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Communication

Uptake of acridinecarboxamide derivatives by L1210 cells[o]

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The uptake of six 9-aminoacridinecarboxamide derivatives by L1210 cells in relation to their lipophilicity and cytotoxic activity was studied. The amount of acridines taken up by cells was estimated by fluorimetric measurements. It was found that the uptake efficiency of this class of compounds by cells depends on the size of carboxamide residue as well as on position of the substituent. The increase of size of carboxamide chain resulted in the loss of capability of acridines to penetrate cell membrane. Cytotoxic effects of acridines were well correlated with the level of drugs accumulated by cells, whereas no clear correlation between uptake and lipophilicity was observed. It is concluded that uptake of 9-aminoacridinecarboxamides is the most important factor determining their antiproliferative activity.

Acridine-4-carboxamides seem to be a potential source of DNA-directed anticancer agents [1]. Some members of this class of compounds presumably act in cell as mixed topoisomerase I/II inhibitors [2, 3]. Structure-activity relationships studies for acridine-4-carboxamides indicated that the electronic properties of the groups substituted into the acridine ring are less important for their activity than the size of the substituents, as derivatives having bulky residues exhibit low cytotoxic activity [3]. The reason for such relationship is not clear as changes in the structure of side chain may affect several properties of acridine derivatives. These are interaction of acridines with intracellular targets

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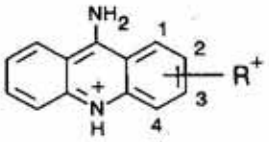
Abbreviations: amino-DACA, *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide; PBS, phosphate buffered saline, pH 7.

(DNA, topoisomerase I/II), lipophilicity, drug metabolism as well as their transport through cell membrane. A great difference was recently reported between the cellular uptake of very cytotoxic acridine amino-DACA (AAC in earlier papers and compound **1** in the current study, Fig. 1) and its poorly cytotoxic derivative with modified side chain [4]. This observation may suggest the efficiency of acridine-4-carboxamides transport through cell membrane to be the major determinant of cellular sensitivity to these drugs.



Acridine	R	Position of R
1	-CO-NH-$(CH_2)_2$-$NH^+(CH_3)_2$	4
2	-CO-NH-$(CH_2)_3$-$NH^+(CH_3)_2$	4
3	-CO-NH-$(CH_2)_4$-$NH^+(CH_3)_2$	4
4	-CO-NH-$(CH_2)_5$-$NH^+(CH_3)_2$	4
5	-CO-NCH_3-$(CH_2)_2$-$NH^+(CH_3)_2$	4
6	-CO-NH-$(CH_2)_2$-$NH^+(CH_3)_2$	3

Figure 1. Structures of acridines tested in this study.

This study was undertaken to answer the question if the structure of carboxamide substituent and its position in the acridine ring affects the uptake of acridinecarboxamides by mouse leukemia cells.

MATERIALS AND METHODS

Drugs and cytotoxicity studies. Structures of acridines are shown in Fig. 1. The compounds tested were synthesized as described earlier [1, 5].

Mouse leukemia L1210 cells were cultured in RPMI 1630 medium (Sigma-Aldrich, U.S.A.) supplemented with 10% of foetal calf serum (Gibco, U.S.A.), gentamycin (50 μg/ml) and 0.02 M Hepes buffer (Gibco, U.S.A.). Cytotoxic effects were assayed by measuring inhibitory effects on proliferation of L1210 cells. In this assay cells were seeded in 2 ml aliquots in 6 ml tissue culture tubes (Corning, U.S.A.) at a concentration of 2×10^4 cells/ml and exposed to drugs for 1 h at 37°C. After 72 h of incubation in fresh growth medium the cell number relative to control was determined by a tetrazolium dye method [6].

Uptake of acridine-4-carboxamides. To study the transport of acridines through cell membranes the procedure of Sullivan *et al.* [7] was used except that drug concentrations were estimated by fluorimetric measurements instead of radioactivity assays. L1210 cells were concentrated to 10^7 cells/ml and treated in growth medium with 2.5–15 μM drugs at 37°C. The aliquots of cell suspension (0.2 ml) were mixed with 10 ml of ice-cold PBS and centrifuged at 1500 r.p.m. for 5 min, then washed twice with ice-cold PBS. Final cell pellets were resuspended in 2.2 ml of PBS. Fluorescence intensity of these suspensions was measured at 20°C by Perkin-Elmer LS 50 B spectrofluorimeter. For compounds **1–5** optimal excitation and emission wavelengths were 260 nm and 454 nm, respectively. For acridine **6** optimal excitation and emission wavelengths were 270 nm and 449 nm, respectively. To estimate the drug concentrations, calibration curves in the concentration range 0.01–0.5 μM were prepared. Due to the fluorescence quenching in the presence of cells, each point of the calibration curves was measured in the presence of 2×10^6 cells.

