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POTENTIAL RADIOSENSITIZING ANTIVIRAL AND ANTICANCER PYRIMIDINE NUCLEOSIDES

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DISCLAIMER

A. Abstract

This program, supported by ERDA from 1974 to May 31, 1978, was concerned with the synthesis of a variety of novel nucleosides as potential antiviral, antineoplastic and radiation sensitizing agents. Perhaps the most significant accomplishment of this support was the development of 5-Iodo-5'-amino-2',5'-dideoxyuridine (AIU, AIdUrd) which is the first compound to require unequivocally, for antiviral activity, a unique activation by an enzyme (thymidine kinase) encoded by the Herpes virus genome. This unusual requirement for activation prevents toxicity to uninfected cells or tissues. The major conceptual contribution is that a non-toxic drug for established viral infections can indeed be developed.

AIU, although not a substrate for E. coli thymidine- or thymidylate kinase, does bind to these enzymes and has a K_i of 240 μM and 59 μM respectively. Both enzymes are sensitized to UV radiations, and the concentrations required for 50% enhancement is roughly equivalent to their respective K_i.

Various other halogenated nucleoside analogs have been prepared and evaluated for radiation sensitization potential. Mammalian enzymes appear to be less sensitive to UV-radiations than bacterial enzymes. In an attempt to overcome this problem 5'-azido nucleoside analogs were prepared, but these were not effective.

B. Summary of Research Activities

1: Synthesis

Analogs of pyrimidine nucleosides have been synthesized as potential sensitizors of neoplastic tissues or topical viral infections to the lethal effects of radiations. The design was based on our working hypothesis that key enzymes concerned with the biosynthesis or possible repair of DNA would be specifically sensitized. The compounds prepared include two general categories: 1. Those that have not been synthesized previously and have the theoretical attributes desired for enzyme binding and radiation sensitization, and 2. Those compound that have been first synthesized by us, have good radiation sensitizing, antiviral or anticancer activity, and merit continued study. All the compounds proposed for synthesis in category 1 have not been prepared, however we have not terminated such studies even though ERDA has discontinued support. This is appropriate because many of the compounds proposed for synthesis may have good antiviral or anticancer activity as well as radiation sensitizing potential. Thus we have recently synthesized 5-azido-methyl-2'-deoxyuridine which we hope will produce good radiation sensitization.

The following published papers described our synthetic accomplishments:

1. J. Carbohydrates.Nucleosides.Nucleotides 2 185-190 (1975) Describes the synthesis of 1-(5-azido-5-deoxy-β-D-arabinofuranosyl)cytosine and 1-(5-amino-5-deoxy-β-D-arabinofuranosyl)cytosine.

2. J. Carbohydrates.Nucleosides.Nucleotides 2 309-313 (1975) Describes the synthesis of 5-iodo-2',5'-dideoxyuridine.

3. J. Carbohydrates, Nucleosides, Nucleotides 5 15-22 (1978) Describes the synthesis and antiviral activity of 5-methylamine-2'-deoxycytidine. The corresponding deoxyuridine analog had been prepared earlier by Dr. Visser. Our objective was to use this compound as a precursor for the synthesis of these two analogs with either a halogen or an azido substituent in the 5'-position in

place of the 5'-hydroxyl moiety.

4. Carbohydrate Res., 62 175-177 (1978) We had shown many years ago that azathymidine decreases the sensitivity of Strepococcus faecalis to UV radiations. This paper describes an improved synthesis of 6-azathymidine. Not only has the yield been increased, but it was obtained in crystalline form for the first time. The availability of crystalline compound afforded the preliminary X-ray diffraction studies by Dr. A Banerjep of the Max Planck Institute for Experimental Medicine in Göttigen, West Germany. Such data may be of value in explaining the modifying influence of this compound when incorporated into DNA on the effects of radiation.

5. J. Medicinal Chemistry, 19, 495-498 (1976) This paper describes the synthesis of various 5- and 5'-substituted thymidine analogs which have radiation sensitization properties to be described in another publication as well as their antiviral activity.

