Diazoacetoacetyl Peptides for Neoplastic Agents

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(received November 26, 2002)

Azaserine was the inhibitor of enzymes with cystein moiety, and used for the chemotherapy of tumor disease. In this view point, we synthesized the short-chained peptides having diazo group. Namely, synthesis of diazoacetoacetyl amino acids from the reaction of acetoacetyl amino acids with tosyl azide in the presense of triethyl amine were successful. Similarly, 2-diazoacetoacetyl dipeptides, tripeptides, and tetrapeptides were prepared by the diazotation of acetoacetyl dipeptides, tripeptides, and tetrapeptides, respectively. Antineoplastic activity of synthesized all medicines was under investigation.

The chemotherapy of neoplastic disease has become increasingly important in recent year. An indication of this importance is the establishment of a medical specialty in oncology, wherein the physician practices various protocols of adjuvant therapy. Most cancer patients now receive some form of chemotherapy, even though it is merely palliative in many cases.

Cancer chemotherapy has recieved no spectacular breakthrough of the kind that the discovery of penicilline provided for antibacterial chemotherapy.

However, there has been substantial progress in many aspects of cancer research. In paticular, an increased understanding of tumor biology has led to elucidation of the mechanisms of action for antineoplastic agents. It also has provided a basis for the more rational design of new agents. Recent advances in clinical techniques, including large cooperative studies, are allowing more rapid and reliable evaluation of new drugs. The combination of these advantages with improved preliminary screening systems is enhancing the emergence of newer and more potent compounds.

There are congent reasons why cancer is more difficult to cure than bacterial infections. One is that there are qualitative differences between human and bacterial cells. Bacterial cells have distinctive cell walls and their ribosomes are different from those of human cells. In contrast, the differences between normal and neoplastic human cells are merely quntitative. Another differences is that immune mechanisms and other host defences are very important in killing bacteria and other foreign cells, whereas they play a negligible role in killing cancer cells. By there very nature, the cancer cells have eluded or overcome the immune surveillance system of body. Thus, it is necessary for chemotherapeutic agents to kill every single clonogenic malignant cell, because even one can reestablish the tumor. This kind of kill is extremely difficult to effect because antineoplastic agent kill cells by first-order kinetics. A potent anticancer drug might reduce this population 10,000-fold, in which case the symptoms would be alleviated and the patient would be in a state of remission. However, the remaining hundred million leukemia cells could readily increase to the original number after cessation of therapy. Furthermore, a higher proportion of resitsnt cells would be present, which would mean the treatment with the same agent would achieve a lessor response than before. For this reason, multiple drug regimens are used to reduce drastically the number of neoplastic cells. Typical protocols for leukemia contain four different anticancer drugs, usually with different modes of action. The addition of immunostimulants to the therapeutic regimen helps the body's natural defense mechanism to identify and eliminate the remaining few cancer cells.

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A further complication to chemotherapy is the relative un-responsiveness of slow-growing solid tumors. Current antineoplastic agents are most effective against cells with a high growth fraction. They act to block the biosynthesis or trans-cription of nucleic acids or to prevent cell division by interfering with mitotic spindles. Cells in the phase of synthesis or mitosis are highly susceptible to these agents. In contrast, cells in the resting state are resisitant to many agents. Slow-growing tumors characteristically have many cells in the resting state.[1]

Toxic effects of sulfur mustard and ethyleneimine on animals were described in the nineteeenth century.[2] Sulfur mustard was shown to be active against animal tumors, but it was too nonspecific for clinical use. A variety of nitrogen mustard, for example mechlorethamine, showed selective toxicity, especially to lymphoid tissue. Success in this area was followed by causious human trials that showed mechlorethamine to be useful against Hodgkin's disease and certain lymphomas.[3] Epoxides have strained ring systems as same as ethyleneimines. The use of epoxides as cross-linking agents in textile chemistry suggested that they be tried cancer chemotherapy. Simple diepoxides such as 1,2:3,4-diepoxybutane showed clinically activity against Hodgkin's disease.[4] Mytomycin C consisting with the benzo-quinone and ethyleneimine groups showed anticancer activity as alkylating agents[5] Some anthraquinone derivatives was reported as antibiotics having clinical anticancer agents.[6] In 1954, azaserine was isolated from a streptmyces species.[7] It was found to antagonaize many of the metabolitic processes involving glutamine, with the most important effect being the conversion of formylglycine ribonucleoside into formylglycinamidine ribonucleoside. Inhibition by azaserine of the enzyme that helps to produce formylglycinamidine ribonucleoside is reversible at first, but upon incubation in the absence of glutamine it becomes irreversible. A study involving incubation with azserinn-^{14}C followed by digestion with proteolytic enzymes and acid hydrolysis produced S-carboxymethyl cysteine-^{14}C, which showed that azaserine had related covalently with a sulfhydryl group of cysteine on the enzyme.

In this view points, author tried to synthesize the short-chained peptides having diazo group as a functional group with the intention of antitumor activity.

