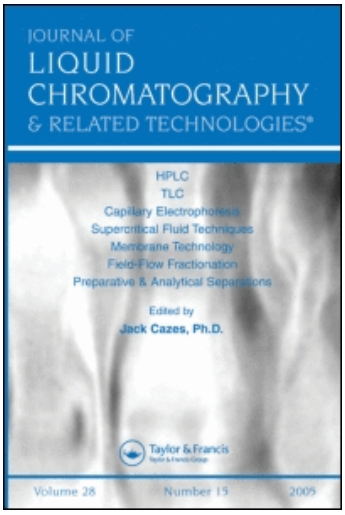



**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Structure-Activity Relationships of 2-Chloro-2'-*arabino*-fluoro-2'-deoxyadenosine and Related Analogues: Protein Binding, Lipophilicity, and Retention in Reversed-Phase LC

V. Reichelova[a]; J. Liliemark[a]; F. Albertioni[a]
[a] Department of Clinical Pharmacology Karolinska Hospital, Stockholm, Sweden

**To cite this Article** Reichelova, V. , Liliemark, J. and Albertioni, F.(1995) 'Structure-Activity Relationships of 2-Chloro-2'-*arabino*-fluoro-2'-deoxyadenosine and Related Analogues: Protein Binding, Lipophilicity, and Retention in Reversed-Phase LC', Journal of Liquid Chromatography & Related Technologies, 18: 6, 1123 — 1135
**To link to this Article: DOI:** 10.1080/10826079508009280
**URL:** http://dx.doi.org/10.1080/10826079508009280

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(6), 1123-1135 (1995)

# STRUCTURE-ACTIVITY RELATIONSHIPS OF 2-CHLORO-2'-*ARABINO*-FLUORO-2'-DEOXY-ADENOSINE AND RELATED ANALOGUES: PROTEIN BINDING, LIPOPHILICITY, AND RETENTION IN REVERSED-PHASE LC

**VIERA REICHELOVA, JAN LILIEMARK, AND FREIDOUN ALBERTIONI***
*Department of Clinical Pharmacology*
*Karolinska Hospital*
*S-171 76 Stockholm, Sweden*



## ABSTRACT

Plasma protein binding of 2-chloro-2'-*arabino*-fluoro-2'-deoxyadenosine (CAFDA), 2-chloro-2'-deoxyadenosine (CdA), 2-fluoro-1-β-D-arabinofuranosyladenine (F-araA) and structurally related analogues 2-chloro-adenosine (2-Cl-Ado), 5'-chloro-5'-deoxyadenosine (5'-Cl-5'-dAdo), as well as parent nucleosides 2'-deoxyadenosine (dAdo), 1-β-D-arabinofuranosyladenine (araA) and adenosine (Ado) was determined and correlated with lipophilicity expressed as the logarithm of partition coefficient in n-octanol/water system (log $P_{O/W}$). Drug binding to human serum albumin (HSA) was utilized since it is considered to exemplify nonspecific binding of small molecules to other macromolecules.

Percentage of drugs bound to HSA increased from 3.5 % to 27 % following the order of increase in lipophilicity (log $P_{O/W}$

* Corresponding author

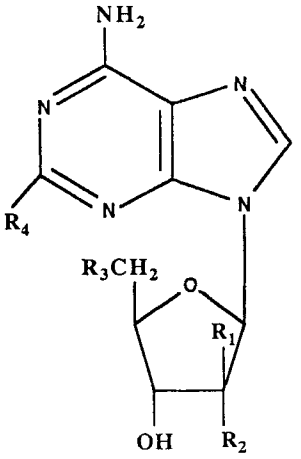
increased from -0.970 to 0.498). A similar correlation was observed when protein binding was correlated with retention in reversed-phase liquid chromatography (RP-LC) (capacity ratios, k', increased from 0.36 to 1.15), but the elution order of some compounds did not follow the parallel increase in either protein binding or lipophilicity.