The 5'-O-p-tolylsulfonyl derivatives of 5-chloro, 5-bromo, and 5-iodo-2'-deoxyuridine were synthesized and converted into the corresponding 5-halo-5'-azido-2',5'-dideoxyuridines. Reduction of 5-chloro-5'-azido-2',5'-dideoxyuridine afforded 5-chloro-5'-amino-2',5'-dideoxyuridine (ACU); however, similar efforts to prepare 5-bromo-5'-amino-2'5'-dideoxyuridine and 5-iodo-5'-amino-2',5'-dideoxyuridine by reduction of the corresponding 5'-azido precursor resulted in the formation of 5'-amino-2',5'-dideoxyuridine. 5-Bromo-5'-amino-2',5'-dideoxyuridine (ABrU) and 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU) were prepared by halogenation of the 5-mercuriacetate of 5'-amino-2',5'-dideoxyuridine. The 5'-amino-5'-deoxy analogs of 5-methyl-, 5-chloro-, 5-bromo-, and 5-iodo-2'-deoxyuridine possess antiviral activity against herpes simplex virus but exhibit no inhibitory activity against sarcoma 180 (murine) or Vero (monkey) cells in culture.

6. J. Medicinal Chemistry, 19, 915-918 (1976) This paper describes the synthesis and biological activities of 5-trifluoromethyl-5'-azido-2',5'-dideoxyuridine and 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine. Their radiations sensitization properties are discussed in another publication.

5-Trifluoromethyl-2'-deoxyuridine (1) was tosylated with p-toluenesulfonyl chloride in dry pyridine at 3° to give 5-trifluoromethyl-5'-0-(p-tolylsulfonyl)-2'-deoxyuridine (2), which was converted to 5-trifluoromethyl-5'-azido-2',5'-dideoxyuridine (3) by reacting with lithium azide in N,N-dimethylformamide at 85-90° for 2 h. Compound 3 was then hydrogenated in ethanol-water (1:1 v/v) at room temperature and 35 psi of hydrogen pressure, using 10% palladium on charcoal as catalyst, to yield 5-trifluoromethyl-5'-amino-2',5'-dideoxyuridine (4). Compound 4 is about fourfold less potent than compound 1 as an antiviral agent but is about 40-fold less toxic to the host Vero cells. Thus the therapeutic index of compound 1 has been improved by a factor of 10 by replacement of the 5'-hydroxyl with an amino group. Compound 1, however, is more than 100-fold more inhibitory to Sarcoma 180 cells in culture relative to compound 4. Compound 3 is markedly less potent than compound 1 or 4 as either antiviral or an antineoplastic compound.

7. J. Medicinal Chemistry, 21, 109-112 (1978) This paper describes the synthesis and biological activity of several azido and amino analogs of thymidine. Their radiation sensitization properties are described in another publication.

3',5'-Diamino-3',5'-dideoxythymidine was synthesized via a nine-step synthesis from thymidine in good overall yield. 3'-Amino-3'-deoxythymidine and 5'-amino-5'-deoxythymidine were prepared with a minor modification of the procedure reported by Horowitz and co-workers. Although the 5'-amino analogue had potent antiviral activity relative to the 3'-amino analogue,

the latter is a potent inhibitor of the replication of both murine sarcoma 180 cells (ED_{50}=5 μM) and of murine L1210 cells (ED_{50}=1 μM) in vitro. Most unexpectedly, however, was the finding of complete lack of either antiviral of antineoplastic activity by the 3',5'-diamino analogue which appears to have acquired the undesirable qualities of both the 3'-amino and 5'-amino analogues of thymidine.

II Biochemistry

The synthesis of 5-iodo-5'-amino-2',5'-dideoxyuridine has produced a compound with rather unique properties of having antiviral activity in the absence of cellular toxicity. The molecular basis for this was investigated in paper 8 and 9.

