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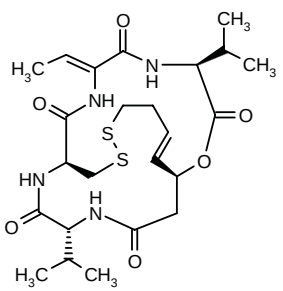
FR901228

Antineoplastic Antibiotic

NSC-630176

(1*S*,4*S*,10*S*,21*R*)-7-[(*Z*)-Ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]tricos-16(*E*)-ene-3,6,9,19,22-pentone

N-[3(*S*)-Hydroxy-7-sulfanyl-4(*E*)-heptenoyl]-D-valyl-D-cysteinyl-2-amino-2(*Z*)-butenoyl-L-valine (4→1)-lactone cyclic (1→2)-disulfide



$C_{24}H_{36}N_4O_6S_2$ Mol wt: 540.7024

CAS: 128517-07-7

EN: 158963

Synthesis

A sequence of reactions has been performed to synthesize FR901228 (1): (i) the formation of a 16-membered cyclic depsipeptide, (ii) the asymmetric construction of the hydroxymercaptoheptenoic acid and (iii) an intramolecular oxidative coupling of the thiols to form a 15-membered disulfide-containing ring.

For the first step, L-valine methyl ester (I) was coupled to *N*-Fmoc-L-threonine using the BOP reagent [(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate], removing the *N*-Fmoc group with Et_2NH and coupling to *N*-Fmoc-(*S*-triphenylmethyl)-D-cysteine to yield the tripeptide (II). The tetrapeptide (III) was prepared by deprotection of the tripeptide (II) and BOP-mediated peptide coupling of the resulting amine with *N*-Fmoc-D-valine. The secondary hydroxyl group was activated as the tosylate and eliminated by treatment with DABCO to produce the internal alkene. After removal of the Fmoc protecting group by addition of Et_2NH to the reaction mixture, the 16-membered depsipeptide (IV) was formed. Scheme 1.

For construction of the hydroxymercaptoheptenoic acid (VIII), cesium triphenylmethylthiolate anion was added to methyl 2,4-pentadienoate (V) which resulted in a β,γ-unsaturated methyl ester that was converted to an α,β-unsaturated ester after exposure to Cs_2CO_3. DIBAL reduction to the primary alcohol followed by Swern oxidation yielded the α,β-unsaturated aldehyde (VI). In the presence of the ligand derived from (*R*)-(–)-binaphthyl amino alcohol, Ti(IV)-catalyzed addition of *O*-benzyl, *O*-TMS ketene acetal to the α,β-unsaturated aldehyde (VI) resulted in the aldol product (VII). Hydrolysis of the benzyl ester with LiOH in aqueous methanol formed the β-hydroxymercaptoheptenoic acid (VIII). Scheme 2.

To complete the synthesis of FR901228, the β-hydroxymercaptoheptenoic acid (VIII) was coupled to the 16-membered cyclic depsipeptide (IV) using the BOP reagent, followed by LiOH-mediated hydrolysis, resulting in the free acid product (IX). Cyclization of the hydroxy acid (IX) with TsOH, DEAD and PPh_3 and oxidation of the bis(*S*-triphenylmethyl)lactone (X) with iodine in methanol solution yielded the depsipeptide FR901228. Scheme 3.

Fermentation and Isolation

Culture of *Chromobacterium violaceum* No. 968, a Gram-negative, motile rod-shaped bacterium with a single polar flagellum, was grown in a stainless steel jar-fermentor with production medium containing 1% glucose, 1% nutrient broth and 0.05% Adekanol LG109 (antifoaming agent) (2). Fermentation was carried out at 30 °C under aeration of 20 l/min, an inner pressure of 1.0 kg/cm^2 and agitation at 200 rpm for 72 h. After fermentation was terminated, cultures were sterilized at 120 °C for 30 min. The cultured broth was passed through a diatomaceous earth filter and the filtrate was extracted twice with EtOAc. The extract was evaporated under reduced pressure to yield an oily residue. The oily residue was mixed with silica gel in methanol. After evaporating the solvent, the dry powder and gel mixture was

