high toxicity, which is particularly manifested in the form of hemorrhagic cystitis, gastrointestinal toxicity and myelosuppression.

A number of camptothecin analogues have been synthesized in order to obtain compounds having low toxicity and high solubility. At present, two drugs are used in clinical practice, namely CPT-11 and topotecan. Other derivatives, such as belotecan, rubitecan, exatecan, gimatecan, pegamotecan, lurtotecan, karenitecin, afeletecan, homocamptothecin, diflomotecan, and many others, are undergoing clinical experimentation. Compound CPT-11 is a highly soluble pro-drug for 10-hydroxy-7-ethylcamptothecin (commonly known as SN-38), approved for the treatment of many solid tumors and ascites (colorectal, skin, stomach, lung, cervice, ovary, non-Hodgkin lymphoma).

Topotecan is a compound soluble in physiological solution, active against the tumors of the lung, stomach, liver, ovary, breast, prostate, esophagus, rectum, soft tissues sarcomas, head and neck, glioblastoma, chronic and acute myelocytic leukemias. Topotecan shows, however, important side effects such as neutropenia and thrombocytopenia.

Lurtotecan is a more soluble derivative, having activity in tumors of the neck, ovary, breast, colo-rectal, and pulmonary microcytoma. However, Lurtotecan also has hematic toxicity.

Rubitecan is a prodrug for the oral use effective against tumors of the pancreas, ovary and breast.

Camptothecin and its analogues, as is the case with all topoisomerase I inhibitors, are effective against tumors resistant to conventional drugs, including topoisomerase II inhibitors; maintain high topoisomerase levels during the whole cell cycle; do not induce multi-drug resistance (Pgo or MRP) or detoxifying metabolism mediated by the enzyme.

Research is now focused on novel inhibitors of the topoisomerase I having lower toxicity than the presently used drugs.

Open-ring camptothecin derivatives show high protein binding (in particular with albumin) and low distribution in the tumor tissues. As a consequence, the product accumulates in the body and tumors are poorly affected.

Conversely, the high lipophilicity of the lactone form promotes the adhesion of camptothecin derivatives to cell membranes, particularly erythrocytes, affecting the tissue/plasma distribution ratio. For this reason, research is being focused towards two alternative approaches: a) design of low protein binding products still having good solubility; b) design of highly potent products having therapeutical effect even at extremely low doses.

Modifications at the 7-, 9-, 10- and 11- positions usually proved well tolerated while not affecting the stability of the DNA-Topoisomerase I-camptothecin ternary complex, the formation of which is responsible for the antitumor activity of the compounds.

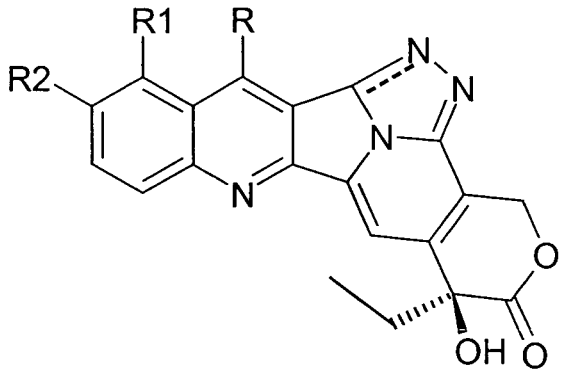
Products with 20R configuration proved either inactive or very less active than the products with 20S configuration - which coincides with the

natural configuration.

As a rule, modifications at the 5- position are considered unfavourable to the formation of the ternary complex, whereas modifications at the pyridone rings D and E have bee reported to be deleterious to the activity of the product.

DISCLOSURE OF THE INVENTION

In a first aspect, the invention relates to camptothecin derivatives of general formula I:



(I)

wherein:

R is alkyl, aminoalkyl, hydroxyalkyl, nitrile, alkoxymino, aryloxymino, silylalkyl;

R1 is hydrogen, hydroxy, alkoxy, aminoalkyl;

R2 is hydrogen, hydroxy, alkoxy, aminoalkyl, optionally protected hydroxyl;

wherein the alkyl, alkoxy, aminoalkyl or alkoxymino groups can contain 1 to 8, preferably 1 to 4 carbon atoms, in a straight or branched chain, whereas the aryloxymino group can contain 5 to 10 carbon atoms;

the pharmaceutically acceptable salts, isomers, enantiomers, diastereomers thereof and corresponding mixtures.

The compounds of the invention show low protein binding and have good solubility and high potency even at very low doses.

The preferred synthetic route for the preparation of the compounds of the invention is illustrated in the following scheme and substantially involves the following steps:

