*Eur J Cancer Clin Oncol.* Vol. 22, No. 5, pp. 557–562, 1986.
Printed in Great Britain.

0277-5379/86$3.00+0.00


# 5-Ethyl-2′-Deoxyuridine: An Explanation for its Lack of Cytotoxic Action *in Vivo*

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**Abstract**—*The aim of this study was to explain why 5-ethyldeoxyuridine (EUdR) showed cytotoxic activity against Ehrlich ascites tumour (EAT) cells* in vitro *but not* in vivo. In vitro *studies showed that EUdR was phosphorylated to nucleotides which inhibit thymidylate synthetase and DNA polymerase. Toxicity in tissue culture appeared to be related to the inhibition of one or both of these enzymes; and could be prevented/reversed by thymidine (TdR).* In vivo *EAT cells also formed active EUdR nucleotides at levels which* in vitro *would have been associated with cytotoxicity but these levels were not maintained. EUdR has been shown to compete with TdR for catabolism by pyrimidine nucleoside phosphorylases from mouse liver and gut. In the ascitic fluid it was found that the level of EUdR fell rapidly while that of TdR and 5-ethyl-uracil increased. It is proposed that competition for catabolism* in vivo *resulted in the rise in TdR which then compromised the antitumour effect of EUdR.*

## INTRODUCTION

IT HAS been well established that modification of natural pyrimidine molecules at the 5 position results in compounds with potent biological activity [1, 2]. The substitution of the methyl group in thymidine (TdR) by an ethyl group resulted in a compound (EUdR) which has been shown both to inhibit cell proliferation *in vitro* [3] and to possess antiviral activity [4, 5]. However, when tested for antitumour activity *in vivo* at doses up to 1000 mg/kg, EUdR was found to be inactive against the same tumour line that was sensitive to it *in vitro* (preliminary report [6]).

The purpose of the present study was to elucidate the molecular mechanisms which might be responsible for the differential action of EUdR *in vitro* and *in vivo* by measuring certain of its biochemical and pharmacokinetic properties.

## MATERIALS AND METHODS

EUdR was synthesised as reported [7]. The radioactive form contained $^{14}C$ at the 2 position of the pyrimidine ring and had a specific activity of 2.48Ci/mmol.

$^{3}H$-methyl-thymidine ($^{3}H$-TdR, 40Ci/mmol), $^{3}H$-deoxyuridine ($^{3}H$-UdR, 5Ci/mmol) and 5-$^{3}H$-deoxyuridine monophosphate ($^{3}H$-dUMP, 20Ci/mmol) were purchased from The Radiochemical Centre (Amersham, U.K.). Biochemicals were obtained from Boehringer Corp. (London) Ltd. (Lewes, U.K.) or Sigma (London, U.K.). Chemical reagents were of the AnalaR grade wherever possible.

*Cell culture*

Ehrlich ascites tumour (EAT) cells were maintained as suspension cultures in RPMI 1640-Hepes buffered medium (Flow Laboratories) supplemented with 2mM glutamine and 10% donor horse serum. For each experiment cells were diluted to $5 \times 10^4$/ml, treated and cell counts were performed after 24 and 48hr incubation at 37°C, using an improved Neubauer haemocytometer.

EAT cells were also routinely passaged in female Balb $C^-$ mice by injection of $10^6$ tumour cells i.p. For experiments using tumour bearing animals $2.5 \times 10^6$ cells were injected and the mice treated 4 days later.

*Radioactive nucleoside incorporation*

EAT cells were incubated at $1.5 \times 10^7$/ml at 37°C in the presence of a radiolabelled nucleoside ($^{3}H$-TdR $1.7 \times 10^{-9}$M, $^{3}H$-UdR $2.7 \times 10^{-8}$M, or $^{14}C$-EUdR $2 \times 10^{-4}$M). At the end of the incubation the cells were washed with cold RPMI 1640 medium, and fractionated by the method of

Accepted 23 October 1985.
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Schneider *et al.* [8]. The nucleotide fraction was analysed on a Dowex 1X8 column using the method of Hurlbert *et al.* [9] as modified by Toth *et al.* [10], or by high performance liquid chromatography.