Lipophilicity calculations. Logarithms of the octanol/water partition coefficients (*logP*) of acridine derivatives in cationic form (+2) were calculated by means of software package Pallas (CompuDrug Chemistry, Hungary). In these calculations $logP_{CDR}$, $logP_{ATOMIC}$ and $logP_{ATOMIC4}$ were obtained using three different sets of additive and constitutive atomic constants reflecting acridine structure [8–

10]. Additionally, the values of $logP_{combined}$ were predicted according to the formula derived as the weighted average of lipophilicity obtained in a three individual procedures:

$$logP_{combined} = 0.313\ logP_{CDR} + \\ + 0.208\ logP_{ATOMIC} + 0.479\ logP_{ATOMIC4}$$

where the $logP_{CDR}$ means that calculated *logP* values have taken into account the lipophilic fragmental constants and its correction terms derived from an extended set of about 1000 experimental n-octanol/water partition coefficients by using the constrained least-squares technique as proposed by Rekker & De Kort [8] and further modified by producer of Pallas software; the $logP_{ATOMIC}$ means that calculated *logP* values have taken into account the additive and constitutive predefined lipophilicity of 222 atomic contributions calculated by applying the Monte Carlo optimization and linear regression analysis to the matrix of experimental values of the n-octanol/water partition coefficients determined for 1868 compounds as reported by Broto *et al.* [9]; the $logP_{ATOMIC4}$ means that calculated *logP* value have taken into account the predefined lipophilicity values of 120 atomic state subtypes (which included their oxidation and hybridized state and the influence of immediately bonded atoms) estimated by using the simple least-squares fitting method to the set of experimental values of the n-octanol/water partition coefficients collected for 893 compounds as described by Viswanadhan *et al.* [10].

RESULTS AND DISCUSSION

The structure of tested acridine derivatives is shown in Fig. 1. Compounds **1–5** vary in size of 4-carboxamide side chain whereas **6** is an isomer of **1** with carboxamide chain substituted in position 3. The uptake of acridinecarboxamides by L1210 cells was linearly dependent on the drug concentration in the growth medium (Fig. 2). The efficiency of drug transport through cell membrane varied among tested acridines. The most efficient transport was observed for compound **1**, the increase in the length of the substituent at position 4 in its homologues **2–4** led to the lowering of acridines uptake (Fig. 2 and Table 1). The analogue **5** having a bulky branched chain at position 4 was taken up by cells the most slowly (Table 1).

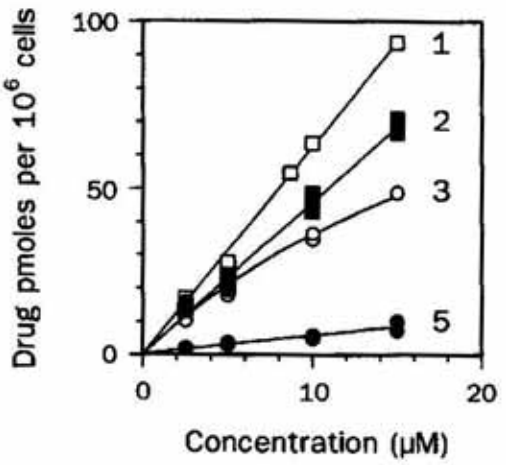


Figure 2. Dependence of uptake of acridinecarboxamides by L1210 cells on drug concentration in growth medium.

Cells were incubated with acridines for 1 h at 37°C. The amount of drugs accumulated in cells was estimated by fluorimetric measurements. Points represent data from individual experiments.

The acridines tested also varied in their cytotoxicity towards L1210 cells and this effect was clearly correlated with cellular uptake of these drugs (Fig. 3). This relationship was described by the significant linear regression equation:

$$\log IC_{50} = 3.237\ (\pm\ 0.287) - \\ - 0.0340\ (\pm\ 0.0051) \times\ Upt \qquad (1)$$

$$n = 6,\ R = 0.9574,\ F_{(1,4)} = 43.9,\ s = 0.38$$

where *Upt* is uptake of the drugs. The high correlation coefficient $R = 0.96$ suggests that

the accumulation of 9-aminoacridinecarboxamides in cell is the main factor influencing cytotoxicity of these drugs.

