dose that causes death in 50% of the test animals within 24 h.

Phase V testing measured the ability of the test compounds to provide protection against seizures induced by ip injection of convulsant doses (CD_{97}) of the following convulsants: bicuculline (2.70 mg/kg), picrotoxin (3.15 mg/kg), and strychnine (1.20 mg/kg). ED_{50} values were determined as in phase II at the time of peak effect.

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Registry No. **3**, 72-44-6; **6a**, 73283-07-5; **6b**, 73283-08-6; **6c**, 73283-14-4; **6d**, 73283-15-5; **6e**, 73283-16-6; **6f**, 73283-17-7; **6g**, 73283-19-9; **6h**, 73283-18-8; **6i**, 73283-12-2; **6j**, 73283-09-7; **6k**, 73283-10-0; **6l**, 73283-11-1; **6m**, 56232-60-1; **7a**, 2385-23-1; **7b**, 22316-59-2; **7c**, 340-57-8; **7d**, 340-94-3; **7e**, 4260-20-2; **7f**, 1788-95-0; **7g**, 25509-06-2; **7h**, 1897-87-6; **7i**, 4260-28-0; **7j**, 35289-03-3; **8a**, 73283-25-7; **8b**, 73283-26-8; **8c**, 73283-27-9; **8d**, 73283-29-1; **8e**, 73283-30-4; **8f**, 73283-31-5; **8g**, 73283-21-3; **8h**, 73283-22-4; **8i**, 73283-23-5; **8j**, 123382-21-8; **8k**, 123382-22-9; **8l**, 73283-33-7; **8m**, 73283-34-8; **8n**, 73283-35-9; **8o**, 123382-23-0; **8p**, 123382-24-1; **8q**, 123382-25-2; **8r**, 123382-26-3; CH_3CO_2H, 64-19-7; CF_3CO_2H, 76-05-1; $C_6H_5CO_2H$, 65-85-0; *p*-$ClC_6H_4CO_2H$, 74-11-3; *p*-$MeOC_6H_4CO_2H$, 100-09-4; 3,4,5-$(MeO)_3C_6H_2CO_2H$, 118-41-2; *p*-$(CH_3CONH)C_6H_4CO_2H$, 556-08-1; *N*-acetylanthranilic acid, 89-52-1; *o*-toluidine, 95-53-4; aniline, 62-53-3; *p*-toluidine, 106-49-0; *o*-chloroaniline, 95-51-2; *m*-chloroaniline, 108-42-9; *o*-bromoaniline, 615-36-1; *p*-bromoaniline, 106-40-1; 2,6-dichloroaniline, 608-31-1; *o*-fluoroaniline, 348-54-9; *o*-anisidine, 90-04-0; *o*-iodoaniline, 615-43-0; 1-adamantanecarboxylic acid, 828-51-3; 2-pyridinecarboxylic acid, 98-98-6; 3-pyridinecarboxylic acid, 59-67-6; 4-pyridinecarboxylic acid, 55-22-1; ethyl 2-pyridinecarboxylate, 2524-52-9; ethyl 3-pyridinecarboxylate, 614-18-6; ethyl 4-pyridinecarboxylate, 1570-45-2.

Supplementary Material Available. Complete anticonvulsant and toxicity screening data for all compounds submitted to the National Institute of Health's Antiepileptic Drug Development (ADD) Program protocol is available from the authors.

Structure–Activity Relationship of Anthracyclines in Vitro

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The cytotoxic activities of several natural and semisynthetic anthracyclines against L1210 leukemia and two human colon tumor cells (Colon 4, HT 29) in vitro were examined after short (1 h) and long (7 days) incubation times and correlated with the water/octanol partition coefficients and the DNA-binding affinity of the compounds. Analysis of equation in which cytotoxicity against L1210 (1-h incubation) was parabolically related to the partition coefficient revealed an almost exclusive correlation ($r = 0.80$) between the cytotoxicity and the parameters, and this correlation was only slightly improved by addition of DNA-binding affinity ($r = 0.85$). On the other hand, cytotoxic activities displayed after continuous incubation were partially related to both partition coefficients (parabolic dependence) and DNA-binding affinities (linear dependence). In this case the correlation between the activity and partition coefficient ($r = 0.67$) was significantly improved by addition of DNA-binding affinity ($r = 0.90$). Similar results were also obtained for human colon tumor cells although the corresponding correlation coefficients were generally of lower value, indicating that cytotoxic activity of anthracyclines against these primary resistant cells may be influenced by additional factors not yet determined.