*Estimation of nucleotides by high performance liquid chromatography (HPLC)*

Following treatment, cell pellets were obtained by centrifugation at 600 ***g*** for 5 min at 37°C. The medium was decanted and nucleotides extracted by the addition of 100μl ice-cold 0.6M perchloric acid per $10^7$ cells. Samples were placed on ice for 1 hr and the precipitate removed by centrifugation at 600 ***g*** for 10 min at 4°C. Fifty-microlitre aliquots of the supernatant were analysed. HPLC was performed on a Waters Associates liquid chromatograph (Boston, MA, U.S.A.) equipped with two model 6000A solvent pumps, a model 660 solvent programmer, a U6K injector and a model 440 u.v. detector. Samples were separated on a μBondapak-$NH_2$ column (Waters Associates) and eluted with ammonium phosphate buffer at a flow rate of 4ml/min. The separation was achieved by gradient elution, from 0.01M ammonium phosphate pH 3.0 to 0.35M ammonium phosphate pH 4.0, using curve 8 on the solvent programmer over 20 min. Two-millilitre aliquots of the eluate were analysed for radioactivity following the addition of 10ml of PCS scintillant (Hopkin and Williams Ltd., Romford, Essex, U.K.).

*Pharmacokinetics of EUdR*

Tumour-bearing mice were treated i.p. with 400mg/kg EUdR. At various times after injection, mice were sacrificed, the tumour cells removed without dilution, and separated from the ascitic fluid by spinning at 600 ***g*** for 10 min. The levels of EUdR, ethyl uracil (EU) and TdR in the ascitic fluid were measured by adapting the method of Taylor *et al.* [11]. The ascitic fluid was treated as described for plasma.

An initial HPLC run was performed using a μBondapak 10μ C18 column (Waters Associates) running isocratically in 0.05M ammonium acetate pH 5.0 for 30 min followed by a 5-min linear gradient to 15% methanol. Fractions collected from this run were subjected to a second HPLC separation using an Apex 5μ C18 column (Jones Chromatography, Glamorgan, Wales) running isocratically in 20% methanol 80% 0.1M acetic acid.

*Enzyme assays*

*Thymidine kinase.* The EAT cells were sonicated in 50 mM Tris, 150 mM KCl pH 7.8, and the sonicate centrifuged at 100,000 ***g*** for 30 min. The supernatant was brought to 30% saturation with ammonium sulphate and the precipitated protein resuspended in 50 mM Tris pH 7.8 and used directly. The assay mixture contained 7.5 mM ATP, 3.5mM $MgCl_2$, 10–100μM $^3$H-TdR (100μCi/μmol), 50 mM Tris pH 7.8. The reaction was carried out at 37°C and stopped by boiling for 5 min. $^3$H-thymidine was separated from its nucleotides on PEI-cellulose plates (Camlab, U.K.) by repeated washing with 5 mM Tris pH 7.8. The $^3$H-nucleotides were eluted from the PEI-cellulose with 1N HCl and counted with an efficiency of 30%. When measuring the phosphorylation of EUdR, $^{14}$C-EUdR (2.48μCi/μmol) was used instead of $^3$H-TdR. All other procedures were the same.

*Pyrimidine nucleoside phosphorylase.* Tumour, liver or intestinal mucosa was homogenised in 50 mM Tris pH 7.5 and the 30,000 ***g*** supernatant used directly. The assay mixture was similar to that of Friedkin and Roberts [12] and contained 50mM arsenate, 33mM succinate, 17mM Tris, 0.5–10 mM $^3$H TdR (2μCi/μmol) pH 5.9. The reaction was carried out at 37°C and stopped by the addition of cold thymine and rapid freezing. Thymine and thymidine were separated on cellulose TLC plates (Merck, Darmstadt) running in a 65 : 5 : 30 mix of ethyl acetate, formic acid and water. The thymine spots were cut out, the thymine eluted with 1 ml water and counted using Cocktail T scintillant (BDH Chemicals Ltd., Poole, U.K.) with an efficiency of 30%.