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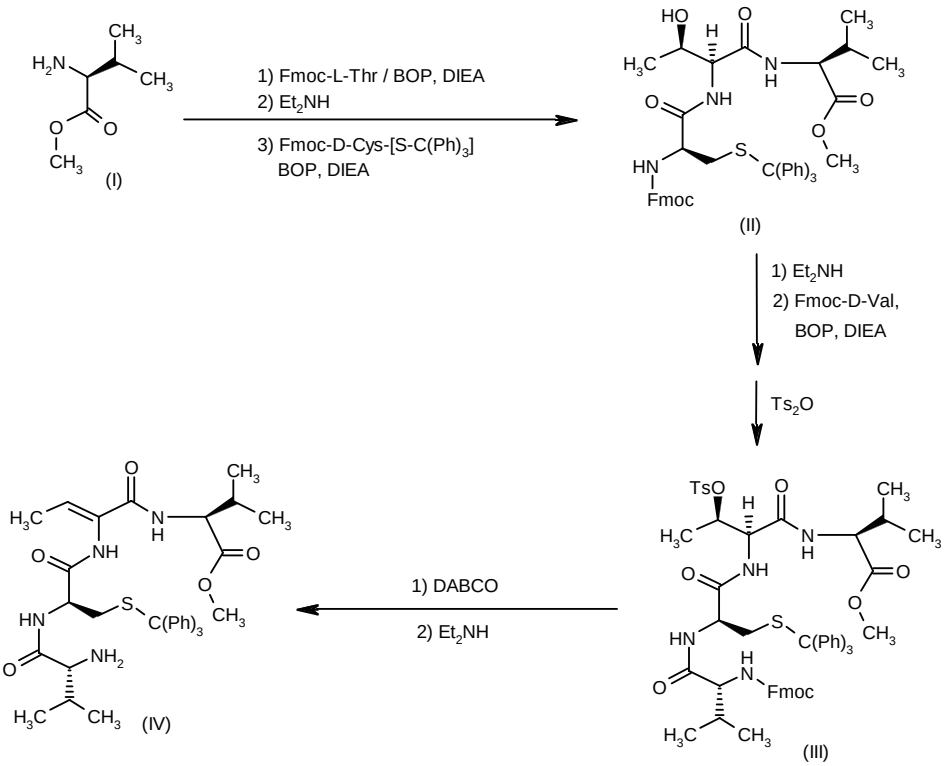
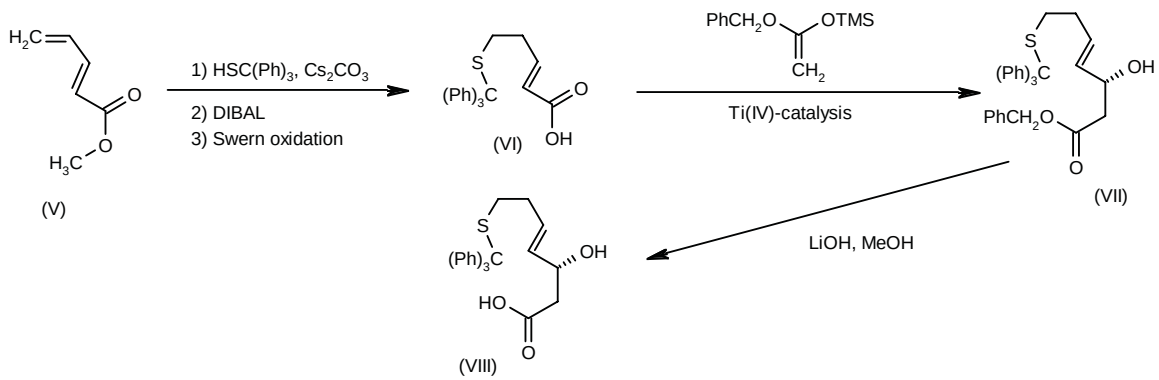
Scheme 1: Synthesis of Intermediate (IV)



Scheme 2: Synthesis of Intermediate (VIII)



separated through a column of silica gel in n-hexane and FR901228 was eluted with EtOAc. Fractions of FR901228 were concentrated under reduced pressure to yield a slightly yellowish powder. The powder of FR901228 was dissolved in a mixture of dichloromethane-methanol-acetonitrile at room temperature which showed colorless prisms of FR901228.

