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Lipidic peptides. XII. Cellular uptake studies of a lipidic amino acid, its oligomers and highly lipophilic drug conjugates on Ehrlich ascites tumour cells

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Summary

Cellular uptake of lipidic amino acid **1a**, oligomers **1b–d**, benzoquinolizine **2a** and conjugates **2b–d** was studied using Ehrlich ascites tumour cells. The lipidic amino acid methyl ester **1a** and benzoquinolizine monomer conjugate **2b** were taken up more readily and to a greater extent than the fully protected dipeptide **1b**, tripeptide **1c**, tetrapeptide **1d** and conjugates **2c** and **2d**. The cellular uptake of lipophilic benzoquinolizine conjugate **2b** was 5–10-fold higher than that of the unconjugated alkaloid **2a**. These experiments demonstrated that conjugation with lipophilic α-amino acids could increase the cellular uptake of otherwise poorly absorbed compounds.

Introduction

Lipidic amino acids and peptides can be covalently conjugated or incorporated into poorly absorbed peptides and drugs to enhance their transport across biological membranes (Toth et al., 1992). Since lipidic amino acids and their homo-oligomers are lipophilic by nature, they are easily absorbed through the cell membrane. Thus, conjugation to poorly absorbed drugs and peptides should facilitate their cellular uptake.

We have previously reported the cellular uptake of lipidic amino acids and oligomers on three cell types, namely, rat erythrocytes, rat hepatocytes and *Escherichia coli* (Toth et al., 1991). We have now extended our investigations with Ehrlich ascites tumour cells in order to establish uptake by transformed mammalian cells.

Ehrlich ascites tumour cells were chosen for this investigation on the basis that they have been

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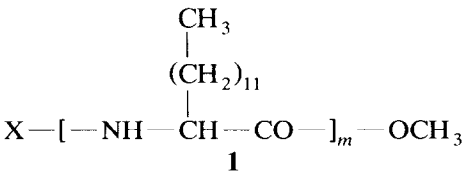

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1	*m*	X
a	1	H
b	2	BOC
c	3	BOC
d	4	BOC

Scheme 1.

used successfully as models to study the uptake of free fatty acids (Spector et al., 1965; Kuhl and Spector, 1970; Spector and Soboroff, 1972), amino acids (Christensen and Riggs, 1952; Christensen et al., 1952) and lipoproteins (Fillerup et al., 1958) into tumour cells.

Studies were carried out to compare the cellular uptake of the non-conjugated benzoquinolizine **2a**, conjugates **2b–d** with the uptake of the underivatised lipidic amino acid **1a** and oligomers **1b–d**.

Materials and Methods

Cells were obtained from Flow Labs. The composition of the medium for culturing was DMEM/10% FCS; DMEM (stock solution × 10 concentration) (50.0 ml); $NaHCO_3$ 7.5% w/v (25.0 ml); foetal calf serum (×10 concentration) (50.0 ml); penicillin/streptomycin antibiotics (5.0 ml), glutamine (stock 200 mM) (2.5 ml); Hepes buffer

