

Relationships between the chromatographic retention data and the effects of nucleoside derivatives in highly metastatic 3LL cells

G. POGÁNY,[†] T. CSERHÁTI,[‡] J. OLÁH[§] and K. VALKÓ^{*||}

[†]Joint Research Organization of the Hungarian Academy of Sciences and Semmelweis Medical University, Department of Molecular Pathology, Budapest, Üllői ut 26, H-1086 Hungary

[‡]Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, P.O. Box 17, H-1525 Hungary

[§]First Institute of Pathology and Experimental Cancer Research, Semmelweis Medical University, Budapest, Hungary

^{||}Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK

Abstract: The effect of 21 nucleoside derivatives on the [³H]-thymidine cellular uptake and on the incorporation into DNA of highly metastatic 3LL (Lewis lung carcinoma) cells has been measured. Hydrophobic and hydrophilic molecular parameters (the adsorption capacity, specific adsorption surface, lipophilicity and specific hydrophobic surface area) have been determined by using thin-layer chromatography.

Stepwise linear regression analysis and principal component analysis have been applied in order to reveal the relationships between the molecular parameters and the effect of the nucleoside derivatives on highly metastatic 3LL cells.

The first principal component obtained from the measured activity data could be attributed to the change of [³H]-thymidine cellular uptake caused by the nucleoside, while the second principal component could be regarded as the measure of the effect on the DNA incorporation of [³H]-thymidine. The effect of the nucleosides on the [³H]-thymidine uptake could be explained by the specific hydrophobic and adsorption surface area of the nucleoside, on the other hand the effect on the DNA incorporation could be described by the adsorption characteristics (specific hydrophilic surface area and adsorption capacity) of the derivatives.

Keywords: Nucleoside derivatives; Lewis lung carcinoma; quantitative structure–activity relationships; chromatographic retention parameters; hydrophobicity.

Introduction

Nucleotides and nucleosides, as the building blocks of DNA, play an essential role in life functions. A great number of nucleoside and nucleotide derivatives have already been synthesized and tested as potential antiviral and anti-tumour agents, as reviewed by De Clerq [1]. Their activity can be related to their influence on the biosynthesis of DNA [2–7]. Pharmaco-biochemical studies of the 5-alkyl-2'-deoxyuridines revealed that the influence of the derivatives on the biosynthesis of DNA was dependent on the substituents and their effect on the molecular mechanism [8, 9]. In order to have a better understanding of the molecular parameters required for the activity of the compounds quantitative structure–activity relationship investigations have been carried

out by using multivariate methods [10, 11]. The physico-chemical parameters of the compounds can be measured by various chromatographic methods [12] which can be used for drug design [13]. Adsorption and reversed-phase thin-layer chromatographic retention behaviour of the nucleoside derivatives has already been described [14]. The specific hydrophobic surface, lipophilicity, specific adsorption surface and adsorption capacity of the derivatives were estimated from the chromatographic retention data [14]. In our previous work the DNA incorporation rate of 5-alkyl-deoxyuridines during *in vitro* polymerase reactions were investigated as a function of various molecular parameters determined by a range of chromatographic methods [15, 16].

The objective of the present work was to study the effect of a series of 5-substituted

* Author to whom correspondence should be addressed.

deoxyuridine and uracil derivatives on the DNA synthesis and degradation in highly metastatic Lewis lung carcinoma (3LL) cells. The physico-chemical parameters of the derivatives were estimated from their chromatographic retention behaviour in order to reveal their importance in the effects of the nucleosides on the tumour cells.