Anthracyclines, especially doxorubicin (adriamycin) and daunorubicin, are of high value in today's cancer chemotherapy, showing activity against some types of leukemia, lymphoma, and soft-tissue carcinoma and also, to a lesser extent, against breast and lung cancer.[1] However, because of the dose-limiting cumulative cardiotoxicity of these compounds and their lack of activity against various kinds of tumors, especially those of the gastrointestinal tract, several anthracycline derivatives have been developed in order to overcome these drawbacks. Some of these derivatives are currently being investigated in clinical trials.[2]

Despite considerable effort, the mechanism of action of anthracyclines has not been fully clarified up to now. The cytotoxic activity was first attributed to intercalation of anthracyclines between adjacent DNA base pairs,[3] but other intracellular events, such as induction of DNA breaks,[4] generation of radicals,[5] inhibition of DNA-related enzymes such as DNA and RNA polymerase[6,7] and topoisomerase II,[8] and enhanced lipid peroxidation,[9] have been suggested to be involved in the mechanism of action of these drugs. It remains to be investigated whether the drug actually has to be taken up by cells to exert its cytotoxic activity or whether the cellular membrane is a target for anthracyclines.[10,11]

Based on analysis of the quantitative structure–activity relationships of anthracyclines, a correlation between biological activity and certain isolated parameters such as

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Table I. Structures for Test Compounds

compound	abbreviation	aglycon	sugar	sugar substitute
doxorubicin	DXR	adriamycinane	7-D	
daunorubicin	DNR	daunomycinone	7-D	
THP-doxorubicin	THP	adriamycinone	7-D	4′-*O*-tetrahydropyranyl
N-acetyldoxorubicin	N-Ac-DXR	adriamycinone	7-D	*N*-acetyl
N-acetyldaunorubicin	N-Ac-DNR	daunomycinone	7-D	*N*-acetyl
aclacinomycin	ACM	aldavinone	7-RN-dF-CA	
β-rhodomycin I	RMN I	β-rhodomycinone	7-D	
β-rhodomycin II	RMN II	β-rhodomycinone	7-RN, 10-RN	
cytorhodin A	CTR A	β-rhodomycinone	7-RN-R-R, 10-RN-R-R	
cytorhodin D	CTR D	β-rhodomycinone	7-RN-Fuci, 10-RN-R-A	
cytorhodin S	CTR S	β-rhodomycinone	7-RN-Fuci, 10-RN	
1-hydroxycytorhodin S	1-OH-CTR S	β-isorhodomycinone	7-RN-Fuci, 10-RN	
cytorhodin X+Y	CTR X+Y	β-rhodomycinone	7-RN-R-CA, 10-RN-R-A[a]	

[a] Contaminated with the isomer 7-RN-R-A, 10-RN-R-CA.[33]

Table II. Partition Coefficient, Binding Affinity, and Cytotoxicity of the Test Compounds against L1210, Colon 4, and HT 29 Cell in Vitro[a]

compound	partition coefficient *P*	binding affinity $K \times 10^{-6}$ M	cytotoxicity: IC_{50} L 1210 1 h	L 1210 cont	Colon 4 1 h	Colon 4 cont	HT 29 1 h	HT 29 cont
DXR	0.9	3.8	0.05	0.03	0.10	0.032	0.33	0.039
DNR	4.5	1.5	0.038	0.028	0.08	0.022	0.35	0.027
THP	49.9	2.7	0.014	0.004	0.031	0.004	0.07	0.006
N-Ac-DXR	22.2	nd	1.00	1.00	nt	nt	nt	nt
N-Ac-DNR	255.0	nd	1.00	0.40	nt	nt	nt	nt
ACM	192.0	2.2	0.26	0.018	0.15	0.024	0.40	0.017
RMN I	14.3	1.0	0.037	0.028	0.11	0.06	0.85	0.11
RMN II	0.4	31.2	0.42	0.04	0.30	0.023	0.78	0.079
CTR A	143.0	24.3	0.032	0.0041	0.022	0.0046	0.13	0.009
CTR D	44.5	13.6	0.017	0.0033	0.023	0.0025	0.09	0.004
CTR S	33.6	25.4	0.012	0.0027	0.024	0.004	0.05	0.0022
1-OH-CTR S	14.0	25.8	0.031	0.0075	0.037	0.0047	0.10	0.002
CTR X+Y	34.5	24.3	0.005	0.0012	0.031	0.0026	0.20	0.010

[a] Abbreviations: nt = not tested, nd = not detectable.

