The Influence of Alkoxymethyl Purine and Pyrimidine Acyclonucleosides on Growth Inhibition of Kirkman-Robbins Hepatoma and Possible Mechanism of Their Cytostatic Activity

Hanna Modrzejewska[a], Janusz Greger[a], Marcin Dramiński[b] and Maciej Rutkowski[b]

[a] Department of Biochemistry, Institute of Physiology and Biochemistry, Medical University of Łódź, Lindleya 6, 90–131 Łódź, Poland
[b] Department of General Chemistry, Institute of Basic Sciences, Military School of Medicine, pl.J.Hallera 1, 90–647 Łódź, Poland

Z. Naturforsch. **51c,** 75–80 (1996), received October 10/November 11, 1996

Hepatoma, Allyloxymethyl Purine and Pyrimidine, Acyclonucleosides, Deoxynucleoside Kinases

Newly synthesized allyloxymethyl purine and pyrimidine acyclonucleosides [Fig. 1, comp. **1–6**] were tested in Syrian hamster, six days after heterotransplantations of Kirkman-Robbins hepatoma and compared with Th5, Th5P and PMT [Fig. 1, comp. **7–9**]. 48 hours after intraperitoneal (i.p.) administration of AMT and Th5 in a dose of 80 mg per kg body weight, these compounds reduced tumor weight by 42%, while AMU (in the same dose) by 30%. The inhibition of tumor weight is accompanied by a decrease in dThd and dGuo kinase activities in tumor cytosol by AMU (36% and 33%, respectively) by AMT (59% and 53%, respectively) and by Th5 (58% and 55%, respectively). AMU, AMT and Th5 are phosphorylated *in vivo* by kinases present in cytosol of growing hepatoma to mono, di and triphosphates, but allyloxymethyl residue of AMU and AMT is first hydrated to hydroxypropoxymethyl residue, having CH_2OH group. The lack of phosphorylation of PMT *in vivo* (having saturated propoxymethyl residue) and phosphorylation of Th5P (when used as a substrate for dNMP kinase) only to Th5 diphosphate suggested that AMU, AMT and Th5 triphosphates are responsible for the inhibition of dTMP and dGMP synthesis.

Introduction

High activity of dThd and dGuo kinases in mammalian tumors (Sakamoto *et al.,* 1993), cytostatic properties of pyrimidine acyclonucleosides inhibiting the growth of experimental tumors by reducing the synthesis of dTMP and dGMP (Greger and Dramiński, 1989), and inhibition of virus replication by ACN's containing unsaturated residue (Hayashi *et al.,* 1988) were the reasons for undertaking a study on newely syntesized purine and pyrimidine ACN's, containing unsaturated residue attached to 1N of the pyrimidine or to 9N of the purine. The study comprised six allyloxymethyl purine and pyrimidine ACN's, obtained by Ozierow *et al.,* (1991) and three newly synthesized thymine ACN's obtained by Dramiński (Rutkowski and Dramiński, 1991). In this work we undertook a research on dNMP synthesis in growing hepatoma subjected to the action of newly synthesized ACN's both *in vitro* and *in vivo*, on their metabolism and supposed mechanism of their cytostatic activity.

Abbreviations: ACN, acyclonucleoside; dAdo, 2'deoxyadenine; dGuo, 2'deoxyguanosine; dCyd, 2'deoxycytidine; dThd, 2'deoxythymidine; dN, 2'deoxynucleoside; dNMP, 2'deoxynucleoside-5'-monophosphate; dNDP, 2'deoxynucleoside-5'-diphosphate; dNTP, 2'deoxynucleoside-5'-triphosphate.

Reprint requests to Dr. H. Modrzejewska.
Fax: (04842) 322347.

