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CCC: 0377-8282/99

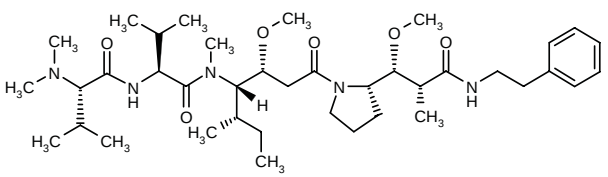
TZT-1027

Antineoplastic

Auristatin PE

3(*S*)-[1-[4(*S*)-[*N*-(*N*,*N*-Dimethyl-L-valyl-L-valyl)-*N*-methylamino]-3(*R*)-methoxy-5(*S*)-methylheptanoyl]pyrrolidin-2(*S*)-yl]-3(*R*)-methoxy-2(*R*)-methyl-*N*-(2-phenylethyl)propionamide

N,*N*-dimethyl-L-valyl-*N*-[2(*R*)-methoxy-4-[2(*S*)-[1(*R*)-methoxy-2(*R*)-methyl-3-oxo-3-(2-phenylethylamino)propyl]-1-pyrrolidinyl]-1(*S*)-[1(*S*)-methylpropyl]-4-oxobutyl]-*N*-methyl-L-valinamide



$C_{39}H_{67}N_5O_6$ Mol wt: 701.999

CAS: 149606-27-9

EN: 227146

Synthesis

TZT-1027 has been obtained by two related ways:

1) The reaction of *tert*-butoxycarbonyl-L-prolinal (I) with benzyl propionate (II) by means of lithium diisopropylamide (LDA) in THF gives a mixture of isomers that is separated by flash chromatography, yielding the (2*R*,3*R*)-isomer (III). The methylation of (III) with diazomethane and boron trifluoride ethearate or NaH and methyl iodide affords the methoxy derivative (IV), which is deprotected with HCl in dioxane, giving (V). The condensation of (V) with tripeptide (VI) by means of diethyl phosphorocyanidate (DEPC) in DMF yields the tetrapeptide benzyl ester (VII), which is finally debenzylated by hydrogenolysis over Pd/C in *tert*-butanol and amidated with 2-phenylethylamine (VIII) and DEPC and triethylamine in DMF (1, 2). Scheme 1.

Intermediate (VI) has been obtained as follows: The condensation of benzyloxycarbonyl-L-isoleucine (IX) with malonic acid monomethyl ester potassium salt (X) by means of carbonyldiimidazole (CDI) and $MgCl_2$ in THF gives the ketoester (XI), which is reduced to the hydroxyester (XII) with $NaBH_4$ in methanol. The methylation of (XII) with methyl iodide and silver oxide in DMF affords the *N*-methyl methoxy ester (XIII). The hydrolysis of (XIII) with NaOH in dioxane/water followed by reesterification with isobutylene gives the *tert*-butyl ester (XIV), which is deprotected by hydrogenation over Pd/C, yielding the amino acid (XV). The condensation of (XV) with benzyloxycarbonyl-L-valine (XVI) by means of DCC in dichloromethane gives the protected dipeptide (XVII), which is debenzylated as usual, yielding (XVIII). The condensation of (XVIII) with *N*,*N*-dimethyl-L-valine (XIX) affords the tripeptide *tert*-butyl ester (XX). Finally, (XX) is hydrolyzed by treatment with trifluoroacetic acid to the free acid intermediate (VI) (1, 2). Scheme 2.

2) The reaction of 3(*R*)-[1-(*tert*-butoxycarbonyl)-2(*S*)-pyrrolidinyl]-3-methoxy-2(*R*)-methylpropionic acid (XXI) with 2-phenylethylamine (VIII) by means of DEPC in dichloromethane gives the amide (XXII), which is deprotected with trifluoroacetic acid to yield the amide (XXIII). Finally, this compound is condensed with the already described intermediate (VI) by means of DEPC in dichloromethane (3, 4). Scheme 3.

Description

Crystals, m.p. 75-8 °C, $[\alpha]_D^{25}$ –38° (c 0.57, MeOH) (1); $[\alpha]_D^{25}$ –38.0° (c 0.566, MeOH) (2); colorless fluffy solid, m.p. 73-9 °C, $[\alpha]_D^{25}$ –37° (c 0.74, MeOH) (3); $[\alpha]_D^{25}$ –36.9° (c 0.74, MeOH) (4).