DNA binding affinity[12-14] and lipophilicity[15,16] was reported. However, this could not be substantiated in recent investigations.[17,18] Therefore, we have investigated the influence of DNA binding affinity and lipophilicity on the biological activity and the structure-activity relationship of a heterogeneous series of anthracyclines, including newly developed drugs. The results of the study provide evidence that both lipophilicity and binding affinity to DNA are critical factors in cytotoxic activity.

Results and Discussion

The intention of the present study was to analyze the structure-activity relationship of a set of anthracycline differing not only in a particular side chain but also in the number of amino sugars and the type of aglycon present. (See Scheme I and Table I for structural details and abbreviations of compound names.) Beside some standard cytotoxic drugs (DXR, DNR, THP, ACM) and derivatives thereof (N-Ac-DXR, N-Ac-DNR), several anthracyclines containing β-rhodomycinone (RMN I, RMN II, CTR A, CTR D, CTR S, CTR X+Y) and 1-hydroxy-β-rhodomycinone (1-OH-CTR S) as an aglycon were included in these studies. Among these compounds, which were either isolated directly[19] or produced semisynthetically[20] from natural products in the fermentation broth of *Strepto-*

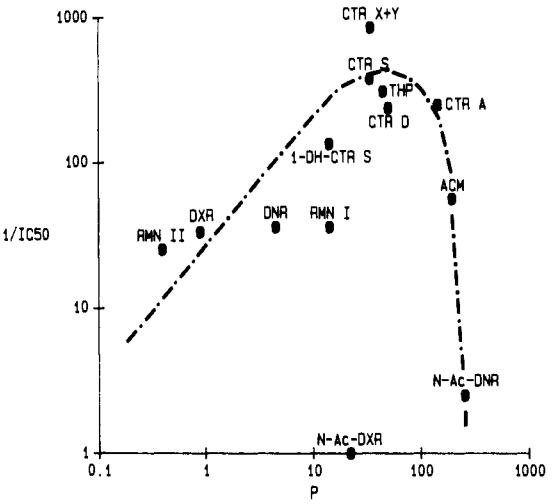

Figure 1. Cytotoxicity of the test compounds (continuous incubation) against L1210 cells as a function of partition coefficient *P*.

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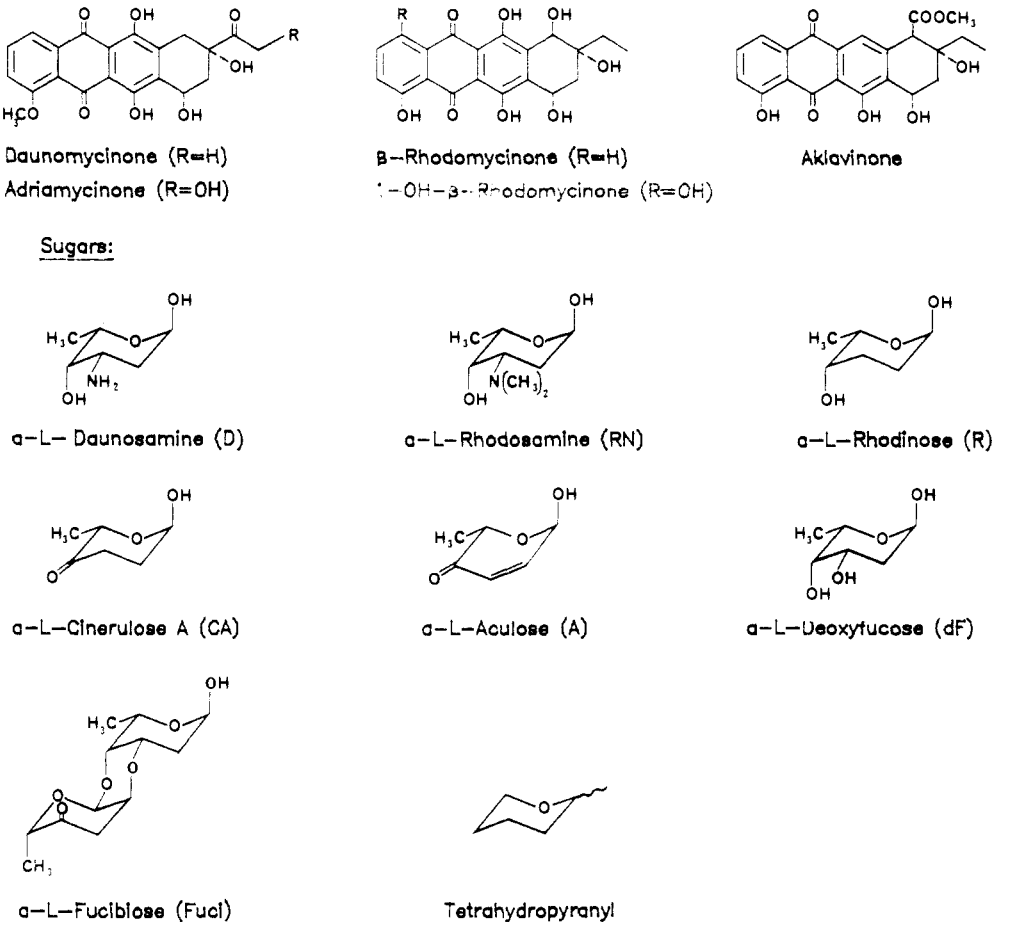
Scheme I. Structures of Test Compounds