Material and Methods

Chemicals

[^{14}C] dN's and [^{32}P]γ ATP were obtained from Amersham corp.[England]. Unlabelled dN's, dNMP's, dNDP's and dNTP's were purchased from Sigma Chemical Company (USA) and from Boehringer (Mannheim, Germany). Other reagents used were highest quality commercially available products from Fluka A.G., Loba-Chemie (Wien), Koch-Light Lab. and POCh (Gliwice, Poland). Allyloxymethyl purine and pyrimidine

0939–5075/96/0100–0075 $ 06.00

ACN's (Fig. 1, comp. **1–6**) were obtained by Ozierov (Ozierov *et al.*, 1991), Th5 and Th5P (Fig. 1, comp. **7,8**) by Dramiński (Rutkowski and Dramiński, 1991). PMT (Fig. 1, comp. **9**) was obtained by the same method as ACN's by use of 1-propanol instead allyl alcohol. Pure material has melting point at 135–136 °C, UV, 1H-NMR and mass spectra confirm the structure of 1-propoxymethylthymine.

Animals and subcellular fraction preparation

A group of four female Syrian hamsters (weighing 70–90 g) were used for each experiment. Kirkman-Robbins hepatoma, one of the fast growing tumors, was maintained as a subcutaneous transplant and was transplanted at 10 days' intervals. The most intensive growth of tumor occurred 6–10 days after transplantation. From the 12–14-th day regressive changes took place and within 2–3 days the tumor was eliminated (Starzyk *et al.*, 1969). For the *in vitro* experiments, six days after the heterotransplantations the animals were sacrificed by sectioning the cervical spinal cord, tumors were excised and homogenized at 0 °C in Potter-Elvehjem apparatus in 25 mM Tris[(-hydroxymethyl)aminomethane]-HCl buffer (pH 7.4), containing 250 mM sucrose, 25 mM KCl and 5 mM $MgCl_2$. Nuclei and mitochondria were spun down at 10 000 x *g* for 20 min and cytozol obtained from the resulting supernatant after centrifugation at 105 000 x *g* for 60 min (Beckman L5–65) was used for the experiments. For the *in vivo* experiments, six days after tumor transplantation the animals were given i.p. respective ACN's (80 mg per kg of body weight), 48 hours later they were sacrificed and cytosol from tumor, obtained as above, was used for the enzyme activity assays.

Chemicals and enzyme assays

dN kinase activities and dTMP kinase activities were assayed as described previously (Greger and Dramiński, 1989). The phosphorylation of ACN's were performed in conditions matching all dN kinase activities, using 0.2 mM ACN (as substrate) and 10 mM [^{32}P]γ ATP (as ^{32}P phosphate donor), 0.5 μ Ci per sample. After incubation in a water bath (37 °C, 40 min), the reaction was terminated by immersion of the tubes in boiling water for 2 min. Denatured protein was removed and phosphorylation products were separated by thin layer ascending chromatography (silica gel plates, Merck Kiselgel, 60F, 0.2 mm) at room temperature in 1-propanol:conc.ammonia water = 22:17 (v/v), with Rf for ACN of 0.6–0.8, ACN monophosphates of 0.21–0.26, ACN diphosphates of 0.18–0.20 and ACN triphosphates of 0.03–0.05. The spots from gel plates were counted in a Packard Tricarb 1600 scintillation counter, using toluene scintillator. Enzyme activities were expressed in μM of dNMP formed per min per mg of protein (ie U/mg of protein) in case of dN kinase activity determination or in μM of Th5 diphosphate formed per min per mg of protein, in case of dTMP kinase activity determination. Protein was assayed by Bradford's method (Bradford, 1976).

HeLa cells culture was grown in Eagle's medium enriched with bovine serum, containing gentamycin and Hepes (pH 7.0). The growth of culture was estimated by calculating the number of cells before and after addition of ACN's.

Results and Discussion

The influence of nine ACN's on the growth of Kirkman-Robbins hepatoma and the dN kinases activities present in cytosol of growing tumor, were investigated in a series of experiments. AMU, AMT and Th5 administered i.p. in animals six days after the tumor transplantation in a dose of 80 mg per kg body weight, reduced tumor weight by 30%±8% (AMU) and by 42%±11% (AMT, Th5) (SD for four experiments). Table I (Influence of ACN presented in Fig. 1 on dN kinase activities *in vitro* and *in vivo*) presents the activities of dN kinases in experiments *in vitro* and *in vivo*. None of the investigated ACN's inhibits the activities of dN kinases *in vitro*, except for AMT which inhibits the synthesis of dTMP by 38%. AMU, AMT and Th5 inhibit the synthesis of dTMP and dGMP *in vivo*, and the percentage of inhibition of dThd and dGuo kinases activities by AMT (59% and 53%, respectively) and by Th5 (58% and 55%, respectively) is practically the same. The inhibition of dTMP and dGMP synthesis by AMU is lower by about a half (36% and 33%). Similar effects are exhibited by the same compounds at the concentration of 0.5 μmol per ml of medium (i.e 91 μg of AMU, 98 μg of AMT and 115 μg of Th5) for the growth inhibition of

