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CCC: 0377-8282/2001

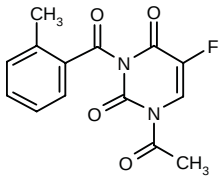
Atofluding

Oncolytic

A-OT

1-Acetyl-3-(*o*-toluyl)-5-fluorouracil

1-Acetyl-5-fluoro-3-(2-methylbenzoyl)pyrimidine-2,4(1*H*,3*H*)-dione



$C_{14}H_{11}FN_2O_4$ Mol wt: 290.2489

CAS: 071861-76-2

EN: 110413

Synthesis*

Atofluding can be prepared by several similar ways: Scheme 1.

a) Acylation of 5-fluorouracil (I) with acetic anhydride in refluxing pyridine gives 1-acetyl-5-fluorouracil (II) (1, 2), which is then acylated with 2-methylbenzoyl chloride by means of either TEA in dioxane at room temperature (3, 4) or pyridine in dioxane at 80 °C (5).

b) Acylation of 5-fluorouracil (I) with 2-methylbenzoyl chloride in pyridine at room temperature yields 5-fluoro-3-(2-methylbenzoyl)uracil (III) (3), which is then acylated with acetic anhydride at 80 °C (4) or with acetic anhydride by means of TEA in cooled dioxane (6).

c) Acylation of 5-fluorouracil (I) with acetyl chloride by means of TEA in dioxane at room temperature provides 1,3-diacetyl-5-fluorouracil (IV) (3), which is then acytated with 2-methylbenzoyl chloride by means of TEA in dioxane (5).

Description

M.p. 141-3 °C (3).

Introduction

5-Fluorouracil (5-FU) is an antitumor agent commonly used as an antimetabolite. Although 5-FU has been clinically proven to be effective against most solid tumors, its plasma half-life is very short (15-20 min) and the drug is usually administered only as a continuous i.v. infusion. In order to overcome this disadvantage, attempts were made to design and synthesize new 5-FU derivatives. One of them, atofluding, is a double prodrug of 5-FU which demonstrated significant antitumor activity and is in phase III clinical trials in China. Following oral administration, atofluding is rapidly metabolized to 3-*O*-toluyl-5-fluorouracil (TFU), which is subsequently slowly metabolized to release 5-FU (7). This metabolic route is shown in Scheme 2.

Pharmacological Actions

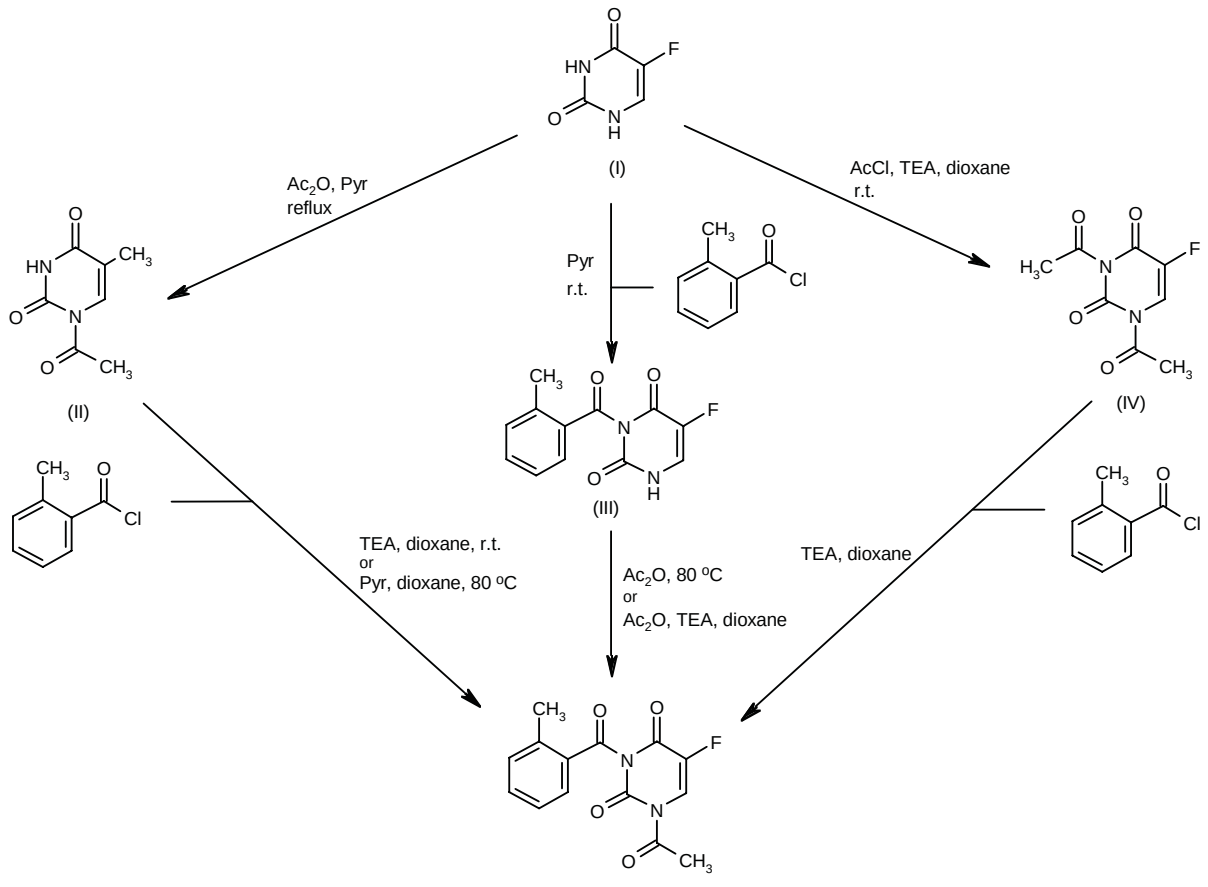
Atofluding demonstrated significant broad-spectrum antitumor activity against Ehrlich, Meth-A, MH-134, L-1210, P-388, H22, S180 and U14 tumor cell lines in mice. Its efficacy was superior to FT-207 and HCFU (3, 8).