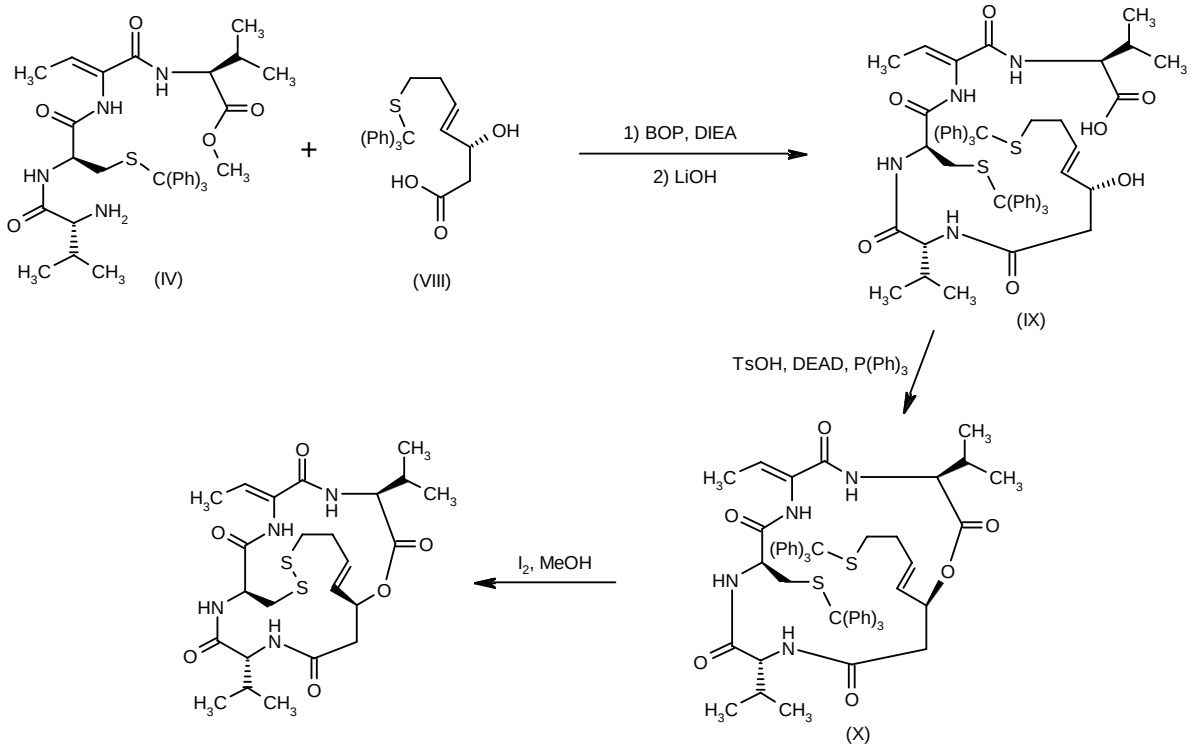
Description

Colorless prisms, m.p. 235-45 °C; $[\alpha]_D$ +39° (c 1.0, $CHCl_3$) (2).

Introduction

FR901228 was isolated from the fermentation broth of *Chromobacterium violaceum* No. 968 and identified as an antitumor agent through efforts in the search for novel agents which selectively reverse the morphological phenotype of *Ras* oncogene-transformed cells (2, 3). Induction of Ras activity has been detected in association with many human tumors and expression of activated Ras results in induction of cancer-related features of transformed cells as seen in tumor progression (4, 5). It is well known that Ras plays a pivotal role in the

Scheme 3: Synthesis of FR901228



intracellular signaling cascade leading to cell proliferation and phenotypic transformation. Blocking Ras-induced signaling pathways has become a major goal for current anticancer drug development.