*Thymidylate synthetase.* EAT cells were homogenised in 10 mM Tris, 250 mM sucrose, 3mM $CaCl_2$, 2 mM dithiothreitol (DTT), pH 7.5. The homogenate was centrifuged at 150,000 ***g*** for 30 min and the supernatant partially purified using saturated ammonium sulphate solution. The protein precipitating between 40 and 60% saturation was redissolved in 10 mM Tris, 10 mM DTT pH 7.5 and dialysed against the same buffer before use. Thymidylate synthetase activity was determined using the method of Calvert *et al.* [13]. The assay contained 200μM tetrahydrofolate, 100 mM NaF, 2 mM formaldehyde, 25–200μM $^3$H-dUMP (10μCi/μmol), 40 mM DTT, 10 mM Tris pH 7.5. $^3H_2O$ was separated from $^3$H-dUMP on Dowex 1X8 chloride columns.

## RESULTS

EUdR inhibited the proliferation of EAT cells growing *in vitro* ($ID_{50}$ at 48 hr $3 \times 10^{-5}$M), but treatment of the tumour growing *in vivo* with up to 1000 mg/kg of EUdR had no effect on the tumour growth (Table 1).

The uptake and incorporation of $^{14}$C-EUdR ($10^{-4}$M) into cellular material was studied *in vitro*. Uptake was rapid but at all times studied >89% was associated with the acid soluble pool (corres-

*Table* 1. *Inhibition by EUdR of cell proliferation* in vitro *or* in vivo

| | *In vitro* no. cells $\times 10^{-4}$/culture | | | *In vivo* no. cells $\times 10^{-7}$/mouse | |
|---|---|---|---|---|---|
| Treatment time: | 24hr | 48hr | | 24hr | 48hr |
| Drug conc. (M) | | | Doses (mg/kg) | | |
| Control | 14.0 ± 1.9 | 27.8 ± 1.5 | Control | 12.0 ± 1.4 | 17.1 ± 2.1 |
| $10^{-6}$ | 14.2 ± 1.7 | 22.1 ± 2.6 | | | |
| $10^{-5}$ | 10.6 ± 0.4 | 12.2 ± 1.8 | 40 | 12.8 ± 1.8 | 18.8 ± 1.9 |
| $10^{-4}$ | 7.7 ± 0.5 | 8.3 ± 0.6 | 200 | 14.1 ± 1.9 | 18.0 ± 2.3 |
| $10^{-3}$ | 5.9 ± 0.3 | 5.7 ± 0.4 | 1000 | 14.6 ± 2.5 | 18.0 ± 2.2 |

ponding to base, nucleoside and nucleotides), <3% with the lipid fraction and <1% with the protein fraction. $^{14}$C-EUdR incorporation into DNA increased with time, and amounted to 33 pmol/$10^6$ cells after 60 min at which time the acid soluble pool contained 500 pmol/$10^6$ cells. This incorporation into DNA was reduced markedly (69%) by the addition of $10^{-5}$M TdR to the culture 5 min before EUdR, but not significantly (14%) by a similar addition of UdR. Analysis of the acid soluble pools showed that the addition of TdR also reduced the amount of EUdR in the nucleotide triphosphate and diphosphate pools (by 75, 42% respectively) while UdR addition only reduced those amounts by 25, 22% respectively.