Tumor-bearing mice were treated with oral atofluding at doses of 30-125 mg/kg/day for 7-9 days. Atofluding doses of 50 and 125 mg/kg/day inhibited hepatic carcinoma (H22) cell growth by 42.7-52.2% (p <0.05) and 83.2-89.3% (p <0.001), respectively. Against S180 sarcoma, growth-inhibitory rates were 19.0-35.0% (p <0.05), 44.2-60.1% (p <0.05) and 72.1-92.4% (p <0.01) with atofluding doses of 30, 61 and 125 mg/kg/day, respectively. Results of experiments using Ehrlich tumor cells showed that atofluding can significantly increase life span. Life span was increased by 10.2-35% (p <0.05), 46.3-62.2% (p <0.01) and 56.2-60.3% (p <0.01) with doses of 30, 61 and 125 mg/kg/day, respectively (9).

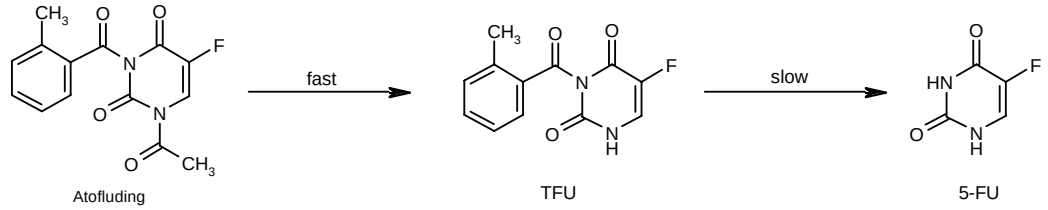
The effects of single oral doses of atofluding (30, 60 and 90 mg/kg) were evaluated in dogs at 30, 60, 120 and 180 min after drug administration. The results showed that there were no significant differences in blood pressure, cardiac rate, TPR and ECG in atofluding-treated

Wenfang Xu[1], Zhen Zhang[1], J. Castañer[2]*. [1]College of Pharmacy, Shandong University, Jinan 250012, China; [2]Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of Atofluding



Scheme 2: Metabolism of Atofluding



dogs as compared to control animals. The effects of consecutive oral doses of atofluding (125 mg/kg/day for 3 days) were evaluated in mice. No significant changes in spontaneous movements were observed in atofluding-treated mice as compared to control animals at 30 min postdosing and on day 4. Spontaneous movement in mice began to decrease at 60 min after drug administration (10).

Toxicology

The acute toxicity of atofluding is low. The LD_{50} values were 2628.92 ± 361.59 mg/kg and 377.79 ± 54.96 mg/kg after oral and i.p. administration, respectively, in mice. In Sprague-Dawley rats, the oral and i.p. LD_{50} values were 1840.8 ± 298.2 mg/kg and 548.5 ± 59.2 mg/kg, respectively. In dogs, the oral LD_{50} was 84.65 ± 40.38 mg/kg (11).

For chronic toxicity studies, Sprague-Dawley rats were divided into 3 groups and administered oral atofluding for 90 days at doses of 40, 70 and 130 mg/kg/day. In the high-dose group (130 mg/kg), 20% of the rats died. There were significant changes in general phenotype, body weight, liver (GPT) and kidney (urea nitrogen) function, viscera coefficients and histopathological findings, as well as a decrease in total number of WBCs. None of the rats in the medium-dose group (70 mg/kg) died, and only slight histopathological changes were observed. No significant changes were seen in the low-dose group (40 mg/kg), and the no-effect dose in rats was determined to be 40 mg/kg/day (12).