Table I. The Infuence of ACN's presented in Fig. 1 on dN kinases activities (U/mg of protein $\times 10^{-5}$) in cytosol of growing hepatoma. *In vitro:* 6 days after tumor transplantation in the presence of 0.2 mM of ACN; *in vivo:* 6 days after tumor transplantation the animals were given respective ACN's (80 mg/kg body weight) and 48 hours later enzyme activities were assayed. Control: dN kinases activities in cytosol of 6–8 days old hepatoma.

	Control	AMU	AMUF	AMT	AMC	AMA	AMG	PMT	Th5	Th5P
In vitro										
dAdo kinase	8.9±1.0	9.1±1.1	8.7±0.9	9.2±1.2	9.4±1.3	9.0±1.0	8.6±0.9	8.8±1.0	9.1±1.2	9.2±1.3
dGuo kinase	30.8±2.7	29.8±2.6	32.0±3.0	27.7±2.5 (10%, NS)	29.7±2.8	33.0±3.1	31.8±2.7	30.4±2.5	33.2±3.2	29.6±2.7
dCyd kinase	1.2±0.2	1.1±0.2	1.0±0.9	1.3±0.3	1.3±0.3	1.4±0.4	1.1±0.2	1.2±0.3	1.4±0.3	1.3±0.3
dThd kinase	91.1±10.2	90.6±9.9	93.0±12	57.2±5.2 (37%, p=0.05)	90.8±10	89.8±9.6	82.2±8.4 (9.8%, NS)	90.3±9.7	73.1±8.0 (19.8%,NS)	92.0±11
In vivo										
dAdo kinase	8.2±0.9	8.6±1.2	8.9±1.3	9.0±1.3	9.0±1.2	8.5±1.2	8.8±1.2	9.1±1.3	8.0±1.2	9.2±1.4
dGuo kinase	31.6±2.8	21.1±1.8 (33%, p=0.05)	29.8±2.2	14.6±1.3 (53.7%, p=0.002)	30.2±2.5	29.0±2.0	32.0±3.0	33.6±3.2	14.1±1.2 (55.2%, p=0.002)	31.8±3.0
dCyd kinase	1.0±0.2	0.8±0.1	1.3±0.3	1.1±0.2	1.2±0.2	0.9±0.2	1.4±0.3	1.1±0.2	1.4±0.4	1.2±0.3
dThd kinase	90.6±9.3	58.0±4.6 (36%, p=0.02)	92.0±11	36.6±2.6 (59.2%, p=0.002)	91.6±11	89.3±9.2	93.0±12	91.8±11	38.1±2.8 (58%, p=0.002)	90.2±9.5

Each value: the mean ± SEM for four experiments. In parentheses percent of inhibition, NS – mean non significant (ie $p<0.05$), p values were calculated using Student's t-test.