Introduction

Dolastatin 10 is a pentapeptide isolated from the marine mollusk *Dolabelia auricularia* and has potent antitumor activity. Among its derivatives, auristatin PE (TZT-1027) was found to exhibit inhibitory effects on the growth of human tumor cells *in vitro* (3) and murine leukemia cells *in vivo* (1), and was selected for further evaluation.

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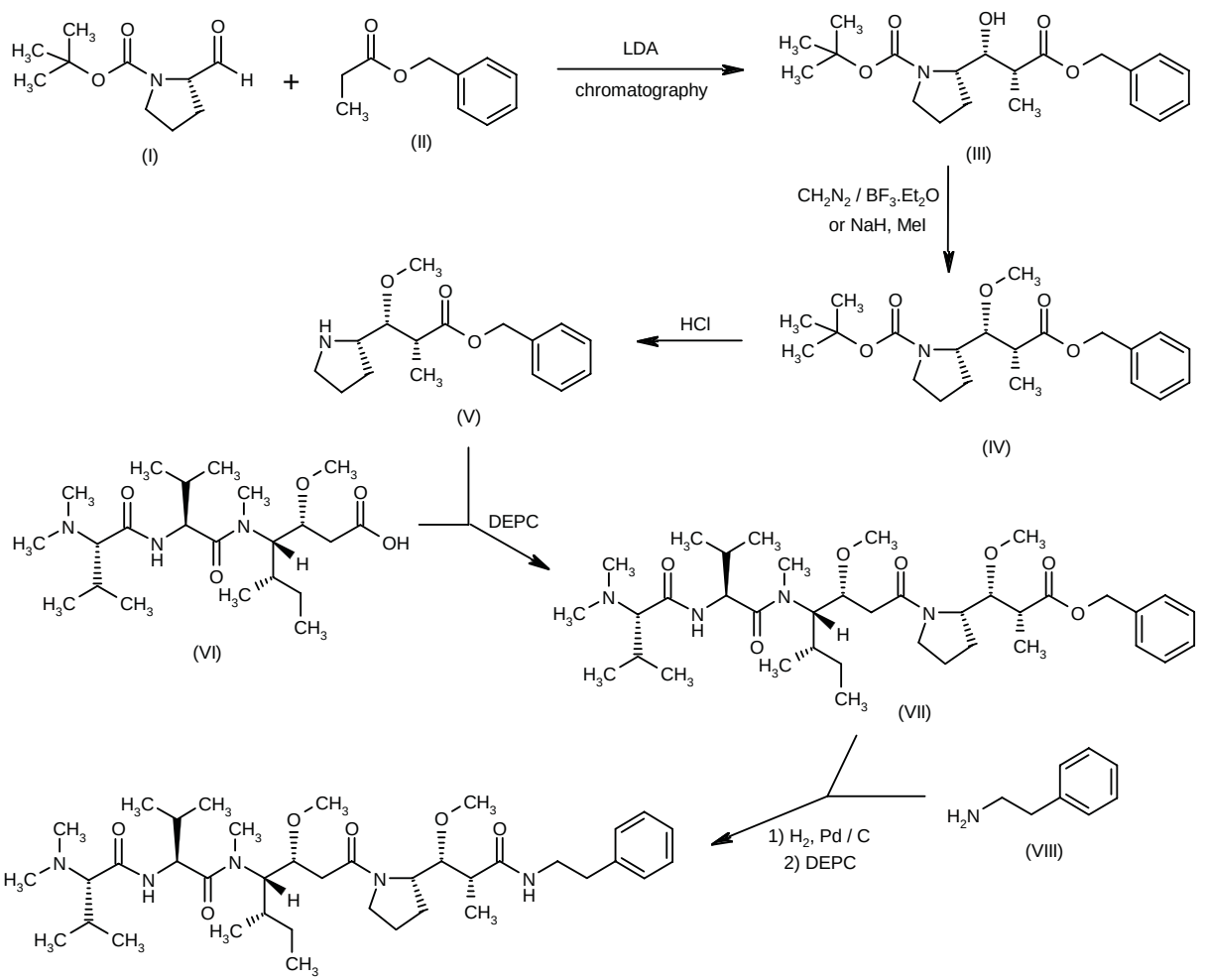
Scheme 1: Synthesis of TZT-1027