myces, high antitumoral activity in vitro and in vivo has already been demonstrated for cytorhodin S (rodorubicin),[21] which has recently been introduced into clinical trials.

Partition Coefficients. Previous work suggests that the lipophilic character of anthracyclines, as determined by the partition coefficient between water and *n*-octanol, plays a critical role on both the cellular uptake and cytotoxicity.[15,22] Cytotoxic activities have been found to be parabolically related to the partition coefficient. This finding might be related to the fact that compounds of optimal lipophilicity pass the cell membrane most rapidly, whereas more hydrophilic or lipophilic compounds enter at a lower speed or accumulate in the cell membrane, respectively, both resulting in low intracellular concentrations. Recently, however, a correlation of cytotoxicity and partition coefficients could not be proven in studies of the quantitative structure–activity relationship.[18]

For the compounds examined in our study, the relationship between cytotoxicity against L1210 leukemia cells, as determined by the clonogenic assay (continuous incubation), and partition coefficients is depicted in Figure 1 (see also Table II). Although very hydrophilic (RMN II) and lipophilic drugs (ACM, N-Ac-DXR) were only marginally cytotoxic, other compounds with intermediate lipophilicity showed a significant deviation from the ideal parabolic relationship between these parameters (given by the line in Figure 2). This lack of correlation was particularly striking for compounds with an approximately equal lipophilicity (1-OH-CTR S, RNM I, N-Ac-DXR),

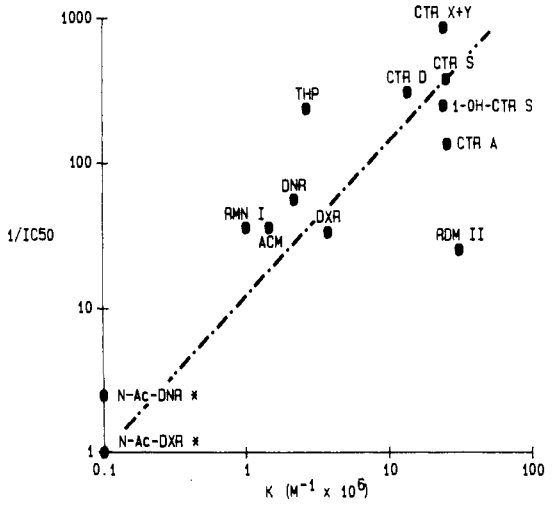


Figure 2. Cytotoxicity of the test compounds (continuous incubation) against L1210 cells as a function of DNA binding affinity *K*. An asterisk indicates that the DNA binding affinity was not detectable.

which nevertheless differed highly in their cytotoxic activities. Accordingly, the quantitative analysis of the structure–activity relationship by equations in which cytotoxic activities are parabolically related to partition coefficients revealed only a moderate correlation between these parameters:

$$-\log IC_{50}(\text{cont}) = 0.91 \log P - 0.29(\log P)^2 + 1.64$$

$$n = 11,\ r = 0.67,\ s = 0.372,\ F_{2,8} = 1.77$$

This correlation was, however, more pronounced when cytotoxic activities determined after a short incubation

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time (1-h incubation) were related to partition coefficients:

$$-\log IC_{50}(1\ h) = 1.40 \log P - 0.63(\log P)^2 + 1.15$$

$$n = 11,\ r = 0.80,\ s = 0.323,\ F_{2,8} = 1.37$$

Under these experimental conditions, lipophilicity becomes a major determinant of cytotoxic activity, most probably because of its predominant influence on the velocity of membrane passage. However, once equilibrium has been reached, cytotoxicity seems to be influenced by additional parameters as indicated by the decrease of correlation between partition coefficients and cytotoxic activities after long-time incubation.

