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MONOGRAPH

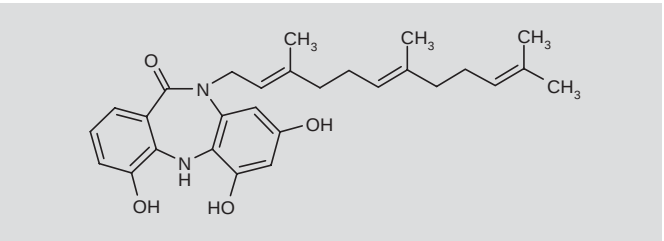
DIAZEPINOMICIN

Apoptosis Inducer
Oncolytic

BU-4664L
ECO-04601
ECO-4601
TLN-4601

4,6,8-Trihydroxy-10-[3,7,11-trimethyl-2(*E*),6(*E*),10-dodecatrienyl]-10,11-dihydro-5*H*-dibenzo[*b,e*][1,4]diazepin-11-one

InChI=1/C28H34N2O4/c1-18(2)8-5-9-19(3)10-6-11-20(4)14-15-30-23-16-21(31)17-25(33)27(23)29-26-22(28(30)34)12-7-13-24(26)32/h7-8,10,12-14,16-17,29,31-33H,5-6,9,11,15H2,1-4H3/b19-10+,20-14+



$C_{28}H_{34}N_2O_4$
Mol wt: 462.5806
CAS: 733035-26-2
EN: 359062

ABSTRACT

Diazepinomicin is a structurally novel farnesylated dibenzodiazepinone discovered using a drug discovery platform (DECIPHER®) designed for the screening of anticancer compounds from actinomycete cultures. Preclinical data suggest that diazepinomicin is a targeted anticancer drug with dual activity: selective binding to the peripheral benzodiazepine receptor (PBR), resulting in tumor apoptosis, and inhibition of the Ras/MAP kinase signaling pathway, which is involved in cellular proliferation and migration. The compound has demonstrated broad-spectrum antitumor activity in cancer cells in vitro and in tumor xenografts in vivo, including leukemia and solid tumors such as melanoma and glioma. It has been shown to be safe and well tolerated when administered as a continuous infusion in primates and in clinical studies in cancer patients. Efficacy has been observed in advanced cancer patients and the compound is under early clinical investigation for the treatment of glioblastoma multiforme.

BACKGROUND

The central benzodiazepine receptor (CBR) is restricted to the central nervous system (CNS) and mediates the anxiolytic and anticonvulsant properties of benzodiazepines (1). The peripheral benzodiazepine receptor (PBR) was identified more recently and is involved in numerous biological functions, including steroid biosynthesis, mitochondrial oxidative phosphorylation, cell proliferation and apoptosis (1, 2). The PBR is a critical component of the mitochondrial permeability transition pore (MPTP), a multiprotein complex located in the contact site between the inner and outer mitochondrial membranes that is involved in the initiation and regulation of apoptosis. An increase in PBR has been documented in many tumor types compared to normal tissues. Moreover, PBR ligands have been shown to induce apoptosis in several tumor types. Tumor cell-targeted therapies, via the induction or enhancement of apoptosis, constitute a promising approach to achieve more specific antitumor efficacy. Moreover, MPTP-mediated regulation of programmed cell death is an apoptosis-inducing factor-independent checkpoint that could be modulated by various conventional cancer therapies, and it has been demonstrated that PBR binding enhances apoptosis induction in many types of tumors (2).

The Ras/Raf/MEK/ERK signaling pathway regulates the expression of a large number of proteins involved in the control of cell proliferation, differentiation and apoptosis. Aberrant activation of this pathway is commonly observed in various cancers. Thus, therapeutic targeting of individual components of the Ras/Raf/MEK/ERK pathway has attracted much attention in the development of anticancer drugs and inhibitors targeting protein kinases of this pathway have demonstrated potential utility as anticancer drugs and are currently in clinical trials (3).

Diazepinomicin is a structurally novel farnesylated dibenzodiazepinone discovered using a drug discovery platform (DECIPHER®) designed to isolate highly potent cytotoxic actinomycete compounds as potential antitumor agents (4). Diazepinomicin represents a unique molecular class composed of a dibenzodiazepine core linked to a farnesyl side-chain. It was isolated from the culture of a marine

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actinomycete of the genus *Micromonospora* and characterized using spectroscopic methods (5-10). Diazepinomicin has been shown to induce the expression of several proteins related to apoptosis induction, including the PBR. The compound has demonstrated broad-spectrum antitumor activity in vitro and in vivo in leukemia and solid tumors including melanoma and glioma. It is undergoing early clinical trials for the treatment of glioblastoma multiforme.

PRECLINICAL PHARMACOLOGY

Diazepinomicin has been shown to have broad-spectrum cytotoxic activity in the low micromolar range when tested in the NCI 60 cell line panel. Potent in vivo antitumor activity was observed against human glioma U-87 MG, hormone-independent breast MDA-MB-231 and prostate cancer PC-3 xenografts. Antitumor efficacy correlated with low and sustained drug plasma levels (4, 11).

Since diazepinomicin has a benzodiazepinone moiety, its binding to benzodiazepine receptors was investigated using radioligand binding assays. The compound was shown to selectively bind the PBR (K_i = 0.257 μM; IC_{50} = 0.291 μM) but not the CBR (IC_{50} > 10 μM) and inhibited the growth of CNS tumor cell lines (4). Since the PBR is involved in apoptosis initiation and regulation, the effects of diazepinomicin on apoptosis were extensively studied. Diazepinomicin induced the production of reactive oxygen species (ROS) and caspase activation in breast MDA-MB-231, prostate PC-3, glioma SF-188 and T-cell leukemia Jurkat tumor cell lines in a concentration-dependent manner, resulting in typical features of apoptosis such as poly(ADP-ribose) polymerase (PARP) cleavage and DNA fragmentation (12).

