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Interaction of taxol and other anticancer drugs with α-cyclodextrin*

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Abstract: The interaction between 23 anticancer drugs and α-cyclodextrin (α-CD) was studied by reversed-phase charge-transrer thin-layer chromatography and the relative strength of interaction was calculated. As α-CD has smaller cavity than β- and τ-CD it interacted only with 10 anticancer drugs proving the relatively poor complex forming capacity of α-CD. The hydrophobicity of host–guest inclusion complex was always different from that of the uncomplexed drug suggesting that the complex formation may influence the uptake, absorption, half-life etc. of the original drug. the inclusion forming capacity of drugs differed considerably according to their chemical structure. The intensity of interaction significantly depended on the hydrophobicity of the guest molecule proving the preponderant role of hydrophobic interactions in inclusion complex formation.

Keywords: *α-cyclodextrin; anticancer drugs; charge-transfer chromatography.*

Introduction

Taxol, a promising anticancer drug was isolated from the bark of various Taxus species such as *Taxus baccata* L *[1], Taxus brevifolia* [2], *Taxus cuspidata* [3] etc. Taxol has been successfully used for the treatment of metastatic breast cancer [4] and ovarian carcinomas [5–7]. Taxol exhibits toxic side effects too such as anaphylactoid reactions, leukopenia, peripherial neuropathy and oropharyngeal mucositis [8]. Due to its high hydrophobicity [9] and the fact that the administration of taxol presents considerable difficulties [10] much efforces have been devoted to the development of less hydrophobic semisynthetic taxol derivatives with better application parameters [11].

Cyclodextrins (CDs) are cyclic oligosaccharides which have the ability to form inclusion complexes with many organic and inorganic compounds of various chemical structures [12, 13]. CDs readily form inclusion complexes with many drugs such as steroids [14, 15], antimycotic agents [16], insulin [17, 18], anticancer drugs [19] etc. The inclusion complex formation modifies the physicochemical characteristics of guest molecules, it improves the performance of intravenous formulation [20], prolongs the pulmonary absorption of sulbutanol [21], sustains the release rate of drugs [22], increases the stability of the guest molecule [23], enhances the peak concentration of several drugs in blood [24], and improves bioavailability [25].

Charge-transfer reversed-phase thin-layer chromatography has been frequently applied to study molecular interactions [26]. This method was used to study the inclusion complex formation of barbituric acid derivatives with crosslinked water-soluble βCD polymer [27] and with hydroxypropyl-β-cyclodextrin [28].

The objectives of this work were to study the interaction of taxol and other anticancer drugs with α-cyclodextrin (α-CD) by means of charge transfer chromatography, to compare their inclusion forming capacity and to elucidate the role of molecular parameters in the inclusion complex formation.

Experimental

Polygram UV_{254} (Macherey-Nagel, Dürren, Germany) plates were impregnated by overnight predevelopment in *n*-hexane–paraffin oil 95:5 (v/v). The IUPAC and common names of

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Table 1
IUPAC and common names of anticancer drugs

