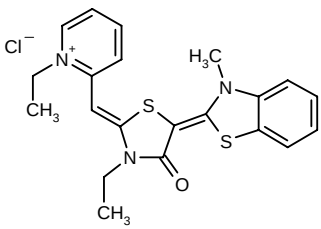
Drugs of the Future 1998, 23(1): 24-27

MKT-077

Antineoplastic

SDZ-MKT-077
FJ-776 (formerly)

1-Ethyl-2-[3-ethyl-5-(3-methyl-2,3-dihydrobenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidenemethyl]pyridinium chloride



$C_{21}H_{22}ClN_3OS_2$ Mol wt: 432.00

CAS: 147366-41-4

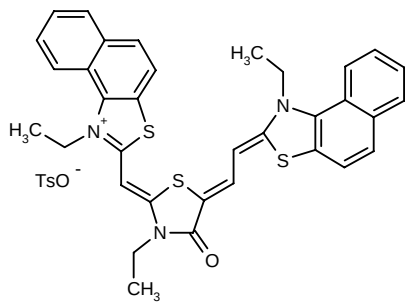
EN: 237755

Synthesis

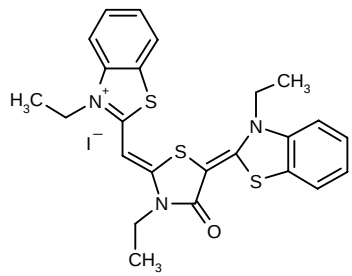
The alkylation of 2-(methylsulfanyl)benzothiazole (I) withmethyl *p*-toluenesulfonate in anisole gives 3-methyl-2-(methylsulfanyl)benzothiazolium *p*-toluenesulfonate (II), which is condensed with 3-ethyl-2-thioxothiazolidin-4-one (III) by means of triethylamine in acetonitrile to yield 3-ethyl-5-(3-methyl-2,3-dihydrobenzothiazol-2-ylidene)-2-thioxothiazolidin-4-one (IV). The methylation of (IV) with methyl *p*-toluenesulfonate in DMF affords 3-ethyl-5-(3-methyl-2,3-dihydrobenzothiazol-2-ylidene)-2-(methylsulfanyl)-4-oxo-4,5-dihydrothiazolium *p*-toluenesulfonate (V) (1), which is condensed with 1-ethyl-2-methylpyridinium *p*-toluenesulfonate (VI) by means of triethylamine in warm acetonitrile giving the *p*-toluenesulfonate of MKT-077 (VII). Finally, this compound is passed through an ion-exchange resin (Amberlyst A-26) in methanol/chloroform (1, 2). Scheme 1.

Introduction

Recent efforts in cancer chemotherapy have focused on the search for anticancer drugs with new mechanisms of action capable of differentiating between tumor cells and normal proliferating cells and with selective toxicity against tumors. Several studies have described that certain π electron-delocalized lipophilic cations, known as DLCs, show notable and specific anticancer potential (3-10). It was suggested that this effect was due to the selective accumulation in negative charged mitochondria in carcinoma cells caused by the electrochemical proton gradient. However, these compounds exhibited toxicity towards normal cells and their clinical use was never fully developed. Two classes of rhodacyanine dyes were later described, class 1 and class 2 [I, II], which showed a potent inhibitory effect on growth *in vitro* on several tumor cell lines and also showed antitumor potential in tumor-bearing nude mice models (10). Structure-activity studies showed that rhodamine and π electron-delocalization were the crucial structural requirements and modifications on the moiety led to a loss of antitumor activity. Following this line of investigation, an extensive screening was conducted of several cationic dye molecules developed for silver halide photographic systems. The results of this screening showed that rhodacyanine dyes had specific antitumor activity against the human carcino-