DNA Binding Affinity. The binding of anthracyclines to DNA by intercalation between adjacent base pairs of the helix has been considered to be of major importance in their mechanism of action.[23] This interaction has been extensively investigated by a number of physicochemical techniques, but its biological significance still has to be elucidated. Rather good correlations between cytotoxic activity and DNA binding affinity have been observed,[12-14] but the finding that certain compounds which do not bind to DNA have high therapeutic activities in vivo[24] has been used as an argument against any significant importance of intercalation in the mechanism of action of anthracyclines.

The DNA binding affinities of the present compounds were found to cover a large range (Table II). Whereas DNA binding, as determined by fluorescence titration and viscometry (data not shown), could not be detected for N-Ac-DXR and N-Ac-DNR, certain compounds exhibited a very high affinity for DNA, being approximately 10-fold enhanced as compared to that of doxorubicin. Interestingly, in each case high-affinity binding corresponds to the presence of two units of the amino sugar rhodosamine, regardless of whether further substituents are connected to the hydroxyl groups of this sugar. Although the geometry of the intercalation complex does not allow any direct ionic interaction between the amino group and the phosphate backbone of DNA, which was deduced from X-ray crystallographic studies of a daunorubicin–oligonucleotide complex,[25,26] recent studies have shown that binding affinity to DNA depends strictly on both the presence and the amount of charge of the amino group.[27,28] The increase of binding affinity, as observed for compounds containing two amino sugars, therefore further demonstrates the stabilizing effect of this functional group on the intercalation complex, although the distribution of hydrogen bondings involved in the interaction with DNA is not known at present.

For the compounds under investigation, cytotoxic activities determined after continuous incubation were only partially related to DNA binding affinity (Figure 2). Whereas lowest cytotoxicity was observed for compounds which apparently do not bind to DNA, an increase of binding affinity did not always result in higher cytotoxic activity. In particular, deviations from the expected linear correlation of these parameters were evident for compounds with comparable affinity to DNA which, on the other hand, differed highly in their cytotoxic activity. According to the quantitative analysis of equations in which cytotoxicity was linearly related to DNA binding affinity, it is evident that under these experimental conditions cytotoxicity is only partially predicted by the affinity to DNA.

$$-\log IC_{50}(\text{cont}) = 0.44 \log K - 0.98$$

$$n = 11,\ r = 0.53,\ s = 0.422,\ F_{1,9} = 5.57,\ F_{1,8}\ (0.05) = 4.43$$

In contrast to the good relationship between cytotoxic activity determined after a short incubation period (1 h) and the partition coefficients (see above), cytotoxic activity was not related to DNA binding affinity.

$$-\log IC_{50}(1\ h) = 0.20 \log K + 0.03$$

$$n = 11,\ r = 0.16,\ s = 0.528,\ F_{1,9} = 156.25,\ F_{1,9}\ (0.05) = 4.03$$

Combined Analysis of Parameters. As cytotoxicity (continuous incubation) was partially related to both lipophilicity and DNA binding affinity, regression equations linking cytotoxic activity to both parameters were analyzed:

$$-\log IC_{50}(\text{cont}) = 0.81 \log P - 0.11(\log P)^2 + 0.41 \log K - 1.29$$

$$n = 11,\ r = 0.90,\ s = 0.219,\ F_{3,7} = 1.02$$

This equation resulted in a significant improvement of the correlation coefficient, suggesting that cytotoxicity was independently influenced by both parameters. Variations in cytotoxic activity observed for compounds with almost equal lipophilicity (N-Ac-DXR, RMN I, 1-OH-CTR S) could therefore well be attributed to the different degree in DNA binding affinity. On the other hand, the simultaneous dependence of cytotoxicity on lipophilicity is particularly evident for compounds with high DNA binding affinity (RMN II, 1-OH-CTR S, CTR A, CTR S, CTR X+Y). Highest activity was observed for compounds with medium lipophilicity (CTR S, CTR X+Y) whereas both a decrease (1-OH-CTR S, RMN II) as well as an increase (CTR A) of lipophilicity resulted in compounds with lower cytotoxic activity, thereby demonstrating a parabolic relationship between the cytotoxicity and lipophilicity.

