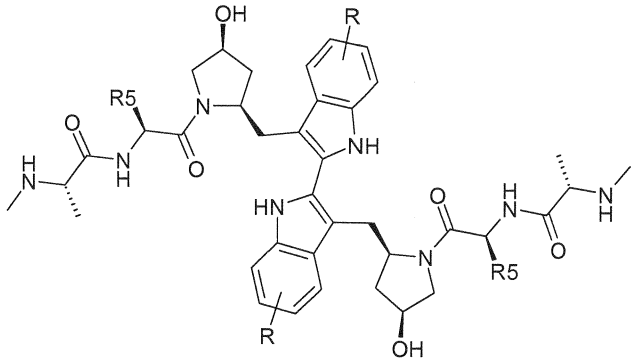
Table 1



Compound	R5	R
15	-CH2CH3	6-F
~~1~~112*	-CH(CH3)CH3	6-F
~~3~~118*	-R-CH(OH)CH3	6-F
4115*	-S-CH(OH)CH3	6-F
~~5~~116*	-R-CH(OCH3)CH3	6-F

*Entries from Example 7 of US2006/0194741

Example 2A. cIAP Degradation Assay

The concentration inducing degradation of cIAP-1 and cIAP-2 by 50% (IC_{50}) for various compounds was determined by monitoring the disappearance of Green Fluorescent Protein (GFP)-signal in A375 cells. Briefly, A375 cell lines expressing GFP-tagged cIAP-1 and cIAP-2 were generated by transfecting HA2xEGFP-pcDNA3 vector containing either cIAP-1 (A375Gc1) or cIAP-2 (A375Gc2) coding region. $2x10^4$ of A375Gc1 or A375Gc2 cells were grown in 96-well plate and treated with various concentrations of test compounds for 2 h. After incubation, cells were collected by trypsinization and suspended in 150 µl of DMEM-10% FBS. A total of 10^4 cells were analyzed using a FACScan (Becton Dickinson). GFP fluorescence was monitored by using an excitation filter at 488 nm and emission was measured with a 530 nm filter. IC_{50} is defined as the concentration of drug at which 50% of GFP signal was inhibited.

Results of the cIAP-1 and -2 degradation assay are shown in Table 2.

Table 2

Compound	GFP-cIAP-1 IC_{50} (nM)	SD	GFP-cIAP-2 IC_{50}	SD	IC50 Ratio cIAP-2/cIAP-1

			(nM)		
15	27 (n=56)	15	174 (n=61)	100	6.4
~~2~~112*	4 (n=3)	0.6	7 (n=3)	0.8	1.8
~~3~~118*	328 (n=3)	83	674 (n=3)	69	2.1
4115*	464 (n=12)	112	604 (n=12)	192	1.3
~~5~~116*	10 (n=41)	2	37 (n=38)	19	3.7

*Entries from Example 7 of US2006/0194741

These data show that Compound 15 has greater relative potency in degrading cIAP-1 relative to cIAP-2 when compared to Compounds ~~2, 3, 4, and 5~~112, 118, 115 and 116 from Example 7 of US2006/0194741.

Example 2B. Caspase-3 Derepression Assay

Exponentially growing MDA-MB-231 tumor cells (ATCC) were harvested by trypsinization and collected by centrifugation in a table top centrifuge at 1000xg for 10 minutes at room temperature. Cell pellet was washed one time by resuspending in 5 mL hypotonic lysis buffer (20 mM HEPES, pH7.5, 10 mM KCl, 1.5 mM $MgCl_2$, 1.0 mM EDTA, 1.0 mM DTT) and recollected by centrifugation. Pellet was next resuspended in 1 volume of hypotonic lysis buffer supplemented with a complete protease inhibitor tablet (Roche) and allowed to swell on ice for 30 minutes. Cells were disrupted by approximately 50 passages through a 27 gauge needle. Lysis was monitored by light microscopy. Lysate was centrifuged at 12000xg for 10 minutes at 4°C to remove membrane fraction, unlysed cells and debris. The soluble fraction was collected for protein concentration determination and subsequent assay analysis.