8. J. Biol. Chem., 251 4833-4838 (1976) 5-Iodo-5'-amino-2',5'-dideoxyuridine (AIdUrd) is a novel thymidine analog which inhibits herpes simplex virus type 1 (HS-1 virus) replication in the absence of detectable host toxicity. When murine, simian or human cells in culture are treated with [^{125}I] AIdUrd for up to 24 hours essentially none of the nucleoside becomes cell-associated. In contrast, upon HS-1 virus infection, significant radiolabel is detected in both nucleotide pools and in DNA. The major acid-soluble metabolite has been shown by enzymic and chromatographic analysis to be the 5'-triphosphate of AIdUrd. DNA from HS-1 virus-infected Vero cells labeled with [^{14}C]thymidine, 5-[^{125}I]iodo-2'-deoxyuridine (IdUrd), or [^{125}I]AIdUrd was isolated by buoyant density centrifugation and subjected to digestion by pancreatic DNase I, spleen DNase II, micrococcal nuclease, spleen and venom phosphodiesterases. Analysis of the digestion products clearly indicate that AIdUrd is incorporated internally into the DNA structure. DNA containing AIdUrd therefore contains phosphoramidate (P-N) bonds, known to be extremely acid-labile. The selective HS-1 virus-induced phosphorylation of AIdUrd and its subsequent incorporation into DNA may account for the unique biological activity of the AIdUrd nucleoside.

9. J. Biol. Chem. 251 4839-4842 (1976). 5-Iodo-5'-amino-2',5'-dideoxyuridine-5'-N'-triphosphate (AIdUTP), a phosphoramidate analog of 5-iodo-2',5'-dideoxyuridine 5'-triphosphate (IdTUP), was synthesized and some of its chemical and biological properties were investigated. Although AIdUTP is stable in alkaline solutions, below pH 8 it undergoes degradation by a novel phosphorylysis

reaction which exhibits first order kinetics. Inclusion of magnesium ion in the reaction mixture decreased the rate of degradation. Protonation of a group on AIdUTP which has a pK_a of 6.10, presumably the secondary ionized oxygen on the γ-phosphate, precedes phosphorylysis. The only detectable reaction products are the nucleoside, 5-iodo-5'-amino-2',5'-dideoxyuridine (AIdUrd), and trimetaphosphate. A mechanism for the acid catalyzed phosphorylysis of AIdUTP is proposed.

AIdUTP, like dTTP, converts *Escherichia coli* thymidine kinase into an inactive dimer with a sedimentation coefficient of 5.78 S. AIdUTP is, however, 60-fold more potent as an allosteric inhibitor than is dTTP at pH 7.8. Although the inhibitory effect of dTTP is markedly reduced at high pH, the activity of AIdUTP is lowered only slightly. The allosteric effects of AIdUTP also differ from those of IdUTP, which is an inhibitor at low pH but a strong activator above pH 7.4. 5-Iodo-2'-deoxycytidine 5'-triphosphate, a potent enzyme activator, cannot completely reverse the AIdUTP inhibition, even when present at a 150-fold molar excess.

The effect of the halogens in the 5-positions and the substitution of a nitrogen for carbon-6 of the pyrimidine moiety of nucleosides as well as the replacement of the 5'-hydroxyl group by an azido moiety on radiation sensitization of enzymes is described in the next two papers:

10. J. Biol. Chem., 251, 6555-6561 (1976) The effect of 5-iodo-2'-deoxyuridine monophosphate (IdUMP), various 5-halogenated-5'-azido-2',5'-dideoxyuridine derivatives, 2'-deoxy-6-azauridine (AzdUrd) and its halogenated analogs on the ultraviolet sensitization of *Escherichia coli* thymidylate kinase has been investigated. Only those compounds iodinated in position 5 enhance the rate of ultraviolet inactivation of this enzyme. However, 5'-azido nucleosides with

iodo, bromo, chloro, or fluoro substituents in position 5 neither protect nor sensitize thymidylate kinase to ultraviolet inactivation. Thymidine 5'-monophosphate partially protects the enzyme against ultraviolet inactivation either in the presence of absence of ultraviolet-sensitizing iodinated analogs. Magnesium ion does not enhance the ultraviolet inactivation of thymidylate kinase by 5-iodinated nucleoside analogs. The kinetic data support an active site-directed enhancement of the enzyme to ultraviolet inactivation by 5-iodo-2'-deoxyuridine monophosphate, since the concentration of IdUMP required to attain 50% maximal enhancement is 0.24 mM which is in good agreement with its K_i of 0.18 mM. When either [^{125}I]IdUMP or [2-^{14}C]IdUMP was irradiated with the enzyme, both radioactivities were associated with the enzyme, however only with the ^{14}C analog was the amount bound at half-saturation essentially equal to the amount required to inactivate the enzyme by 50%. These data support the hypothesis that the active entity in the enhancement by IdUMP of thymidylate kinase inactivation during ultraviolet irradiation is the uridylate free radical which is formed photochemically from IdUMP.