Number	Common name	IUPAC name	Provenience
1	Ftorafur	N-(2-furanidyl)-5-fluorouracil	Medexport (Russia)
2	Bicnu	N,N-bis(2-chloroethyl)-N-nitrosourea	Laboratoire BRISTOL (France)
3	Leukeran	4-[bis(2-chloroethyl)amino]benzenebutanic acid	Wellcome Foundation Ltd (UK)
4	Vincristin	22-oxo-(3α,14β,16α)-14,15-dihydro-14-hydroxy-eburnamenine-14-carbocyclic acid methyl ester	Richter Gedeon Ltd (Hungary)
5	Vinblastine	(3α,14β,16α-14,15-dihydro-14-hydroxyeburnamenine-14-carbocyclic acid methyl ester	Richter Gedeon Ltd (Hungary)
6	Vumon	4-O-demethyl-1-O(4,6-O-2-thenylidene-β-D-glucopyranosyl)epipodophyllotoxin	Bristol–Arzneimittel (Germany)
7	Provera	17-α-acetoxy-6-α-(methyl)progesterone	Upjohn Limited (UK)
8	Bleogin	N^1-[3-dimethyl(sulfonio)propyl]bleomycin amide	Nippon Kayaku (Japan)
9	Paraplatin	9,11,15-trihydroxy-15-methylprosta-5-13-dienoic acid	Bristol–Arzneimettel (Germany)
10	Zitazonium	2-(4-(2-chloro-1,2-difenilethynile)fenoxi]-N,N-diethyl-ethamine-citrat	EGIS Pharm. Works (Hungary)
11	Farmorubicin	(8S-*cis*)-10-[(3-amino-2,3,6-trideoxy-α-L-arabino-hexopyranosyl)oxy]-7,8,9,10-tetra-hydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione	Farmitalia (Italy)
12	Adriblastine (Doxorubicine)	10-(3-(amino-2,3,6-trideoxy-α-L-hexapyranosyl)oxy]-7,8,9-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5-12-naphthacene-dione	Farmitalia (Italy)
13	Natulan	N-(1-methylethyl)4-((2-methylhydrazino)methyl]-benzamide	Roche (Switzerland)
14	Alexan	4-amino-1-β-D-arabifuranosyl-2(14)-pyrimidine	Mack (Germany)
15	Mitomycin C Kyowa	[1-aR]-6-amino-8-[(aminocarbonyl)oxymethyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-azirino-[2′,3′:3,4]pyrrolo[1,1a]indole-4,7-dione	Kyowa (Japan)
16	Cytoxan	2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate	Bristol–Myers (Germany)
17	Estracyt	Estra-1,3,5-(10)-triene-3,17-diol-3-[bis-chloroethyl)carbamate	Aktiebolaget (Sweden)
18	Deticene	5-(3,3-dimethyl-1-triazenyl)-1-H-imidazole-4-carboxamide	Rhone–Poulenc (France)
19	Metotrexate	2,4,-diamino-10-methyl-pteroylglutamic acid	Lachema (Czech Republic)
20	Myelobromol	1,6-dibrom-1,6-bis(dezoxy)-D-mannit	Chinoin (Hungary)
21	Zitostop	1,2,5,6-tetramezil-D-mannit	EGIS Pharm. Works (Hungary)
22	Elobromol	1,6-dibrom-1,6-bis(dezosy)-D-dulcit	Chinoin (Hungary)
23	Taxol	[2aR-[2aα,4β,6β,9α(aR*,βS*),11α,-12α,12aα,12bα]]-β-(Benzoylamino)-α-hydroxybenzenepropanoic acid 6,12b-bis(acetyoxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,-13 tetramethyl-5-oxo-7,11-methano-14-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester	Sigma Chemie GmbH (Germany)