The analysis of regression equations linking cytotoxic activity displayed after a short incubation time (1 h) to both lipophilicity and binding affinity also revealed a good correlation between the cytotoxicity and the parameter examined:

$$-\log IC_{50}(1\ h) = 1.41 \log P - 0.64(\log P)^2 + 0.23 \log K - 0.48$$

$$n = 11,\ r = 0.85,\ s = 0.280,\ F_{3,7} = 2.17$$

The low coefficient associated with binding affinity indicates that under these experimental conditions the contribution of DNA binding affinity to cytotoxic activity is low. This conclusion is further supported by the observation of a poor direct correlation between cytotoxicity and binding affinity. Nevertheless, in comparison to cytotoxic activity displayed after continuous incubation, the observed correlation between cytotoxicity displayed after 1-h incubation and the physicochemical parameters is of a lower value. Whether this finding is simply due to possible variances in the determination of cytotoxicity or whether under these experimental conditions cytotoxic activity is influenced by other parameters is not known

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at present and will be further investigated.

Cytotoxic Activity on Human Colon Tumor Cells. The structure–activity relationship of the compounds was further examined with two human colon tumor cell lines (HT 29, Colon 4). These cell lines were 2–40-fold less sensitive against the compounds tested (Table II) than L1210. Therefore, with respect to chemosensitivity, the cell lines resemble quite well the characteristics common to human colon tumors, which are usually primarily resistant against chemotherapeutic treatment.

Quantitative analysis of the structure–activity relationship revealed that the cytotoxic activity of the compounds against these colon tumor cells is also dependent on both lipophilicity and binding affinity. Again, best correlations were obtained by equations relating activity to both parameters:

Colon 4

$$-\log IC_{50}(\text{cont}) = 0.83 \log P - 0.30(\log P)^2 + 0.46 \log K = -1.49$$

$$n = 11,\ r = 0.86,\ s = 0.245,\ F_{3,7} = 3.30$$

$$-\log IC_{50}(1\text{ h}) = 0.86 \log P - 0.32(\log P)^2 + 0.24 \log K - 0.75$$

$$n = 11,\ r = 0.87,\ s = 0.181,\ F_{3,7} = 1.53$$

HT 29

$$-\log IC_{50}(\text{cont}) = 1.16 \log P - 0.43(\log P)^2 + 0.35 \log K - 0.90$$

$$n = 11,\ r = 0.79,\ s = 0.350,\ F_{3,7} = 2.35$$

$$-\log IC_{50}(1\text{ h}) = 0.83 \log P - 0.3(\log P)^2 + 0.2 \log K - 0.93$$

$$n = 11,\ r = 0.71,\ s = 0.280,\ F_{3,7} = 1.99$$

As far as cytotoxic activity determined after 1-h incubation is concerned, the coefficient associated with binding affinity was very low for both cell lines. As was already observed for L1210, the cytotoxic activity against human colon tumor cells measured after short incubation time is therefore predominantly influenced by the lipophilicity of the compounds. On the other hand, binding affinity becomes of more importance as the incubation time is increased thus allowing equilibrium to be reached between intra- and extracellular concentrations of the compounds.

However, in comparison to L1210, the correlation between cytotoxic activity against colon tumor cells and the evaluated physicochemical properties of the compounds is significantly lower. It might therefore be suggested that the cytotoxic activity of anthracyclines against these cells is influenced by additional factors not yet determined. At present, however, these factors, which probably are related to the mechanism of primary resistance of colon tumors, are not known and have to be elucidated.

Conclusion

The present study about the structure–activity relationship of anthracyclines provides further evidence that the cytotoxic activity of these compounds is partially related to both lipophilicity and binding affinity to DNA. In addition, if both parameters are taken into account simultaneously, a high correlation with cytotoxic activity of the compounds is observed, although their relative contribution varies with incubation time. This correlation was not only observed for L1210 leukemia cells, which are relatively sensitive to anthracyclines, but also for two human colon tumor cell lines, which are much less sensitive to chemotherapeutic drugs, both in vitro and in vivo. The optimization of anthracyclines with regard to both lipophilicity and DNA binding affinity may well serve as a guideline in the design of new anthracycline derivatives with improved antitumoral activity. Thus cytorhodin S (rodorubicin), which is of intermediate lipophilicity and which exhibits high binding affinity to DNA, was recently selected for clinical trials because of its high activity on human tumors both in vitro and in vivo. The results of this study, however, indicate that further investigations about the factors involved in the mechanism of resistance are needed. These investigations might hopefully lead to a guide in the development of compounds being active on tumors that are not sensitive against currently available chemotherapeutic agents.