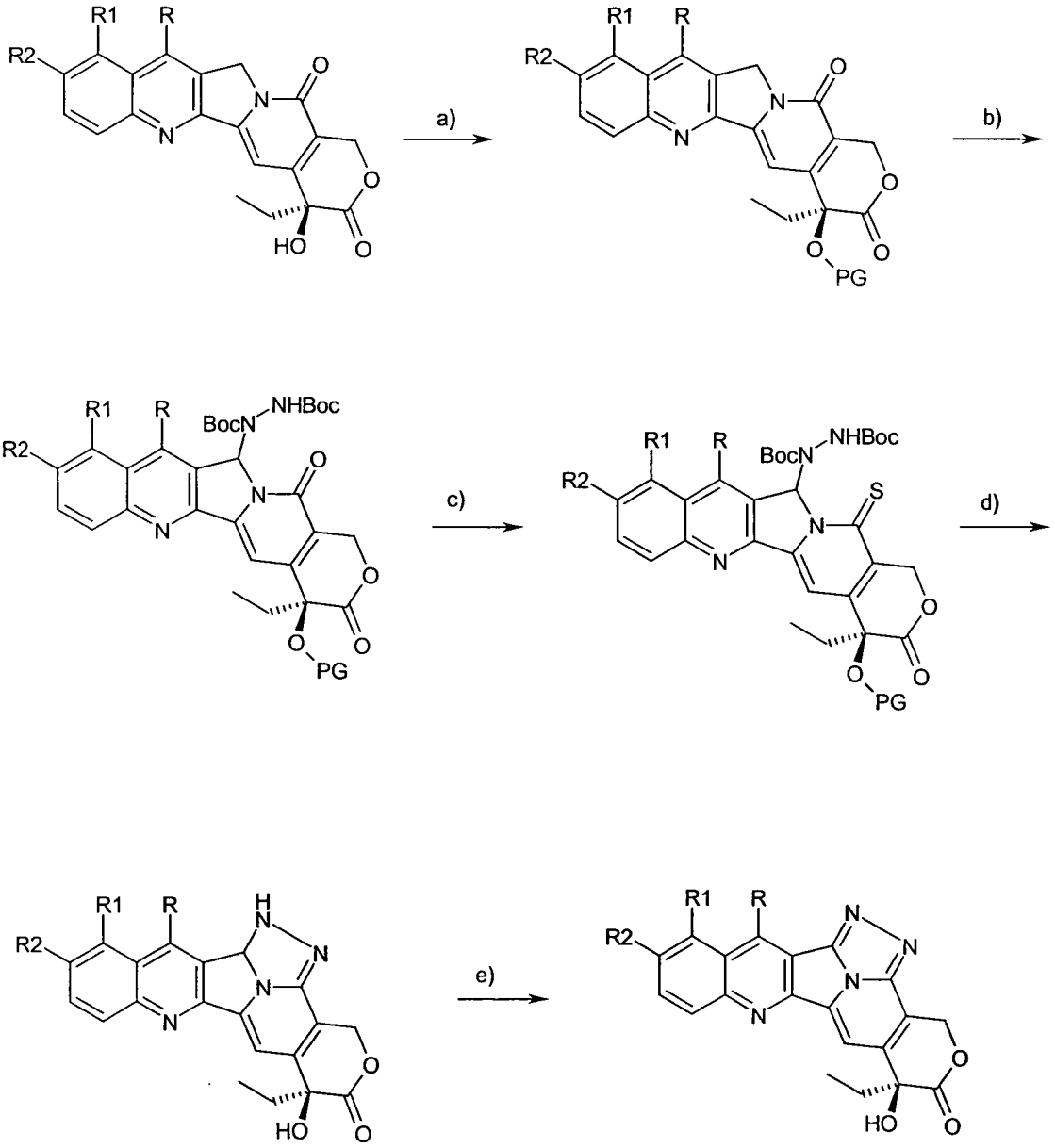
a) protection of the precursor hydroxy groups

b) derivatization at 5- with N,N-diprotected hydrazine

c) optional conversion of the pyridone ring to thiopyridone ring

d) removal of the protective groups with concomitant cyclization

e) optional aromatization of the pyrazole ring



In the Scheme, R, R1 and R2 have the meanings described above, and PG is a hydroxy-protecting group.

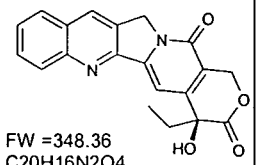
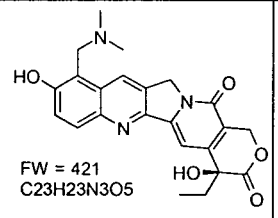
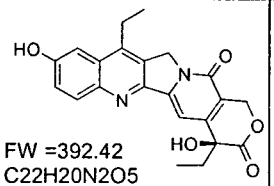
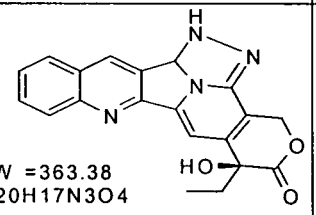
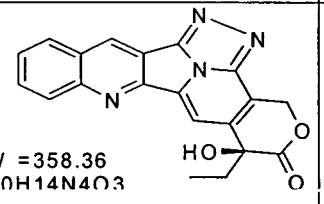
Hydroxyls are preferably protected by means of easily cleavable acyl groups, preferably trichloroacetate and Troc, or silyl groups, preferably triethylsilyl.

Derivatization at 5- with protected hydrazine can be obtained by treating the precursor with a strong organic base, such as LiHMDS, and reacting the resulting carbanion with an aza dicarboxylate, such as di-t-butoxy aza dicarboxylate or dibenzyloxy aza dicarboxylate. Conversion of the pyridone ring to thiopyridone ring can be obtained by reaction with 2,4-bis(4-methoxyphenyl)-1,2,3,4-dithiaphosphethane-2,4-disulfide (commonly known as Lawesson's reagent) (Cava P.M. et al., Tetrahedron 1985, 41, 5061; Cherkasov RA et al Tetrahedron 1985 41, 2567; Ghattas AAG et al, Sulfur Lett. 1982, 1, 69; Yde B et al, Tetrahedron 1984, 40. 2047) or with an equivalent reagent. Lawesson's reagent is preferred.