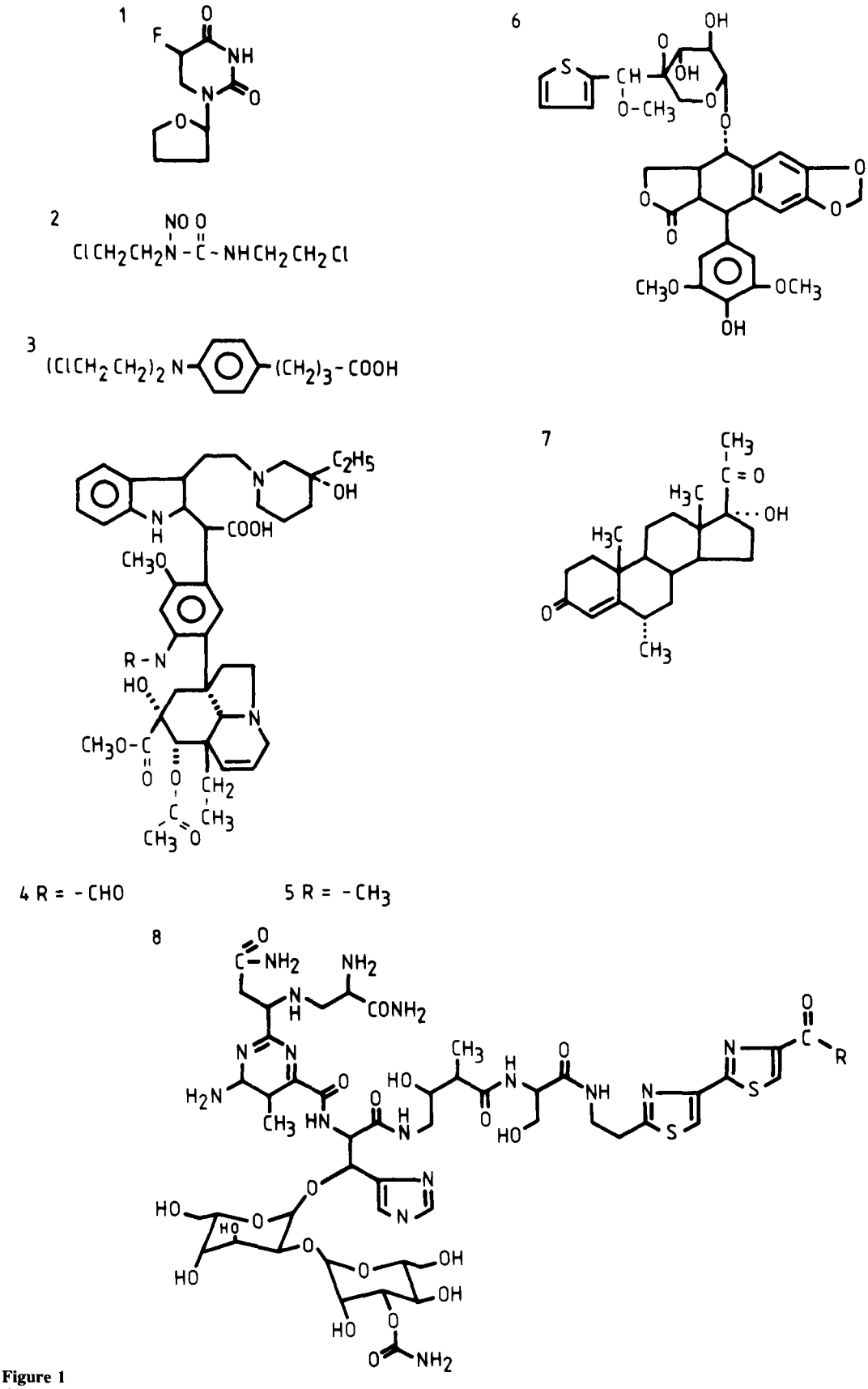

Figure 1
Chemical structures of anticancer drugs.

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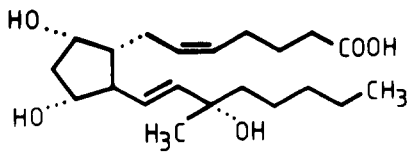

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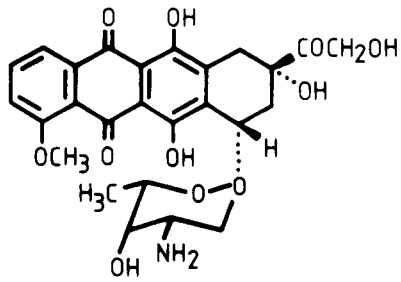

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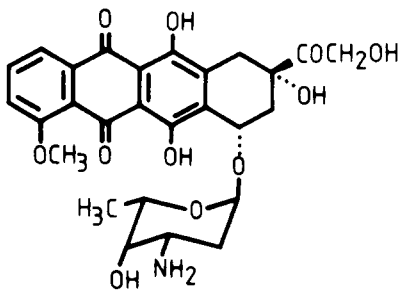

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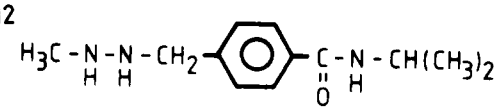

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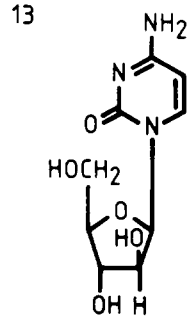