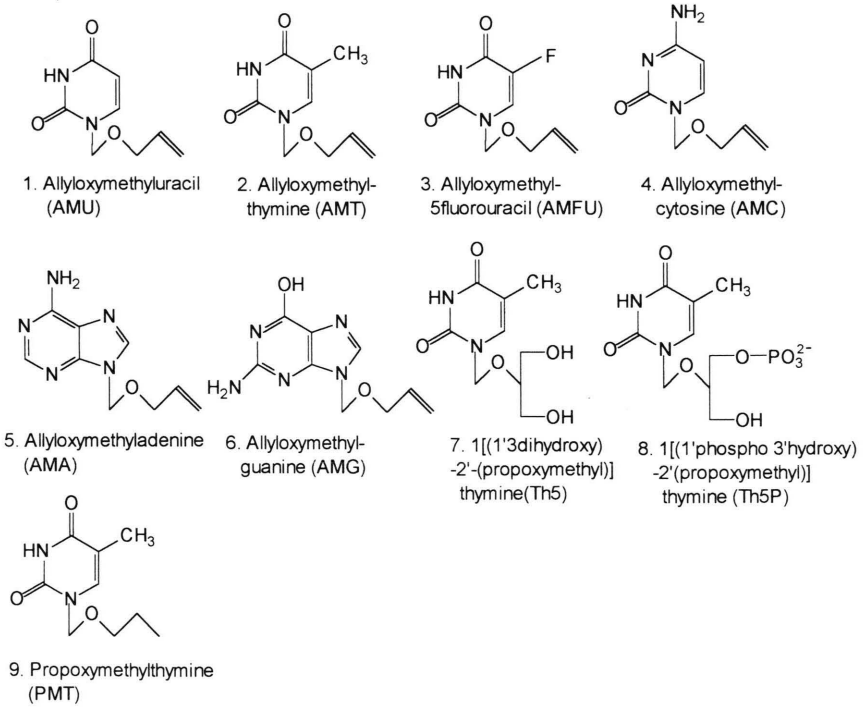

Figure 1

Fig. 1. Structural formula of the investigated acyclonucleosides.

HeLa cells of 40%±9%, 60%±12% and 65%±13%, respectively (SD for eight experiments) (Modrzejewska *et al.*, 1994).

Aiming at elucidating differences between the activities of ACN's *in vitro* and *in vivo*, phosphorylation of AMU, AMT and Th5, catalyzed by kinases present in cytosol of growing hepatoma was investigated. For reasons which will be explained later the investigation comprised also Th5P and PMT, despite the fact that these compounds do not exhibit any activity *in vivo* (Table I). The results of phosphorylation of five ACN's mentioned above are presented in Table II (The phosporylation of ACN by kinases present in cytosol of six days growing hepatoma). AMU, AMT and Th5 are phosphorylated to mono, di and triphosphates. The highest phosphorylation of AMU, AMT and Th5 obtained by a method used for determination of dThd kinase activity allows to presume that the synthesis of AMU, AMT and Th5 monophosphates is catalyzed by dThd kinase. This fact is confirmed by other authors who found that phosphorylation of pyrimidine ACN's *in vivo* is mainly catalyzed by this enzyme (Neyts and De Clerq, 1994; Wang *et al.*, 1994). If phosphorylation of Th5, containing two CH_2OH groups in the residue, is comprehensible, then phosphorylation of AMU and AMT may take place only when allyloxymethyl residue binding uracil (AMU) or thymine (AMT) is hydrated *in vivo*. Hydration of allyloxymethyl residue ($-CH_2-O-CH_2-CH=CH_2$) to hydroxypropoxymethyl residue ($-CH_2-O-CH_2-CH_2-CH_2OH$) containing CH_2OH group, transforms AMU and AMT into substrates for appropriate kinase. The fact that AMU and AMT phosphorylation is catalyzed with (almost) 5-fold (AMU) and 3-fold (AMT) lower activity, as compared to Th5 phosphorylation, evidences that AMU and AMT allyloxymethyl residue hydration maybe the rate limiting reaction of AMU and AMT phosphorylation. The enzymatic hydration of unsaturated binding of the ribosyl moiety of S-adenosyl-L-homocysteine inhibitor was demonstrated by Jarvi *et al.* (1991). The lack of this reaction *in vivo* in case of 1(ethoxymethyl)-6-(phenylselenenyl)pyrimidine ACN's inhibiting the reverse transcriptase of HIV 1 was revealed by Goudgaon *et al.* (1992), who have also found these ACN's not to be phosphorylated *in vivo*. Recently, it has also been reported that 1[(2- hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT), a selective inhibitor of the same virus (Tanaka *et al.*, 1994), does not require phosphorylation in order to inhibit HIV 1 reverse transcriptase. Consequently, the presence of a hydroxy group in HEPT is not necessary for its anti HIV activity (Fossey *et al.*, 1994). In order to confirm our presumptions that *in vivo* allyloxymethyl residue of AMU and AMT is hydrated, phosphorylation of PMT was subject to a separate investigation. Saturated propoxymethyl residue ($-CH_2-O-CH_2-CH_2-CH_3$) of PMT after hydroxylation (or hydration) is transformed into hydroxypropoxymethyl residue, which means PMT becomes a compound identical with AMT after hydration of allyloxymethyl residue. If such a transformation of PMT really occurs *in vivo*, then PMT should exhibit the same or similar properties as AMT. Complete lack of both biological activity *in vivo* (Table I) and phosphorylation of PMT (Table II) indicate that this situation results from the presence of saturated alkyl substituent in this ACN which does not undergo hydroxylation (or hydration) *in vivo*. Data presented in Table II also markedly indicate that hydratase

