International Journal of Pharmaceutics, 87 (1992) 141–147

IJP 02889

Lipidic peptides. XIII: Synthesis, structure elucidation and in vitro toxicity assessment of chlorambucil conjugates with lipidic acids, lipidic amino acids and their oligomers

Ian P. Wood [a], Istvan Toth [a], Jane L. Holley [b] and William A. Gibbons [a]

[a] *Department of Pharmaceutical Chemistry and* [b] *Department of Pharmacology / Toxicology, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX (UK)*

(Received 11 February 1992)
(Modified version received 5 May 1992)
(Accepted 7 May 1992)

Key words: Lipidic amino acid; Lipidic acid; Lipidic peptide; Transmembrane delivery; Cytotoxicity; ADJ/PC6 plasmacytoma cell; Chlorambucil

Summary

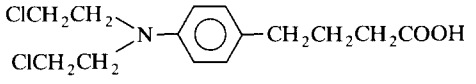
A series of lipidic amides of chlorambucil **3a–c**, oligomer **3d**, lipidic esters of chlorambucil **5a–c** and a chlorambucil conjugate containing both ester and amide linkage **7** were synthesized. The lipophilic character of the conjugates was altered by varying the number of lipidic acid units and the length of the alkyl chain. In vitro, the chlorambucil conjugates exhibited varying degrees of toxicity to ADJ/PC6 plasmacytoma cells. Their toxicity appeared to be related to their lipophilicity and the nature of the conjugating linkage.

Introduction

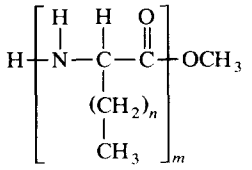
4-[4-Bis(2-chloroethyl)aminophenyl]butanoic acid (chlorambucil, **1**) has been in clinical use for over 30 years, and along with the related nitrogen mustards, is still among the most useful drugs in the fight against malignant diseases (Freckman et al., 1964; Lebhertz et al., 1965; McElwain et al., 1977; Savitsky et al., 1977; Lister et al., 1978). Although chlorambucil is extensively used, factors underlying transport, action, metabolism and resistance to the compound are incompletely understood. Chlorambucil acts intracellularly by alkylating DNA primarily at the N-7 position of guanine residues (Kohn et al., 1987), damaging macromolecular structure and resulting in cell death. The activity is dependent on plasma membrane permeability enabling it to reach its site of action. Investigations using Yoshida ascites sarcoma cell lines (Harrap and Hill, 1970; Hill et al., 1971; Hill, 1972) and chronic lymphocytic leucocytes (Hill and Harrap, 1972) indicated that uptake occurs by passive diffusion; however the

Correspondence to: I. Toth, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, U.K.

plasma membranes of some tumours have reduced permeability resulting in resistance to some antitumour agents. The lipidic amino acid based delivery system which possesses a high degree of membrane-like character (Toth et al., 1992) was utilised in the synthesis of a series of novel chlor-

