Antiviral Activity of Bezoxazinone Derivatives Having Amino Acid Moiety

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Condensation of anthranilic acid and N-protected amino acids by means of active ester method gave N-amino-acetylated anthranilic acids which were cyclized under acidic condition to afford the corresponding 2-substituted 3,1-benzoxazin-4-one derivatives. When N-protecting group of amino acids was acetoacetyl group, the target benzoxazinone was not obtained. In the case of N-benzyloxycarbonyl (Z) amino acids such as Z-Gly, Z-Ala, and Z-Phe, the benzoxazinone were obtained in 88%, 76%, and 76% yields, respectively. Each compounds were tested against RSV, HIV, FluV-A, and HSV, however, antiviral active compounds were not found out in this investigation.

The chemotherapy of viral disease is today at about the same stage of development as was the chemotherapy of bacterial infections prior to the discovery and development of the sulfonamides. Viral diseases such as smallpox and poliomyelitis are at present controlled by public health measures and immunization. With few exceptions, treatment of viral disease consists of making the condition tolerable for the patient and ensuring that a secondary bacterial infection does not develop.

The two major obstacles to effective antiviral chemotherapy are, first, the close relationship that exists between the multiplying virus and the host cell, and second, the fact that many viral-caused diseases can be diagnosed and recognized only after it is too late for effective treatment. In the first case, an effective antiviral agent must prevent completion of the viral growth cycle in the effected cells without being toxic to the surrounding normal cells. One encouraging development is the discovery that some virus-specific enzymes are elaborated during multiplication of the virus particles and this may be a point of attack by a specific enzyme inhibitor. However, recognition of the disease state too late for effective treatment would render antiviral drugs useless, even if they were available. Thus, until early recognition of the impending disease state is provided, most antiviral chemotherapeutic agents will have their greatest value as prophylactic agents.

Two approaches to the development of prophylactic antiviral agents that show promise involve the discovery of substances which either prevent penetration of virus particles into host cells or induce the in vivo synthesis of interferon, an antiviral protein produced by host cells in response to viral infection. The first approach has led to the introduction into therapy of amantadine for the prevention of certain forms of influenza.

Most attempts to inhibit virus multiplication without causing damage to the host have been unsuccessful, probably because virus multiplication is so intimately dependent on host cell metabolism. The first antiviral agent as antimetabolite is the pyrimidine nucleoside, iodoxuridine,[1] and a second antiviral agent is the purine nucleoside, vidarabine.[2] Ribavirin has broad spectrum antiviral activity in vitro against DNA and RNA viruses.[3] Its clinical efficiency against cutaneous herpes infections has been clearly demonstrated, but its activity against hepatitis and influenza infections is more doubt-

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ful. Acyclovir is more significantly more active than vidarabine against herpes virus and is marketed for the treatment of herpes infection.[4] Some interferon inducers with broad spectrum antiviral activity have been discovered,[5,6] but none of these substances has achieved clinical acceptance to date.

Quinazolinones, being similar to nuleoside base in the skeleton, are biologically active compounds having anticancer,[7] antiinflammatory,[8] antiallergic,[9] anticonvulsant,[10] sedative-hypnoic,[11] and antihypertensive activities.[12]

We focused the simple synthetic approach of quinazolinones via benzoxazinone having amino acid moiety for the invention of novel antiviral agents.

Materials and Methods

Chemicals

Acetoacetyl amino acids are easily prepared by the reaction of amino acids and diketene in a sodium hydrocarbonate aqueous solution in good yields following the ethyl acetate extraction of the acidified reaction mixture in usual manner.