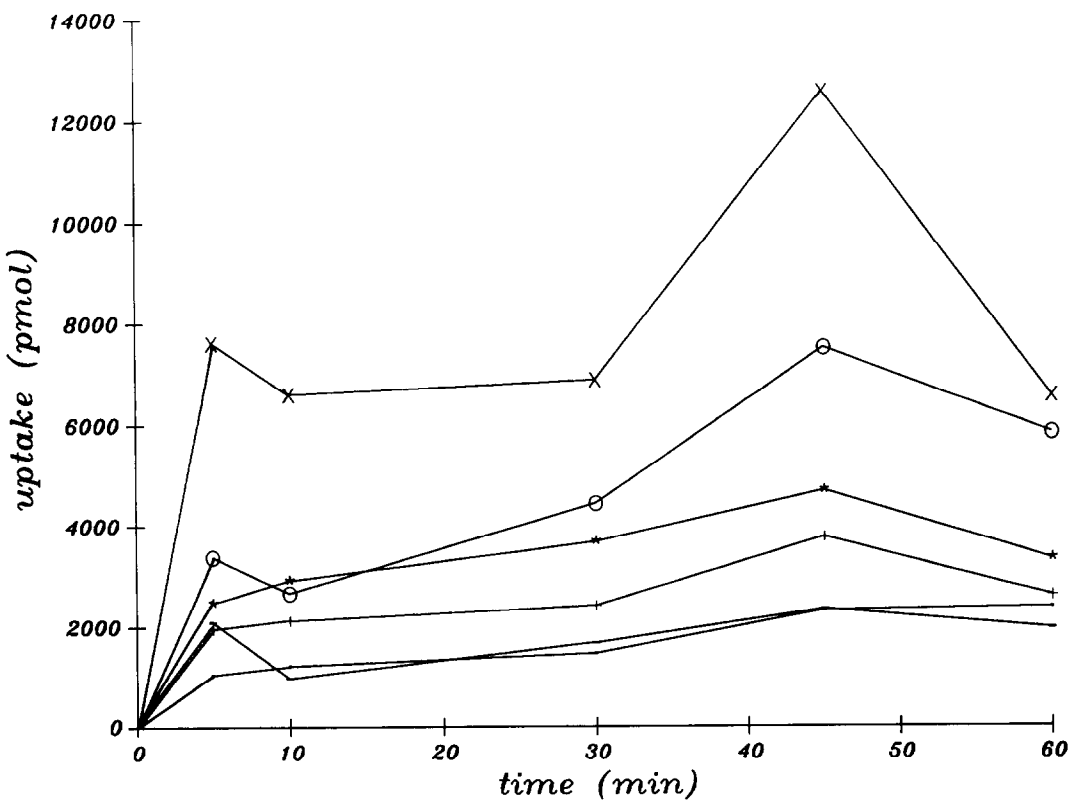
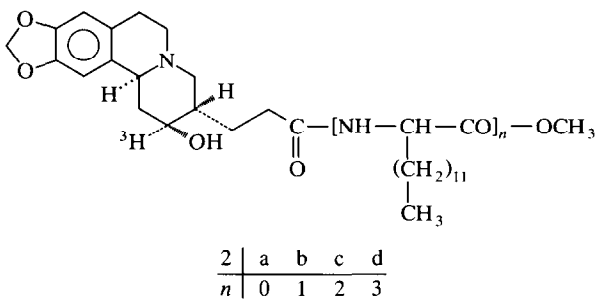


Fig. 1. Cellular uptake of **1a**. (×) 200 μM, (○) 100 μM, (*) 70 μM, (+) 49 μM, (−) 34 μM, (●) 24 μM.



2	a	b	c	d
n	0	1	2	3

Scheme 2.

(12.5 ml) and sterile distilled water (355.0 ml) were used.

Cell preparation The Ehrlich ascites tumour cells (EATC) were maintained under sterile conditions (5% CO_2, 37°C) in Dulbecco's modification of Eagles medium (DMEM) supplemented with 10% foetal calf serum (FCS) and glutamine. The cells were harvested by trypsinisation (0.25% trypsin in 0.9% NaCl, 10 min, 37°C) followed by the addition of 10 ml DMEM/10% FCS. The cells were centrifuged (1000 rpm, 5 min), the medium removed and the cells resuspended in DMEM/10% FCS to give a final density of 1×10^5 cells/ml; 0.5-ml aliquots were added to the wells of 24-well multiwell culture plates and incubated overnight to form monolayers.