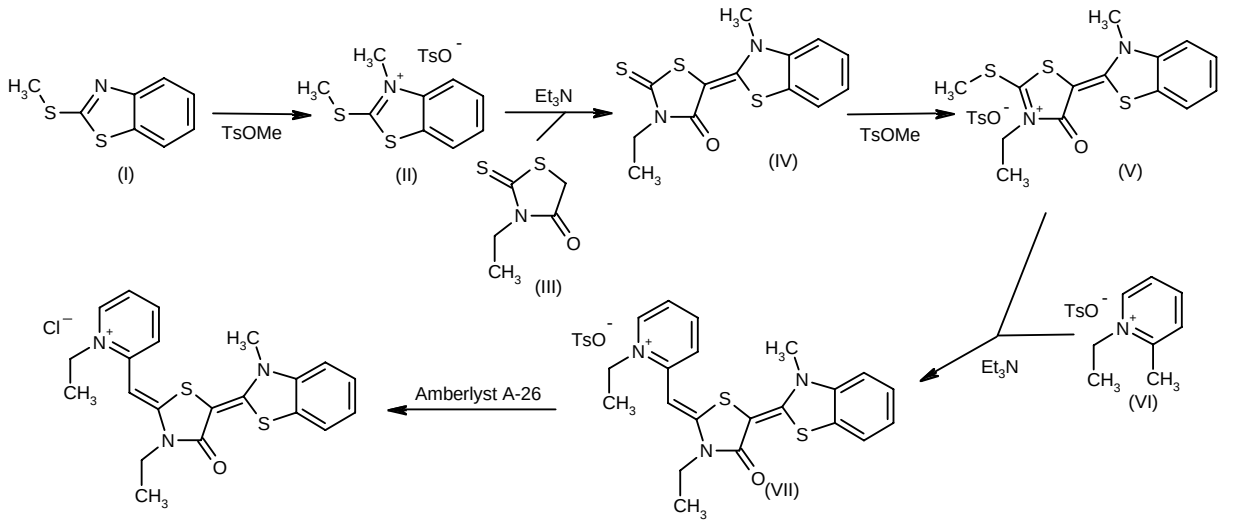
[I]



[II]

T. Wrobleski, A. Graul, J. Castañer. Prous Science Publishers, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of MKT-077



ma cell line, CX-1, compared to a normal epithelial cell line, CV-1. Considering these findings, a wide spectrum of rhodacyanine dyes were designed and synthesized, and their biological and chemical properties such as *in vitro* activity, *in vivo* efficacy and toxicity, stability and solubility were studied (1). These investigations led to the recognition of the rhodacyanine dye MKT-077, which has potent antitumor activity and is presently under development.

Antitumor Activity

MKT-077 is a rhodacyanine dye with potential to cause selective mitochondrial damage, leading to marked and selective antitumor activity (1). An *in vitro* study demonstrated that the compound inhibits respiratory activity in a dose-dependent manner in isolated intact mitochondria and electron transport activity in freeze-thawed mitochondrial membrane fragments. Half-maximal inhibition of ADP-stimulated respiration was 4-fold higher in mitochondria isolated from a normal monkey kidney epithelial cell line, CV-1 (15 μg MKT-077/mg protein) than from a human colon carcinoma cell line, CX-1 (4 μg MKT-077/mg protein). Treating cells with MKT-077 (3 μg/ml for up to 3 days) induced selective loss of mitochondrial DNA in CX-1 and pancreas carcinoma CRL1420 cells but not in CV-1 cells, while nuclear DNA was unaffected in all cell lines (11).

Another *in vitro* study assessed the antineoplastic activity of MKT-077 on a human colon cancer cell line, CX-1, and a normal monkey kidney epithelial cell line, CV-1. CV-1 cells took up small amounts of the compound and released it rapidly, while CX-1 cells retained a significant amount even after 20 h. In a growth assay with MTT as endpoint, the antineoplastic activity of MKT-077 correlated to contact time and concentration and its IC_{50} values in tumor cells ranged from 2.0-10.1 mg/ml, compared to 47.1 ± 12.4 mg/ml in human splenic cells (12). In another *in vitro* assay, MKT-077 gave IC_{50} values of 0.81 and > 69.0 μM in K_B epidermoid and CV-1 cells, respectively (1).