The purpose of the optional conversion to thiopyridone is to promote ring closure once hydrazine has been deprotected. It has however been observed that said closure reaction is spontaneous and immediate even without activation of the pyridine carbonyl for example as thiocarbonyl.

When the hydroxy-protecting groups are silyls and those at the nitrogen are carbamates, they are usually removed with trifluoroacetic acid. In an alternative procedure, steps b) and c) can be reversed.

The compounds of the invention were tested in a cytotoxicity assay on a wide spectrum of tumour cells. By way of example, the cytotoxicity data on the NCI-H460 cell line (NSCL cancer) concerning two compounds of formula (I) are reported, using camptothecin and the drugs Topotecan and SN-38 as references:

Name	Formula	NCI-H460 IC50 (µg/mL) Cell count
Camptothecin	 FW =348.36 C20H16N2O4	0.115 ± 0.0174
Topotecan	 FW = 421 C23H23N3O5	0.63 ± 0.44
SN38	 FW =392.42 C22H20N2O5	0.0865 ± 0.0049
IDN 6132	 FW =363.38 C20H17N3O4	17 ± 4.25
IDN 6137	 FW =358.36 C20H14N4O3	3.8 ± 1.08

The most active compounds were evaluated in a DNA cleavage assay measuring the active concentration and damage persistence (see the section 'Examples'). The derivatives of formula (I) surprisingly show higher

persistence in blocking DNA replication than the reference standards (particularly topotecan and camptothecin), while maintaining an effective cytotoxic activity.

In a further aspect, the invention relates to pharmaceutical compositions containing a compound of formula (I) together with pharmaceutically acceptable carriers and excipients. The pharmaceutical forms suitable to the oral or parenteral administration of the compounds (I) can be solid, preferably capsules, tablets and granules, or liquid, preferably injectable or infusion solutions.

The suitably formulated compounds of the invention can be used for the treatment of solid tumors and leukemias, in particular tumors of the lung, ovary, breast, stomach, liver, prostate, soft tissue sarcomas, head and neck, esophagus, pancreas, colon, rectum, glioblastoma, chronic and acute myelocytic leukemias.

EXAMPLES

EXAMPLE I - 20-OTES-camptothecin

Camptothecin (0.100 g, 0.287 mmol), is suspended in anhydrous dimethylformamide (3 mL), under inert atmosphere, and the resulting suspension is added with imidazole (0.980 g, 1.44 mmol). The mixture is stirred for 10' minutes, subsequently triethylsilyl chloride (TES-Cl) (0.193 mL, 1,15 mmol) is dropped therein, followed by addition of 4-dimethylamino pyridine (DMAP) (0.040 g 0.287 mmol). After 46 h, the reaction mixture is evaporated under vacuum, (TLC control of the complete disappearance of the reagent, eluent CH_2Cl_2/MeOH = 30/1). The solid is subsequently redissolved in CH_2Cl_2 and washed with H_2O and saturated NH_4Cl. The aqueous phase is extracted with CH_2Cl_2 (2 X 10 mL). The organic phases are combined and dried over Na_2SO_4, filtered and concentrated under vacuum, thereby obtaining the desired product (0.133 g, 0.287 mmol) as a pale yellow

solid.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.37 (s, 1 H, Ar, H-7), 8.25 (d, 1 H, *J* = 8.4 Hz, Ar), 7.92 (d, 1 H, *J* = 8.0 Hz, Ar), 7.82 (t, 1 H, *J* = 8.0 Hz, Ar), 7.65 (t, 1 H, *J* = 8.4 Hz, Ar), 7.57 (s, 1 H, H-14), 5.67 (d, 1 H, *J* = 16.4 Hz, H-17), 5.29 (s, 2 H, H-5), 5.25 (d, 1 H, *J* = 16.4 Hz, H-17), 2.00-1.84 (m, 2 H, H-19), 1.03-0.93 (m, 12 H), 0.80-0.71 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.7, 157.6, 152.5, 151.5, 149.0. 145.9, 130.9, 130.4, 130.0. 128.4, 128.1, 128.0. 127.9, 118.9, 94.4, 75.3, 66.0. 50.0. 33.2, 7.9, 7.2, 6.4.

EXAMPLE II - Preparation of 5-di-t-butoxycarbonylhydrazino-20-OTES-camptothecin

Camptothecin 20-OTES (0.100 g, 0.216 mmol) is dissolved in anhydrous THF (6 mL) with stirring under inert atmosphere, then cooled to a temperature of -78°C and a 1.0 M LiHMDS solution in THF (0.281 mL, 0.281 mmol) is dropped therein. After 20', di-tert-butylazo dicarboxylate (DTBAC) (0.075 g, 0.324 mmol) in anhydrous THF (2 mL) is added. After 4 h at -78°C, the disappearance of the reagent is monitored by TLC (Hexane/AcOEt = 3/1). Formation of the two diastereomers is observed. The reaction is quenched by addition of saturated NH_4Cl. The aqueous phase is extracted with CH_2Cl_2 (3 x 15 mL) and the organic phases are combined, dried over Na_2SO_4, filtered and concentrated under vacuum. The residue is purified by flash chromatography (SiO_2, Hexane/AcOEt = 3/1), thereby obtaining a mixture of the two isomers (0.145 g, 0.210 mmol, 97%). The two isomers are separated by further chromatography. In order of elution:

1st diastereomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.80 (br s, 1 H, Ar), 8.23 (d, 1 H, *J* = 8.4 Hz, Ar), 8.01 (br d, 1 H, Ar), 7.90-7.71 (m, 2 H, Ar), 7.70-7.45 (m, 2 H, Ar + H-14), 6.52 (br s, 1 H, H-5), 5.61 (d, 1 H, *J* = 16.8 Hz, H-17), 5.23 (d, 1 H, *J* = 16.8 Hz, H-17), 2.03-1.81 (m, 2 H, H-19), 1.79-1.08 (br s, 18 H), 1.06-0.92 (m, 12 H), 0.80-0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100

MHz) δ 171.7, 157.8, 155.5, 155.5, 152.0. 152.0. 151.2, 149.4, 145.0. 132.1, 130.6, 130.0. 128.7, 128.4, 127.9, 119.9, 98.2, 82.7, 81.5, 79.7, 75.2, 65.7, 33.2, 28.3, 27.6, 7.7, 7.2, 6.4.

2nd diastereomer: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.79 (br s,1 H, Ar), 8.23 (d, 1 H, *J* = 8.4 Hz, Ar), 8.01 (br d, 1 H, Ar), 7.85-7.76 (m, 2 H, Ar), 7.65 (br t, 1 H, *J* = 8.4 Hz, Ar), 7.52 (s, 1 H, H-14), 6.54 (br s, 1 H, H-5), 5.61 (d, 1 H, *J* = 16.8 Hz, H-17), 5.22 (d, 1 H, *J* = 16.8 Hz, H-17), 2.03-1.82 (m, 2 H, H-19), 1.76-1.08 (br s, 18 H), 1.04-0.92 (m, 12 H), 0.80-0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 157.9, 155.5, 155.5, 152.3, 152.0. 151.2, 149.4, 145.1, 132.1, 130.6, 130.0. 128.7, 128.4, 127.9, 119.9, 98.2, 82.9, 81.5, 79.6, 75.2, 65.8, 33.3, 28.3, 27.4, 7.8, 7.2, 6.4.

EXAMPLE III - Preparation of 5-di-*t*-butoxycarbonylhydrazino-20-OH-camptothecin 1st diastereomer

5-di-*t*-Butoxycarbonylhydrazino-20-OTES-camptothecin (0.050 g, 0.072 mmol) first diastereomer is dissolved in anhydrous THF (4 mL) with stirring under inert atmosphere, subsequently $Et_3N\bullet 3HF$ (0.088 mL, 0.542 mmol) is dropped therein. The reaction mixture is reacted for 35 h at room temperature, monitoring by TLC the disappearance of the reagent (Hexane/AcOEt = 3/2). The solvent is evaporated off under vacuum and the residue is purified by flash chromatography (SiO_2, Hexane/AcOEt = 3/2), thereby obtaining the desired compound (0.041 g, 0.071 mmol, 98%) as a pale yellow solid.

The product is further purified by crystallization from CH_2Cl_2/Pentane = 1/50.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.77 (br s, 1 H, Ar), 8.16 (br d, 1 H, *J* = 8.0 Hz, Ar), 7.97 (br s, 1 H, Ar), 7.86-7.50 (m, 4 H, Ar), 6.51 (br s, 1 H, H-5), 5.66 (d, 1 H, *J* = 16.4 Hz, H-17), 5.24 (d, 1 H, *J* = 16.4 Hz, H-17), 3.86 (br s, 1 H, OH), 2.00-1.80 (m, 2 H, H-19), 1.79-1.13 (br s, 18 H), 1.03 (t, 3 H, *J* =

7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 157.9, 155.5, 155.5, 152.1, 151.3, 150.7, 149.6, 145.7, 132.3, 130.7, 129.9, 128.7, 127.9, 127.6, 120.0. 97.9, 82.8, 81.6, 79.7, 72.7, 66.1, 31.8, 28.3, 27.7, 7.7.

EXAMPLE IV - Preparation of 5-di-*t*-butoxycarbonylhydrazino-20-OH-camptothecin 2nd diastereomer

5-di-*t*-Butoxycarbonylhydrazino-20-OTES-camptothecin (0.050 g, 0.072 mmol) 2nd diastereomer is dissolved in anhydrous THF (4,5 mL) with stirring under inert atmosphere, subsequently $Et_3N \bullet 3HF$ (0.088 mL, 0.542 mmol) is dropped therein. The reaction mixture is reacted for 35 h at room temperature, monitoring by TLC the disappearance of the reagent (Hexane/AcOEt = 3/2). The solvent is evaporated off under vacuum and the residue is purified by flash chromatography (SiO_2, Hexane/AcOEt = 3/2), thereby obtaining the desired compound (0.040 g, 0.069 mmol, 96%) as a pale yellow solid.

The product is further purified by crystallization from CH_2Cl_2/Pentane = 1/50.