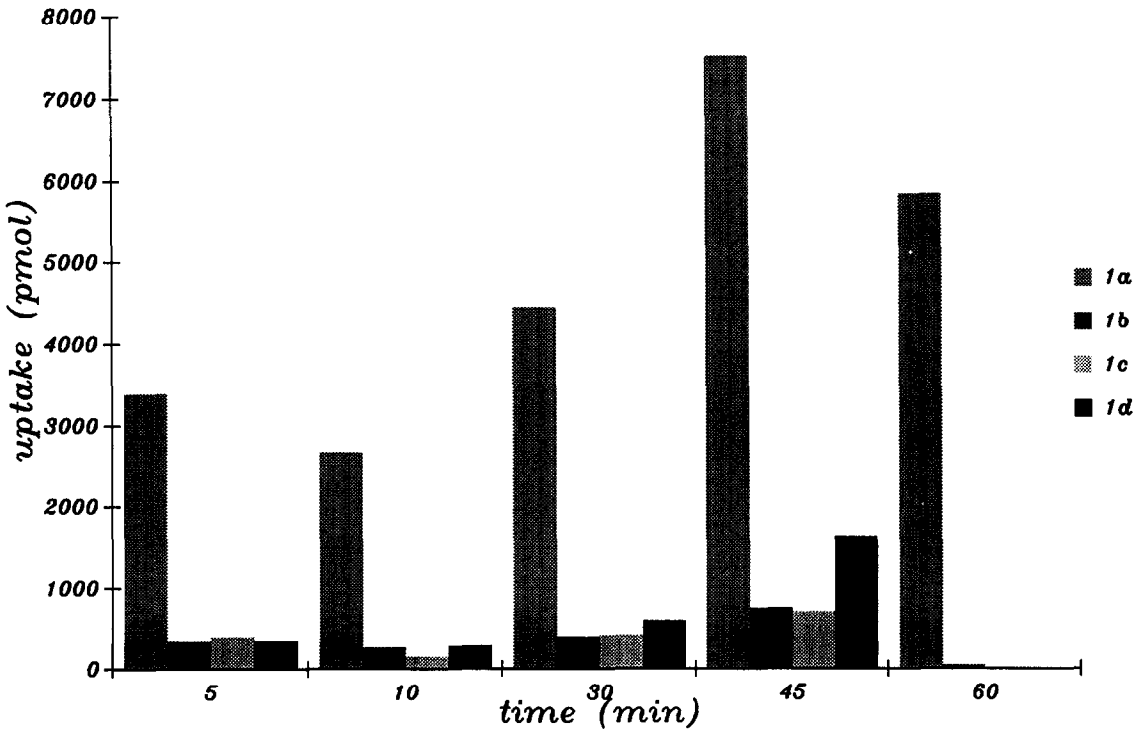


Fig. 2. Cellular uptake of **1a–d** (100 μM).