Anthranilic acid and acetoacetyl amino acids were reacted by means of the active ester method. Namely, acetoacetyl amino acids were treated with N-hydoxysuccinimide (HO-SUI) in the presence of dicyclohexylcarbodiimide (DCC) in dioxan solution to give active ester intermediates which, in situ, were added to the alkaline solution of sodium salt of anthranilic acid. The reaction mixture was stirred at room temperature overnight, and then acidified with conc.HCl and extracted with ethyl acetate. The acylated anthranilic acids (I) were obtained (L-Phe, mp 169-171°C, Yield 21%, $[\alpha]_D+4.4°$; D-Phe, mp 165-170°C, Yield 24%, $[\alpha]_D+1.0°$; L-Val, mp 170-172°C, Yield 23%, $[\alpha]_D-3.6°$), however, in the case of Leu and Pro, the acylated products were not produced.

When benzyloxycarbonyl (Z) amino acids were used instead of acetoacetyl amino acids in the same reaction condition, the expected acylated anthranilic acids (II) were similarly obtained (Gly, mp 147-148°C, Yield 35%; L-Phe, mp 183-184°C, Yield 27%, $[\alpha]_D-59°$; L-Ala, mp 149-151°C, Yield 25%, $[\alpha]_D-41°$; L-Val, mp 194-196°C, $[\alpha]_D-12°$ Yield 8%; L-Leu, mp 188-190°C, Yield 6%, $[\alpha]_D-9°$).

In the cyclization reaction of acylated anthranilic acids by the treatment of excess acetic anhydride (Ac_2O) on heating, acetoacetyl aminoacylated anthranilic acids gave none of the sole product after the evaporation of excess Ac_2O. On the other hand, in the same reaction condition, Z-aminoacylated anthranilic acids afforded the corresponding 3,1-benzoxazin-4-one derivatives (III) substituting 2-position with amino acid moiety in good yield (Gly, mp 99-101°C, Yield 88%; L-Phe, mp 101-102°C, Yield 76%; L-Ala, mp 94-99°C, Yield 76%). All compounds were identified by spectral measurements and elemental analyses. The ring transformation of 3,1-benzoxazinones into quinazolones is under investigation.