^1H NMR ($CDCl_3$, 400 MHz) δ 8.79 (br s, 1 H, Ar), 8.22 (br d, 1 H, *J* = 8.4 Hz, Ar), 7.99 (br s, 1 H, Ar), 7.88-7.50 (m, 4 H, Ar), 6.53 (br s, 1 H, H-5), 5.65 (d, 1 H, *J* = 16.4 Hz, H-17), 5.26 (d, 1 H, *J* = 16.4 Hz, H-17), 3.80 (br s, 1 H, OH), 2.00-1.80 (m, 2 H, H-19), 1.79-1.13 (br s, 18 H), 1.03 (t, 3 H, *J* = 7.2 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.6, 157.9, 155.4, 155.4, 152.1, 151.3, 150.8, 149.5, 145.6, 132.3, 130.8, 129.8, 128.7, 127.9, 127.8, 119.8, 98.0. 83.0. 81.5, 79.7, 72.7, 66.3, 31.8, 28.3, 27.7, 7.8.

EXAMPLE V - Preparation of 5-dibenzyloxycarbonylhydrazino-20-OTES-camptothecin

Camptothecin 20-OTES (0.100 g, 0.216 mmol) is dissolved in anhydrous THF (6 mL) with stirring under inert atmosphere, then cooled to a temperature of -78°C and a 1.0 M LiHMDS solution in THF (0.281 mL, 0.281

mmol) is dropped therein. After 20', dibenzyl azodicarboxylate (0.097 g, 0.324 mmol) in anhydrous THF (2 mL) is added. After 3 h at -78°C, temperature is left to raise to 25°C and the disappearance of the reagent is monitored by TLC (Hexane/AcOEt = 3/1). Formation of the two diastereomers is observed. After 90 min at room temperature, the reaction is quenched by addition of saturated NH_4Cl. The aqueous phase is extracted with CH_2Cl_2 (3 x 15 mL) and the organic phases are combined, dried over Na_2SO_4, filtered and concentrated under vacuum. The residue is purified by flash chromatography (SiO_2, Hexane/AcOEt = 4/1 then 7/2), thereby obtaining a pale yellow solid (0.161 g, 0.212 mmol, 98%). The two isomers are separated by further chromatography. In order of elution:

1st diastereomer: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.70 (br s, 1 H, Ar), 8.39 (br s 1 H, Ar), 8.22 (br d, 1 H, *J* = 7.6 Hz, Ar), 7.95 (br d, 1 H, *J* = 7.6 Hz, Ar), 7.83 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.65 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.64-7.00 (m, 11 H, Ar + H-14), 6.49 (br s, 1 H, H-5), 5.57 (d, 1 H, *J* = 16.4 Hz, H-17), 5.47-4.44 (m, 5 H), 1.98-1.82 (m, 2 H, H-19), 1.02-0.89 (m, 12 H), 0.80-0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6, 158.0. 156.3, 156.3, 153.0. 152.2, 151.0. 149.6, 144.8, 135.3, 132.1, 130.6, 130.0. 128.6-127.8 (11 C), 119.9, 98.4, 79.5, 75.2, 68.4, 67.9, 65.6, 33.0. 7.9, 7.2, 6.4.

2nd diastereomer: ^1H NMR ($CDCl_3$, 400 MHz) δ 8.85 (br s, 1 H, Ar), 8.58 (br s 1 H, Ar), 8.20 (br s, 1 H, Ar), 7.93 (br s, Ar), 7.81 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.63 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.56-6.90 (m, 11 H, Ar + H-14), 6.52 (br s, 1 H, H-5), 5.55 (d, 1 H, J = 16.8 Hz, H-17), 5.44-4.71 (m, 5 H), 1.98-1.80 (m, 2 H, H-19), 1.05-0.90 (m, 12 H), 0.81-0.70 (m, 6 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 157.9, 156.4, 156.4, 152.9, 152.4, 150.9, 149.4, 144.8, 135.3, 132.1, 130.6, 129.9, 128.6-127.8 (11 C), 119.9, 98.5, 79.3, 75.2, 68.4, 67.8, 65.6, 32.9, 7.8, 7.2, 6.4.

EXAMPLE VI - Preparation of 5-dibenzyloxycarbonylhydrazino-20-OH-

camptothecin 1st diastereomer

5-Dibenzyloxycarbonylhydrazino-20-OTES-camptothecin 1st diastereomer (0.140 g, 0.184 mmol) is dissolved in anhydrous THF (6 mL) with stirring under inert atmosphere, subsequently $Et_3N \bullet 3HF$ (0.225 mL, 1.380 mmol) is dropped therein. The reaction mixture is reacted for 52 h at room temperature, monitoring by TLC the disappearance of the reagent (Hexane/AcOEt = 1/3). The solvent is evaporated off under vacuum and the residue is purified by flash chromatography (SiO_2, Hexane/AcOEt = 1/1 then 2/3), thereby obtaining (0.113 g, 0.175 mmol, 95%) of the desired compound as a pale yellow solid. The product is further purified by crystallization from CH_2Cl_2/Pentane = 1/50.

^1H NMR ($CDCl_3$, 400 MHz) δ 8.67 (br s, 1 H, Ar), 8.39 (br s 1 H, Ar), 8.12 (br d, 1 H, *J* = 7.6 Hz, Ar), 7.95 (br s, 1 H, Ar), 7.74 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.65-6.66 (m, 12 H, Ar + H-14), 6.48 (br s, 1 H, H-5), 5.55 (d, 1 H, *J* = 16.0 Hz, H-17), 5.42-4.44 (m, 5 H), 3.86 (br s, 1 H, OH), 1.92-1.72 (m, 2 H, H-19), 0.95 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.5, 158.0. 156.2, 156.0. 153.0. 150.9, 150.9, 149.5, 145.3, 135.4, 132.2, 130.7, 129.8, 128.7-127.8 (11 C), 119.9, 98.2, 79.6, 72.7, 68.5, 68.0. 65.9, 31.6, 7.8.