FR901228 is a bicyclic depsipeptide with a structure unrelated to any known class of cyclic peptides. An unusual disulfide bond connects a thiol and D-cysteine. Cleavage of the disulfide bond by reduction abolished the anticancer activity of FR901228 (Wang and Chan, unpublished data). The disulfide bond may be a redox-controlled conformational switch. FR901228 is soluble in chloroform, EtOAC and DMSO (2, 6) and is sparingly soluble in methanol and ethanol (2). It is insoluble in water and *n*-hexane (2). The hydrophobic property of FR901228 may orientate it primarily to the cytoplasmic membrane. Treatment of Ras-transformed cells with FR901228 reversed morphologic transformation and inhibited Ras-induced myc expression (3), indicating that FR901228 blocked the Ras-induced mitogen-activated signaling pathway. The target site in the mitogen-activated signaling pathway for FR901228 may be upstream of the Raf (Wang *et al*., unpublished data). In addition, FR901228 was found to act as an inhibitor of histone deacetylase (7). FR901228 appears to have multiple molecular targets for its anticancer activity.

Pharmacological Actions

Antimicrobial activity

FR901228 at 100 μg/ml exhibited weak antimicrobial activity against *Shizosaccharomyces pombe*, *Aureobasidium pullulans* IFO 4466 and *Aspergillus niger*, but was not effective against *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* or *Escherichia coli* (2).

In vitro *antitumor activity*

The cytotoxic activity of FR901228 was determined by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (2). It exhibited potent cytotoxic activity against human lung adenocarcinoma A549 and PC-9, human squamous cell carcinoma PC-1 and PC-10, human small cell lung carcinoma ADH and LX-1, human stomach adenocarcinoma MKN28 and MKN74, human mammary adenocarcinoma MCF-7 and ZR-75-1 and human colon adenocarcinomas Colo201 and SW480 at IC_{50} concentrations of 0.3-3.2 ng/ml (2, 6). FR901228 showed cytotoxic effects against the human normal endothelial cell HE-9 and mouse fibroblasts

Balb/c3T3 and NIH3T3 cells at IC_{50} concentrations of 2.7-9.2 ng/ml (2). However, FR901228 was not cytotoxic to human and mouse primary normal fibroblasts at a concentration of 500 ng/ml (2).

In vivo *antitumor activity*

The antitumor activity of FR901228 was evaluated on human solid tumors and murine ascitic and solid tumors (2, 8). FR901228 had a dose-dependent inhibitory effect on the growth of human mammary adenocarcinoma MCF-7 and MX-1, lung adenocarcinoma A549, large cell lung carinoma Lu-65 and LC-6 and stomach adenocarcinoma SC-6 cells in both immunosuppressed and nude mice at 0.56-5.6 mg/kg (2, 8). FR901228 inhibited the growth of mouse solid tumor cells in normal mice, including Colon 38 carcinoma, M5076 reticulum cell sarcoma and Meth A fibrosarcoma at doses of 1.0-5.6 mg/kg; it did not inhibit Colon 26 adenocarinoma (8). FR901228 also showed inhibitory effects on mouse ascitic leukemia P388 and L1210 cells and melanoma B16 cells in mice at 0.056-0.56 mg/kg (8). FR901228 appears to be a new type of antitumor drug which may be clinically effective on tumors which are refractory to antitumor drugs such as mitomycin, cisplatin, cyclophosphamide, vincristine and 5-fluorouracil (8).