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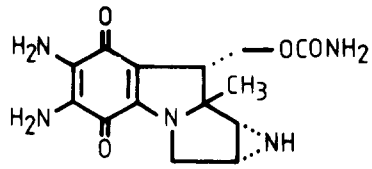

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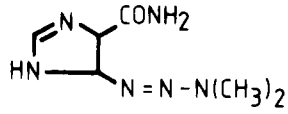

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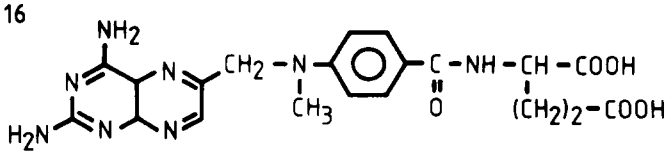

Figure 1
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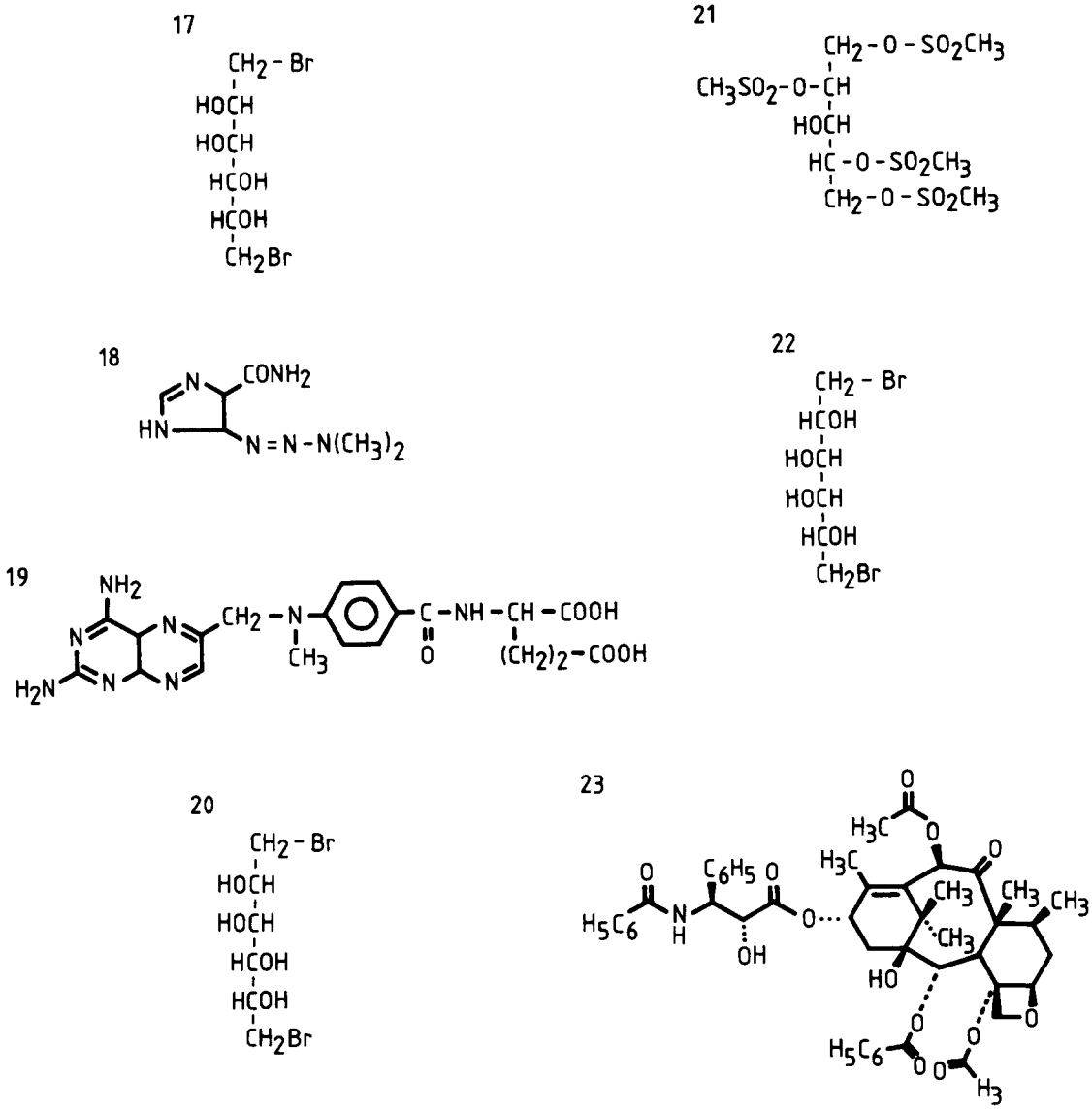