EXAMPLE VII - Preparation of 5-dibenzyloxycarbonylhydrazino-20-OH-camptothecin 2nd diastereomer

5-Dibenzyloxycarbonylhydrazino-20-OTES-camptothecin 2nd diastereomer (0.140 g, 0.184 mmol) is dissolved in anhydrous THF (6 mL) with stirring under inert atmosphere, subsequently $Et_3N \bullet 3HF$ (0.150 mL, 0.921 mmol) is dropped therein. The reaction mixture is reacted for 55 h at room temperature, monitoring by TLC the disappearance of the reagent (Hexane/AcOEt = 3/2). The solvent is evaporated off under vacuum and the residue is purified by flash chromatography (SiO_2, Hexane/AcOEt = 1/1),

thereby obtaining the desired compound (0.113 g, 0.175 mmol, 95%) as a pale yellow solid. The product is further purified by crystallization from CH_2Cl_2/Pentane = 1/50.

^1H NMR ($CDCl_3$, 400 MHz) δ 8.71 (br s, 1 H, Ar), 8.34 (br s 1 H, Ar), 8.18 (br s, 1 H, Ar), 7.94 (br s, 1 H, Ar), 7.79 (br t, 1 H, *J* = 7.6 Hz, Ar), 7.70-6.70 (m, 12 H, Ar + H-14), 6.52 (br s, 1 H, H-5), 5.53 (d, 1 H, *J* = 16.4 Hz, H-17), 5.44-4.48 (m, 5 H), 3.87 (br s, 1 H, OH), 1.90-1.70 (m, 2 H, H-19), 0.99 (t, 3 H, *J* = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.4, 158.0. 156.3, 156.1, 153.0. 151.0. 150.9, 149.6, 145.3, 135.5, 132.3, 130.8, 129.8, 128.7-127.8 (11 C), 119.8, 98.4, 79.5, 72.7, 68.5, 67.8, 66.0. 31.6, 7.7.

EXAMPLE VIII - Preparation of 4,5-dihydro-triazole[5,4-c]16a-deoxocamptothecin TFA salt

5-di-*t*-Butoxycarbonylhydrazino-20-OTES-camptothecin (0.225 g, 0.324 mmol, 1:1 diastereomeric mixture) is dissolved in anhydrous 1,2-dichloroethane (DCE) (8 mL) with stirring under inert atmosphere, subsequently trifluoroacetic acid (TFA) (0.895 mL, 11.67 mmol) is dropped therein. The reaction mixture is reacted for 20 h at room temperature, monitoring by TLC the disappearance of the reagent (Hexane/AcOEt = 1/3), then refluxed for 4 h. The solvent is evaporated off under vacuum and the residue is purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH = 30/1), thereby obtaining the desired compound (0.084 g, 0.178 mmol, 55%) as the trifluoroacetate salt. The 1:1 mixture of the two diastereomers is further purified by flash chromatography (SiO_2, Toluene/AcOEt = 1/1).

^1H NMR ($CDCl_3$, 400 MHz) δ 10.61 (br s, 0.5 H, N5-NH=C16a), 10.39 (br s, 0.5 H, N5-NH=C16a), 8.67 (s, 1 H, Ar, H-7), 8.22-8.15 (m, 1 H, Ar), 7.96-7.92 (m, 1 H, Ar), 7.88-7.78 (m, 1 H, Ar), 7.69-7.60 (m, 2 H, Ar), 6.38-6.36 (m, 1 H, Ar, H-5), 5.72-5.62 (m, 1 H, Ar, H-17), 5.32-5.20 (m, 2 H, Ar, H-17 + N5H), 4.08-3.86 (br s, 1 H, OH), 1.96-1.74 (m, 2 H, H-19),

1.05-0.98 (t, 3 H, J = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8 (0.5 C), 173.4 (0.5 C), 159.1, 159.0. 156.7 (q CF_3COOH), 156.5 (q CF_3COOH) 151.5, 151.3, 150.7, 150.5, 150.1, 149.9, 144.8, 144.7, 134.0. 133.8, 131.6, 131.5, 129.9, 129.8, 128.7, 128.7, 128.4, 128.4, 128.2, 128.2, 127.1, 126.9, 120.5, 120.3, 99.1(2 C), 78.9, 78.6, 72.7, 72.7, 66.0 (2 C), 31.7 (2 C), 7.7, 7.7.

EXAMPLE IX - Preparation of triazole[5,4-c]16a-deoxocamptothecin

The 4,5-dihydro-triazole[5,4-c]16a-deoxocamptothecin TFA salt (0.020 g, 0.042 mmol) is dissolved in anhydrous CH_2Cl_2 (4 mL) with stirring under inert atmosphere, subsequently 2,3-dichloro-5,6-diciano-p-benzoquinone (DDQ) (0.025 mg, 0.110 mmol) is added thereto. The reaction mixture is reacted for 31 h at room temperature, monitoring by TLC the disappearance of the reagent (CH_2Cl_2/MeOH = 30/1). The reaction is quenched by addition of H_2O. The aqueous phase is extracted with CH_2Cl_2 (3 x 15 mL) and the organic phases are combined, dried over Na_2SO_4, filtered and concentrated under vacuum. The residue is purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH = 45/1), thereby obtaining a yellow solid (0.014 g, 0.039 mmol, 94%).