Table II. The phosphorylation of ACN's (0.2 mM) by kinases present in cytosol of six days growing hepatoma (U/mg of protein $\times 10^{-5}$).

Substrate	ACN^{32}P monophosphate formed	ACN^{32}P diphosphate formed	ACN^{32}P triphosphate formed	Di/tri phosphate ratio
AMU	11.2±1.4	0.78±0.12 (6.2%)	0.65±0.12 (5.14%)	1.2
AMT	19.8±2.6	1.47±0.20 (6.5%)	1.19±0.16 (5.29%)	1.23
Th5	52.0±7.0	3.57±0.48 (6.1%)	3.11±0.38 (5.3%)	1.15
Th5P	0.0	31.8±3.2 (99.4%)	0.18±0.03 (0.56%)	176.6
PMT	0.0	0.0	0.0	–

Each value: the mean ± SD for eight experiments. In parentheses percent of phosphorylation. Besides AMU, AMT, Th5 and Th5P, none of ACN's presented in Table I is phosphorylated by kinases of cytosol of growing tumor.

present in the hepatoma cells catalyzes the hydration of the allyloxymethyl residue only when this residue is uracil or thymine-bound.

If Th5 monophosphate quantitatively prevails among ACN's monophosphates presented in Table II, then percentage phosphorylation of AMU, AMT and Th5 monophosphates to appropriate diphosphates is, practically, the same. Similarly, phosphorylation products of AMU, AMT and Th5 diphosphates to corresponding triphosphates occur, proportionally, in the same quantities (Table II). The situation changes when Th5P, applied similarly as AMU, AMT and Th5 in concentration of 0.2 mM (Table II), becomes the substrate for dNMP kinase (probably dTMP kinase). Th5 diphosphate which is then formed in quantities exceeding 9 to 40 times AMU, AMT and Th5 diphosphates formed as a consequence of phosphorylation of AMU, AMT and Th5, probably inhibits dNDP kinase (or dNMP kinase when Th5P is phosphorylated to dTh5 *bis*-monophosphate) making the phosphorylation of Th5 diphosphate to Th5 triphosphate impossible. Inhibition of the enzyme activity by ACN diphosphates was revealed already in 1990 by Krenitsky *et al.* (1990), who stated that this very acyclovir diphosphate is the inhibitor of purine nucleoside phosphorylase. The lack of phosphorylation of Th5P to Th5 triphosphate and the remaining results of phosphorylation included in Table II indicate univocally that among the studied ACN's, only these are biologically active which are *in vivo* phosphorylated to corresponding triphosphates which probably inhibit dTMP and dGMP synthesis. This view is confirmed by the idea postulated by other authors that biologically active ACN's inhibiting the growth of tumors or virus replication are their triphosphates (Wang *et al.*, 1994; Balazarini *et al.*, 1994), although other mechanisms of cytostatic activity of ACN's have also been described (Greger and Dramiński, 1989; Goudgaon *et al.*, 1992). Among the studied ACN's , AMT is the most promising one. This compound inhibits the synthesis of dTMP and dGMP *in vivo* to the same extent as Th5 (Table I), although the phosphorylation of AMT by kinases present in cytosol of growing tumor results in a 3-fold smaller quantity of AMT triphosphate as compared with the synthesis of Th5 triphosphate (Table II). A 37% inhibition of dTMP synthesis by AMT *in vitro* (Table I) does not seem to be of great significance. AMT is a weak competitive inhibitor of dThd kinase (K_i= 740 μM, Fig. 2), which does not exclude the synergistic effect of AMT and AMT triphosphate on the synthesis of dTMP. It should be expected that the final cytostatic effect exerted by AMU, and especially by AMT and Th5, is correlated, beside the inhibition of dTMP and dGMP synthesis, with the inhibition of pyrimidine and purine nucleoside phosphorylase activity, as it is in case of other ACN's (Drabikowska *et al.*, 1987; Naguib *et al.*, 1993; Bzowska *et al.*, 1994). In the phosphorylation chain leading from AMU, AMT and Th5 to their biologically active triphosphates, the inhibition of