The introduction of a fluorine at the 2'-arabino position of CdA not only increased the acid stability of CAFDA, but also resulted in a higher binding to HSA (27.0% for CAFDA versus 24.3% for CdA) and much higher lipophilicity (log P of 0.498 for CAFDA compared to 0.025 for CdA).

## INTRODUCTION

2-Chloro-2'-*arabino*-fluoro- 2'-deoxyadenosine (CAFDA) (Fig. 1) is an acid-stable 2'-*arabino*-fluoro-derivative of CdA (Cladribine, Leustatin), a newly developed anticancer drug currently in phase II trials in the treatment of lymphoprolipherative disorders. CdA is acid-labile and has only approximately 50 % oral bioavailability (1). The synthesis of CAFDA was based on the rationale that the introduction of a fluorine at 2'-arabino (up) position of 2',3'-dideoxyadenosine increased the chemical stability of the glycosidic bond of the compound to acidic or enzymatic hydrolysis (2, 3). This was confirmed by the study of Carson et al (4), where the in vitro antilymphocytic activity of CAFDA was demonstrated. The results suggested that CAFDA might substitute for CdA as an effective oral drug.

The importance of physico-chemical properties including ionization constants, $pK_a$, and lipophilicity for drug absorption has been widely recognized. The interaction of drugs with plasma proteins (i.e. human serum albumin (HSA), $\alpha_1$-acid -glycoprotein (AGP),...) may have important pharmacokinetic implications as regards drug disposition and action. Though it has long been thought that only the free drug in plasma was available for diffusion into tissues, recent studies have shown that a part of the drug bound in plasma can be dissociated in capillaries and thus becomes available for transfer (5).




| Compound | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Ado | H | OH | OH | H |
| 2-Cl-Ado | H | OH | OH | Cl |
| 5'-Cl-5'-dAdo | H | OH | Cl | H |
| dAdo | H | H | OH | H |
| CdA | H | H | OH | Cl |
| CAFDA | F | H | OH | Cl |
| ara A | OH | H | OH | H |
| F-ara A | OH | H | OH | F |

FIGURE 1. Structural formulae of studied compounds.



The goal of our study was to assess the relationship between protein binding and lipophilicity, as a prerequisite for drug absorption, disposition and also penetration into CNS, of CAFDA, clinically used CdA and F-araA, and structurally related analogues 2-Cl-Ado, 5'-Cl-5'-dAdo, as well as parent nucleosides dAdo, araA and Ado. The binding to HSA was correlated with lipophilicity expressed as the logarithm of partition coefficient (log $P_{O/W}$) in n-octanol/water system. The use of capacity ratios (k') in the reversed-phase LC to predict hydrophobicity of studied compounds was assessed and the correlation between retention behaviour and plasma protein binding was compared with the above mentioned.

## EXPERIMENTAL

### Materials

CdA was synthesized by Dr. Zygmunt Kazimierczuk (Foundation for the Development of Diagnostics and Therapy, Warsaw, Poland). CAFDA was a gift from Dr. Howard Cottam (University of California, San Diego, CA, USA) and F-araA was a gift from Dr. Ze've Shaked (Berlex, Alameda, CA, USA). The purity of CdA, CAFDA and F-araA was more than 99 % as checked by HPLC and mass spectrometry. The nucleosides Ado, dAdo, 2-Cl-Ado, araA and 5'-Cl-5'-dAdo, human serum albumin (99% purity) and 1-octanol were obtained from Sigma (St. Louis, MO, USA). Methanol was of HPLC grade (J.T.Baker, Deventer, Netherlands). Analytical-reagent grade potassium dihydrogen phosphate and potassium hydroxide were purchased from Merck (Darmstadt, Germany).