^1H NMR ($CDCl_3$, 400 MHz) δ 8.89 (s, 1 H, Ar, H-7), 8.20 (d, 1 H, J = 8.4 Hz, Ar), 8.00 (d, 1 H, J = 8.4 Hz, Ar), 7.88 (t, 1 H, J = 8.4 Hz, Ar), 7.79 (s, 1 H, Ar H-14), 7.69 (t, 1 H, J = 8.4 Hz, Ar), 5.70 (d, 1 H, J = 17.2 Hz, H-17), 5.28 (d, 1 H, J = 17.2 Hz, H-17), 3.83 (br s, 1 H, OH), 2.00-1.74 (m, 2 H, H-19), 1.08 (t, 3 H, J = 7.6 Hz, Me). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.6, 157.4, 152.5, 150.8, 148.9, 143.7, 134.9, 132.5, 132.4, 130.0. 129.5, 128.7, 127.5, 122.6, 121.4, 101.2, 72.4, 66.0. 31.6, 7.7.

EXAMPLE X - Cell growth inhibition assay

H460 Cells from human large cell lung tumor were cultured in RPMI-1640 medium containing 10% foetal calf serum. Cell sensitivity was

determined by cell growth inhibition assay after 1 or 72 hr drug exposure. The cells in logarithmic growth were collected and seeded in duplicate in 6-wells plates. Twenty-four hours after seeding, cells were exposed to the drugs and counted with a Coulter conter 72 hours after exposure to the drugs for the determination of IC_{50}s. IC_{50} is defined as the concentration inhibiting by 50% cell growth compared with untreated controls growth.

EXAMPLE XI - Topoisomerase-I - dependent DNA rupture assay

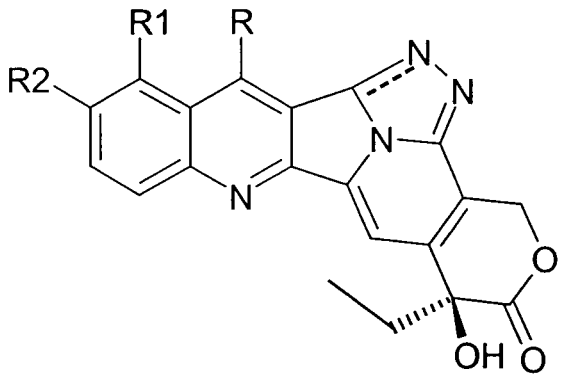
DNA ruptures were determined using a 751-bp BamHI-EcoRI DNA SV40 purified gel (Beretta GL, Binaschi M, Zagni AND, Capuani L, Capranico G. Tethering a type IB topoisomerase to a DNA site by enzyme fusion to a heterologous site-selective DNA-binding protein domain. Cancer Res 1999; 59:3689-97). DNA fragments were only labeled at 3'. The DNA rupture reaction (20,000 cpm/sample) was carried out in 20 ml of 10 mM Tris-HCL (pH 7.6), 150 mM KCl, 5 mM $MgCl_2$, 15 µg/mL BSA, 0.1 mM thiothreitol, and the human recombinant enzyme (full length top1) for 30 min at 37°C. The reactions were blocked using 0.5% SDS and 0.3 mg/mL K proteinase for 45 min. at 42°C. DNA damage persistence was tested at different times adding 0.6M NaCl after 30 min. incubation with 10 µM of the drug. After precipitation, DNA was resuspended in denaturation buffer (80% formamide, 10 mM NaOH, 0.01 M EDTA and 1 mg/mL dye) before seeding in denaturating gel (7% polyacrylamide in TBE buffer). All of DNA rupture levels were measured by means of a Phospholmager model 425 (Molecular Dynamics) (Dallavalle S, Ferrari A, Biasotti B, et al. Novel 7-oxyiminomethyl camptothecin derivatives with potent in vitro and in vivo antitumor activity. J Med Chem 2001; 44:3264-74).

Persitence of DNA damage (%)

Compounds	Time (min)			
	0	1	5	10
Topotecan	100	65	20	10
Camptothecin	100	58	23	20
SN38	100	60	33	28
IDN 6132	100	45	32	20

CLAIMS

1. Compunds of general formula (I):



(I)

wherein:

R is alkyl, aminoalkyl, hydroxyalkyl, nitrile, alkoxymino, aryloxymino, silylalkyl;

R1 is hydrogen, hydroxy, alkoxy, aminoalkyl;

R2 is hydrogen, hydroxy, alkoxy, aminoalkyl, optionally protected hydroxyl;

wherein the alkyl, alkoxy, aminoalkyl or alkoxymino groups can contain 1 to 8, preferably 1 to 4 carbon atoms, in a straight or branched chain, whereas the aryloxymino group can contain 5 to 10 carbon atoms;