Photochemical studies of 6-azauracil (AzUra), 2'-deoxy-6-azauridine and 5-iodo-2'-deoxy-6-azauridine (IAzdUrd) were performed. Photolysis of IAzdUrd in the presence of a hydrogen donor yields AzdUrd which upon further photolysis yields the photohydrate. The photohydrate of AzdUrd when incubated in the dark at pH 5.2 is 90% converted back to AzdUrd, whereas the photohydrate of AzUra is only partially (20%) converted to AzUra. The rate of deiodination of IAzdUrd is 2.1-fold greater than that of IdUMP. Although the K_i of IdUMP and IAzdUrd is similar, the increased photosensitivity of the aza analog accounts for the much greater enhancement of ultraviolet inactivation of thymidylate kinase. The ability of a compound to enhance the ultraviolet inactivation of deoxythymidylate kinase is correlated with the potential of the compound to produce a free radical rather than a photohydrate when the enzyme substrate analog complex is irradiated.

The rationale for incorporation of an azido moiety in place of the 5'-phosphate of IdUMP was the hope that by this modification the ultraviolet sensitization phenomenon would be markedly augmented by virtue of formation, during irradiation, of a nitrene radical in the 5' position in addition to the free radical in position 5 of the pyrimidine moiety. Since the K_i of IdUMP and its 5'-azido analog are 180 and 120 μM respectively, no hindrance to the formation of an enzyme analog complex is exerted by the azido moiety, nevertheless the sensitization of dTMP kinase to ultraviolet radiation is not only not augmented by virtue of the azido moiety, but rather is eliminated. The K_i for 5-bromo-5'-azido-2',5'-dideoxyuridine is similar to that of the corresponding iodo analog, and as expected the bromo derivative also showed neither protection nor enhancement of the ultraviolet inactivation of the enzyme. Thus the presence of an azido group in the 5' position of nucleosides does not enhance radiation sensitization of *E. coli* dTMP kinase. Azido derivatives of metabolic substrates have been reported to indeed covalently interact with the appropriate biopolymer. However, most nitrenes undergo intramolecular rearrangement rather than insertion into a nearby C-H bond. Another possibility that may explain the failure of the azido analog to enhance the hoped for radiation sensitization is that the moiety in the 5' position whether a phosphate or an azido group is not directly involved in the binding of the compound to the enzyme. Thus even if the nitrene were produced by ultraviolet irradiation it may not be in sufficient proximity to a susceptible molecular linkage at the active site of the enzyme to afford the interaction required to effect enzyme inactivation.

A major concern is the absence of a relationship between the K_i of the various nucleoside analogs iodinated in position 5 of the pyrimidine moiety and their ability to sensitize thymidylate kinase to ultraviolet radiations. Thus whereas AIdUrd and IdUMP exert a positive sensitization, IdUrd and the 5'-azido analog of IdUrd are essentially inert even though the K_i values of the latter two compounds have a value intermediate between AIdUrd and IdUMP.

11. Biochemistry 16 3310-3315 (1977). The effect of various halogenated and nonhalogenated allosteric effectors on the sensitivity of *Escherichia coli* thymidine kinase to ultraviolet radiations (UV, 253.7 nm) was investigated. All naturally occurring dNTPs convert the monomeric form of the enzyme into the dimeric form which is less sensitive to UV inactivation. Whereas 5-iodo-2'-deoxycytidine triphosphate (IdCTP) and 5-iodo-2'-deoxyuridine triphosphate (IdUTP) enhance the UV inactivation of the enzyme, 5-bromo-2'-deoxyuridine triphosphate and 5-bromo-2'-deoxycytidine triphosphate exert a protective effect similar to that produced by the corresponding naturally occurring effectors dTTP and dCTP. The enhanced UV inactivation by IdUTP is prevented totally by dTTP, but only partially by dCTP or dThd, whereas the enhanced sensitization by IdCTP is prevented almost totally by dCTP, partially by dTTP, and not at all by dThd. The UV sensitization of thymidine kinase by IdCTP appears to be at the regulatory site since a maximum saturation effect is observed, and the concentration required to exert a 50% maximal UV sensitization is similar to K_m for enhancement of catalytic activity. When the enzyme was irradiated in the presence of either [2-^{14}C]IdUTP or [2-^{14}C]IdUrd, zone sedimentation analysis in sucrose density gradients showed the sedimentation coefficient of the radioactive labeled proteins to be the same, 3.8 S. Hence, UV irradiation of the effector-induced dimer resulted in not only dissociation to the monomer, but also complete loss of catalytic activity. The substitution of an azido group for the 5'-OH group of 5-iodo-, 5-bromo-, 5-chloro-, or 5-fluorodeoxyuridine greatly decreased their affinity for thymidine kinase, and in addition the kinetics of inhibition changed from a competitive to a noncompetitive pattern. The presence of the azido moiety in the 5' position of the halogenated nucleosides did not enhance the rate of UV inactivation of the enzyme.