The effects of EUdR on $^3$H-TdR and $^3$H-UdR uptake and incorporation were then studied (Table 2). EUdR pretreatment had no effect on the formation of TdR nucleotides whereas it decreased the $^3$H-UdR labelling of the total nucleotide pool. Furthermore EUdR specifically decreased the $^3$H-UdR labelling of the di- and tri-phosphate but not the monophosphate pool. The incorporation of both labelled nucleosides into nucleic acids was decreased, the effect on $^3$H-UdR being more pronounced as would be anticipated from the reduced labelling of the triphosphate pool.

The activity of EUdR against thymidine kinase and pyrimidine nucleoside phosphorylase was measured as these enzymes are important in the metabolism of pyrimidine deoxynucleosides. In addition, the activity of EdUMP against thymidylate synthetase was also measured, as inhibition of this enzyme could account for the differential effect of EUdR on $^3$H-TdR and $^3$H-UdR uptakes. The results are given in Table 3. EUdR competitively inhibited thymidine kinase ($K_i$ 61μM) by virtue of it being a substrate for the enzyme with a $K_m$ considerably higher than that of TdR (53 μM v. 1.0 μM) and with a Vm somewhat higher (96 v. 71 nmol/hr/mg protein). EdUMP was found to be a competitive inhibitor of thymidylate synthetase

*Table* 2. *Incorporation of* $^3$H-*TdR or* $^3$H-*UdR into cellular material in the presence or absence of EUdR*

| | $^3$H-TdR | | $^3$H-UdR | |
|---|---|---|---|---|
| | Control | EUdR | Control | EUdR |
| DNA | 95,500* ± 3000 | 26,250 (27%) ± 1570 | 117,600 ± 530 | 4000 (3%) ± 24 |
| Acid soluble | 9000 ± 540 | 9920 (110%) ± 770 | 15,750 ± 1350 | 6870 (44%) ± 470 |
| Ratio N/M/D/T† | 2.3/1/5.2 | 2.7/1/6.5 | 2.5/1/4.6 | 4.8/1/2.9 |

Cells were incubated for 10 min with $^3$H-TdR 1.7nM (40Ci/mmol) or $^3$H-UdR 26.7 nm (5Ci/mmol) with or without a 20 min preincubation with 200μM EUdR. Cell fractionation was carried out as described in Materials and Methods.

* Results given as cpm/$10^6$ cells ± S.D., with the number in parentheses being the result expressed as a percentage of the appropriate control.

† Ratio of cpm in nucleoside/monophosphates/diphosphates/triphosphates fraction.

*Table 3. Kinetic parameters for enzymes important in pyrimidine metabolism*

| Enzyme source | EAT | EAT | phosphorylase | |
|---|---|---|---|---|
| Enzyme | Thymidine kinase | Thymidylate synthetase | Liver Pyrimidine | Gut nucleoside |
| $K_m$ substrate | 1.0 ± 0.5μM* | 0.6 ± 0.7μM | 1.7 ± 0.2mM | 0.5 ± 0.1mM |
| $V_{max}$ substrate | 71 ± 7† | 3.0 ± 0.1 | 377 ± 14 | 796 ± 50 |
| $K_i$ EUdR | 61 ± 9μM | – | 4.5 ± 0.5mM | 0.6 ± 0.03mM |
| $K_i$ EdUMP | – | 9 ± 11μM | – | – |
| $K_m$ EUdR | 53 ± 7μM | – | – | – |
| $V_{max}$ EudR | 96 ± 5 | – | – | – |

Enzyme preparations and assays as described in Materials and Methods.
Kinetic parameters were calculated by non-linear regression using program BMDX85 (17).
* Mean ± S.E.
† nmol/hr/mg protein.

with $K_i$ 9μM compared with a $K_m$ for dUMP of 0.6μM. Pyrimidine nucleoside phosphorylase activity was considerably lower in the tumour preparation than in the gut or liver preparations (95 v. 796, 377 nmol/hr/mg protein) and so no kinetic studies were carried out with the tumour extracts. However, the pyrimidine nucleoside phosphorylase activities in both gut and liver were competitively inhibited by EUdR, this inhibition being more pronounced for the gut preparation ($K_i$ 0.6mM cf. $K_m$ TdR 0.5mM) than for the liver preparation ($K_i$ 4.5 mM cf. $K_m$ TdR 1.7 mM).