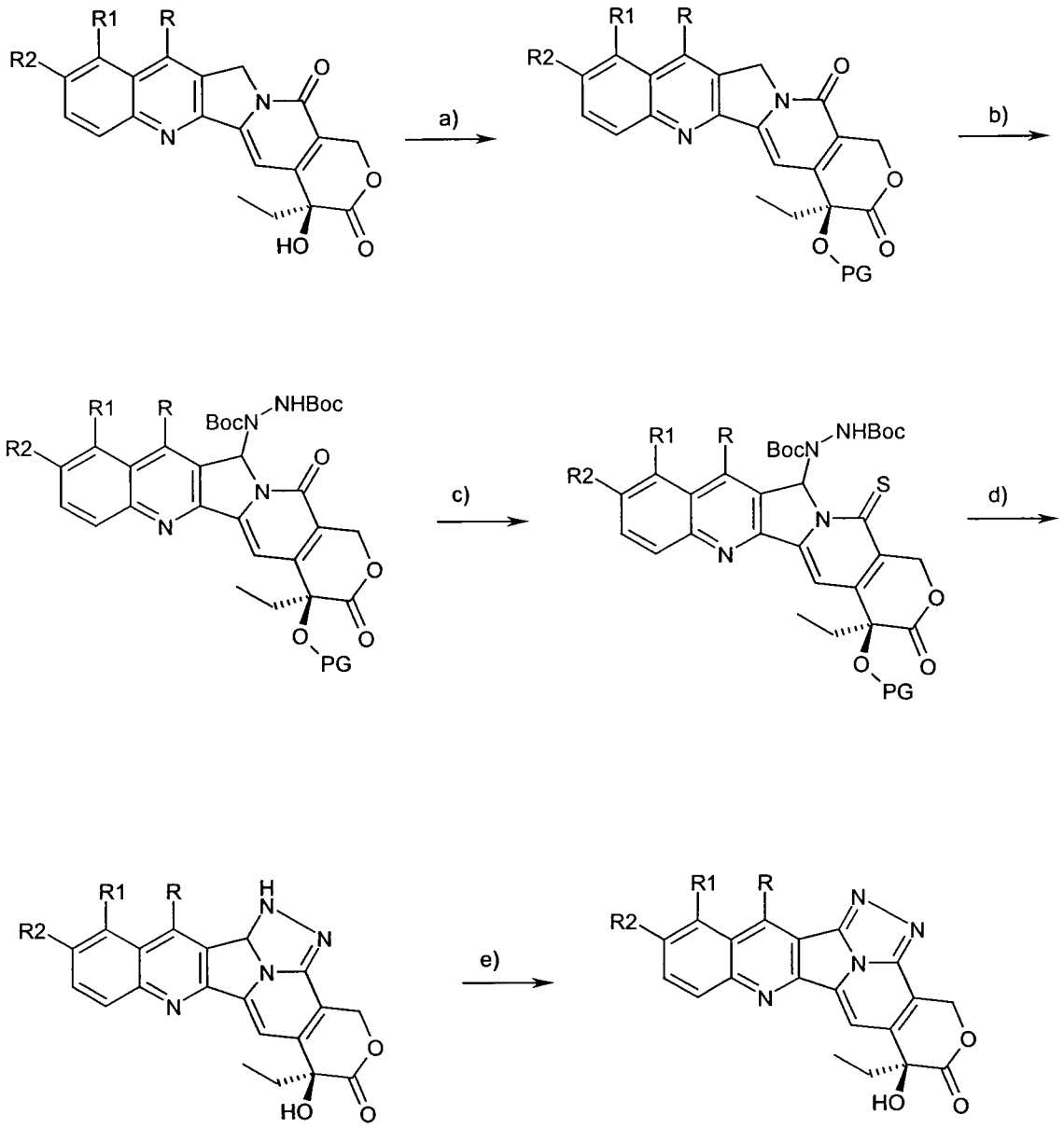
the pharmaceutically acceptable salts, isomers, enantiomers, diastereomers thereof and corresponding mixtures.

2. A compound of formula (I) as claimed in claim 1, which is selected from the group consisting of:

a) 4,5-dihydro-triazole[5,4-c]16a-deoxocamptothecin,

b) triazole[5,4-c]16a-deoxocamptothecin.

3. A process for the preparation of the compounds of formula (I), which process substantially comprises steps (a)-(e) shown in the following scheme:



wherein:

a) protection of precursor hydroxy groups;

b) derivatization at 5- with N,N-diprotected hydrazine;

c) optional conversion of the pyridone ring to thiopyridone ring;

d) removal of the protective groups with concomitant cyclization;

e) optional aromatization of the pyrazole ring;

and wherein R, R1 and R2 have the meanings defined above, while PG is a hydroxy-protective group.

4. The process for the preparation of compounds of formula (I) as claimed in claim 3, in which the order of the steps (b) and (c) is reversed.

5. A pharmaceutical composition containing a compound of formula (I) together with pharmaceutically acceptable carriers and excipients.

6. A pharmaceutical composition as claimed in claim 5, which is in a form suited to the oral or parenteral administration.

7. The use of a compound as claimed in claims 1-2 or of a composition as claimed in claims 5-6 for the preparation of a drug for the treatment of tumors.

8. The use as claimed in claim 7, wherein said drug is used for the treatment of solid tumors and leukemias, in particular tumors of the lung, ovary, breast, stomach, liver, prostate, soft tissues sarcomas, esophagus, pancreas, head and neck, glioblastoma, chronic and acute myelocytic leukemias.