The hypotonic lysate (25 µg protein), 50 µg/mL cytochrome *c* and 10 mM dATP were combined in a microcentrifuge tube to a final volume of 9 µl in hypotonic lysis buffer followed by addition of test compound and incubated for 30 minutes at room temp. Following incubation 50 µl of hypotonic lysis buffer containing 5 µM of pro-fluorescent rhodamine-$110_{(2)}$ based caspase-3 substrate zDEVD-$R110_{(2)}$ was added and fluorescence intensity was monitored over time. Activation of the lysate by addition of cytochrome *c* and dATP results in apoptosome formation and subsequent activation of caspases-9 and -3. Endogenous XIAP inhibits much of this activity and addition of test compound to the activated lysate results in more caspase activity than is generated by activated lysate alone as measured by increase in fluorescence intensity upon cleavage of zDEVD-$R110_{(2)}$ by caspase-3. IC_{50} values were calculated using GraphPad Prism by plotting increase in fluorescence intensity vs. different concentrations of compounds tested and the results are shown in Table 3.

Table 3

Compound	XIAP Caspase-3 Derepression IC_{50} (nm)	SD
15	24.3 (n=3)	2.0
~~2~~112*	13.4 (n=3)	4.3
~~3~~118*	0.2 (n=3)	0
~~4~~115*	0.35 (n=3)	0.2
~~5~~116*	0.36 (n=3)	0.05

*Entries from Example 7 of US2006/0194741

These data show that Compound 15 has a lower potency for antagonizing XIAP function in comparison to Compounds ~~2, 3, 4, and 5~~112, 118, 115 and 116 from Example 7 of US2006/0194741.

Example 3 - Cytotoxicity

SKOV-3 ovarian tumor cell cytotoxicity data were generated substantially as follows. The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is an example of an assay that has been used for measuring cell growth as previously described (Hansen, M. B., Nielsen, S. E., and Berg, K. (1989) *J. Immunol. Methods* **119,** 203–210) and incorporated herein by reference in its entirety. Briefly, SK-OV-3 cells were seeded in 96-well plates in McCoy's medium containing 10% fetal bovine serum albumin (5,000 per well) and incubated overnight at 37°C. The next day, test compounds were added at various concentrations (0.003-10 μM) and the plates were incubated at 37°C for an additional 72 hrs. This incubation time was optimal for measuring inhibitory effects of different analogs. Fifty microliters of 5mg/mL MTT reagent to each well was added and the plates were incubated at 37°C for 3 hours. At the end of the incubation period, 50 microliters of DMSO was added to each well to dissolve cells and the optical density (OD) of the wells was measured using a microplate reader (Victor^2 1420, Wallac, Finland) at 535 nm. Cell survival (CS) was calculated by the following equation:

CS = (OD treated well/ mean OD control wells) **X** 100%

The CC_{50}, defined as the drug concentration that results in 50% CS, was derived by calculating the point where the dose-response curve crosses the 50% CS point using GraphPad Prism. These results suggest that Smac mimetics that bind to cIAP-1 can be used in the treatment of cancer either as monotherapy or in combination with chemotherapeutics.

The results of SKOV-3 cytotoxicity assays for the compounds tested in this assay are shown in Table 4.

Table 4

Compound	CC_{50} (nM)	Std Dev (nM)
15	0.14 (n=76)	0.02
~~2~~112*	0.5 (n=6)	0.1
~~3~~118*	13 (n=4)	4
4115*	2 (n=23)	0.3
~~5~~116*	0.13 (n=76)	0.1

*Entries from Example 7 of US2006/0194741

These data indicate that Compound 15 has equivalent potency to Compound ~~5~~ 116 and is more potent than Compounds ~~2, 3, and 4~~112, 118, and 115 from Example 7 of US2006/0194741.

Example 4 – Toxicity

Body weight loss (BWL), mortality and additional toxicity data were generated substantially as follows. Sprague-Dawley rats were dosed daily (QDx4, i.v. bolus slow push) with Compounds 15, ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741. Body weights were taken on day 4 and are shown as percent change from day 1. Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 were administered at 0.3 mg/Kg, 1 mg/Kg, or 3 mg/Kg; Compound 15 was administered at 1, 5, or 10 mg/Kg.

The results of the BWL assay are shown in Fig 1.

Mortality. Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 were not tolerated at 3 mg/Kg, and caused the animals to die at this dose. No mortality was observed with Compound 15 at 5 mg/Kg (mortality was observed at 10 mg/Kg.)

Clinical Results. There were no clinical signs noted at 1 mg/kg/day with Compound 15 after 4 days of administration. Animals treated with Compound 15 at 5 mg/kg/day exhibited clinical signs similar to Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 at 1 mg/kg/day such as lethargy, increased/irregular respiration and increased heart rate. Rats treated with 1 mg/kg Compound ~~5~~116 from Example 7 of US2006/0194741 exhibited additional clinical observations including dehydration, ungroomed appearance, chromorhinorrhea, alopecia (head), and excessive scratching from days 2 to 4.