Possible explanations for the lack of activity of EUdR *in vivo* were then investigated. In order to determine if EUdR was taken up into EAT cells and phosphorylated *in vivo*, tumour bearing mice were treated with $^{14}$C-EUdR (60mg/kg, i.p.). Subsequently the tumour was removed and the nucleotide profile analysed by HPLC. All classes of nucleotide were labelled (Table 4) and the total amount was comparable to that achieved *in vitro* at a cytotoxic dose ($2 \times 10^{-4}$M). However, this total decreased considerably between 20 and 40 min after injection suggesting rapid turnover of EUdR and its metabolites.

The pharmacokinetics of EUdR and its congeners in the ascitic fluid of tumour-bearing mice were followed after treatment with EUdR at a higher dose (400 mg/kg, i.p.) (Fig. 1). The concentration

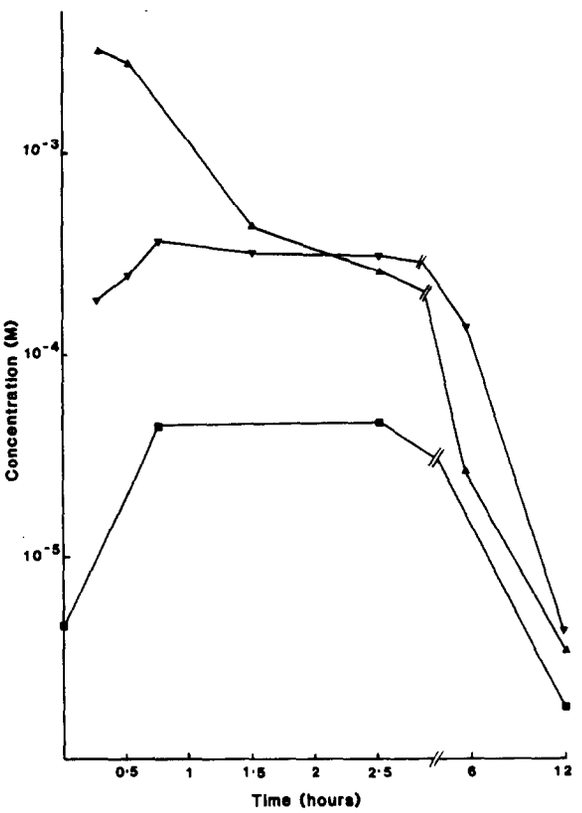


*Fig. 1. The concentration of TdR (■), EUdR (▲) and EU (▼) in the ascitic fluid of EA tumour bearing mice following i.p. administration of* 400 mg/kg *of EUdR.*

*Table 4. Uptake of $^{14}$C-EUdR into EAT cells* in vivo, *and its distribution between the nucleotide classes*

| Time post inf. | Total EUdR in acid soluble fraction (pmol/$10^6$ cells) | Nucleoside and base | Mono-phosphates | % Total Diphos-phates | Triphos-phates |
|---|---|---|---|---|---|
| 20 min | 1030 | 74.7 | 17.4 | 5.2 | 2.7 |
| 40 min | 500 | 67.6 | 17.3 | 12.2 | 2.9 |

Tumour-bearing mice were given $^{14}$C-EUdR (60mg/kg, 2.48mCi/mmol) i.p. At the appropriate time, the tumour was removed and the acid soluble fraction obtained and analysed by HPLC as described in Materials and Methods.

of EUdR fell rapidly, but remained above a level shown to be cytotoxic *in vitro* ($10^{-5}$M) for at least 6 hr. The base EU accumulated. The most interesting observation was the elevation in TdR which was maintained for at least 2 hr.