Experimental

Materials

The structures of the investigated compounds are shown in Table 1. The general structures are shown in Fig. 1. The syntheses of the compounds have been described elsewhere [3, 4, 17, 18]. The compounds were chromatographically pure. The thin-layer chromatographic procedure for the determination of the adsorption capacity, specific adsorption surface, lipophilicity and specific hydrophobic surface have already been described in detail [14]. RPMI-1640 medium and foetal calf serum

were obtained from Flow Laboratories (UK). [³H]-thymidine (spec. act.: 1.48–2.22 GBq mmol⁻¹) were purchased from UVVVR (Prague, Czechoslovakia). Highly metastatic variants of 3 Lewis lung carcinoma (3LL) cells were selected and cultured as described previously [19, 20]. The cultures were maintained in RPMI-1640 medium containing 10% heat-inactivated foetal calf serum at 37°C [21].

Methods

A. Measurement of [³H]-thymidine cellular uptake. The continuous treatment of the 3LL cells maintained in RPMI-1640 medium by the nucleoside derivatives with 1, 10 and 100 µm ml⁻¹ concentrations (dissolved in the medium) was carried out for 48 h. After the treatment the cells were labelled with 37 kBq ml⁻¹ (1 µCi ml⁻¹) [³H]-thymidine for 1 h. Then the culture medium was removed by suction and the adherent cells were washed twice with 1 ml serum-free medium in the culture dishes. Then 2 ml ice-cold ethanol (4°C) was added to them and kept for 18 h in order to precipitate the macromolecules [22]. The suspensions were centrifuged at 500*g* for 25 min. The radioactivity of the supernatant (i.e. the ethanol soluble pool fractions) was measured in a toluene based cocktail using a Beckman LS 100C scintillation counter. The measured activity was always related to the activity of the control which was obtained by using the same procedure without adding the nucleoside

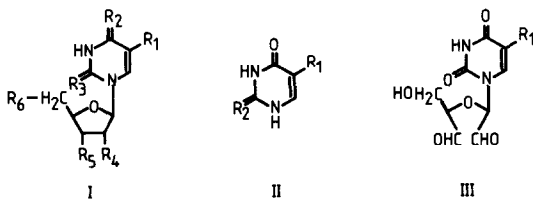


Figure 1
Chemical structure of nucleoside derivatives.

Table 1
Chemical structure of the investigated nucleoside derivatives

Serial no.	Structure in Fig. 1	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆
1	I	C ₂ H ₅	O	O	H	OH	OH
2	I	CH(CH ₃) ₂	O	O	H	OH	OH
3	I	C ₄ C ₉	O	O	H	OH	OH
4	I	C ₃ H ₁₁	O	O	H	OH	OH
5	I	C ₆ H ₁₃	O	O	H	OH	OH
6	I	C ₇ H ₁₅	O	O	H	OH	OH
7	I	C≡C—C ₃ H ₇	O	O	H	OH	OH
8	I	C≡C—C ₄ H ₉	O	O	H	OH	OH
9	I	C≡C—C ₅ H ₁₁	O	O	H	OH	OH
10	I	C ₆ H ₁₃	O	R ₃ —O—R ₄	H	CH ₃ COO	CH ₃ COO
11	I	C ₆ H ₁₃	O	O	H	CH ₃ COO	CH ₃ COO
12	I	C ₆ H ₁₃	O	O	H	C ₂ H ₅ COO	C ₂ H ₅ COO
13	I	C ₆ H ₁₃	NH	O	H	OH	OH
14	I	H	O	O	OH	OH	OH
15	I	C ₈ H ₁₇	O	Cl	H	OH	OH
16	I	I	O	O	H	OH	OH
17	I	C ₆ H ₁₃	O	O	OH	OH	OH
18	II	F	O				
19	II	C ₃ H ₇	S				
20	II	C ₄ H ₉	S				
21	III	C ₆ H ₁₃					

derivatives to the cell culture. The activity was expressed as the percentage relative to the control.