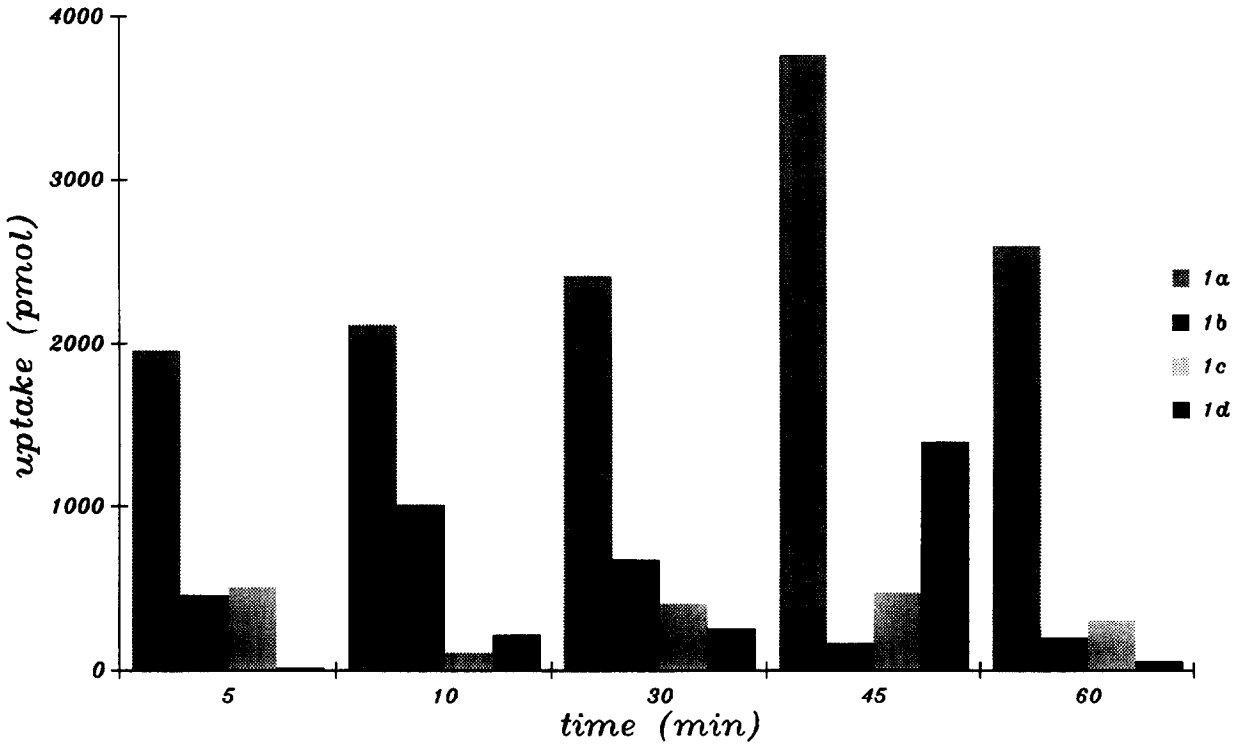


Fig. 3. Cellular uptake of **1a–d** (49 μM).

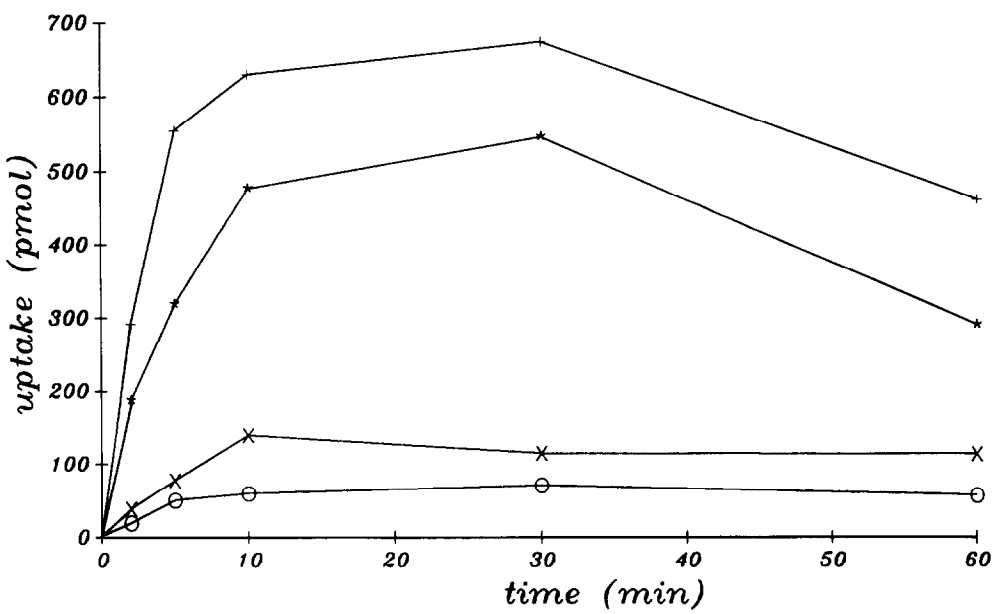


Fig. 4. Cellular uptake of **2a**. (+) 70 μM, (*) 37 μM, (×) 6 μM, (○) 4 μM.

Uptake experiments Compounds **1a–d** (Toth et al., 1991) and **2a–d** (Toth et al., 1992) were dissolved in DMSO (0.1 ml). Solutions of varying concentrations were prepared (from 4 μM–200 μM of known specific activity) by dilution with DMEM/10% FCS. The solutions of the conjugates (0.2 ml) were added to the wells and incubated for set times (each well was washed with 1 ml of a 1 mM solution of unlabelled drug made up in 4% bovine serum albumin using non-sterile 0.9% NaCl solution). After the incubation period, 400 μl of 1 M NaOH was added to each well, and left overnight. 300 μl of solution was taken from the wells into a scintillation vial, neutralised with 300 μl of 1 M HCl, Aquasol scintillation fluid (4 ml) was added and the radioactivity determined. The radioactivity counts were converted to pmoles.