III Animal Experiments

12. Invest. Opthamol. 15 470-478 (1976). This paper is our initial study in animals and concerns the antiviral activity of AIU in herpes keratitis. Studies

are in progress on the effect of AIU in cutaneous herpes and herpes genitalis in experimental animals in which the effect of radiations will be more appropriate.

The efficiency of 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU) in the therapy of experimental herpes keratitis in rabbits has been examined. Virus infections were established bilaterally in 40 animals using herpes simplex, type 1 (NIH strain 11124). Twenty-four hours after infection the rabbits were divided into five matched groups of eight and each group was treated, double-blind with topical drugs at four-hour intervals for a total of 72 hours. The solutions instilled were: (1) saline; (2) IdUrd, 1 mg. per milliliter; (3) AIU, 1 mg. per milliliter; (4) AIU, 4 mg. per milliliter; and (5) AIU, 8 mg. per milliliter. Each eye was examined daily for 12 days and graded independently by two opthamologists. Although IdUrd and AIU (8 mg. per milliliter) were effective therapeutically, IdUrd had a greater effect. The AIU at 1 and 4 mg. per milliliter were less active, but showed more rapid healing than the saline control. Viral recovery studies are consistent with the clinical observations. A second independent experiment, similar to that described above, gave essentially identical results. Although less potent than IdUrd, AIU does provide effective therapy for herpes keratitis.

IV Review and General Manuscripts

A number of review papers concerning compounds prepared in the past by support from ERDA have been presented.

13. Ann. N.Y. Acad. Sci. 255 332-341 (1975) Synthesis and Biological Activity of Some Novel Analogs of Thymidine, Yung-Chi Cheng, John P. Neenan, Barry Goz, David C. Ward, and William H. Prusoff.

14. Biochem. Pharmacol. 25 1233-1239 (1976) Nucleoside Analogs with Antiviral Activity. William H. Prusoff and David C. Ward.

15. Ann. N.Y. Acad. Sci. 284 335-341 (1977) Recent Studies on the Antiviral and Biochemical Properties of 5-Halo-5'-Amino-Deoxyribonucleosides. William H. Prusoff, et al.

16. Pharmacol. and Therapeutics, In press. Antiviral Iodinated Pyrimidine Deoxyribunucleosides: 5-Iodo-2'-deoxyuridine; 5-Iodo-2'-deoxycytidine; 5-Iodo-5'-amino-2',5'-dideoxyuridine. William H. Prusoff, Ming S. Chen, Paul Fischer, Tai-Shun Lin, George T. Shiau, Raymond F. Schinazi and Jamieson Walker.

17. In "Antimetabolites in Biochemistry, Biology and Medicine", Ed. J. Skoda and P. Langen; Pergamon Press. Molecular Basis for Serendipitous Development of Antiviral and Anticancer Aminonucleosides. William H. Prusoff, Ming S. Chen, Paul Fischer, Tai-Shun Lin, George T. Shiau, Raymond F. Schinazi and Jamieson Walker. In press.

18. In "Chemistry and Biology of Nucleosides and Nucleotides", Ed. Harmon, Academic Press. Synthesis, Antiviral Activity and Mechanism of Action of A Novel Series of Pyrimidine Nucleoside Analogs. William H. Prusoff, Tai-Shun Lin, Ming-Shen Chen, George T. Shiau and David C. Ward.