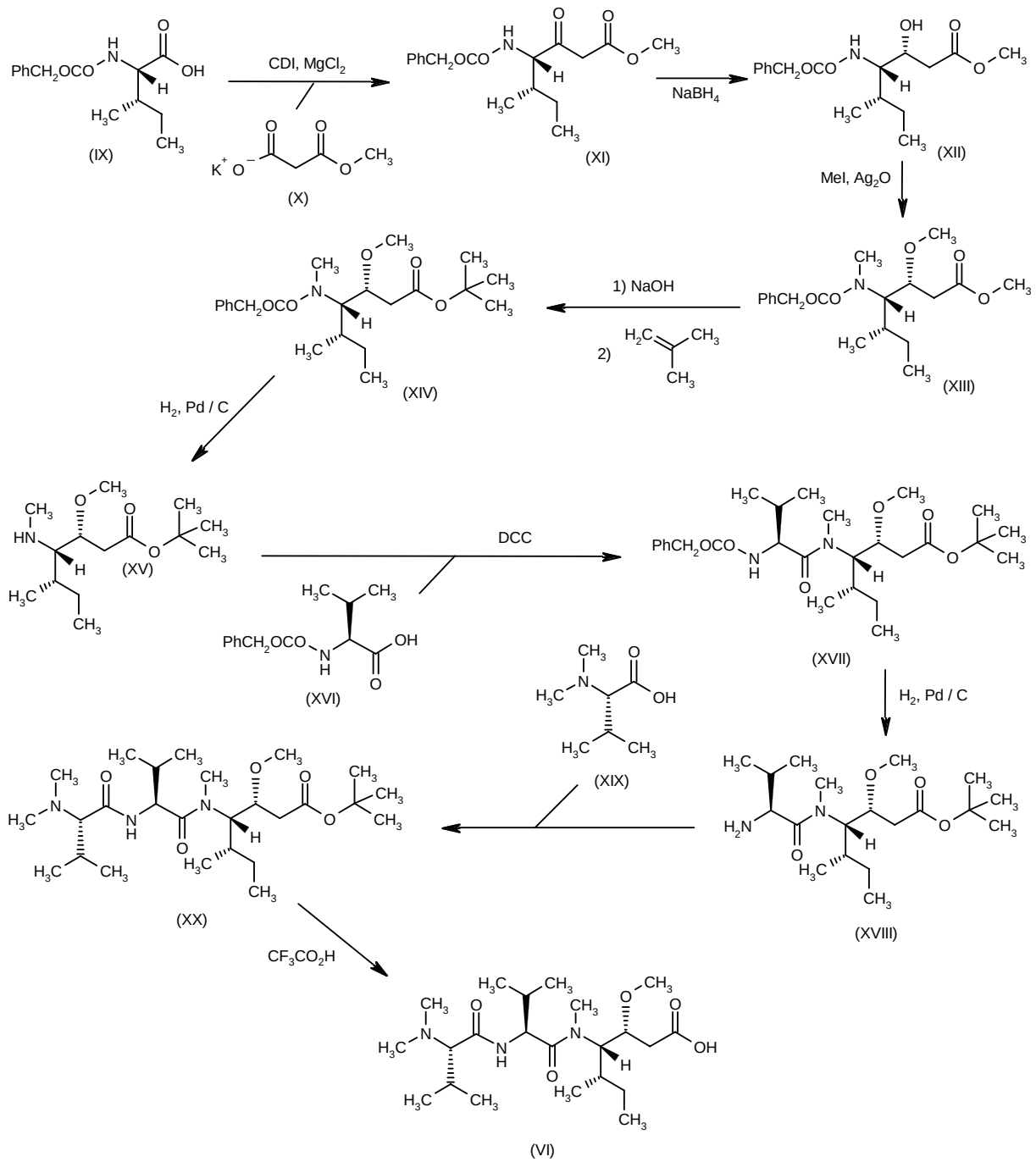


Pharmacological Actions

TZT-1027 inhibited the growth of various human tumor cells *in vitro*, including OVCAR-3, SP-295, A498, NCI-H460, KM20L2 and SK-MEL-5 cells. Its activity was similar to that of dolastatin 10 (3). TZT-1027 and several derivatives also had activity against murine leukemia P388 *in vivo*, which again was similar to that of dolastatin 10 (1). In studies against murine solid tumors, the compound had potent activity on tumor regression and/or growth inhibition of colon 26 adenocarcinoma, B16 melanoma and M5076 sarcoma. The optimal dosing schedule was intravenous administration every 4 days for 5-6 times (5-7). In other studies, TZT-1027 was shown to inhibit the growth of HL-60, K562, MKN45 and MCF-7 human tumor cell lines (8), as well as Waldenstrom's macroglobulinemia (9). The compound also showed activity against various tumors xenografted in nude mice, including stomach, breast, colon, lung, renal, ovarian and liver carcinomas when administered intravenously on a q7dx4 schedule (10).