We have shown above that pretreatment with TdR reduced the incorporation of EUdR into nucleotides and DNA *in vitro*, but it was not known if TdR could rescue the cells from the cytotoxicity of EUdR when added after the compound. To test this, TdR was added at different times to cells exposed to EUdR. A time-dependent rescue was achieved (Table 5).

*Table* 5. *The reversal of cytotoxic action of EUdR by thymidine in Ehrlich ascites tumour cells growing* in vitro

| | No. cells × $10^{-4}$/ml ± S.D. after 48 hr |
|---|---|
| Control | 24 ± 2.0 |
| EUdR $10^{-5}$M | 13 ± 0.5 |
| TdR $10^{-5}$M | 29 ± 3.0 |
| EUdR + TdR 30 min later | 23 ± 0.8 |
| EUdR + TdR 3 hr later | 20 ± 4.0 |
| EUdR + TdR 8 hr later | 17 ± 1.0 |
| EUdR + TdR 24 hr later | 12 ± 0.3 |

## DISCUSSION

The present studies indicated that EUdR acted as a nucleoside antimetabolite, competing with TdR for both TdRK and pyrimidine nucleoside phosphorylase. In EAT cells EUdR was phosphorylated by thymidine kinase to EdUMP which inhibited thymidylate synthetase from this source and has also been reported to be an inhibitor of the enzyme from L1210 cells [14]. The inhibition of thymidylate synthetase by EdUMP leading to a reduced conversion of $^{3}$H-UdR to $^{3}$H-TMP can explain the reduction in di- and tri-phosphates and the increase in monophosphates from $^{3}$H-UdR observed following EUdR treatment.

In addition, EdUMP was further phosphorylated to EdUDP and EdUTP. The EdUTP formed could act as a substrate for DNA polymerase [15] and so account for the reduced incorporation of $^{3}$H-TdR in the presence of EUdR and also for the incorporation of $^{14}$C-EUdR into nucleic acids reported here. The incorporation of the analogue is not thought to contribute towards cell death as EUdR has no mutagenic activity and does not alter chromosome morphology [2, 16]. Thus, *in vitro* it appears EUdR has two loci of action: TS and DNA polymerase, either or both of which may be important for its cytotoxic activity.

*In vivo* activation of EUdR to potentially cytotoxic levels of EdUMP and EdUTP occurred after even a low dose (60 mg/kg), however, these levels did not persist. The EUdR was rapidly broken down to its base (EU), probably by pyrimidine nucleoside phosphorylases, but following a dose of 400 mg/kg cytotoxic levels of EUdR were maintained for at least 6 hr. At higher doses the peak level and time of exposure to cytotoxic concentrations would be anticipated to be extended proportionately. However, no antitumour activity was observed in mice receiving 1000 mg/kg.

Associated with the rapid degradation of EUdR was a rise in TdR in the ascitic fluid. This presumably resulted from the inhibition of TdR breakdown in the liver and gut as a consequence of competition between EUdR and TdR for the same nucleoside phosphorylases. Our *in vitro* studies showed that low levels of TdR reverse the cytotoxic effects of EUdR, suggesting that the elevation in TdR observed *in vivo* could compromise any antitumor activity of EUdR.

In man it remains to be demonstrated that EUdR metabolism and excretion is as rapid as in the mouse, and whether the competition between EUdR and TdR for catabolism would generate sufficient TdR to compromise the antitumour efficacy of EUdR in man, given that the normal plasma levels of UdR and TdR are approximately 10-fold lower than those found in mice [18].