C. Progress in Field

Earlier studies in our laboratory have been concerned with the mechanism of UV photosensitization of 5-iodouracil (Rupp and Prusoff, Nature 202, 1288, 1964; Biochem. Biophys. Res. Commun. 18, 145, 158 (1965) and it was established that free radical formation in the 5-position of the pyrimidine occurred which underwent a variety of subsequent reactions dependent on the composition of the reaction mixture: hydrogen abstraction to form uracil, rearrangement to a 5-membered ring, or cleavage of a disulfide to form a 5-thioether of uracil.

A dissapointment in our research has been the failure of the azido derivatives of thymidine or of the 5-halo-analogs of thymidine to sensitize thymidine kinase or thymidylate kinase to UV radiation.

The rationale for incorporation of an azido moiety in place of the 5'-phosphate of IdUMP was the hope that by this modification the ultraviolet sensitization

phenomenon would be markedly augmented by virtue of formation, during irradiation, of a nitrene radical in the 5'-position, in addition to the free radical in position 5 of the pyrimidine moiety. Since the K_i of IdUMP and its 5'-azido analog are 180 and 120 μM respectively, no hindrance to the formation of an enzyme analog complex is exerted by the azido moiety. Nevertheless, the sensitization of dTMP kinase to ultraviolet radiation is not only not augmented by virtue of the azido moiety, but rather is eliminated. The K_i for 5-bromo-5'-azido-2',5'-dideoxyuridine is similar to that of the corresponding iodo analog, and as expected the bromo derivative also showed neither protection nor enhancement of the ultraviolet inactivation of the enzyme. Thus the presence of an azido group in the 5'-position of nucleosides did not enhance radiation sensitization of dTMP kinase.

We have recently found that the irradiation of 5-N_3CH_2-2'-deoxyuridine with UV light, caused the compound to undergo a very rapid photochemical reaction to yield a spectrum with three isobestic points (227 nm, 250 nm, and 277 nm) and two maximum (208 nm and 302 nm). The original spectrum has two maximum (214 nm and 266 nm) and one minimum (236 nm). The rate of photochemical reaction of 5-N_3CH_2-2'-deoxyuridine was found to be at least 2 orders of magnitude faster than 5-iodo-2'-deoxyuridine. 5-N_3CH_2-2'-deoxyuridine was found to be a competitive inhibitor against thymidine (HSV 1 encoded deoxypyrimidine kinase) with a K_i of 0.8 μM. It has the same affinity as the substrate (thymidine) itself. It was also found to be a potent antiviral agent. Experiments with this compound are in progress because it is a very good photochemical reactive agent, has excellent affinity to the HSV-1 deoxypyrimidine kinase, and is a potent antiviral agent.

Perhaps the most important direction being pursued in several laboratories is the development of compounds that will sensitize hypoxic cells to the lethal effects of radiations. A serious limitation in radiotherapy of neoplasms in man, is the relative resistance of hypoxic neoplastic cells in a tumor population. Some degree of success has been achieved with nitroimidazole analogs which sen-

sitize hypoxic cells to the lethal effects of X-ray radiations. Unfortunately, some of these radiosensitizers are really more toxic to hypoxic cells relative to well oxygenated cells. Hence one sees an additive rather than a synergistic effect.

Nevertheless, preferential toxicity to a hypoxic cell is a desirable objective and a continued search for agents that will sensitize hypoxic cells to radiations should be supported. No doubt with appropriate modification of the structure, compounds will evolve that do indeed sensitize hypoxic cells to radiation.

D. List of Publications

1. Lin, T.S. and Prusoff, W.H., Synthesis of 1-(5-azido-5-deoxy-β-D-arabinofuranosyl)cytosine and 1-(5-amino-5-deoxy-β-D-arabinofuranosyl)cytosine. J. Carbohydrates, Nucleosides, Nucleotides, 2, 185-190, 1975.
2. Lin, T.S. and Prusoff, W.H., Synthesis of 5-Iodo-2',5'-dideoxyuridine, J. Carbohydrates, Nucleosides, Nucleotides, 2, 309-313, 1975.
3. Cheng, Y.C., Neenan, J.P., Goz, B., Ward, D.C., and Prusoff, W.H., Synthesis and Biological Activity of Some Novel Analogs of Thymidine, Ann. N.Y. Acad. Sci. 255, 332-341, 1975.
4. Lin, T.-S., Neenan, J.P., Cheng, Y.-C., Prusoff, W.H. and Ward, D.C., Synthesis and Antiviral activity of 5- and 5'-Substituted Thymidine Analogs. J. Med. Chem., 19, 495-498, 1976.
5. Prusoff, W.H. and Ward, D.C. Nucleoside Analogs with Antiviral Activity. Biochem. Pharmacol., 25, 1233-1239, 1976.
6. Lin, T.S., Chai, C. and Prusoff, W.H. Synthesis and Biological Activities of 5-Trifluoromethyl-5'-azido-2',5'-dideoxyuridine and 5'-trifluoromethyl-5'-amino-2',5'-Dideoxyuridine. J. Med. Chem., 19, 915-918, 1976.
7. Albert, D.M., Lahav, M., Bhatt, P.M., Reid, T.W., Ward, R.E., Cykiert, R.C., Lin, T.S., Ward, D.C. and Prusoff, W.H. Successful Therapy of Herpes