Body Weight. At 1 mg/Kg, animals receiving Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 lost weight whereas animals receiving Compound 15 at 1 mg/kg/day gained weight. At 5 mg/kg/day with Compound 15, a treatment related mean body weight loss of approximately 8% was noted

from day 1 to day 4. A treatment-related mean body weight loss of approximately 4 % and 6 % was noted in animals treated with Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741, respectively, at 1 mg/kg/day.

Pathology. Assessment of anatomic pathology after treatment with Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 at 1 mg/kg/day resulted in the following findings. There was marked to severe bone marrow hypocellularity of the erythroid series, mild to moderate hypercellularity of the myeloid series, and mild to moderate hypertrophy and hyperplasia of megakaryocytes in the tibia and sternum when Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 were administered at 1 mg/kg/day. For Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 the lungs had dose-related mild to moderate diffuse hypertrophy/hyperplasia of Type 2 pneumocytes which were accompanied by an increase in alveolar macrophages, hypertrophied bronchiolar epithelium, proliferating perivascular mononuclear cells and hypertrophied visceral pleural cells. In contrast, assessment of anatomic pathology following treatment with Compound 15 at the same dose (1 mg/kg/day) identified minimal to mild hypocellularity of erythroid cells, minimal to mild hypercellularity of myeloid cells, and minimal Type 2 pneumocyte hypertrophy in the lungs.

The data described above indicate that Compound 15 is approximately 5-fold better tolerated in the rat as compared to Compounds ~~4 and 5~~115 and 116 from Example 7 of US2006/0194741 on a dose per dose basis.

Example 5 – Tumor Volume Reduction and Body Weight Change

MDA-MB-231 xenograft data were generated substantially as follows. MDA-MB-231 human breast tumor cells were injected into the mammary fat pad of female nude mice and dosing initiated twelve days later at an average tumor volume of approximately 148 mm^3. No tumor burden was associated with this model based on lack of weight loss or animal morbidity in control groups. Mice were injected subcutaneously into the mammary fat pad with $1x10^7$ cells suspended in 200µl of a 1:1 HBSS: Matrigel solution ; injected cells were within nine passages of the original lot. Pre-study tumor volumes were recorded beginning approximately one week prior to the estimated start date. When tumors reached approximately 150 mm^3 animals are matched by tumor volume into treatment and control groups and dosing initiated (Day 0); mice are tagged and followed individually throughout the experiment. Animals were dosed by weight (0.01 mL per gram; 10 ml/Kg). Beginning on Day 0, animals were observed daily and weighed twice weekly using a digital scale (Ohaus SP601); data including individual and mean gram weights (Mean We ± SD), mean percent weight change versus Day 0 ($\%vD_0$) and mean percent weight change versus prior measurement ($\%vD_{-x}$) were recorded for each group and plotted at study completion.

Compound ~~5~~116 from Example 7 of US2006/0194741 was administered by i.p. injection alone at 15 mg/Kg on a q3dx5 schedule. A T-C value of 21 days was calculated for this group which was found to be statistically significant compared with control (p=0.002). In this group 3/10 mice were considered long-term survivors and partial tumor regression was reported in five mice. The efficacy of this dose level produced half the number of long term survivors as 20 mg/Kg of Compound 15.

The results of the MDA-MB-231 xenograft assay are shown in Figures 2A and 2B. Compound 15 at 20 mg/Kg had comparable anti-tumor activity to Compound ~~5~~116 from Example 7 of US2006/0194741 at 15 mg/Kg. Subsequent studies have shown that the minimal effective dose of Compound 15 in this model is less than 1 mg/Kg. Weight loss was greater in the mice administered Compound ~~5~~116 from Example 7 of US2006/0194741 at 15 mg/Kg when compared to the mice dosed with Compound 15 at 20 mg/Kg. Thus, Compound 15 has comparable efficacy with less toxicity relative to Compound ~~5~~116 from Example 7 of US2006/0194741 and therefore has an improved therapeutic index.