Figure 1
Continued.

anticancer drugs as well as their chemical structures are shown in Table 1 and in Fig. 1, respectively. The drugs were separately dissolved in methanol at a concentration of 3 mg ml^{-1} and 2 μl of the solutions were plotted on the plates. To study the inclusion complex formation of the anticancer drugs α-CD (CYCLOLAB Research and Development Laboratory, Budapest, Hungary) was added to the eluents at 0, 20 and 40 mg ml^{-1} concentrations. Water–methanol mixtures were used as eluents, the methanol concentration ranging from 0 to 70 vol.%. As the object was to study the complex formation between the solutes and α-CD and not the study of the effect of α-CD on the separation of solutes, they were separately spotted on the plates. In this way the ratio α-CD:solute was the same for each compound. Methanol was chosen as the organic solvent miscible with water because it forms only weak inclusion complexes with cyclodextrins [29, 30]. The application of this wide range of methanol concentration was motivated by the highly different hydrophobicity of anticancer drugs. Developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, the distance of development being about 16 cm. After development the plates were dried at 105°C and the spots of anticancer drugs were revealed

by their visible and UV spectra, by iodine vapour and by phosphormolibdenic acid reagent. Each experiment was run in quadruplicate.

The R_M value characterizing the molecular hydrophobicity in reversed-phase thin-layer chromatography was calculated for each drug in each eluent from

$$R_M = \log(1/R_f - 1), \quad (1)$$

where R_f is the distance of the solute from the start divided by the distance of the eluent front from the start. When the coefficient of variation of the parallel determinations was higher than 8% the R_M value was omitted from the following calculations.

To separate the effects of methanol and α-CD on the hydrophobicity of anticancer drugs the following equation was fitted to the experimental data

$$R_M = R_{M0} + b_1.C_1 + b_2.C_2, \quad (2)$$

where R_M = hydrophobicity (R_M) value for a drug determined at given methanol and α-CD concentrations; R_{M0} = R_M value extrapolated to zero methanol and α-CD concentrations (considered as the best estimation of the molecular hydrophobicity); b_1 = decrease in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of drugs) [31]; b_2 = decrease in the R_M value caused by 1 mg ml^{-1} concentration change of α-CD in the eluent (related to the relative strength of interaction); C_1 and C_2 = concentrations of methanol and α-CD, respectively. Equation 2 was applied separately for each anticancer drug.

To test the validity of the hypothesis that in the case of homologous series of solutes the slope (specific hydrophobic surface area, b_1) and intercept (hydrophobicity, R_{M0} in equation 2) are strongly intercorrelated [32, 33], linear correlation was calculated between the two physicochemical parameters

$$R_{M0} = A + B.b_1, \quad (3)$$

where A and B are the slope and intercept values of the equation without any concrete physicochemical meaning.

To find the physicochemical parameters of anticancer drugs significantly influencing their complex forming capacity stepwise regression analysis was applied [34]. The relative strength of interaction (b_2) was the dependent variable whereas the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of equation 2 were the independent variables, respectively. The number of accepted independent variables was not limited and the acceptance limit was set to the 95% significance level. In the common multivariate regression analysis the presence of independent variables exerting no significant influence on the change of dependent variable considerably decreases the significance level of the equation. Stepwise regression analysis eliminates from the selected equation the dependent variables having no significant impact on the dependent variable

Table 2
Parameters of linear correlations between the hydrophobicity (R_M) of anticancer drugs and the methanol (C_1) and α-cyclodextrin concentration (C_2) in the eluent. n = number of observations