In a study using several human tumor cell lines including PC3 (prostate), OVCAR3 (ovarian), HCT116 (colorectal), T47D (breast) and A375 (melanoma), MKT-077 had an IC_{50} of < 5 μM and its cytotoxic effects persisted for up to 48 h after drug withdrawal. Multiple short exposures for 2 h had approximately the same effect as continuous exposure for up to 72 h, as demonstrated by the IC_{50} values(13).

In a comparative *in vitro* study with rhodamine-123, MKT-077showed equal cytotoxicity when tested on human prostate cancer DU145 and PC-3 cells at concentrations of 0.25-8 μg/ml, while at 16-64 μg/ml MKT-077 showed more cytotoxicity than rhodamine-123. PC-3 cells appeared to be more sensitive than DU145 cells. When tested on two nontumorigenic cells (NPF-209 prostate and NF-2 foreskin) at concentrations of 0.25-4 μg/ml, neither rhodamine-123 nor MKT-077 showed cytotoxicity and the cells remained viable 1-5 days after exposure. However, differential sensitivity between the two cell types was observed at 8-16 μg/ml (14).

The antitumor activity of MKT-077 was evaluated *in vivo* using several human tumor xenografts including Co-4 and HT29 (colon cancer), St-4 and MKN45 (gastric cancer), and CRL1420 and LS174T (pancreatic cancer) serially transplanted in nude mice. Treatment with MKT-077 began once the tumors had entered exponential growth at doses of 7.5 mg/kg/day x 14 days i.p. or at 7.5, 20, 30 and 40 mg/kg/day by continuous s.c. injection for 7 or 14 days. Maximum tolerated dose appeared to be 20 mg/kg/day when administered continuously and the antineoplastic activity of the compound appeared to be dose-

dependent when evaluated in terms of T/C ratio of tumor weight. Of the cell lines tested, St-4, Co-4 and CRL1420 appeared to be sensitive to MKT-077 (15).

In nude mice with implanted human melanoma LOX cells, the compound gave a T/C value of > 344% when administered at a dose of 5 mg/kg/day i.p. for 4 days. It inhibited the growth of CA755 adenocarcinoma subcutaneous allografts in BDF1 mice by 75.5% at the dose of 3 mg/kg/day i.v. x 4 days (1).

Pharmacological Actions

An *in vivo* study assessed the effects MKT-077 on selected rat organs. The compound was administered by bolus i.v. injection (15 mg/kg) daily for a period of 5 days. Examination of the organs selected indicated that the drug had no apparent effects on rat heart and kidney mitochondrial respiration, but a decreased rate of liver mitochondrial respiration was observed. This effect appeared to be reversed 3 days after drug withdrawal. Kidney and liver mitochondrial DNA appeared not to be affected by the treatment, but levels of heart mitochondrial DNA were lower than those in controls. Again, the levels of heart mitochondrial DNA were partially recovered after 10 days postdrug withdrawal and completely recovered after 30 days. The study concluded that MKT-077 had minimal effects on mitochondrial function in rat heart, liver and kidney (16).

Toxicity

In toxicity studies in mice, MKT-077 gave LD_{50} values of 20 mg/kg i.v. and 50 mg/kg i.p. (1, 17). No myelotoxicity was observed in rats, dogs or humans (18).

Clinical Studies

MKT-077 is presently undergoing evaluation in phase I/II clinical trials for the treatment of solid tumors (1, 16).

In one phase I trial, MKT-077 has been administered to 30 patients with refractory carcinomas at the dose of 48 mg/m^2 by 30-min infusion on days 1, 3 and 5, followed by a 3-week recovery period. No serious adverse effects (*e.g.*, myelotoxicity or cardiotoxicity) had been observed at the time the results were reported (18).

Manufacturer

Novartis Pharma AG (CH), licensed from Fuji Photo Film Co., Ltd. (JP).

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