The compound of Formula 1 is particularly well tolerated and well suited for use in a pharmaceutical composition, as well as in a method for treating a proliferative disorder or an autoimmune disorder. In particular, the pharmaceutical composition of the invention for the treatment of a proliferative disorder, which comprises an effective amount of Compound 15 in addition to at least one pharmaceutically acceptable excipient, can improve therapeutic index by reducing toxicities. The reduced toxicities include, e.g., one of, or any combination of one or more of:

- reduced body weight loss,
- decreased incidence of mortality,
- reduced bone marrow hypocellularity of the erythroid series,
- reduced hypercellularity of the myeloid series,
- reduced hypertrophy and hyperplasia of megakaryocytes,
- reduced diffuse hypertrophy/hyperplasia of Type 2 pneumocytes
- decreased lethargy,
- more regular respiration,
- lessened increase in heart rate.

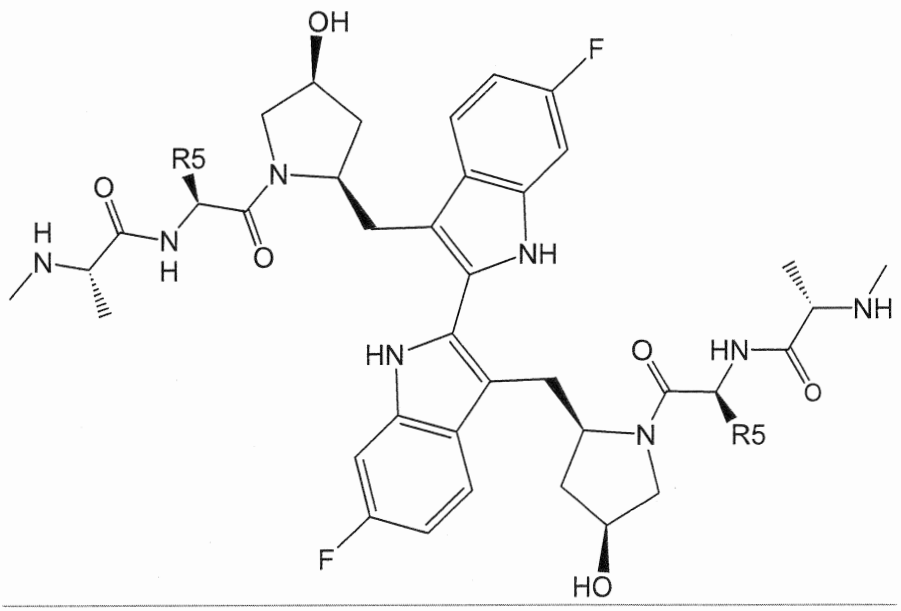
The reduced toxicities listed above are those observed in the animals tested. Similar, additional, or different reduced toxicities will be observed in humans. The reductions are relative, e.g., relative to the extent that toxicities would be observed following internal administration of a pharmaceutical composition in which the active pharmaceutical ingredient is an analog of Compound 15, e.g., one or more of the analogs in which R5 is –CH2CH3, -CH(CH3)CH3, -*R*-CH(OH)CH3, -*S*-CH(OH)CH3, and -*R*-CH(OCH3)CH3, e.g., at the same dose or at a dose of comparable potency.

4. The pharmaceutical composition of claim 2 for use in the treatment of a cancer.

5. The pharmaceutical composition of claim 2, 3, or 4 which is a sterile liquid for injection.

6. The pharmaceutical composition of claim 2, 3, 4, or 5 which is in a unit dose form.

7. The use of Compound 15, or a pharmaceutically acceptable salt thereof, of the following structure:



wherein R5 is $-CH_2CH_3$, in the manufacture of a medicament for the treatment of a proliferative disorder in a mammal in need thereof, wherein said medicament is to be administered internally to the mammal.

8. The use of claim 7 wherein the proliferative disorder is a cancer selected from the group consisting of: ~~lung adenocarcinoma,~~ pancreatic cancer, ~~colon cancer,~~ ovarian cancer, breast cancer, mesothelioma, peripheral neuroma, ~~bladder cancer,~~ glioblastoma, melanoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, ~~breast cancer,~~ cervical cancer, chronic myeloproliferative disorders ~~(e.g., chronic lymphocytic leukemia, chronic myelogenous leukemia)~~, colon cancer, ~~endocrine cancers,~~ endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, ~~extragonadal germ cell tumors,~~ extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic

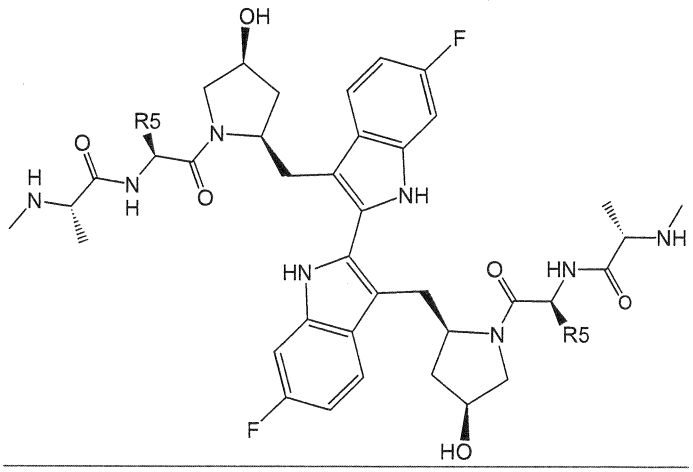
tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, ~~intraocular melanoma,~~ islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, ~~acute lymphoblastic leukemia, acute myeloid leukemia,~~ lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, melanoma, Merkel cell carcinoma, metastatic squamous neck cancer, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, oropharyngeal cancer, bone cancers, ~~including osteosarcoma and malignant fibrous histiocytoma of bone, ovarian epithelial cancer, ovarian germ cell tumors, ovarian low malignant potential tumors, pancreatic cancer,~~ paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, ~~skin cancer,~~ small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional

cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

9. The use of claim 7 wherein the proliferative disorder is a cancer selected from the group consisting of: sarcomas, bladder cancer, ovarian cancer, breast cancer, brain cancer, pancreatic cancer, colon cancer, blood cancer, skin cancer, lung cancer, and bone cancer.

10. The use of claim 7 wherein the cancer is selected from colorectal cancer, renal carinoma, ovarian carcinoma, pancreatic carcinoma, prostate carcinoma, breast carcinoma, melanoma, gliobastoma, acute myeloid leukemia, small cell lung cell carcinoma, non-small cell lung carcinoma, rhabdomyosarcoma, and basal cell carcinoma.

11. Use of Compound 15 or a pharmaceutically acceptable salt thereof, of the following structure:

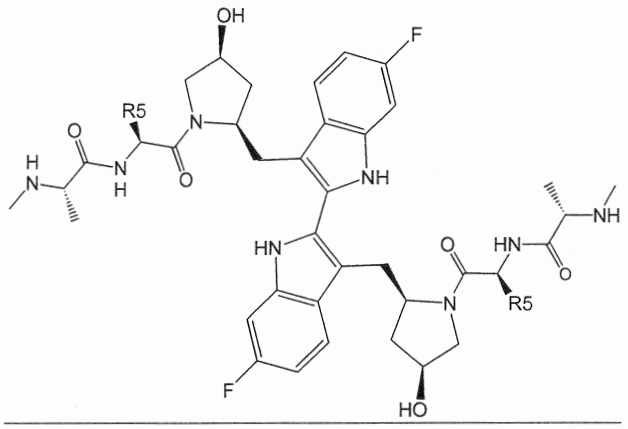


wherein R5 is $-CH_2CH_3$, in the manufacture of a medicament for inducing apoptosis in a cell.

12. The use of claim 11 wherein the cell is a cancer cell.

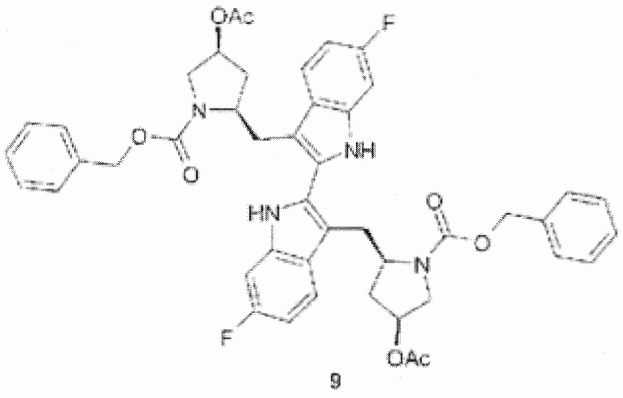
13. The use of any one or more of claims 7 through 12 wherein Compound 15 is to be administered in combination with a second cancer therapy selected from radiation, chemotherapy, immunotherapy, photodynamic therapy, and combinations thereof.

14. The use of Compound 15 or a pharmaceutically acceptable salt thereof, of the following structure:



wherein R5 is $-CH_2CH_3$, in the manufacture of a medicament for the treatment of an autoimmune disease, in a mammal in need thereof, wherein the autoimmune disease is one in which the condition is caused or exacerbated by abnormal regulation of apoptosis and is selected from the group consisting of: systemic lupus erythematosus, psoriasis, and idiopathic thrombocytopenic purpura (Morbus Werlhof), and wherein said medicament is to be administered internally to the mammal.