Hominis Keratitis in Rabbits by 5-Iodo-5'-amino-2',5'-Dideoxyuridine (AIU). Invest. Opthamol., 15, 470-478, 1976.

8. Chen, M.S., Ward, D.C. and Prusoff, W.H. Specific Herpes Simplex Virus Induced Incorporation of 5-Iodo-5'-amino-2',5'-dideoxyuridine into Deoxyribonucleic Acid. J. Biol. Chem., 251, 4833-4838, 1976.

9. Chen, M.S., Ward, D.C. and Prusoff, W.H. 5-Iodo-5'-amino-2',5'-dideoxyuridine-5'-N'-triphosphate: Synthesis, Chemical Properties and Effect on E. coli Thymidine Kinase Activity. J. Biol. Chem., 251, 4839-4842, 1976.

10. Chen, M.S., Chang, P.K. and Prusoff, W.H. Photochemical Studies and Ultraviolet Sensitization of E. coli Thymidylate Kinase by Various Halogenated Substrate Analogs. J. Biol. Chem., 251, 6555-6561, 1976.

11. Prusoff, W.H. and Ward, D.C. Nucleoside Analogs with Antiviral Activity. Biochem. Pharmacol., 25, 1233-1239, 1976.

12. Prusoff, W.H., Ward, D.C., Lin, T.S., Chen, M.S., Shiau, G.T., Chai, C., Lentz, E., Capizzi, E., Idriss, J., Ruddle, N.H., Black, F.L., Kumari, H.L., Albert, D., Bhatt, P.N., Hsiung, G.D., Strickland, S., and Cheng, Y.C. Recent Studies on the Antiviral and Biochemical Properties of 5-Halo-5'-Amino-Deoxyribonucleosides. Ann. N.Y. Acad. Sci., 284, 335-341, 1977.

13. Chen, M.S. and Prusoff, W.H. Kinetic and Photochemical Studies and Alteration of Ultraviolet Sensitivity of *Escherichia coli* Thymidine Kinase by Halogenated Allosteric Regulators and Substrate Analogues. Biochemistry, 16, 3310-3315, 1977.

14. Lin, T.S. and Prusoff, W.H. Synthesis and Biological Activity of Several Amino Analogues of Thymidine. J. Med. Chem., 21, 109-112, 1978.

15. Lin. T.S. and Prusoff, W.H. Synthesis and Antiviral Activity of 5-Methylamino-2'-deoxycytidine. J. Carbohydrates, Nucleosides, Nucleotides, 5, 15-22, 1978.

16. Shiau, G.T. and Prusoff, W.H. An Improved Synthesis of 6-Azathymidine. Carbohydrate Res., 175-177, 1978.

17. Prusoff, W.H., Chen, M.S., Fischer, P., Lin, T.S., Shiau, G.T., Schinazi, R.F., and Walker, J. Antiviral Iodinated Pyrimidine Deoxyribonucleosides: 5-Iodo-2'-deoxyuridine; 5-Iodo-2'-deoxycytidine; 5-Iodo-5'-amino-2',5'-dideoxyuridine. Pharmacol. and Therapeut., In press.

18. Prusoff, W.H., Chen, M.S., Fischer, P., Lin, T.S. Shiau, G.T., Schinazi, R.F., and Walker, J. In "Antimetabolites In Biochemistry, Biology and Medicine". Ed. J. Skoda and P. Langen, Pergamon Press. In press.