Materials and Methods

Acetoacetylamino acids, which were easily prepared from the reaction of sodium salts of amino acids and diketene followed by acidification, were bonded with other amino acids in usual manner for the synthesis of peptides via active ester method using N-hydroxysuccinimide and dicyclohexylcarbodiimide to give acetoacetyl di-, tri-, and terta-peptides.

Acetoacetyl amino acids were treated with the equimolar amount of p-toluensulfonyl azide in the presence of excess amount of triethylamine to afford the expected 2-diazoacetoacetyl amino acids. Yields of the diazo-amino acids were as follows: Gly 61%, Ala 44%, Val 85%, Leu 74%, Ile 68%, Pro 70%, L-Phe 70%, D-Phe 78%.

2-Diazocetoacetyl dipeptide were similarly synthesized by the same treatment descibed above. Yields of 2-diazo-dipeptides as follows: Ala-Val 75%, Gly-Phe 84%, Phe-Leu 75%.

2-Diazoacetoacetyl tripeptides were obtained by the same method described above. Yields of 2-diazo-tripeptieds were as follows: Ala-Val-Gly 44%, Gly-Phe-Phe 77%, Phe-Leu-Gly 70%.

2-Diazoacetoacetyl tetrapeptides were obtained by the same treatment described above. Yields of 2-diazo-tetrapeptides were as follows: Gly-Leu-Phe-Gly 70%, Gly-Phe-Phe-Gly 76%.

All compounds were identified by spectral measurements and elemental analyses

The P388 leukemia cells and colon 26 cells were incubated for 48 h at 37° in a medium containing n-butyric acid (4 mmol), 12-O-tetradecanoylphorbol-13-acetate (TPA) (32 mmol) and various amounts of test compounds.

Smears were made from the cell suspension, Epstein-Barr virus early antigen (EBV-EA) inducing cells were stained by means of an direct immunofluorescence technique.[8] The details of

the in vitro assay on EBV-EA activation have been reported.[9] In each assay, at least 500 cells were counted and the number of stained cells (positive cells) among them was recorded. Triplicate assays were preformed for each data point.

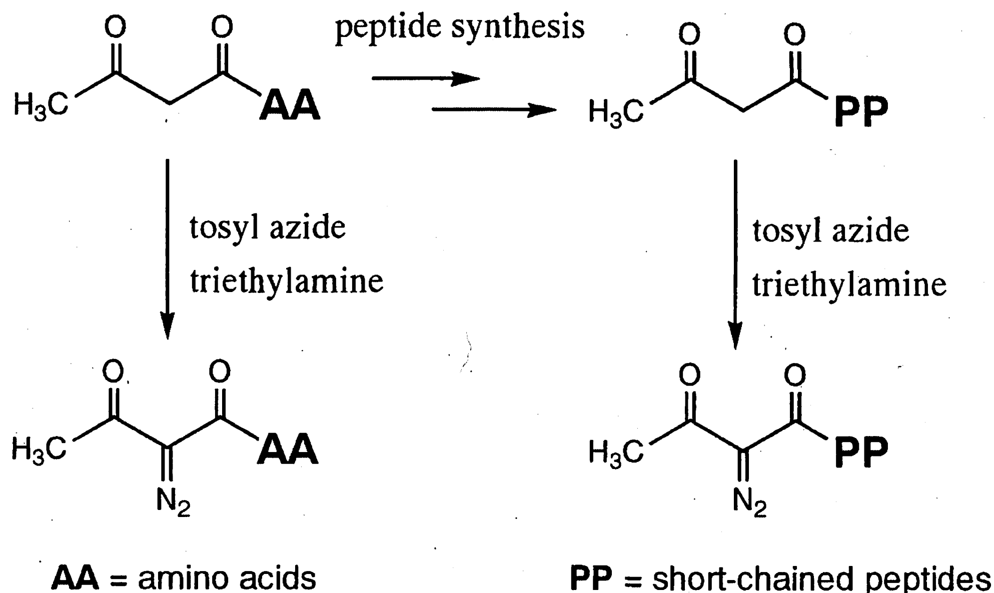
P388 leukemia cells and Colon 26 cells were used as tumor cells. At 72 h in contact with P388 leukemia cells and Colon 26 cells, the maintenance medium was withdrawn, the cells were washed twice with phosphate buffered saline (PBS, pH 7.2) and fixed with acetone for 10 min at room temperature. After staining, the cells were washed with PBS and mounted with 20% glycerol in PBS. Immunofluorescence was analysed under a fluorescent microscope (Nikon Optiphot + EFD2, Nikon Industrial Co., Tokyo, Japan).

Results and Discussion

In general, diazo compounds were active and unstable to decompose into the brown red residue, however, in the presence of the neighboring carbonyl group diazo compounds were stable. Scince the obtained diazo amino acids and diazo short-chained peptides were also having the neighboring carbonyl group, these diazo products were stable compounds with the significant diazo group absorption on 2133 cm^{-1} in the IR (infrared) spectra.

The inhibitory mechanism of these 2-diazoacetoacetyl amino acids and short-chained peptides on antitumor activity and their structure-activity relationships are now being studied.

Scheme. Synthetic Pathway of Diazo-Peptides



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