### Determination of protein binding

In vitro protein binding of the studied compounds was measured in triplicate by equilibrium dialysis, at 37°C, in the dark under continuous agitation for 6 hours. The apparatus consisted of Lucite cells using SPECTRA/POR molecular porous membranes (Spectrum Medical Industries Inc., LA, U.S.A.) (m.w. cut off 3500). Two hundred µl of 1 µmol/L solutions of drugs dissolved in either human serum albumin (40 g/L) or in human plasma (pooled from 6 healthy volunteers) were introduced on one side of the dialysis membrane and an equal volume of saline solution (pH 7.4) to the other side. To prevent the deamination during dialysis, the adenosine deaminase inhibitor, deoxycoformycin (dCF), was incubated with HSA or blank plasma samples for 30 min at 37°C in the concentration of 5 µmol/L before solutions of Ado, dAdo and araA

were added. At equilibrium, the volumes of both compartments were measured to assess the possible volume shift and the concentrations of studied compounds were determined from 100 µl in both compartments by reversed-phase LC. Samples from the buffer compartment were injected directly and those from the protein compartment were diluted 10 times before the analysis. Nonspecific binding to the filter was assessed by comparing the concentrations of the drugs in a saline buffer alone, before and after dialysis, and was consistently less than 2%. The bound fraction was calculated as $(C_1 - C_2)/C_1$, where $C_1$ is the concentration in the protein compartment and $C_2$ the concentration in the buffer compartment at the end of the dialysis.

## High performance liquid chromatography

The chromatographic system consisted of a Shimadzu LC-9A pump (Shimadzu Corp., Kyoto, Japan), a CMA-240 Carnegie autosampler (Carnegie Medicine, Stockholm, Sweden) and a Milton Roy variable wavelength detector (Milton Roy, LDC Division, USA). A Macintosh Classic computer (Apple Inc., Chicago, IL, USA) equipped with Chromac 3.1. software (Drew Ltd, London, UK) was used for collecting the HPLC data. The capacity factors k' were determined isocratically on a high speed $C_{18}$ column (80 x 4.6 mm, 3 mm, Perkin-Elmer, Norwalk, CT, USA) at 265 nm and 22°C. An aqueous mobile phase of 0.01 M $KH_2PO_4$ with 20 % of methanol, pH 7 at the flow-rate of 1 ml/min was used. The pH was adjusted with a few drops of potassium hydroxide before methanol was added using a PHM 62 standard pH meter (Radiometer, Copenhagen, Denmark). The k' values were calculated as $(t_r-t_o)/t_o$, where $t_r$ is the retention time of an individual compound and $t_o$, the retention time of an unretained compound determined as the time from injection to the first distortion of the baseline.

Determination of $pK_a$ and lipophilicity (log P)

$pK_a$ was determined spectrophotometrically and lipophilicity by shake-flask method as described in our previous study (6).

## RESULTS AND DISCUSSION

The binding of the studied compounds to HSA and plasma proteins is reported in Table 1. The percentage of drugs bound to HSA and plasma proteins was in the range of 3.5% to 27% and 14% to 47.1%, respectively. A good correlation (polynomial equation, $y = 24.3 + 5.2*x - 5.2*x^2 + 10.8*x^3$, $r = 0.989$) was observed between binding to HSA and lipophilicity expressed as log P (Fig. 2), while no correlation was found between log P and the total plasma protein binding (Table 1). The spectrophotometrically determined $pK_a$ of CAFDA was higher than that of CdA (1.75 for CAFDA versus 1.28 for CdA).

We have recently reported on the effects of structural changes in the molecules of Ado, dAdo and araA on ionization constants ($pK_a$), lipophilicity (log P) and retention in RP-LC (log k') (6). It was confirmed that the introduction of a halogen atom (chlorine, fluorine) into a molecule increases the lipophilic character of the compound, with CdA as the most lipophilic in the studied group. There was a good linear correlation observed between log P and log k' and, in agreement with other studies, it was concluded that lipophilicity of new nucleoside analogues could be predicted from their retention in RP-LC. Thus, CAFDA, a new derivative of CdA, which was the most retained in RP-LC in the present study (Fig. 3), was supposed to be the most lipophilic from all studied structurally related purine analogues. This was confirmed by the log P value determined in n-octanol/water system (log P of 0.498).