Due to its farnesylated moiety, the effect of diazepinomicin on the Ras signaling pathway has also been investigated. Several studies showed that diazepinomicin is a potent inhibitor of the Ras/MAP kinase pathway. Diazepinomicin inhibits Ras signaling events such as Raf-1 and ERK1/2 phosphorylation in human breast MCF7, glioma U-87 MG and prostate PC-3 cancer cells in vitro. In these cells, Ras inhibition was not mediated by prenylation and diazepinomicin did not inhibit epidermal growth factor receptor (EFGR, ErbB-1), Raf-1, MEK or ERK1/2 kinase activities (13-15). Interestingly, diazepinomicin decreased the amount of Ras associated with the cell membrane (14) and induced Raf-1 degradation through the proteasome (15).

Diazepinomicin is also a 5-lipoxygenase inhibitor and this effect has been related to potential inhibition of tumor cell migration and metastasis. Diazepinomicin inhibited EGF-induced cell migration and lamellipodia formation in human epidermoid carcinoma A-431 cells in vitro though a mechanism involving the inhibition of leukotriene C_4 (LTC_4) (16). Moreover, it was shown that diazepinomicin induced glioma cell migration in both wild-type EGFR and mutant EGFRvIII U-87 MG cells (17).

PHARMACOKINETICS AND METABOLISM

The plasma protein binding of diazepinomicin was found to be similar across species (71%, 71% and 67%, respectively, in rats, monkeys and humans). In Sprague–Dawley rats the pharmacokinetics following a single i.v. bolus of 30 mg/kg followed two phases and were best described by a 2-compartment model. The half-life values for the two phases were 4.8 min for phase α and 2.5 h for phase β, with an average $AUC_{0\text{-}12h}$ of 37,804 ng/mL.h. In cynomolgus monkeys the equivalent surface area dose of diazepinomicin (15 mg/kg) administered as a single i.v. bolus injection showed a similar biphasic pharmacokinetic profile, with short half-lives for each phase ($t_{1/2\alpha}$ = 20 min and $t_{1/2\beta}$ = 8 h). Metabolism studies in cryopreserved hepatocytes showed a single glucuronide as the major metabolite in rats, monkeys and humans. Further biodistribution analysis of diazepinomicin in rats and monkeys detected the glucuronide in liver and kidneys, with larger amounts of this metabolite in urine. In monkey feces relatively high concentrations of diazepinomicin were detected, suggesting that the compound is excreted unchanged through the bile. Altogether these data suggest that diazepinomicin has a short half-life in the vascular compartment, where it binds to plasma proteins. The compound does not accumulate in tissues, but it is mainly metabolized to a glucuronide and subsequently eliminated in the urine or eliminated unchanged in the feces. The free compound fraction available to diffuse from the plasma into organs, as well as the metabolite products, were similar across species (rats, monkeys, humans) (18).

Pharmacokinetic studies in xenograft models using s.c., i.p. and i.v. bolus showed that the antitumor efficacy of diazepinomicin was associated with AUC and sustained drug levels rather than C_{max} (4, 11).

Since antitumor activity is dependent on continuous exposure, a target plasma diazepinomicin concentration of 2 μM was determined for phase I clinical trials. In a phase I clinical trial conducted in cancer patients a dose of 30 mg/m^2/day by continuous i.v. infusion gave a mean plasma concentration of 0.50 μM, with moderate interpatient variability. Diazepinomicin concentrations increased linearly as a function of dose escalation, while clearance, half-life and volume of distribution were independent of dose. Doses of 270, 360 and 480 mg/m^2/day resulted in plasma concentrations of 2.4, 3.1 and 4.7 μM, respectively. After the infusion ceased, diazepinomicin was rapidly eliminated from the bloodstream within 24 h at all dose levels, with a mean $t_{1/2\alpha}$ of 9.4 h and a $t_{1/2\beta}$ of < 30 min. When the 2-week continuous infusion cycle was repeated after 1 week off, the pharmacokinetic parameters were maintained, indicating that no drug accumulation had occurred (19).

SAFETY

An adequate margin of safety for diazepinomicin was seen in a 2-week continuous infusion study in monkeys. Occasional lack of appetite, a modest degree of reversible anemia with no other hematological abnormalities, elevations in serum cholesterol and triglycerides, a decrease in albumin, diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes in the spleen were observed (20).

Diazepinomicin has been shown to be safe and well tolerated at doses resulting in plasma levels with substantial anticancer activity in cancer patients. Adverse effects seen with diazepinomicin include anaphylaxis, fatigue, nausea, vomiting, rash and hemoglobin decrease (21).

CLINICAL STUDIES

Results from phase I studies in patients with advanced solid tumors showed stable disease in 7 of 12 patients evaluable after 3 cycles of treatment (5 colorectal, 1 ovarian and 1 duodenal cancer) (21, 22).

Diazepinomicin is currently being evaluated in a phase II clinical trial in patients with recurring glioblastoma multiforme (23). The aim of this study is to assess the safety and efficacy of diazepinomicin in patients who recur/progress after receiving first-line systemic therapy after surgery or radiotherapy.

SOURCE

Thallion Pharmaceuticals, Inc. (CA).

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