The antitumor activity of TZT-1027 has been characterized *in vivo* in mice. Schedule-dependent antitumor activity was observed in tumor-bearing mice, with intermittent injections being more effective than single or repeated injections. The compound was active against P388 leukemia when given both i.p. and i.v. However, although it was active when given i.v. against murine solid tumors, when given i.p. it was only effective against colon 26 adenocarcinoma. Marked antitumor activity was observed in mice bearing colon 26 adenocarcinoma, B16 melanoma and sarcoma M5076 after i.v. administration of TZT-1027, its activity being comparable or superior to that of other anticancer agents such as dolastatin 10, cisplatin and 5-FU. The compound showed good activity against cisplatin-resistant P388 leukemia, moderate activity

Scheme 2: Synthesis of Intermediate (VI)

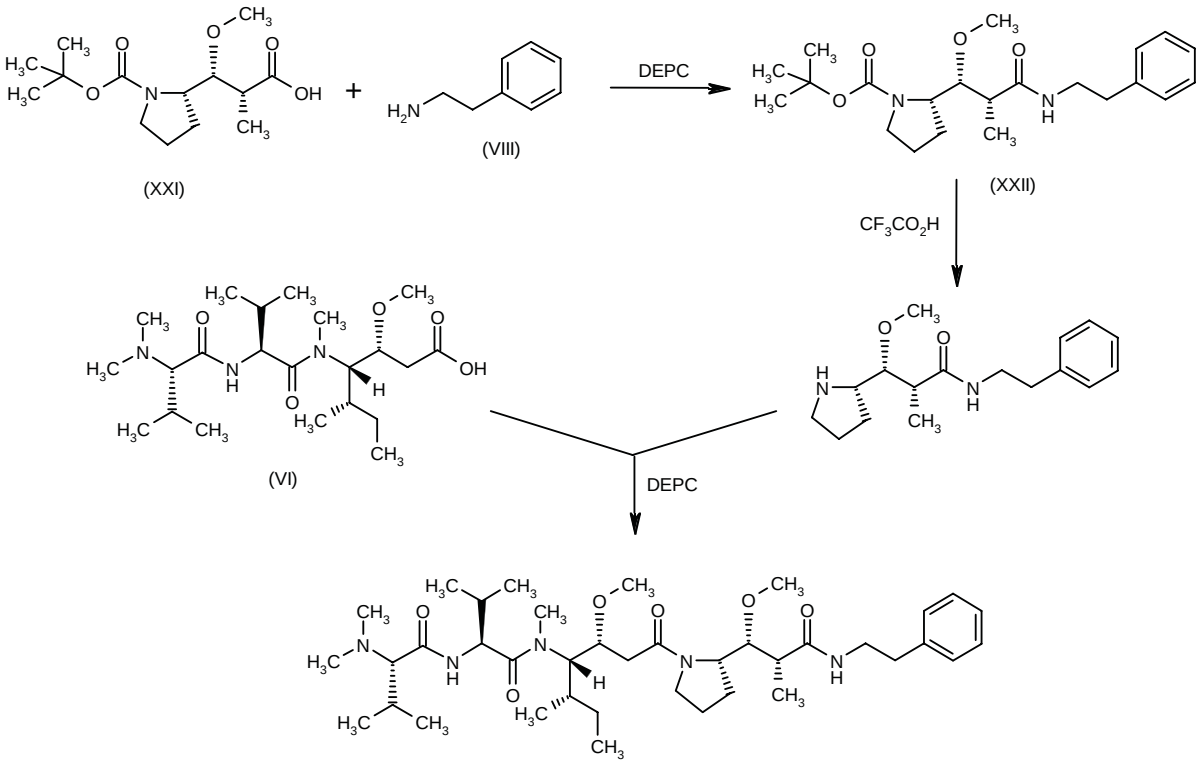


against vincristine- and 5-FU-resistant P388, but no activity against doxorubicin-resistant P388 leukemia. Tumor regressions were obtained in mice bearing human breast cancer MX-1 and lung carcinoma LX-1 xenografts. Its mechanism of action appears to involve inhibition of microtubule assembly (11, 12).