The binding of CAFDA to HSA was higher than that of CdA (27.0% for CAFDA versus 24.3% for CdA) and it was the highest in the group of studied compounds. While binding to HSA followed the

TABLE 1

Lipophilicity, Protein Binding and Retention in RP-LC
of CAFDA and Related Nucleoside Analogues

| Compound 1 µmol/L | Log P | % B ± SD HSA | % B ± SD Plasma | Log k' |
|---|---|---|---|---|
| Ado + 5 µmol/L dCF | -0.970* | 3.5 ± 0.1 | 22.4 ± 1.2 | 0.48 |
| ara A + 5 µmol/L dCF | -0.955* | 6.6 ± 0.0 | - | 0.36 |
| F-ara A | -0.801* | 9.1 ± 0.8 | 14.0 ± 3.5 | 0.52 |
| dAdo + 5 µmol/L dCF | -0.611* | 18.9 ± 0.1 | 22.4 ± 3.2 | 0.58 |
| 2-ClAdo | -0.368* | 20.2 ± 6.0 | 30.1 ± 3.3 | 0.96 |
| 5'-Cl-5'-dAdo | -0.055* | 24.4 ± 3.1 | 22.4 ± 9.5 | 1.10 |
| CdA | 0.025* | 24.3 ± 1.2 | 21.1 ± 8.7 | 1.01 |
| CAFDA | 0.498 | 27.0 ± 2.0 | 47.1 ± 7.6 | 1.15 |

*Log P values were taken from Reichelova et al (Ref. 6), protein binding was determined using equilibrium dialysis and drug concentrations were analyzed on HPLC using $C_{18}$ column (80 x 4.6 mm, 3 µm), mobile phase, 0.01 M $KH_2PO_4$ with 20% MeOH pH=7.0, flow rate 1 ml/min, detection 265 nm
Results for % bound drugs are the mean (±SD) of 3 experiments

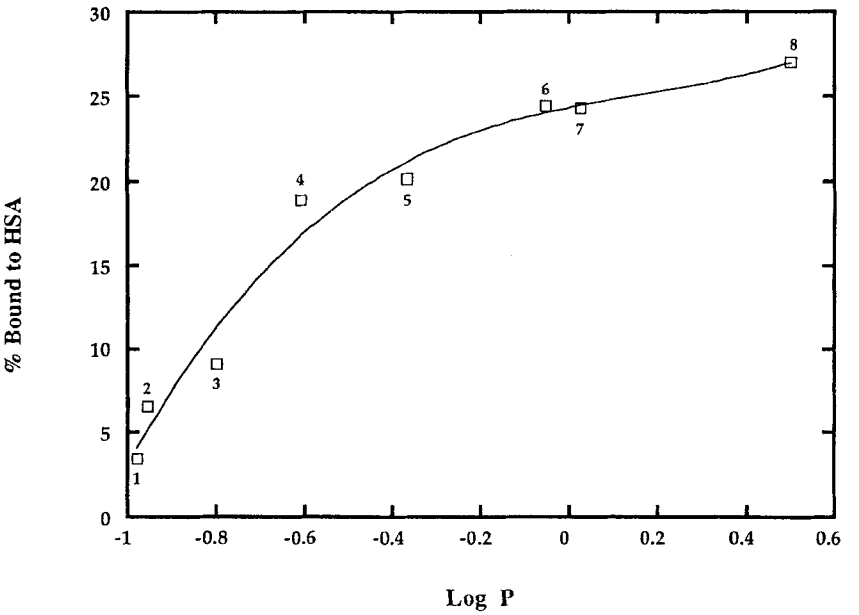



FIGURE 2. Correlation between percentage of drugs bound to HSA and logarithm of partition coefficients (log P) in n-octanol/water system. (1) Ado, (2) ara A, (3) F-ara A, (4) dAdo, (5) 2-ClAdo, (6) 5′-Cl-5′-dAdo, (7) CdA and (8) CAFDA.



order of increase in lipophilicity expressed as log P, it did not follow the elution order (increase in log k') so rigidly (Fig. 4). The explanation can be found in the differences of interactions of protein binding, partitioning and retention in RP-LC. Binding to HSA and liquid-liquid partitioning are both nonspecific processes, while retention in RP-LC is of a mixed character involving both partitioning and adsorptive interactions (7). Though HPLC has often been used for determination of the lipophilicity of drugs our results show that log P determined by a traditional liquid-liquid partitioning predicts the binding to HSA better than log k'.