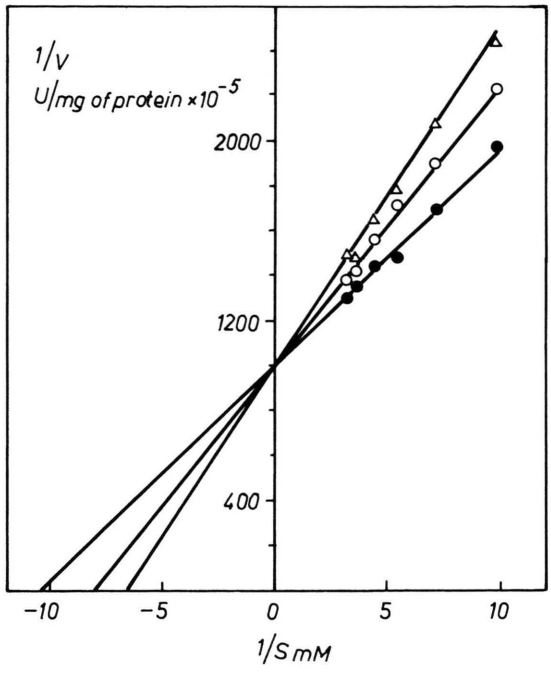

Fig. 2. Lineweaver – Burk plot of dThd kinase activity in cytosol of six days growing hepatoma (●-●-●-) in the presence of 0.2 mM (-○-○-○-) and 0.4 mM (-△-△-△-) AMT. K_m calculated from this plot equals 98 μM, K_{mi} at 0.2 mM and 0.4 mM AMT are 124 μM and 151 μM, respectively. K_i at 0.2 mM and 0.4 mM AMT calculated from these values are 736 μM and 742 μM, respectively. Regression coefficients (B) and correlation coefficients (r) values are: for dThd kinase, B = 102.13, r = 0.99; for dThd kinase in the presence of 0.2 mM AMT, B = 107.00, r = 0.96; for dThd kinase in the presence of 0.4 mM AMT, B = 152.02, r = 0.99.

phosphorylase activity could significantly facilitate the first step of phosphorylation. An investigation on the influence of AMU, AMT and Th5 on the activity of pyrimidine nucleoside phosphorylase will be the subject of further research.

Acknowledgements

The authors are grateful to Dr. J. Bartkowiak and Dr. G. Graczyk for performing tumor transplantations.

Balzarini J., Bohman C., Walker R.T. and De Clerq E. (1994), Comparative cytostatic activity of different antiherpes drugs against herpes simplex virus thymidine kinase gene transfected tumor cells. Molec. Pharmacol. **45,** 1253–1258.

Bradford M. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein – dye binding. Anal. Biochem. **72,** 248–254.

Bzowska A., Ananiev A.V., Ramzaeva N., Alksins E., Maurins A., Kulikowska E. and Shugar D. (1994), Purine nucleoside phosphorylase: inhibition by purine N (7) and N (9) acyclonucleosides and substrate properties of 7-β-D-ribofuranosylguanine and 7-β-D-ribofuranosylhypoxandine. Biochem. Pharmacol. **48,** 937–947.

Drabikowska A.K., Lissowska L., Dramiński M., Zgit-Wróblewska A. and Shugar D. (1987), Acyclonucleoside analogues consisting of 5 and 5,6 substituted uracil and different acyclic chains: inhibitory properties versus *E.coli* uridine phosphorylase. Z. Naturforsch. **42c,** 288–296.