B. Measurement of [^3H]-thymidine incorporation into DNA. The ethanol was removed from the cell pellet sediment under nitrogen flow and the pellet was extracted twice with 1 ml 0.5 N perchloric acid at 80°C. After centrifugation of the pooled extracts the UV absorbance of the supernatant was measured at 260 nm in order to determine the DNA content of cells [23]. The radioactivity of the so obtained hot perchloric acid extracts (i.e. the hydrolysed nucleic acids) was also measured in a toluene-based cocktail using a Beckman LS 100C scintillation counter. The incorporated [^3H]-thymidine was expressed as the percentage of the measured radioactivity relative to the control. Each determination was run in triplicate.

Principal component analysis [24] (PCA) was applied for the evaluation of the effect of nucleoside derivatives on 3LL cells obtained at the three concentrations and the two types of activity data referring to the ethanol soluble (A) and hot perchloric acid soluble (B) fractions, respectively. A point was considered to be zero when the measured radioactivity value did not differ from the control at the 95% significance level. For a better understanding of the results of the principal component analysis, the nonlinear map of the principal component loadings and variables was also calculated [25]. The calculation was run to 99.9% variance explained (i.e. as many principal components were calculated as needed to explain more than 99.9% of the inherent variance of the original data matrix). For establishing quantitative relationships between the activity data and the chromatographically measured physico-chemical parameters of the compounds stepwise linear regression analysis was used. The DrugideaTM program package (Chemicro Ltd, Budapest, Hungary) was run on an IBM AT compatible personal computer.

Results and Discussion

The measure of the [^3H]-thymidine cellular uptake reflects the effect of the nucleoside derivatives on the transport of the thymidine. When it is higher than 100%, it means that the derivatives did not inhibit the cellular uptake but inhibit the DNA incorporation and/or

promote the DNA degradation process. When it is lower than 100%, it means that the applied derivative inhibits the cellular uptake and/or promotes the DNA incorporation of thymidine.

When the DNA incorporation of [^3H]-thymidine is higher than 100% in the presence of a modified nucleoside, it means that it promoted the DNA incorporation and/or it inhibited the DNA degradation process.

The compounds generally inhibited the [^3H]-thymidine uptake and incorporation, however in some cases stimulation was also observed as it can be seen in Figs 2 and 3, respectively. The concentration dependence of the effect was investigated by using stepwise regression analysis for each derivative. The parabolic, logarithmic and reciprocal functions of the concentration on the radioactivity have been fitted. The concentration dependence of the

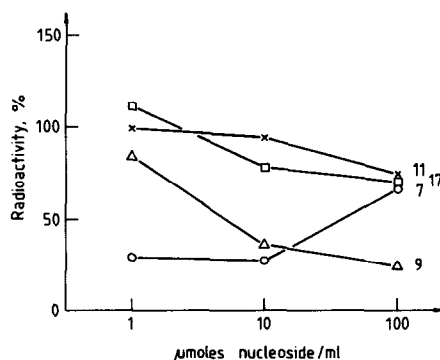


Figure 2
Effect of some synthetic nucleosides on the cellular uptake of [^3H]-thymidine obtained from the radioactivity in the low molecular weight pool fractions of 3LL cells as a function of their concentration (method A). Numbers refer to the compounds in Table 1.

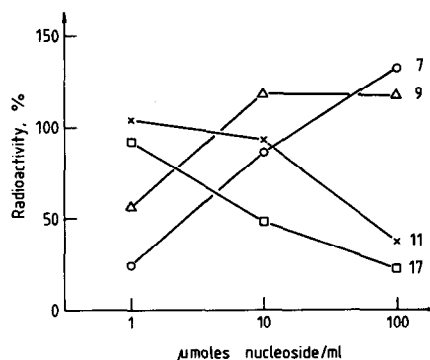


Figure 3
Effect of some synthetic nucleosides on [^3H]-thymidine incorporation into the DNA of 3LL cells as a function of the concentration (method B). Numbers refer to the compounds in Table 1.