Results and Discussion

The results of cellular uptake experiments on EATC correlate with those obtained previously on rat erythrocytes, rat hepatocytes and *Escherichia coli* (Toth et al. 1991). The absorption was dependent upon the concentration of the compounds, incubation time and nature of the lipidic amino acids and oligomers. Cellular uptake of the lipidic amino acid **1a** was substantially greater than that of the dimer **1b**, trimer **1c** and tetramer **1d**. This trend was observed at all concentrations (Figs 1–3). The uptake of **1a** increased over 45 min (Fig. 1) after which either the monomer was lost from the cell or bound activity decreased due to decomposition. This 45 min turning point was also evident in the uptake of the dimer **1b**, trimer **1c** and tetramer **1d** (Figs 2 and 3). The cellular uptake studies of drug conjugates **2b–d** and the parent drug **2a** showed interesting results. The parent benzoquinolizine **2a** was taken up, but the absorption was low (Fig. 4). The uptake decreased after 60 min of incubation time, indicating either removal of compound **2a** from the cell or metabolism, then removal from the cell, or cell lysis. The cellular uptake of monomer conjugate **2b** was greater (Fig. 5), the uptake increasing by 5–10-fold compared with

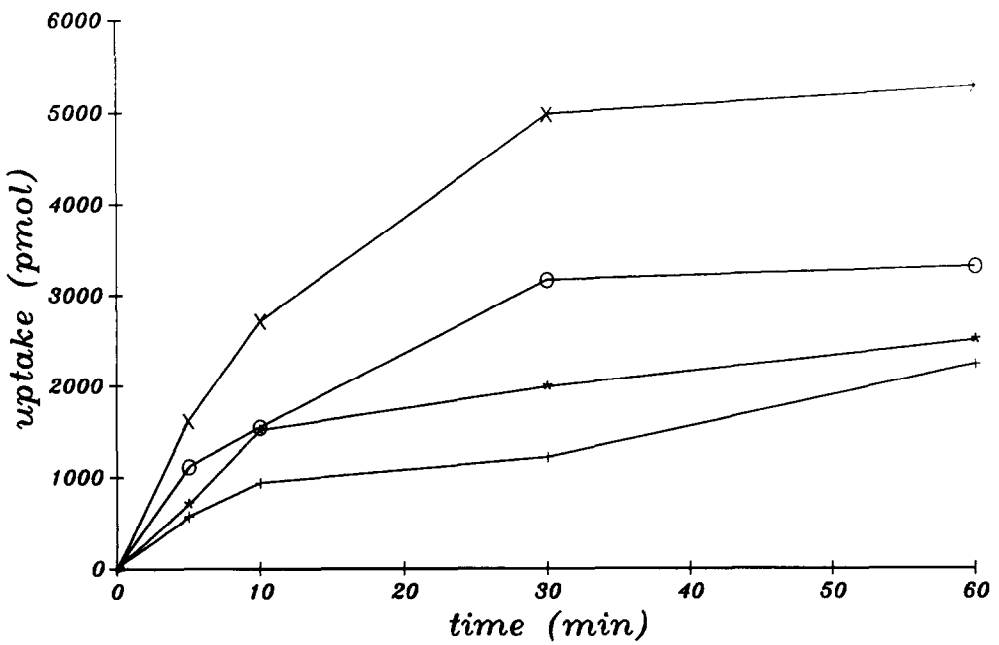


Fig. 5. Cellular uptake of **2b**. (×) 81 μM, (○) 57 μM, (*) 40 μM, (+) 28 μM.