## REFERENCES

1. Heidelberger C, Ansfield FJ. Experimental and clinical use of fluorinated pyrimidines in cancer chemotherapy. *Cancer Res* 1963, **23**, 1226.
2. Prusoff WH, Ward DC. Nucleoside analogs with antiviral activity. *Biochem Pharmacol* 1976, **25**, 1233.
3. Swierkowski M, Kamac J, Chlopkiewicz B. The antitumour activity of certain substituted nucleosides. *Pol J Pharmacol Pharm* 1976, **28**, 403.
4. Gauri KK, Malorny G. Chemotherapie der Herpes-Infektion mit neuen 5-alkyl-uracildesoxyribosiden. *Arch Exp Pathol Pharmacol* 1967, **257**, 21.
5. Swierkowski M, Shugar D. A non-mutagenic thymidine analog with antiviral activity 5-ethyldeoxyuridine. *J Med Chem* 1969, **12**, 533.
6. Ötvös L, Lapis K, Szabolcs A, Kemeny V, Jeney A, Kopper L. Biochemical–pharmacological characterisation of the antitumour action of 5-alkyl-2'-deoxyuridines. XII *Congresso Internacional del Cancer Resumenses* 2. No 23. Impresso en Argentina, 1978.

7. Szabolcs A, Sagi J, Otvös L. Synthesis of 5-alkyl-2′-deoxyuridines. *J Carbohydrates Nucleosides, Nucleotides* 1975, **2,** 197.
8. Schneider WC, Hogeboom GH, Ross HE. Intracellular distribution of enzymes. VII. The distribution of nucleic acids and adenosine-triphosphatase in normal mouse liver and mouse hepatoma. *J Natl Cancer Inst* 1950, **10**, 977.
9. Hurlbert RB, Schmitz H, Brumm AF, Potter VR. Nucleotide metabolism II. Chromatographic separation of acid-soluble nucleotides. *J Biol Chem* 1954, **209**, 23.
10. Toth M, Zakar T, Antoni F. Inhibitory effect of prostaglandin E2 on the incorporation of $^{3}$H-thymidine into the DNA of thymus cells. *Acta Biochim Biophys Acad Sci Hung* 1975, **10**, 161.
11. Taylor GA, Dady PJ, Harrap KR. Quantitative high-performance liquid chromatography of nucleosides and bases in human plasma. *J Chromatog* 1980, **183**, 421.
12. Friedkin M, Roberts D. The enzymatic synthesis of nucleosides 1. Thymidine phosphorylase in mammalian tissue. *J Biol Chem* 1954, **207**, 245.
13. Calvert AH, Jones TR, Dady PJ, Grzelakowska-Sztabert B, Paine RM, Taylor GA, Harrap KR. Quinazoline antifolates with dual biochemical loci of action. Biochemical and biological studies directed towards overcoming methotrexate resistance. *Eur J Cancer* 1980, **16**, 713.
14. De Clercq E, Balzarini J, Torrence PF, Mertes MP, Schmidt CL, Shugar D, Barr PJ, Jones AS, Verhelst G, Walker RT. Thymidylate synthetase as target enzyme for the inhibitory activity of 5-substituted 2′-deoxyuridines on mouse leukaemia L1210 cell growth. *Molec Pharmacol* 1981, **19**, 321.
15. Sagi J, Nowak R, Zmudzka B, Szemzo A, Övös L. A study of substrate specificity of mammalian and bacterial DNA polymerases with 5-alkyl-2′-deoxyuridine 5′-triphosphates. *Biochim Biophys Acta* 1980, **606**, 196.
16. Singh S, Willers I, Goedde HW. 5-Ethyl-2′-deoxyuridine: absence of effects on the chromosomes of human lymphocytes and fibroblasts in culture. *Humangenetik* 1974, **24**, 135.
17. BMD Biomedical Computer Programs. *X-Series Supplement, BMDX*85 – *Non Linear Least Squares*, 1970, 177.
18. Taylor GA, Jackman AL, Calvert AH, Harrap KR. Plasma nucleoside and base levels following treatment with the new thymidylate synthetase inhibitor CB 3717. In: De Bruyn CHMM, Simmonds HA, Muller MM, eds. *Purine Metabolism in Man.* New York, Plenum Press, 1984, Vol. IVB, 379.