In spite of a better correlation between the binding to HSA and the log P we would like to emphasize the importance of using

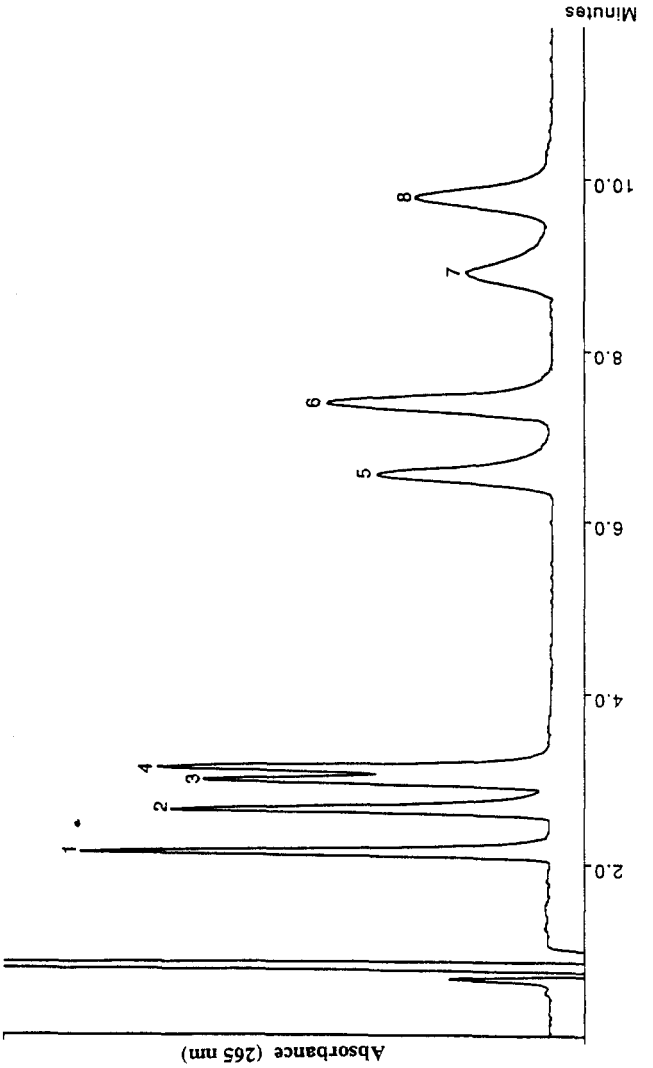



FIGURE 3. Reversed-phase chromatographic separation of (1) ara A, (2) Ado, (3) F-ara A, (4) dAdo, (5) 2-ClAdo, (6) CdA, (7) 5′Cl-5′-dAdo, and (8) CAFDA. Column, high-speed C18 (3 μm) (80 x 4.6 mm I. D.); mobile phase, 0.01 M $KH_2POH4$ (pH 7.0) with 20% of methanol; flow-rate, 1 ml/min; detection 265 nm.