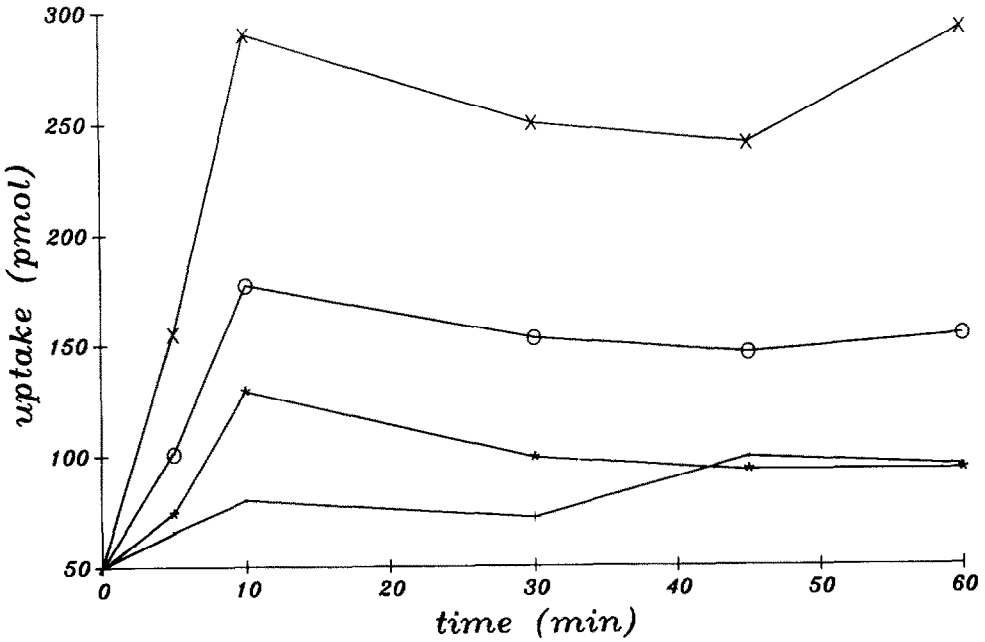


Fig. 6. Cellular uptake of **2c**. (×) 101 μM, (○) 52 μM, (∗) 36 μM, (●) 26 μM.

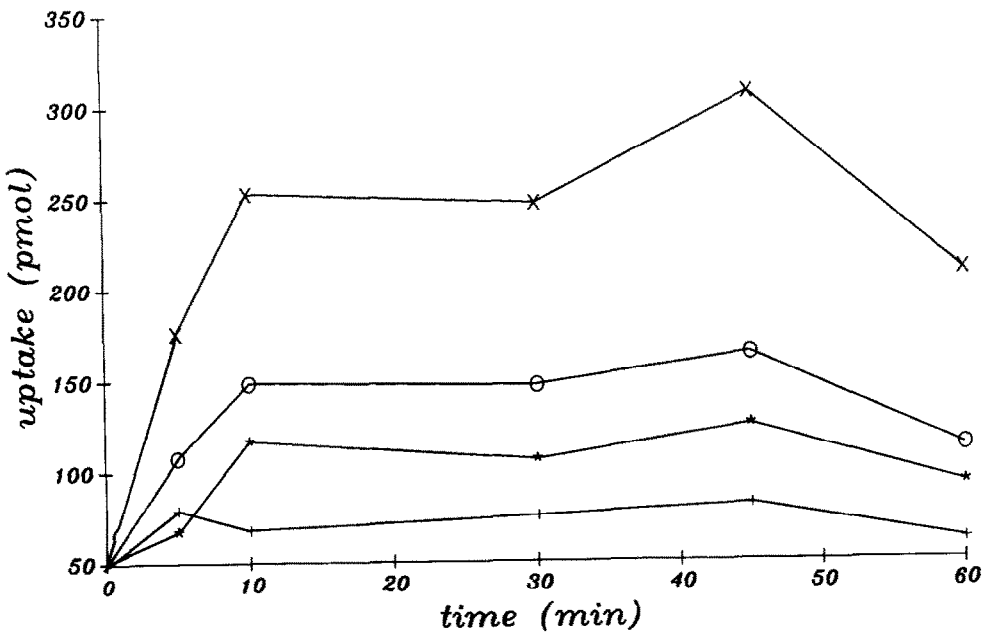


Fig. 7. Cellular uptake of **2d**. (×) 118 μM, (○) 40 μM, (∗) 34 μM, (+) 27 μM.