Biological and molecular activity

FR901228 induced cell growth arrest and apoptosis of cells in culture in a dose- and time-dependent manner. Flow cytometric analysis showed that FR901228 treatment of normal and H-*Ras*-transformed mouse embryo fibroblast NIH3T3 cells induced a growth arrest of cells in the G_0/G_1 phase of the cell cycle (3). Treatment of H-*Ras*-transformed NIH3T3 cultures with FR901228 resulted in a reversion to a morphology indistinct from normal cells and reduction of c-myc expression, indicating blockade of the Ras-induced signaling pathway by FR901228 (3). Human mammary adenocarcinoma MCF-7 and MDA-MB231 cultures which were treated with FR901228 showed an increased population of cells accumulated in the G_2/M phase of the cell cycle (6, 7). Treatment of MCF-7 and MDA-MB231 cultures resulted in increased expression of $p21^{Cip1}$ and phosphorylation of Bcl-2 that may contribute to cell growth inhibition and apoptosis (6). On the other hand, treatment of human B cell chronic lymphocytic leukemia cells with FR901228 resulted in induction of apoptosis, increased expression of Bax and decreased level of $p27^{Kip}$ in a dose-dependent manner (9). FR901228 also showed inhibitory effects against histone deacetylase, resulting in accumulation of acetylated histone species in cells that may play a critical role in growth arrest of cells and morphological reversion of transformed cells (7, 10). In contrast, pretreatment of cultures of T cell hybridomas blocked CD3 activation-induced apoptosis and suppression of c-myc and Fas ligand expression (11). Since both c-myc and Fas ligand expression are important in the induction of apoptosis (12-17), their suppression may contribute to the effect of FR901228 in blocking CD3-induced apoptosis of T cells. The discrepancy between the effect of FR901228 on induction and blockade of apoptosis of different types of cells requires additional studies to clarify the compound's precise mechanism of action.

Toxicity

Results of an acute toxicity study in 5-week-old, female BDF1 mice showed that the LD_{50} values of FR901228 were 6.4 mg/kg i.p. and 10.0 mg/kg i.v. (2).

Pharmacokinetics

The pharmacokinetics of single doses of FR901228 were studied in rat plasma using a liquid chromatographic/mass spectrometric method (LC/MS) that quantified FR901228 with concentrations of 1-5000 ng/ml in rat (18). For the intravenous study, FR901228 was dissolved in 40% ethanol, 5% polyethylene glycol 400 (PEG 400) and 55% normal saline. Each rat was given FR901228 at 10 mg/kg. Beginning at 2 h after administration of the drug, blood samples were collected for LC/MS analysis. Plasma FR901228 concentrations in rats declined from 2000 ng/ml to 13 ng/ml within 3 h in a biphasic manner. The mean initial half-life was 6 min and the mean terminal half-life was 87 min. The mean total clearance was 425 min/kg and the mean V_{dss} was 22.3 l/kg. The mean V_{dss} value indicated a large volume of distribution consistent with the lipophilic property of FR901228. For the oral study, FR901228 was formulated in 64% ethanol, 2% PEG400 and 34% normal saline and given to rats at 50 mg/kg by gavage. Using a non-crossover AUC method, the oral bioavailability of FR901228 up to 6 h was measured, yielding a mean value of 15.6 ± 7.3% (SD, n = 3). Although oral absorption of FR901228 was found to be more than 15%, the authors indicated that the accuracy of bioavailability needed to be clarified (18).

The pharmacokinetics of FR901228 in human are being determined in two phase I dose-escalation studies involving cancer patients. Results are not yet available.

Clinical Studies

Two phase I trials with FR901228 are ongoing. The agent is being evaluated as a single therapy or in combination with other agents. Patients are given FR901228 via a 4-h continuous infusion on days 1 and 5, every 3 weeks. The studies have not yet been concluded.

Manufacturer

Fujisawa Pharmaceutical Co., Ltd. (JP).

References

1. Li, K.W., Wu, J., Xing, W., Simon, J.A. *Total synthesis of the antitumor depsipeptide FR901228.* J Am Chem Soc 1996, 118: 7237-8.

2. Ueda, H., Nakajima, H., Hori, Y., Fujita, T., Nishimura, M., Goto, T., Okuhara, M. *FR901228, a novel antitumor bicyclic depsipeptide produced by Chromabacterium violaceum No. 968: I. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity.* J Antibiot 1994, 47: 301-10.