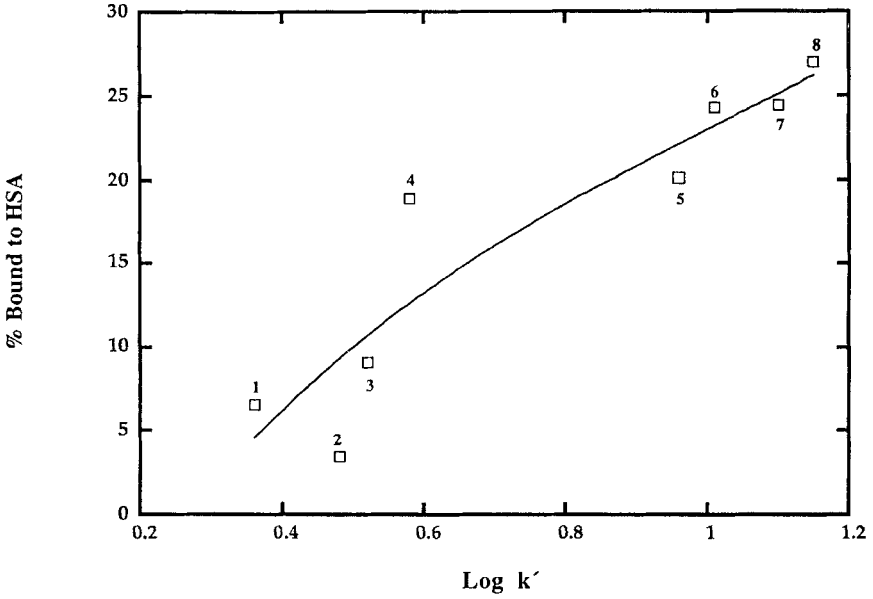



FIGURE 4. Correlation between percentage of drugs bound to HSA and logarithm of capacity factors in reversed-phase LC (log k') of (1) ara A, (2) Ado, (3) F-ara A, (4) dAdo, (5) 2-ClAdo, (6) CdA, (7) 5′Cl-5′-dAdo, and (8) CAFDA.



retention characteristics of drugs in RP-LC in order to predict the binding of metabolites and analogues. Our results lend support to the conclusion of a similar study of correlation between retention in RP-LC and plasma protein binding of betaxolol and its analogues (8). Although the retention behaviour may not always quantitatively predict the binding in plasma it can give a prediction of potential binding interactions. In this view, the reversed-phase LC is considered to be a useful tool for the estimation of both lipophilicity (log P) and protein binding. It is simple and easy to use and only small amounts of drugs are required compared to equilibrium dialysis and shake-flask method.

No correlation was observed between log P and total plasma protein binding. The composition of plasma and the binding

mechanisms may explain this lack of correlation. Human plasma contains over 60 proteins with HSA and AGP as the most abundant and most studied. While the hydrophobic interaction governs the binding to HSA, the involvement of electrostatic interactions in the binding to AGP must be considered (9).

The importance of plasma protein binding and lipophilicity as determinants of transport across the blood-brain barrier was confirmed by a study of brain uptake of benzodiazepines (10). The relationship between lipophilicity and protein binding, as well as its effects on anti-HIV activity was recently reported in a series of anti-HIV agents (11). Again, lipophilicity was regarded an important factor which may affect the entry of the compounds into the CNS. In this respect, the higher lipophilicity of CAFDA compared to CdA makes us assume a good penetration of CAFDA into CNS since CdA itself was shown to penetrate the blood-brain barrier (12, 13).

Recently, in addition to deoxycytidine kinase (dCK), another enzyme, deoxyguanosine kinase (dGK), was observed to contribute to CdA phosphorylation in crude extracts of malignant human brain tissue (14). In the view of a similar metabolism of CAFDA and CdA our results are of a clinical importance not only for the oral administration, but also in the case if the therapeutic role of CdA in the treatment of brain malignancies is proved.



Acknowledgements

This work was supported by grants from Swedish Children Cancer Foundation, Swedish Cancer Foundation, Jenny Foundation and The Swedish Association for Cancer and Trafic Victims.

## REFERENCES

1. J. Liliemark, F. Albertioni, M. Hassan and G. Juliusson, On the bioavailability of oral and subcutaneous 2-chloro-2'-deoxyadenosine in humans: alternative routes of administration. J. Clin. Oncol., 10: 1514-8, 1992.

2. V. E. Marquez, C. T.-H. Tseng, J. A. Kelley, H. Mitsuya, S. Broder, J. S. Roth and J. S. Driscoll, Rapid Communications. 2',3'-Dideoxy-2'-Fluoro-AraA. An Acid-Stable Purine Nucleoside Active against Human Immunodefficiency Virus (HIV). Bioch. Pharmacol., 36: 2719-2722, 1987.