15. A compound selected from the group consisting of Compound 9 represented by the formula:



9

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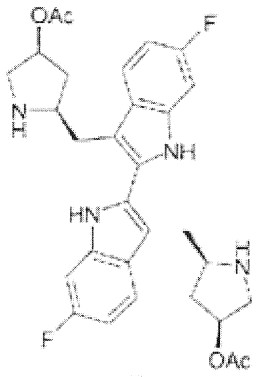
Compound 10 represented by the formula:



10

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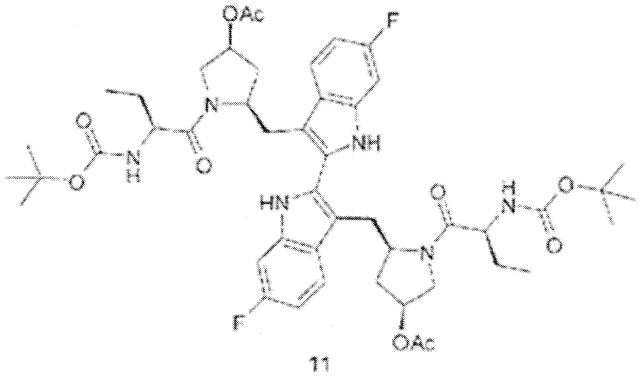
Compound 11 represented by the formula:



11

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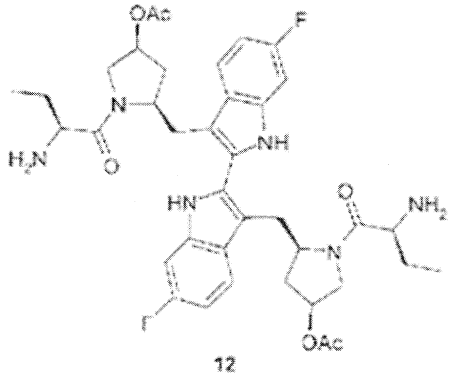
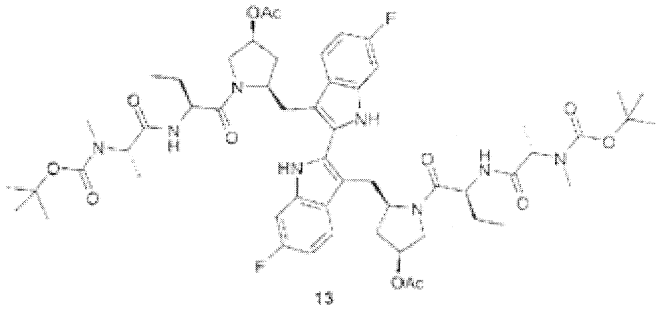
Compound 12 represented by the formula:



12

,

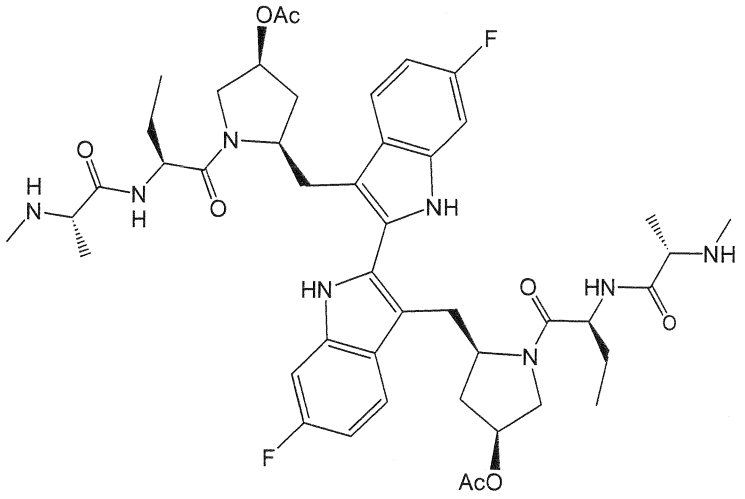
Compound 13 represented by the formula:



13

, and

~~Protected~~ Compound ~~15~~14 represented by the formula:

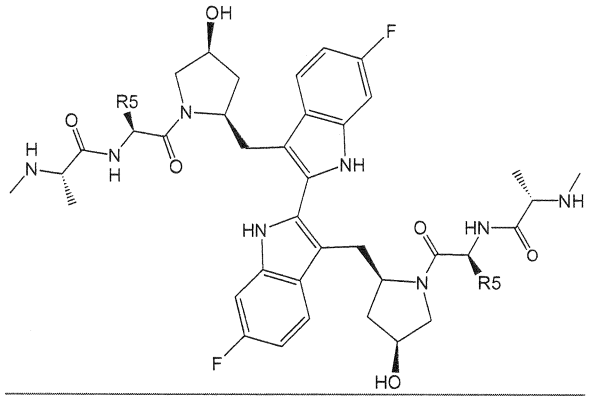


14

.

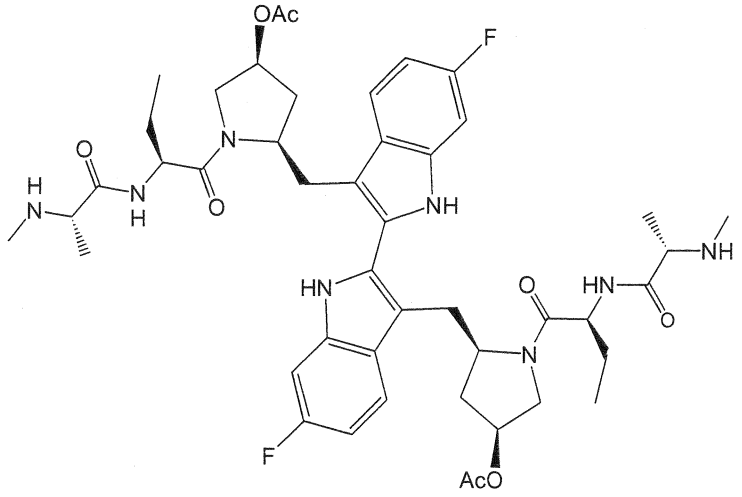
~~16. The compound of claim 15 that is Compound 14.~~

~~17~~16. A process for preparing Compound 15, or a pharmaceutically acceptable salt thereof, of the following structure:



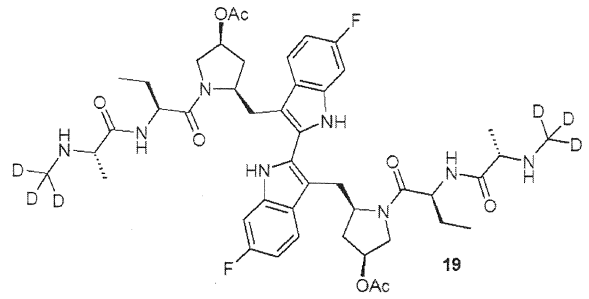
wherein R5 is $-CH_2CH_3$, ~~that~~ wherein the process comprises deprotecting a Protected Compound 15.

~~18~~17. The process of claim ~~17~~16 wherein the Protected Compound 15 is Compound 14 represented by the formula:

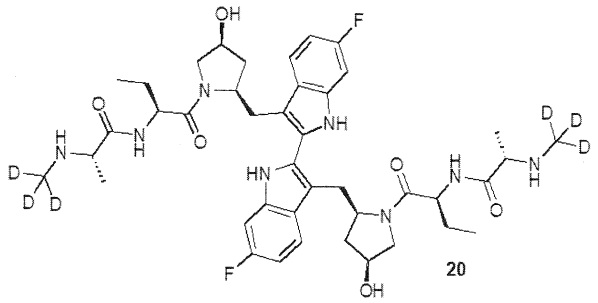


14

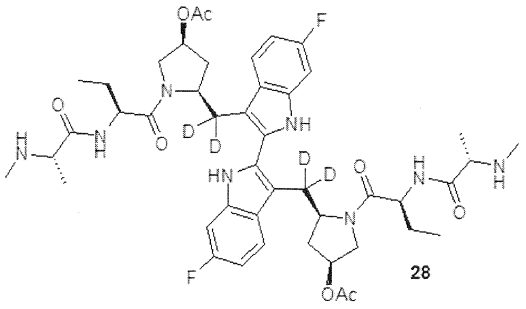
~~19~~18. A compound selected from the group consisting of Compound 19 represented by the formula:



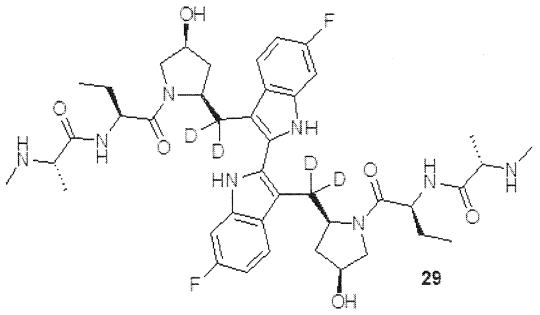
~~,~~Compound 20 represented by the formula:



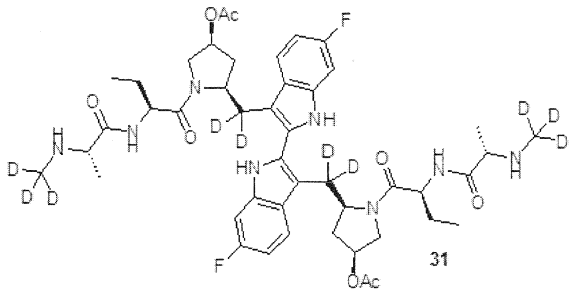
~~,~~Compound 28 represented by the formula:



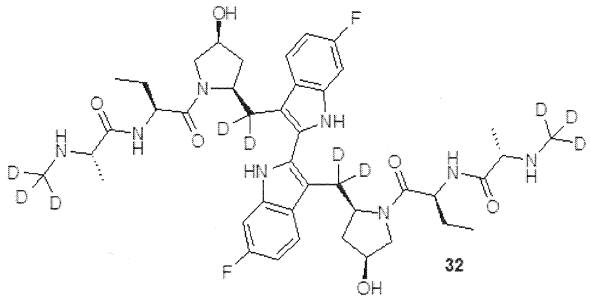
~~,~~Compound 29 represented by the formula:



,Compound 31 represented by the formula:



,and Compound 32 represented by the formula:.



2019. An *in vitro* method for inducing apoptosis in a cell comprising contacting the cell with Compound 15 or a pharmaceutically acceptable salt thereof.

2120. The method of claim 2019 wherein the cell is a cancer cell.

2221. The compound of claim 1 for use in treating a proliferative disorder, wherein the proliferative disorder is a cancer selected from the group consisting of: lung adenocarcinoma, pancreatic cancer, colon cancer, ovarian cancer, breast cancer, mesothelioma, peripheral neuroma, bladder cancer, glioblastoma, melanoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, breast cancer, cervical cancer, chronic myeloproliferative disorders (e.g., chronic lymphocytic leukemia, chronic myelogenous leukemia), colon cancer, endocrine cancers, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extragonadal germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric

cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, ~~intraocular melanoma,~~ islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, ~~acute lymphoblastic leukemia, acute myeloid leukemia,~~ lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, melanoma, Merkel cell carcinoma, metastatic squamous neck cancer, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, oropharyngeal cancer, bone cancers, ~~including osteosarcoma and malignant fibrous histiocytoma of bone, ovarian epithelial cancer, ovarian germ cell tumors, ovarian low malignant potential tumors, pancreatic cancer,~~ paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, ~~skin cancer,~~ small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional

cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

~~23~~22. The compound of claim 1 for use in treating a proliferative disorder, wherein the proliferative disorder is a cancer selected from the group consisting of: sarcomas, bladder cancer, ovarian cancer, breast cancer, brain cancer, pancreatic cancer, colon cancer, blood cancer, skin cancer, lung cancer, and bone cancer.

~~24~~23. The compound of claim 1 for use in treating a proliferative disorder, wherein the proliferative disorder is a cancer selected from colorectal cancer, renal carcinoma, ovarian carcinoma, pancreatic carcinoma, prostate carcinoma, breast carcinoma, melanoma, gliobastoma, acute myeloid leukemia, small cell lung carcinoma, non-small cell lung carcinoma, rhabdomyosarcoma, and basal cell carcinoma.

~~25~~24. The compound of any of claims ~~22~~21 to ~~24~~23, wherein said compound is for administration in combination with a second cancer therapy selected from radiation, chemotherapy, immunotherapy, photodynamic therapy, and combinations thereof.

~~26~~25. The compound of claim 1 or a pharmaceutically acceptable salt thereof for use in treating an autoimmune disease, wherein the autoimmune disease is one in which the condition is caused or exacerbated by abnormal regulation of apoptosis and is selected from the group consisting of: systemic lupus erythematosus, psoriasis, and idiopathic thrombocytopenic purpura (Morbus Werlhof).