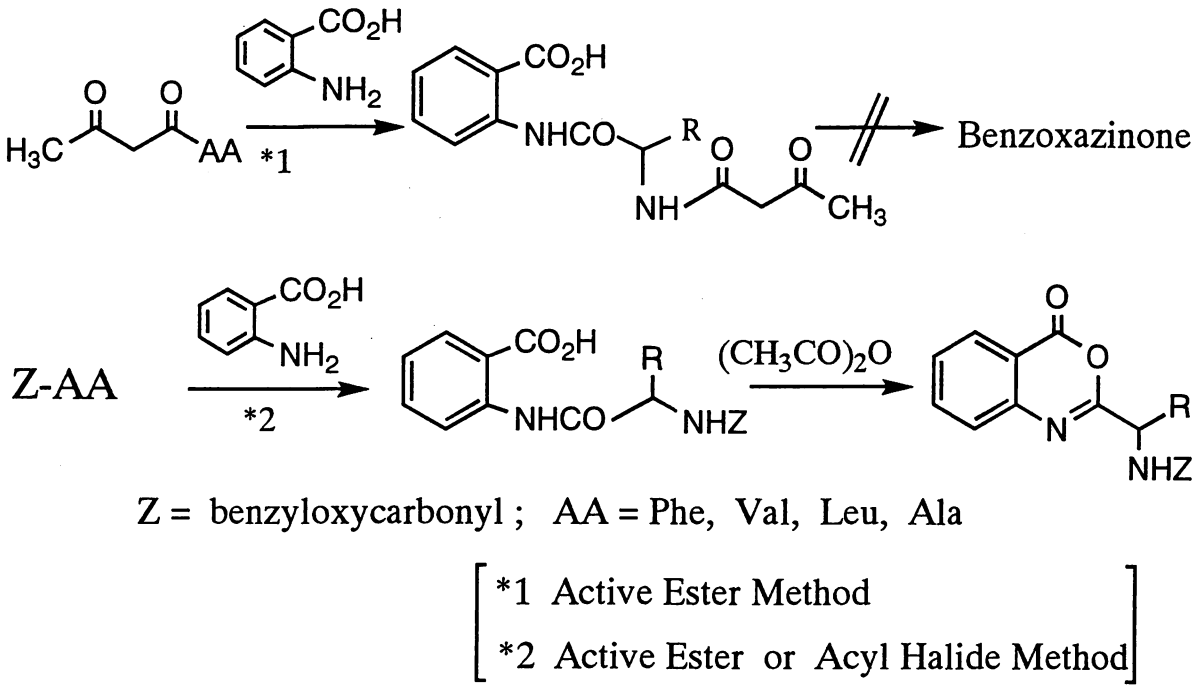
Cells and Viruses

RSV (Long strain) was provided from Sendai National Hospital. FLUV-A/Ishikawa/7/98 (H_3N_2) was laboratory strain which was passed more than 10 times in hen's embryonated eggs and 3 times in MDCK cells. HIV typel and HIV type 2 were obtained from the culture supernatant of HUT-78 cell lines peristently infected with HTLV-IIIB and CEM cells peristently infected with LAV-2, respectively. HSV (KOS strain) was propagated in human embryonic fibroblasts (HEF).

Cytotoxicity of the compounds was determined by measuring of viability of infected each host cells by the corresponding viruses.[13,14] The concentration of compounds that reduced the viability of infected host cells to 50% of the control was estimated as the 50% cytotoxic concentration (CC_{50}).

Results and Discussion

The 3,1-benzoxazin-4-one is the intra-type active ester compound. When nucleophiles such as amines present in the same circumstances, the



Z = benzyloxycarbonyl ; AA = Phe, Val, Leu, Ala

[*1 Active Ester Method
*2 Active Ester or Acyl Halide Method]

Scheme 1. Synthetic pathway of 3, 1-Benzoxazin-4-ones

benzoxazinone ring is easily opened to give the acylated anthranilic amide derivatives. We presumed that this chemical reactivity could be applied for the antiviral activity.

Unfortunately, acetoacetyl aminoacyl anthranilic acids did not afford the destinated benzoxazinones. On the other hand, Z-aminoacyl anthranilic acids gave the corresponding benzoxazinones in good (76-88%) yields.

These compounds (III) did not show the antiviral activity against RSV, FLUV-A, HIV, and HSV. The values of cytotoxicity of these benzoxazinones were over 200 μg/ml.

The continuous study, a ring transformation of benzoxazinone into quinazolinone having the similar skeleton to nucleic acid, is under investigation.

References

1. L.J. Ravin, J.J. Gulesich, J. Am. Pharm. A. NS4 : 122 (1964).
2. D. Pavan-Langston, Adenosine Arabinoside : An Antiviral Agent, Raven Press, N.Y., 1975.
3. R.W. Sidwell, et al., Science, **177**, 705 (1972).
4. H.J. Schaeffer, et al., Nature, **272**, 583 (1978).
5. A.K. Field, Proc. Nat. Acad. Sci. U.S. **58**, 1004 (1967).
6. G.D. Mayer, R.F. Krueger, Science, **169**, 1213 (1970).
7. I. Hermecz, et al., Advances in Heterocyclic Chemistry, Vol.39 281 (1986).
8. J.B. Jiang, et al., J. Med. Chem., **33**, 1721 (1990).
9. C.F. Schwender, et al., J. Med. Chem., **22**, 114 (1979).
10. J.F. Wolfe, et al., J. Med. Chem., **33**, 161 (1990).
11. S. Buyuktimkin, Pharmazie, **40**, 393 (1986).
12. M.-H. Yen, et al., J. Pharm. Pharmacol., **48**, 90 (1996).
13. J. Balzarini, et al., Biochem. Biophys. Res. Commun., **136**, 64 (1986)..
14. C.B. Hall, et al., J. Am. Med. Assoc., **95**, 183 (1983).