The increase in the size of alkyl residue of substituent affects the lipophilic character of the molecules (Table 1). For the homologous series of acridines **1–4** a systematic loss of cytotoxic activity as well as lowering of uptake were observed when lipophilicity increased. However, no lipophilicity-cytotoxicity or lipophilicity-uptake relationships exist for the whole set of the compounds tested (Fig. 4). Steric properties of the molecule seem to be a more important factor influencing acridinecarboxamides uptake and cytotoxicity than is lipophilicity. Compounds **1** and **6** varying in the position of side carboxamide chain possess similar lipophilicity but they differ greatly in uptake (Table 1). The presence of bulky branched substituent (compound **5**) also resulted in the loss of capability to penetrate cell membrane and in cytotoxicity.

Depending on the class of acridine derivatives different relationships between lipophilicity and biological activity have been described [11–15]. In the case of proflavine derivatives and 9-anilinoacridines a positive correlation between toxicity for bacterial cells and lipophilic character of molecules was found [11, 12]. In contrast, toxicity of these classes of acridines towards mammalian cells was not related to lipophilicity [11, 12]. In the case of mammalian cells a clear activity-lipophilicity relationship was described for 8-hydroxy derivatives of imidazoacridinones [13]. Earlier studies on structure-activity rela-

Table 1. Lipophilicity, cytotoxic activity and uptake of acridinecarboxamides by L1210 cells

Acridine	Lipophilicity (*log P*)*	Cytotoxic activity IC_{50} (μM)	Uptake (drug pmoles/10^6 cells)
1	1.38	0.37	93.5
2	1.52	17	69.5
3	2.05	56	54.5
4	2.55	68	44.0
5	1.46	630	9.5
6	1.46	420	11.7

LogP, logarithm of the octanol/water partition coefficient of the cationic (+2) form of the drug. IC_{50}, the acridine concentration effective in inhibiting 50% of the cell growth after 1 h exposure of L1210 cells to the drug. Accumulation of acridines in L1210 cells incubated for 1 h with 10 μM drug in growth medium at 37°C.

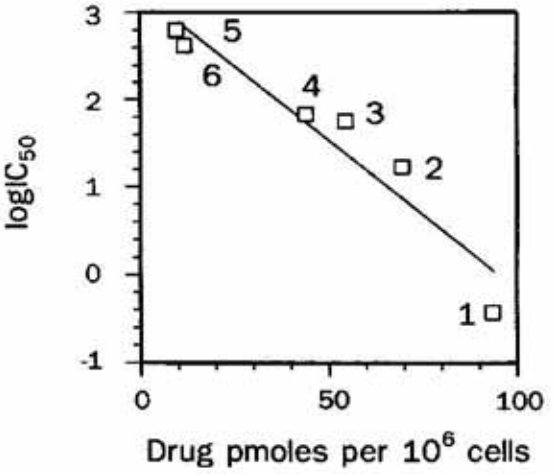


Figure 3. Relationship between cytotoxic activity and uptake of acridinecarboxamides as revealed by equation (1).

Logarithms of IC_{50} values were plotted against accumulation of acridines in L1210 cells which were incubated for 1 h with 10 μM drugs in growth medium at 37°C (data from Table 1).

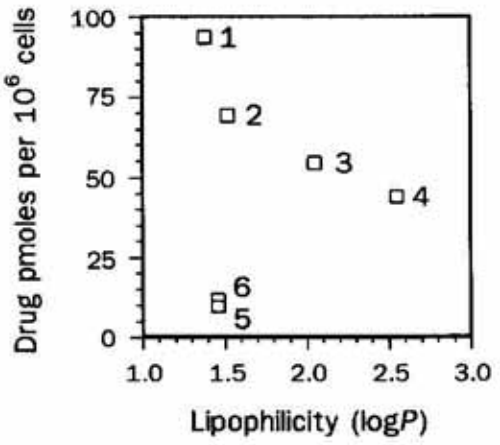


Figure 4. Accumulation of acridines in L1210 cells plotted against lipophilicity (*logP*) of the drugs (data from Table 1).

tionship of numerous acridinecarboxamides have shown that there are derivatives similar in lipophilic character and DNA binding which, however exhibit striking differences in biological activity [14, 15]. The detailed mechanism of cytotoxic action of acridine-4-carboxamides is not clear. Woynarowski *et al.* [16] suggested that these compounds block topoisomerase II, whereas more recent papers pointed to the existence of an additional target e.g. topoisomerse I [2–4]. Although our preliminary studies are limited to six derivatives, it seems reasonable to assume that, at least for 9-aminoacridinecarboxamides, their uptake by target cells is the most important factor determining their biological activity.

The mechanism of transport of acridinecarboxamides through cell membrane as well as their cellular localisation are not known. As tested acridines are protonated at pH 7 and carry a total +2 charge on their molecules (Fig. 1) it is unlikely that they can pass through cell membrane by simple diffusion. Lack of relationship between uptake and lipophilicity as well as strong steric effect may suggest that these drugs are transported by a specific mechanism.

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