	Compound no.							
Parameter	1	2	3	4	5	6	7	8
n	20	21	16	12	15	19	22	21
R_{M0}	0.31	1.07	1.97	2.23	2.26	1.77	3.76	1.31
$-b_1.10^{-2}$	1.54	2.05	1.75	2.69	2.79	1.94	5.22	1.35
$s_{b1}.10^{-3}$	1.09	1.04	3.22	4.23	3.47	2.95	1.97	2.39
$-b_2.10^{-2}$	0.52	—	0.84	—	—	−2.11	–	0.99
$s_{b2}.10^{-3}$	1.29	—	2.22	—	—	3.23	–	2.81
b'_1 (%)	77.55	—	59.09	—	—	50.15	–	61.61
b'_2 (%)	22.25	—	40.91	—	—	49.85	–	38.39
$F_{calc.}$	111.29	391.60	29.83	40.24	64.57	43.91	703.05	23.29
r^2	0.9291	0.9537	0.8211	0.8009	0.8324	0.8459	0.9723	0.7213

R_M = hydrophobicity (R_M) value for a drug determined at given methanol and α-CD concentrations; R_{M9} = R_M value extrapolated to zero methanol and α-CD concentrations (considered as the best estimation of the molecular hydrophobicity); b_1 = decrease in the R_M value caused by 1% increase in methanol concentration in the eluent (related to the specific hydrophobic surface area of drugs); b_2 = decrease in the R_M value caused by 1 mg ml^{-1} concentration

increasing in this manner the reliability of the calculation.

Results and Discussion

Compounds 4, 9 and 20–22 were near to the front in each eluent system and over the α-CD front that means that these drugs are highly hydrophilic and their interaction with α-CD cannot be determined under the used experimental conditions.

The R_M values of drugs decreased in each instance with increase in methanol concentration, i.e. these compounds do not show any anomalous retention behavior in this concentration range that would invalidate the evaluation using equation 2. In some instances an increase in α-CD concentration also caused a modification in R_M values, indicating the complex (probably inclusion complex) formation. This finding suggests that the biological properties (adsorption, uptake, half-life etc). of drug — α-CD complexes may be different from that of uncomplexed drug resulting in modified effectivity. We have to stress that the inclusion complex formation of drug not necessarily results in the modification of biological activities. It is only a possibility which has to be investigated for each drug — cyclodextrin pair.

The parameters of equation 2 are compiled in Table 2. Blank sites in Table 2 indicate that these independent variables did not influence significantly the R_M value of the anticancer drug. The equation fits the experimental data well, the significance levels in each instance being over 99.9% (see calculated F values). The ratios of variance explained were about 72–97% (see r^2 values). Some anticancer drugs interact with α-CD (b_2 values differ significantly from zero) that means that in pharmaceutical formulations containing both anticancer drugs and α-CD their possible interaction has to be taken into consideration. The parameters of equation 2 show high variations between the drugs proving that the hydrophobicity (R_{M0}), specific hydrophobic surface area (b_1) and their capacity to form inclusion complexes with α-CD (b_2) differ considerably. This finding suggests also that the inclusion complex formation may influence differently the biological effect of individual anticancer drugs. As the calculations proved taxol is a highly hydrophobic anticancer drug. This finding explains its low solubility in water and in various infusions. Taxol forms inclusion complex with α-CD indicating that the solubility of taxol can be modified by α-CD. The taxol — α-CD complex may have advantageous application parameters its elucidation needs further investigations. It is highly improbable that large molecules such as taxol can enter the relatively small cavity of α-CD. We assume that the various substructures of taxol and other large anticancer drugs can insert more or less deeply in the cavity of α-CD. This interaction results in the decrease of hydrophobicity.