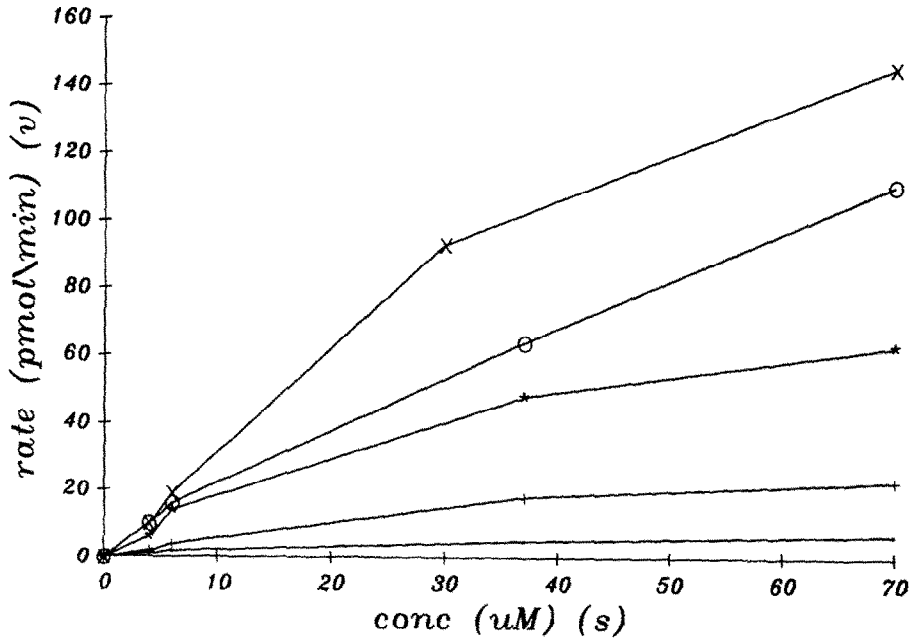


Fig. 8. Rate of cellular uptake of **2a**. **2a**: (×) 2 min, (○) 5 min, (∗) 10 min, (+) 30 min, (●) 60 min.

that of the parent **2a**. The uptake increased steadily, indicating that accumulation of the monomer occurred in the cell either as the conjugate or as a metabolite. The cellular uptake of the dimer conjugate **2c** and trimer conjugate **2d** was similar to that of the parent **2a** (Figs 6 and 7).

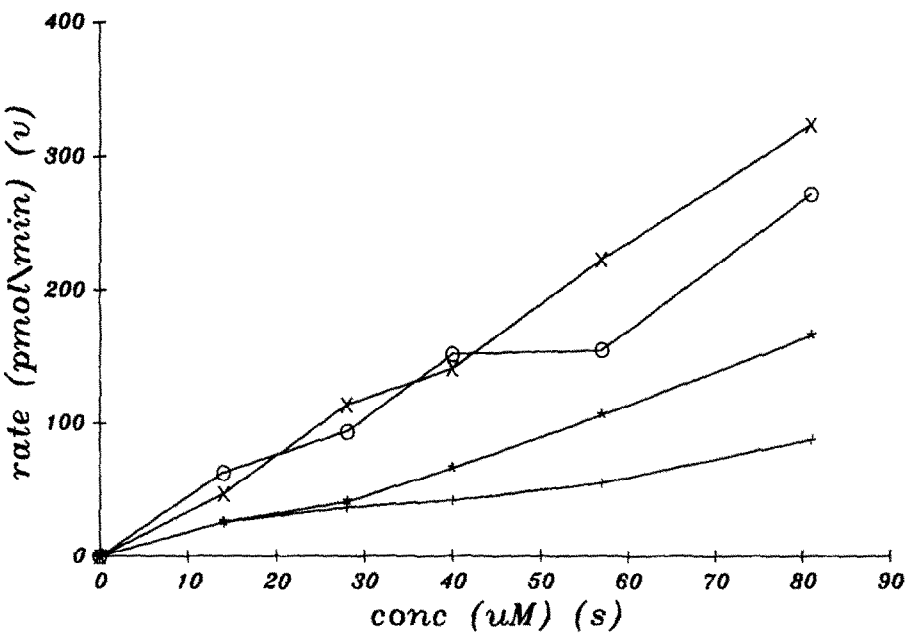


Fig. 9. Rate of cellular uptake of **2b**. **2b**: (×) 5 min, (○) 10 min, (∗) 30 min, (+) 60 min.

As the cells were washed with cold compound after uptake, it is believed that the majority of surface compound is displaced and therefore the results reflected the actual cellular uptake.

The rates of cellular uptake of compounds **2a** and **2b** are summarised in Figs 8 and 9. Both compounds showed a linear diffusion pattern, but the rate of uptake of **2b** was significantly higher than that of compound **2a**.

Conclusions

The monomer **1a** was taken up much more rapidly and extensively than the larger dimer **1b**, trimer **1c** or tetramer **1d**. We suggest that the monomer **1a** was transported across the membrane via a simple diffusion process. The dimer **1b**, trimer **1c** and tetramer **1d**, due to the increased lipophilicity of their structures, are retained in the plasma membrane as a result of lipophilic interactions. Conjugation of the lipophilic system to a drug (benzoquinolizine) could increase the uptake of that drug. This was apparent in the case of conjugate **2b**, however, conjugation with lipidic dimer (**2c**) or trimer (**2d**) caused a slight decrease in uptake.

Acknowledgement

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