Experimental Section

Drugs. Doxorubicin (adriamycin) and daunorubicin were obtained from Farmitalia (Milan, Italy), aclacinomycin was from Sanraku Ocean Co., Ltd. (Tokyo, Japan), and 4-*O*-tetrahydropyranyldoxorubicin was from Meiji Saeka Kaisha Ltd. (Tokyo, Japan). CTR A, CTR D, CTR S, 1-OH-CTR S, CTR X+Y, RMN II, and RMN I, *N*-acetyldoxorubicin, and *N*-acetyldaunorubicin were prepared at Hoechst AG (Frankfurt, FRG) and Behringwerke AG (Marburg, FRG). All other chemicals were obtained commercially.

Tissue Culture. L1210 cells were passaged and maintained in RPMI 1640 supplemented with 15% (v/v) Ficoll calf serum, penicillin (100 units/mL), and streptomycin (100 μg/mL). HT 29 cells were grown in monolayer in tissue-culture flasks (Nunc GmbH, Wiesbaden, FRG), in RPMI 1640 supplemented as above. Colon 4 cells were maintained in tissue-culture flasks in Dulbecco's medium containing 10% Ficoll calf serum, 4 mmol of Glutamine, penicillin (100 units/mL) and streptomycin (100 μg/mL). All cells were kept in humidified atmosphere of 5% CO_2 at 37 °C. Adherent cells were harvested with a trypsin (0.1% w/v)/EDTA (0.1% w/v) solution.

Clonogenic Assay. Drug sensitivity was determined according to the clonogenic assay of Hamburger and Salmon.[29] Briefly, after replacement of the culture medium by McCoy's medium, cells were incubated with the drugs for 1 h at 37 °C, washed, and plated in a mixture of 0.3% (w/v) agarose in CRML over a layer of 0.3% (w/v) agarose in plastic dishes. L1210 cells were plated at a concentration of 1000 cells/plate and HT 29 and Colon 4 cells were plated at a concentration of 5000 cells/plate. In addition, parallel experiments were performed with continuous incubation by admixing the drug to the upper layer prior to plating. Plates were stored in an incubator with 5% CO_2 and 95% humidity for 7 days, and thereafter colonies were counted by using an automatic colony counter (Bausch and Lomb).

Partition Coefficient. Partitioning was measured by shaking 1 mL of the drug solution (10 μM, drug dissolved in 1 mL of *n*-octanol-saturated 10 mM Tris–HCl, pH 7.0) with an equal volume of buffer-saturated *n*-octanol for at least 1 h. The phases were then separated by centrifugation and the drug level in the aqueous phase was determined by fluorimetry.

Viscometry. Viscometric measurements were performed in 10 mM NaCl, 2 mM HEPES, 20 μM EDTA, pH 7.0 (SHE), buffer according to the method of Revet.[30] The drug solution (100 μM) was added in increments of 50–100 μL to 1.5 mL of PM2-DNA (200 μM base pairs) and the flow was measured in a capillary viscosimeter, which was maintained at 25 °C. For calculation of reduced viscosities, v_{app} (mol of drug/mol of DNA at maximal viscosity), the dilution caused by addition of the drug, was taken into account.

Binding Measurements. Binding measurement was done by fluorescence titration according to Blake and Peacock.[31] Briefly, calf thymus DNA (2 μmol of base pairs) was incubated for 1 h with various concentrations (C_t) of the drug (0.1–1.0 μM in 10 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, pH 7.0) and

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thereafter the fluorescence of the drug in the presence and absence of DNA was recorded. The amount of drug bound to DNA (C_b) was determined according to the equation

$$C_b = C_t\,[(I_{obs} - I_f)/I_f(V - 1)]$$

where I_{obs} and I_f represents the fluorescence intensities in the presence and absence of DNA, respectively. The term V, representing the ratio of fluorescence of totally bound drug/fluorescence of free drug, was determined at a ratio of drug to base pairs of 200. Binding constants were calculated according to the neighbor-exclusion model of McGee and Von Hippel.[32]

Registry No. DXR, 23214-92-8; DNR, 20830-81-3; THP, 123639-68-9; N-Ac-DXR, 69299-74-7; N-Ac-DNR, 32385-10-7; ACM, 66676-88-8; RMN I, 1404-52-0; RMN II, 23666-50-4; CTR A, 95599-38-5; CTR D, 100630-83-9; CTR S, 96497-67-5; 1-OH-CTR S, 123639-69-0.