Table 2

The results of the principal component analysis obtained on the activity data of nucleoside derivatives on the [^3H]-thymidine cellular uptake and DNA incorporation by 3LL cells

Serial number of principal component			Eigenvalue	Explained variance (%)		
PC 1			2.56	42.70		
PC 2			1.46	24.36		
PC 3			0.86	14.25		
PC 4			0.67	11.13		

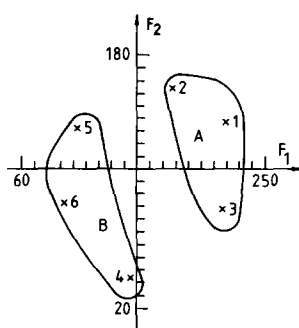
Activity on	Conc. ($\mu\text{mol ml}^{-1}$)	Variables	Principal component loadings			
			PC 1	PC 2	PC 3	PC 4
Uptake	1	1	0.85	-0.38	-0.16	-0.11
Method A	10	2	0.83	-0.40	-0.14	0.16
	100	3	0.72	0.23	0.14	-0.60
DNA incorporation	1	4	0.17	0.93	0.01	-0.06
Method B	10	5	0.59	0.49	-0.45	0.38
	100	6	0.53	0.09	0.77	0.34

activity however, could not be described by any of the functions. Therefore principal component analysis was carried out on the data. The results are compiled in Table 2. The first two principal components account for about 67% of the total variance. This means that two background variables describe the majority of the information content of the six types of activity data (cellular uptake and DNA incorporation each at three concentrations). It must be emphasized that the two hypothetical variables need not have any concrete biochemical meaning. The calculation only proves their mathematical possibility. The first principal component had the highest loading in the cellular uptake, while the second one had the highest loading at the DNA incorporation. The two-dimensional nonlinear map of PC loadings can be seen in Fig. 4. Two clusters can be

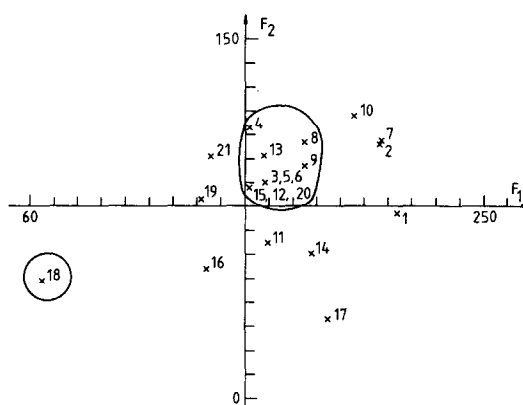
observed which refer to the cellular uptake and the DNA incorporation, respectively.

On the two-dimensional nonlinear map of the principal component variables (Fig. 5) the nucleosides do not form clusters. This finding implies that in the case of [^3H]-thymidine incorporation each substituent has a similar impact. 5-Fluoro-uracil (Compound 18) clearly differed from the other compounds. Considering only the group of 5-alkyl substituted deoxyuridine derivatives (Compounds 1–6), the 5-ethyl-deoxyuridine (Compound 1) diverged from the others. This is in agreement with our previous finding that the 5-ethyl-deoxyuridine derivative showed cytotoxic activity *in vitro* [9], but the structurally related compounds did not [10].

The chromatographically determined physico-chemical parameters of the derivatives

**Figure 4**

Two-dimensional nonlinear map of principal component loadings. Number of iterations was 35. Maximum error is 5.03×10^{-2} . 1, 2 and 3 represent the effects on the cellular uptake (method A) of the modified nucleosides at 1, 10 and 100 $\mu\text{mol ml}^{-1}$ concentrations. 4, 5 and 6 represent the effects on the DNA incorporation (method B) of the nucleosides at 1, 10 and 100 $\mu\text{mol ml}^{-1}$ concentrations.

**Figure 5**

Two-dimensional map of the principal component variables. Number of iterations was 190. Maximum error is 2.27×10^{-2} . The numbers refer to the compounds listed in Table 1.