Significant linear correlation was found between the intercept (hydrophobicity) and slope (specific hydrophobic surface area) values of anticancer drugs (Fig. 2). This finding indicates that from a chromatographic point of view these drugs behave as a homologous

Compound no.									
10	11	12	13	15	16	17	18	19	23
19	16	15	16	20	21	19	21	19	16
3.67	2.16	2.24	1.47	1.20	1.29	5.20	0.86	0.99	4.00
4.71	3.06	3.10	3.33	2.92	2.51	7.29	1.73	3.55	6.05
4.28	3.68	3.50	3.01	2.18	1.02	5.29	1.71	3.22	7.27
−0.61	—	—	1.02	0.72	0.64	—	0.80	—	−1.11
2.42	—	—	2.07	2.58	1.20	—	2.01	—	4.32
81.48	—	—	69.29	82.82	82.17	—	71.79	—	76.34
18.52	—	—	30.71	17.18	17.83	—	28.21	—	23.66
65.50	69.21	78.77	95.16	96.74	323.63	190.16	61.85	121.82	86.23
0.8912	0.8317	0.8583	0.9361	0.9192	0.9729	0.9179	0.8730	0.8775	0.9299

change of α-CD in the eluent (related to the relative strength of interaction); b' = dimensionless numbers indicating the relative impact of the independent variable on the dependent variable; $F_{calc.}$ = calculated F value relating to the fitness of equation to the experimental results; r^2 = ratio of variance explained by the independent variables.

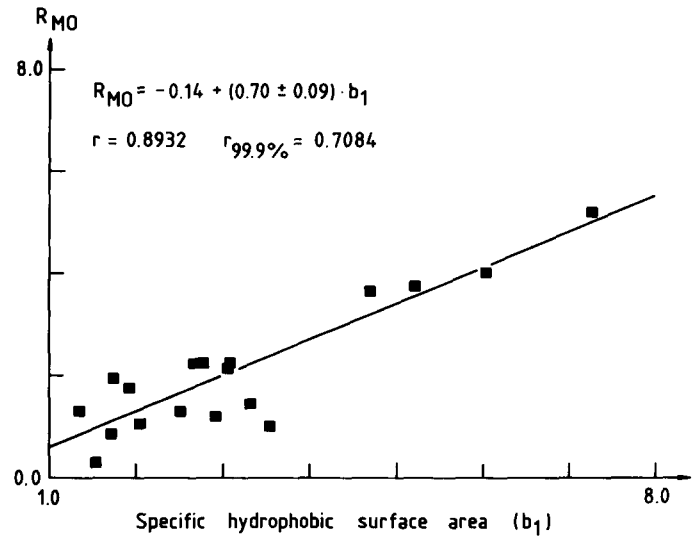

Figure 2
Relationship between the hydrophobicity (R_{M0}) and specific hydrophobic surface area (b_1) of anticancer drugs.

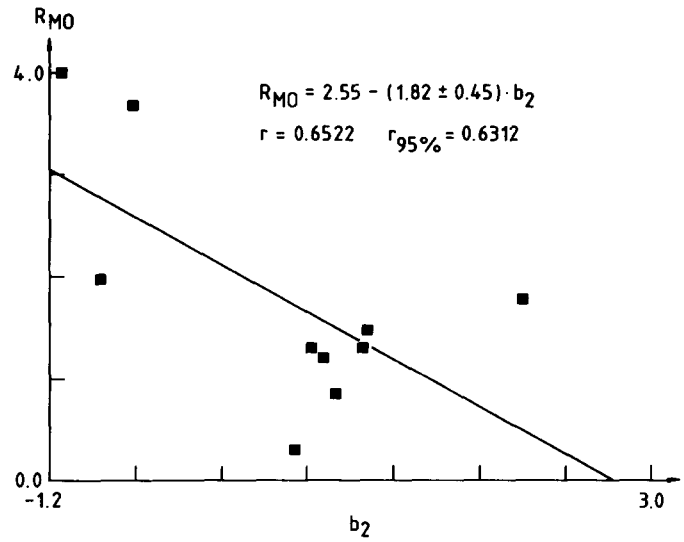

Figure 3
Relationship between the hydrophobicity (R_{M0}) and complex forming capacity (b_2) of anticancer drugs.

series of compounds, although their chemical structures are considerably different.

Significant linear relationship was found between the hydrophobicity (R_{M0}) and complex forming capacity of anticancer drugs (b_2) (Fig. 3) proving that hydrophobic forces are involved in the binding of these drugs to the inner wall of cyclodextrin cavity.

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