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Synthesis and Characterization of Iodobenzamide Analogues: Potential D-2 Dopamine Receptor Imaging Agents

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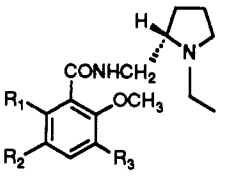
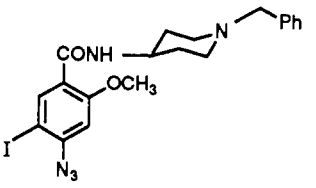
(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide ([^{123}I]IBZM) is a central nervous system (CNS) D-2 dopamine receptor imaging agent. In order to investigate the versatility of this parent structure in specific dopamine receptor localization and the potential for developing new dopamine receptor imaging agents, a series of new iodinated benzamides with fused ring systems, naphthalene (INAP) and benzofuran (IBF), was synthesized and radiolabeled, and the in vivo and in vitro biological properties were characterized. The best analogue of IBZM is IBF (21). The specific binding of [^{125}I]IBF (21) with rat striatal tissue preparation was found to be saturable and displayed a K_d of 0.106 ± 0.015 nM. Competition data of various receptor ligands for [^{125}I]IBF (21) binding show the following rank order of potency: spiperone > IBF (21) > IBZM > (+)-butaclamol > (±)-ADTN,6,7 > ketanserin > SCH-23390 ≫ propanolol. The in vivo biodistribution results confirm that [^{125}I]IBF (21) concentrated in the striatal area after iv injection into rats. The study demonstrates that [^{123}I]IBF (21) is a potential agent for imaging CNS D-2 dopamine receptors.

A variety of substituted benzamide derivatives possessing antipsychotic and antiemetic properties have been reported.[1-5] The pharmacological effects of these agents are assumed to be induced by blocking the central nervous system (CNS) D-2 dopmaine receptor. In this series of benzamide derivatives, agents with an (*S*)-(-)-*N*-ethyl-2-(aminomethyl)pyrrolidinyl group appear to be the most attractive antagonists—showing the best selectivity and the highest affinity for the CNS D-2 dopamine receptor. Raclopride[6] and eticlopride[7,8] are two excellent examples which show specific D-2 antagonistic activity, with high affinity in rat striatum tissue preparations and low nonspecific binding (Table I). Radioactive iodinated benzamides are not only potentially useful as imaging agents (labeled with ^{123}I, $T_{1/2}$ = 13 h, γ-ray energy = 159 keV) but are also very valuable as pharmacological tools for probing the D-2 dopamine receptor under in vitro and in vivo conditions (labeled with ^{125}I, $T_{1/2}$ = 60 days, γ-energy = 30–65 keV). Several iodinated benzamide derivatives, iodosulpiride,[9] iodoazidoclebopride,[10] iodopride,[11] and IBZM,[12-14] have been reported as showing very high affinity and selectivity to the D-2 dopamine receptor in the same striatal membrane preparation.

Imaging studies of CNS D-2 dopamine receptor in humans with [^{11}C]raclopride (labeled at the *N*-ethyl group), in conjunction with positron emission tomography (PET), have been reported.[15-20] A high ratio of specific striatal to nonspecific cerebellar binding in living human brain was observed. With use of an equilibrium model and Scatchard plots, the affinity constant (K_d = 7.1 nM, B_{max} = 15 pmol/mL) in living human brain was measured by PET.[19,20] The values for the dopamine D-2 receptor density were comparable to those determined earlier using a different imaging agent, *N*-methylspiperone, NMSP (K_d = 0.097 nM, B_{max} = 16.6 pmol/g).[21-23] Planar imaging studies in humans with (*S*)-[^{123}I]IBZM (*S*—the active

Table I. Chemical Structures and in Vitro Binding Constants of Benzamides[a]





iodoazidoclebopride

compound	R_1	R_2	R_3	K_d, nM
iodosulpiride[c]	H	SO_2NH_2	H	1.5
raclopride[d,e]	OH	Cl	Cl	1.1
eticlopride[d,e,f]	OH	C_2H_5	Cl	0.17
IBZM[g]	OH	I	H	0.43
BZM[g]	OH	H	H	31.1
iodopride[h]	H	I	H	3.0[h]
iodoazidoclebopride[i]				14

[a] Unless otherwise stated, all of the benzamides contain a *S*-(-)-*N*-ethyl-2-(aminomethyl)pyrrolidinyl group. [b] IC_{50} against [^{3}H]spiperone binding of rat striatal tissue preparation. [c] See ref 9. [d] See ref 4. [e] See ref 6. [f] See ref 7. [g] See ref 14. [h] See ref 11. [i] See ref 10.

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