Table 3

Chromatographically determined physico-chemical parameters of the nucleoside derivatives from ref. 14. R_{ma} = adsorption capacity, b_a = specific adsorption surface, R_{mo} = lipophilicity, b_l = specific hydrophobic surface

Serial no. of compounds	R_{ma}	b_a	R_{mo}	b_l
1	1.92	4.42	0.30	4.86
2	1.72	4.30	0.52	4.89
3	1.65	4.13	1.10	4.28
4	1.67	4.39	1.49	4.23
5	1.56	4.17	1.75	3.77
6	1.59	4.40	2.46	4.71
7	Not determined			
8	1.47	4.06	1.58	4.81
9	1.42	4.00	2.11	5.43
10	0.80	2.67	2.79	5.19
11	-0.16	3.17	3.47	5.83
12	-0.35	3.04	4.22	7.07
13	2.65	2.40	1.81	4.75
14	2.45	3.97	-0.56	3.76
15	-0.01	3.69	4.48	7.27
16	1.55	3.97	0.11	4.29
17	1.92	4.48	1.88	5.54
18	1.15	3.09	-0.64	2.80
19	-0.23	2.18	0.36	3.32
20	-0.32	2.47	0.78	3.12
21	1.30	3.96	1.60	3.63

Table 4

The results of the stepwise regression analysis between the activity data (the first two principal components) and the physico-chemical parameters of the nucleoside derivatives. Equation (1): $Y_1 = a + b_1.X_1 + b_2.X_2$; equation (2): $Y_2 = a + b_1.X_2 + b_2.X_3 + b_3.X_4$. Y_1 are the first principal component variables; Y_2 are the second principal component variables; X_1 are the specific hydrophobic surface areas; X_2 are the specific adsorption surface areas; X_3 are the quadrates of the adsorption capacity values; X_4 are the lipophilicity values

Parameter	Equation (1)	Equation (2)
a	25.33	-35.12
b_1	-2.33	16.48
s_{b1}	± 0.69	± 2.82
Path coeff. %	43.36	32.94
b_2	-63.01	-9.40
s_{b2}	± 14.29	± 1.22
Path coeff. %	56.64	47.60
b_3	—	-5.18
s_{b3}	—	± 1.56
Path coeff. %	—	19.46
r^2	0.6157	0.8202
$F_{calc.}$	13.62	24.33
$F_{99, 9\%}$	10.97	9.34

s = standard deviation; r = multiple regression coefficient; F = Fisher-test value.

[14] are listed in Table 3. Stepwise regression analysis was applied to reveal the quantitative relationships between the first two principal components and the molecular properties of the derivatives. The parameters of the obtained regression equations are compiled in

Table 4. About 62% of the variance of the first principal component variables could be explained by the specific hydrophobic and adsorption surface areas of the compounds. The larger were the specific hydrophobic and adsorption surface areas the greater extent the compounds inhibited the [3H]-thymidine cellular uptake. The relatively low regression coefficient (but it is significant at 99.9% level) can be explained by the complex nature of the cellular uptake. The effect of the compounds on the DNA incorporation (i.e. the second principal component) could be described by the optimum function of the specific adsorption capacity, the specific adsorption surface area and the lipophilic character of the compounds. Taken together, the equations suggest how the maximum effect can be obtained with optimum hydrophobic and hydrophilic properties and optimum adsorption capacity of the compounds.

In conclusion, the first principal component obtained from the measured activity data could be attributed to the change of [3H]-thymidine cellular uptake caused by the nucleoside, while the second principal component could be regarded as the measure of the effect on the DNA incorporation of [3H]-thymidine. The effect of the nucleosides on the [3H]-thymidine uptake could be explained by the specific hydrophobic and adsorption surface area of the nucleoside. On the other hand the effect on the DNA incorporation could be described by the adsorption characteristics (specific adsorption surface area and adsorption capacity) of the derivatives.

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