3. Ueda, H., Nakajima, H., Hori, Y., Goto, T., Okuhara, M. *Action of FR901228, a novel antitumor bicyclic depsipeptide produced by Chromabacterium violaceum No. 968, on Ha-ras transformed NIH3T3 cells.* Biosci Biotech Biochem 1994, 58: 1579-83.

4. Cerutti, P., Hussain, P., Pourzand, C., Aguilst, F. *Mutagenesis of the H-ras protooncogene and the p53 tumor suppressor gene.* Cancer Res 1994, 54(Suppl.): 1934-8S.

5. Spaargaren, M., Bischoff, J.R., McCormick, F. *Signal transduction by Ras-like GTPases: A potential target for anticancer drugs.* Gene Expr 1995, 4: 345-56.

6. Rajgolikar, G., Chan, K.K., Wang, H-C.R. *Effects of a novel antitumor depsipeptide FR901288 on human breast cancer cells.* Breast Cancer Res Treat 1998, 51: 29-38.

7. Nakajima, H., Kim, Y.B., Terano, H., Yoshida, M., Horinouchi, S. *FR901228, a potent antitumor antibiotic, is a novel histone deacetylase inhibitor.* Exp Cell Res 1998, 241: 126-33.

8. Ueda, H., Manda, T., Matsumoto, S., Nishigaki, F., Kawamura, I., Shimomura, K. *FR901228, a novel antitumor bicyclic depsipeptide produced by Chromabacterium violaceum No. 968: III. Antitumor activities on experimental tumors in mice.* J Antibiot 1994, 47: 315-23.

9. Byrd, J.C., Shinn, C., Ravi, R., Willis, C.R., Waselenko, J.K., Flinn, I.W., Dawson, N.A., Grever, M.R. *Depsipeptide (FR901228): A novel therapeutic agent with selective, in vitro activity against human B-cell chronic lymphocytic leukemia cells.* Blood 1999, 94: 1401-8.

10. Yoshida, M., Horinouchi, S., Beppu, T. *Trichostatin A and trapoxin: Novel chemical probes for the role of histone acetylation in chromatin structure and function.* Bioessays 1995, 17: 423-30.

11. Wang, R., Brunner, T., Zhang, L., Shi, Y. *Fungal metabolite FR901228 inhibits c-myc and Fas ligand expression.* Oncogene 1998, 17: 1503-8.

12. Evan, G.I., Wyllie, A.H., Gilbert, C.S., Littlewood, T.D., Land, H., Brooks, M., Waters, C.M., Penn, L.Z., Hancock, D.C. *Induction of apoptosis in fibroblasts by c-myc protein.* Cell 1992, 69: 119-28.

13. Shi, Y., Glynn, J.M., Guilbert, L.J., Cotter, T.G., Bissonnette, R.P., Green, D.R. *Role for c-myc in activation-induced apoptotic cell death in T cell hybridomas.* Science 1992, 257: 212-4.

14. Janicke, R.U., Lee, F.H., Porter, A.G. *Nuclear c-myc plays an important role in the cytotoxicity of tumor necrosis factor α in tumor cells.* Mol Cell Biol 1994, 14: 5661-70.

15. Klefstrom, J., Vastrik, I., Saksela, E., Valle, J., Eilers, M., Alitalo, K. *c-Myc induces cellular susceptibility to the cytotoxic action of TNF-α.* EMBO J 1994, 13: 5442-50.

16. Walker, P.R., Saas, P., Dietrich, P.Y. *Role of Fas ligand (CD95L) in immune escape: The tumor cell strikes back.* J Immunol 1997, 158: 4521-4.

17. Solary, E., Micheau, O., Dimanche-Boitrel, M.T., Martin, F. *The Fas/Fas-ligand system: Implications in the antitumor immune response and in the activity of cytotoxic agents.* Bull Cancer 1998, 85: 685-94.

18. Chan, K.K., Bakhtiar, R., Jiang, C. *Depsipeptide (FR901228, NSC-630176) pharmacokinetics in the rat by LC/MS/MS.* Invest New Drugs 1997, 15: 195-206.