3. V. E. Marquez, C. T.-H. Tseng, H. Mitsuya, S. Aoki, J. A. Kelley, H. Ford, J. S. Roth, S. Broder, D. G. Johns and J. S. Driscoll, Acid-Stable 2'-Fluoro Purine Dideoxynucleosides as Active Agents against HIV. J. Med. Chem., 33: 978-985, 1990.

4. D. A. Carson, D. B. Wasson, L. M. Esparza, C. J. Carrera, T. J. Kipps and H. B. Cottam, Oral antilymphocyte activity and induction of apoptosis by 2-chloro-2'-arabino-fluoro-2'-deoxyadenosine. Proc. Natl. Acad. Sci. USA., 89: 2970-2974, 1992.

5. F. Herve, S. Urien, E. Albengres and J.-C. Duche, Drug Binding in Plasma. A Summary of Recent Trends in the Study of Drug and Hormone Binding. Clin. Pharmacokinet., 26: 44-58, 1994.

6. V. Reichelova, F. Albertioni and J. Liliemark, Hydrophobicity Parameters of 2-Chloro-2'-Deoxyadenosine and Some Related Analogues and Retention in Reversed-Phase Liquid Chromatography. J. Chromatogr., 667: 37-45, 1994.

7. D. J. Minick, J. J. Sabatka and D. A. Brent, Quantitative Structure-Activity Relationships using Hydrophobicity Constants measured by High-Pressure Liquid Chromatography: A Comparison with Octanol-Water Partition Coefficients. J. Liq. Chromatogr., 10: 2565-2589, 1987.

8. J. Ganansia, G. Bianchetti and J. P. Thenot, Correlation Between Reversed-Phase High-Performance Liquid Chromatography and Plasma Protein Binding. J. Chromatogr., 421: 83-90, 1987.

9. T. Maruyama, M. A. Furuie, S. Hibino and M. Otagari, Comparative Study of Interaction Mode of Diazepines with Human Serum Albumin and -Acid Glycoprotein. J. Pharm. Sciences., 81: 16-20, 1992.

10. D. R. Jones, S. D. Hall, E. K. Jackson, R. A. Branch and G. R. Wilkinson, Brain Uptake of Benzodiazepines: Effects of

Lipophilicity and Plasma Protein Binding. J. Pharm. Exp. Therapeutics., 245: 816-822, 1988.

11. M. Baba, S. Yuasa, T. Niwa, M. Yamamoto, S. Yabuuchi, H. Takashima, M. Ubasawa, H. Tanaka, T. Miyasaka, R. T. Walker, J. Balzarini, E. De Clerq and S. Shigeta, Effect of Human Serum on the *In Vitro* Anti-HIV-1 Activity of 1-[(2-Hydroxyethoxy)Methyl]-6-(Phenylthio)Thymine (HEPT) Derivatives as Related to Their Lipophilicity and Serum Protein Binding. Bioch. Pharmacol., 45: 2507-2512, 1993.

12. A. Saven, H. Kawasaki, C. J. Carrera, T. Waltz, B. Copeland, J. Zyroff, M. Kosty, D. A. Carson, E. Beutler and L. D. Piro, 2-Chlorodeoxyadenosine Dose Escalation in Nonhematologic Malignancies. J. Clin. Oncol., 11: 671-678, 1993.

13. J. Liliemark and G. Juliusson, On the Pharmacokinetics of 2-Chloro-2'-Deoxyadenosine (CdA) in Cerebrospinal Fluid (CSF). Blood., 80: 471a, 1992.

14. L. Wang, A. Karlsson, T. Mathiesen and S. Eriksson, 2-Chloro-2'-Deoxyadenosine Phosphorylation by Deoxyadenosine Kinase in Crude Extracts of Malignant Human Brain Tissue. In: Recent Advances in Chemotherapy, 18th International Congress of Chemotherapy, Sweden, 1993, J. Einhorn, C. E. Nord and S. R. Norrby, eds., American Society for Microbiology, 1994, 919-921.



Received: August 16, 1994
Accepted: November 17, 1994