In primary cultures, TZT-1027 (0.2 μg/ml) inhibited the growth of human tumors, including osteosarcoma (77%), renal carcinoma (67%), non-small cell lung carcinoma (61%) and soft tissue sarcoma (58%) (13).

Results of studies on human diffuse large cell lymphoma (WSU-DLCL2) and B-cell chronic lymphocytic

Scheme 3: Synthesis of TZT-1027



leukemia (WSU-CLL) cell lines demonstrated that TZT-1027 (50 pg/ml) was more effective in inhibiting cell growth *in vitro* than dolastatin 10 (500 pg/ml). In SCID mice bearing the WSU-DLCL2 and WSU-CLL cell lines, TZT-1027 administered in combination with bryostatin 1 resulted in complete cures in 2/5 and 5/5 animals, respectively, whereas a combinatioin of dolastatin + bryostatin resulted in no cures and complete cures in 2/5 mice, respectively. Results from these studies indicated that the synergistic effect between these agents was more apparent with the TZ-1027 + bryostatin combination than with dolastatin + bryostatin (14, 15).

In SCID mice implanted with human pancreatic adenocarcinoma, combination treatment with gemcitabine (2.5 mg/kg i.p.) and TZT-1027 (2.0 mg/kg i.v.) was shown to be more effective than treatment with either agent alone. Mean pancreatic weight in mice treated with the combination was significantly lower (0.84 ± 0.639 g) than that of the control group (2.91 ± 1.19 g) and the gemcitabine alone group (1.84 ± 0.796) (16).

In mice transplanted with B16 melanoma, treatment with TZT-1027 resulted in a decrease in tumor size, followed by induction of apoptosis which lasted for at least 48 h (17, 18).

TZT-1027 appears to act by inhibiting tubulin polymerization and has demonstrated potent and broad-spectrum antitumor activity against human tumor xenografts in nude mice, including refractory ovarian and renal cancers. More detailed investigation of its mode of action indicated both a high-affinity and a low-affinity binding site on tubulin. The tubulin binding site was similar to that of vinblastine but different from that of colchicine (19-21).

A number of preclinical studies with TZT-1027 have been presented. The compound was reported to exert its antitumor activity by inhibiting microtubule polymerization (22, 23). In mice bearing colon 26 tumors, TZT-1027 was shown to destroy tumor vasculature and to have enhanced antitumor activity relative to vincristine (24). *In vitro* and *in vivo* studies in rats and rabbits indicated a low liability for neurotoxicity, including peripheral neuropathy (25, 26).

TZT-1027 has been assessed for potential synergy in combination with ara-C in L1210 cells. TZT-1027 is thought to exert its antitumor effects by inhibiting tubulin polymerization. Exposure to TZT-1027 (0.6 nM) followed 3 h later by addition of ara-C (0.1 μM) provided synergistic cytotoxicity (about 50% decrease in cell count at 24 h), whereas exposure to ara-C followed 3 h later by TZT-1027 was only slightly more effective than ara-C alone. The synergistic schedule also resulted in a significant accumulation of cells in the G_2/M phase. It is suggested that the synergy observed with this combination and

schedule is due to inhibition of the M phase by TZT-1027 via its inhibitory effect on tubulin polymerization, and to inhibition of the S phase by ara-C via inhibition of DNA synthesis (27).

Clinical Studies

The results from single-dose phase I trials have also been reported. TZT-1027 was administered by 1-h i.v. infusion starting at 0.15 mg/m^2 and escalating according to a modified Fibonacci's scheme up to 1.35 mg/m^2. Dose-limiting toxicity was myelosuppression (leukopenia and neutropenia), with a maximum allowable dose (MAD) of over 1.35 mg/m^2; alopecia was also observed. One patient with soft tissue sarcoma had a partial response, and a greater than 50% reduction in primary tumor or metastatic lesion size was obtained in 3 patients with non-small cell lung cancer. Phase I trials using an intermittent schedule of administration on days 1, 8 and 15 are in progress (28).

TZT-1027 is currently in phase I trials in Japan for the treatment of a variety of solid tumors (29).

Manufacturer

Teikoku Hormone Mfg. Co., Ltd. (JP).

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