In chronic oral toxicity studies in dogs, atofluding was administered for 114 days at doses of 0.31, 2.0 and 8.30 mg/kg/day. Dogs in the high-dose group (8.30 mg/kg) experienced changes in ECG, urine and peripheral blood, and decreases in appetite, movement and body weight, as well as tissue necrosis of the testes, indicating that this was the toxic dose in dogs. Some animals in the medium-dose group (2.0 mg/kg) had slight changes in urine and peripheral blood, indicating low toxicity. No toxic effects were observed in the low-dose group (0.31 mg/kg) as compared with untreated control animals (12).

In the Ames test to determine gene mutations, 4 different strains of *Salmonella typhinium* (TA97, TA98, TA100 and TA102) were used at drug concentrations of 0.005-0.5 μg/dish. Bone marrow micronucleus test of polychromatic erythrocytes was carried out in mice following oral doses of 215, 435 and 2175 mg/kg atofluding. Chromosome teratogenicity test of human peripheral lymphocytes was carried out at doses of 0.25, 1, 5 and 10 μg/ml. All results showed that atofluding was not mutagenic and had no potential carcinogenic activity (13).

Fertility toxicity studies were carried out in Wistar rats. Pregnant rats were divided into two groups and received oral atofluding for 5 days at doses of 50 or 200 mg/kg/day. Embryotoxicity and teratogenesis were observed in animals in the high-dose (200 mg/kg) group (p <0.01). There were no significant differences in skeletal and organ growth of fetus in the low-dose (50 mg/kg) group as compared to normal control rats (p <0.05) (14).

Pharmacokinetics

Atofluding was rapidly metabolized to TFU and 5-FU after oral administration in mice. Tissue distribution studies showed that the drug was detected 30 min after administration. Maximum concentrations of 5-FU were obtained between 0.5-6 h in most tissues. The highest concentrations were found in stomach, kidney and intestines, with lower concentrations found in spleen and testes. The maximum concentration of TFU in all tissues was reached 30 min after administration and decreased rapidly 2 h later. TFU could not be detected in most tissues 6 h after drug administration.

Drug excretion experiments revealed that after oral administration of atofluding in mice, biliary excretion accounted for 0.59-2.23% of the compound within 12 h; urinary and fecal excretion accounted for 14.16-22.75% within 48 h.

Atofluding was metabolized rapidly to TFU and 5-FU after oral administration in rabbits. The metabolism of TFU and 5-FU *in vivo* essentially followed a first-order, one-compartment model. After oral administration of 25, 50 and 100 mg/kg atofluding, the active metabolites TFU and 5-FU were absorbed rapidly in blood. The $t_{1/2\alpha}$ values of 5-FU for the three dose groups were 0.59, 0.51 and 0.39 h, respectively, and those of TFU were 0.67, 0.33 and 0.47 h, respectively. The elimination of 5-FU in rabbits was relatively slow, with $t_{1/2}$ values of 7.90, 7.81 and 7.55 h for the 25, 50 and 10 mg/kg doses. In contrast, the elimination of TFU was fast, with $t_{1/2}$ values of 1.82, 1.49 and 1.78 h, respectively, for the three dose groups (15).

TFU was detected in all patients after single oral doses of 800, 1000 and 1200 mg atofluding. The drug concentration-time curve followed a one-compartment model. 5-FU was not detected in any of the patients after single-dose administration. After multiple dosing (800 mg every 6 h), steady-state concentrations of TFU in blood (9.97 ± 0.7 μg/ml) were reached at 30 h. 5-FU was detected in most of the patients. Steady-state concentrations of 5-FU were lower than those of TFU and were reached at 50-52 h (16). These results showed that orally administered atofluding was rapidly metabolized to TFU, which was then slowly metabolized to 5-FU. Concentrations of the drug, like sustained release agents, remained in the body up to 52 h.

Clinical Studies

A total of 420 patients from 10 Chinese hospitals were enrolled in a multicenter trial, of whom 100 were randomized to receive oral atofluding alone 400 mg t.i.d. (750 mg/m^2/day) for 6-8 weeks. Of the remaining patients, 213 received oral atofluding 200 mg q.i.d. (500 mg/m^2/day) in combination with other drugs and 107 patients served as controls and received FT-207 200 mg q.i.d. (500 mg/m^2/day) in combination with other drugs. The results showed that in patients with gastrointestinal tumors, the complete remission rate for atofluding alone was 25%. No immunosuppression was observed during treatment. The hematological toxicity of atofluding was minimal. No grade IV bone marrow depression was observed. Grade III reductions in WBC count were reported in 2% of the patients. The most frequent nonhematological toxicity was grade I-II but was tolerable. The efficacy of treatment with atofluding in combination with other drugs was not significantly better than treatment with atofluding alone. The results of phase II clinical studies have shown that atofluding is effective in several types of cancer, including gastric, intestinal and esophageal carcinomas, with only mild adverse events. Phase III clinical trials of oral atofluding are currently in progress in China (17).

Manufacturer

Xian Lijun Pharmaceutical Co. Ltd. (CN).

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