Fossey C., Laduree D. and Robba M. (1994), Synthesis of acyclic thieno (3,2-d) pyrimidine nucleosides and azido derivatives as potential anti HIV agents. Nucleosides & Nucleotides **13,** 883–889.

Goudgaon N.M., McMillan A. and Schinazi R.F. (1992), 1-(Ethoxymethyl)-6-(phenyl selenenyl) pyrimidines with activity against human immunodeficiency virus types 1 and 2. Antiviral Chem. & Chemother. **3,** 263–265.

Greger J. and Dramiński M. (1989), Growth inhibition of Kirkman-Robbins hepatoma by 1(1',3'dihydroxy-2-propoxymethyl)5,6 tertramethyleneuracil and possible mechanism of its biological activity. Z. Naturforsch. **44c,** 985–991.

Hayashi S., Phadtare S., Zemlicka J., Matusakura M., Mitsuya H. and Broder S. (1988), Adenallene and cytallene: acyclic nucleoside analogues that inhibit replication and cytopathic effect of human immunodeficiency virus *in vitro.* Proc. Natl. Acad. Sci. USA **85,** 6127–6131.

Jarvi E.T., McCarthy J.R., Mehdi S., Matthews D.P., Edwards M.L., Prakash N.J., Bowlin T.L., Sunkara P.S. and Bey P. (1991), 4',5'Unsaturated 5 halogenated nucleosides. Mechanism-based and competitive inhibitors of S-adenosyl-L-homocysteine hydrolase. J. Med. Chem. **34,** 647–656.

Krenitsky T.A., Tuttle J.V., Miller W.H., Moorman A.R., Orr G.F. and Beauchamp L. (1990), Nucleotide analogue inhibitors of purine nucleoside phosphorylase. J. Biol. Chem. **265,** 3066–3069.

Modrzejewska H., Greger J., Lewandowska U. and Fidek W. (1994), *In vivo* phosphorylation of allyloxymethyl purine and pyrimidine acyclonucleosides and the inhibitory effect of these compounds on thymidine and deoxyguanosine kinases. Acta Biochim. Polon. **41,** 185–187.

Naguib F.N.M., Levesque D.L., Wang E.C., Panzica R.P. and El Kouni M.H. (1993), 5-Benzylbarbituric acid derivatives, potent and specific inhibitors of uridine phosphorylase. Biochem. Pharmacol. **46,** 1273–1283.

Neyts J. and De Clerq E. (1994), Mechanism of action of acyclic nucleoside phosphonates against herpes virus replication. Biochem. Pharmacol. **47,** 39–41.

Ozierov A.A., Novkov M.C., Brel A.K., Andrejeva O.T., Vladyko G.V., Boreko E.I., Korbachenko L.V. and Vervetchenko C.G. (1991), The synthesis and antiviral activity of newly synthesized unsaturated pyrimidine acyclonucleosides. Chim. Pharm. **25,** 44–47.

Rutkowski M. and Dramiński M. (1991), Phosphorylation of acyclonucleosides by nucleoside phosphotranferase from higher plants and bacteria. Acta Biochim Polon. **38,** 449–457.

Sakamoto S., Ebuchi M. and Iwama T. (1993), Relative activities of thymidylate synthetase and thymidine kinase in human mammary tumors. Anticancer Res. **13,** 205–207.

Starzyk H., Rzucidlo Z., Djaczenko W. and Granska J. (1969), Some morphologic, biologic and metabolic properties of transplantable Kirkman-Ribbins hepatoma in hamsters. Arch. Immunol. Ther. Experimental. **17,** 523–535.

Tanaka H., Baba M., Takahashi E., Matsumoto K., Kittaka A., Walker R.T. and De Clerq E. (1994), Design and synthesis of regioisomeric analogues of a specific anti-HIV-1 agent 1[(2 hydroxyethoxy) methyl]-6-(phenylthio) thymine (HEPT). Nucleosides & Nucleotides **13,** 155–162.

Wang E.C., Chen H.Y. and Tzega C.L. (1994), Acyclic nucleosides synthesis of 1[(hydroxy-2-propoxy) methyl] thymine, 6-azathymine, uracil and 6-azauracil as potential antiviral agents. Nucleosides & Nucleotides **13,** 1201–1213.