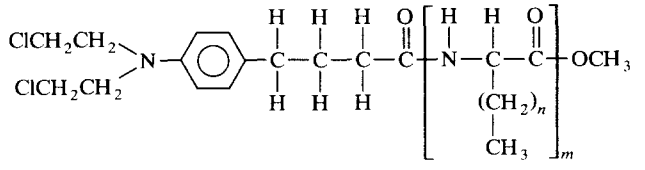


1



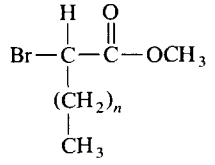
2	n	m
a	7	1
b	11	1
c	11	1
d	13	2

2



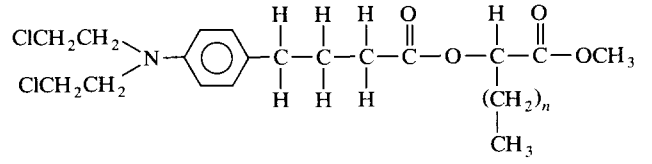
3	n	m
a	7	1
b	11	1
c	13	1
d	13	2

3



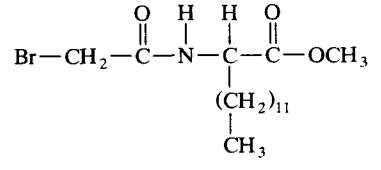
4	n
a	5
b	11
c	13

4

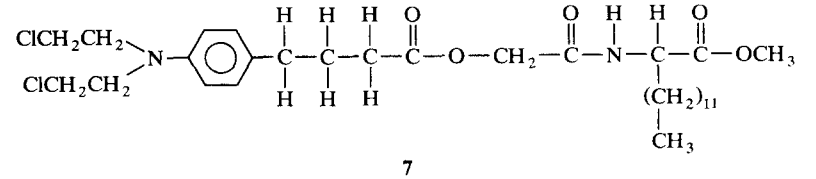


5	n
a	5
b	11
c	13

5



6



7

ambucil lipidic conjugates to modify the delivery and cytotoxicity of chlorambucil. A series of chlorambucil conjugates **3a–d**, **5a–c** and **7** were synthesised. Their in vitro toxicity to ADJ/PC6 plasmacytoma cells (Tisdale and Phillips, 1975) was used as an indication of the amount of active compound alkylating the DNA. It was envisaged that chlorambucil lipidic conjugates **3a–d**, **5a–c** and **7** would be absorbed onto/into target cell membranes, then would be hydrolysed by cellular esterases or amidases respectfully, releasing the active parent chlorambucil molecule within the target cell. It was anticipated that the conjugates **3a–d**, **5a–c** and **7** themselves would not be able to react with DNA in the cells, as they were unable to react with naked DNA in preliminary studies (unpublished reports).

Materials and Methods

Infrared spectra were recorded with a Perkin-Elmer 841 spectrophotometer. ^{1}H-NMR spectra were obtained with Varian XL-300 and Bruker AM500 instruments operating at fields of 300 and 500 MHz, respectively; chemical shifts are reported in ppm downfield from internal TMS. Mass spectra were run with a VG analytical ZAB-SE instrument, using the fast-atom bombardment (FAB) techniques: 20 kV Cs^+ bombardment, with 2 μl appropriate matrix, either 3-nitrobenzyl alcohol or thioglycerol, with NaI (MeOH solution) added when necessary to produce nitrated species where no protonated molecule ions were observed. Reaction process was monitored by thin-layer chromatography (TLC) on Kieselgel PF_{254} using solvent (A) CH_2Cl_2/MeOH (10:1) or solvent (B) CH_2Cl_2/benzene (6:4) as stated. Purification was achieved by TLC using Kieselgel $PF_{254+366}$ (Merck) on 20 × 20 cm plates 1.5 mm thick with the aforementioned solvent systems, or column or flash chromatography through Kieselgel G using CH_2Cl_2/MeOH (10:0.5) as the eluent. Solvents were evaporated under reduced pressure with a rotary evaporator.

In vitro toxicity

Approx. 5×10^4 ADJ/PC6 tumour cells were seeded into wells of 24-well multiwell culture dishes together with different concentrations of the conjugates **3a–d**, **5a–c** and **7** being tested. The final volume in each well was 1 ml, and all incubations were carried out in DMEM/10% FCS (Dulbecco's modification of Eagles medium supplemented with 10% foetal calf serum) in a 5% CO_2 atmosphere at 37°C, under sterile conditions. The cells were incubated with the conjugates for 70 h: then 0.5 μCi [^{3}H]thymidine was added to each well and incubated for a further 2 h. Cells were transferred to 75 × 12 mm tubes, centrifuged at 1000 rpm to spin down the cells and the radioactive supernatant discarded. The cells were washed twice with 0.9% NaCl containing 1 mM thymidine (unlabelled) to remove unbound radiolabelled thymidine. 10% trichloroacetic acid (1 ml) was added to the cell pellet to precipitate protein (4°C, 10 min) and then centrifuged at 2000 rpm for 5 min. The supernatant was discarded and the precipitate dissolved in 300 μl of 1 M NaOH. The solution was neutralised with 300 μl of 1 M HCl, transferred to scintillation vials and 4 ml Aquasol (scintillation fluid) added to each vial. After capping and mixing the samples were analyzed on the β-counter. The amount of labelled [^{3}H]thymidine incorporated into the DNA of the cells gave an indication of their viability. Results for each concentration of conjugate were expressed as a percentage of the radioactivity incorporated into untreated control cells. A 72 h incubation was used to enable the cells to go through a number of cell cycles, allowing the DNA damage inflicted by the conjugates to be manifested as cell death.

Method A

*α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}-ω-methoxy[imino-(1-octyl-2-oxo-1,2-ethanediyl)] (**3a**)* Chlorambucil **1** (150 mg, 0.49 mM), triethylamine (0.5 mg, 0.98 mM), 1-hydroxybenzotriazole hydrate (67 mg, 0.49 mM), methyl-α-aminodecanoate hydrochloride (119.3 mg, 0.49 mM) and dicyclohexylcarbodiimide (102 mg, 0.49 mM) were stirred in dichloromethane at 0°C for 1

h then at 20°C for a further 4 h. The reaction mixture was filtered, the solvent removed in vacuo, the residue washed with ethyl acetate, then filtered and the ethyl acetate removed in vacuo. The crude material was purified on TLC using solvent system (A). Yield: 64%.

^{1}H-NMR ($CDCl_3$): $\delta = 7.06$ (2H, d, aro.H), 6.65 (2H, d, aro.H), 5.86 (H, d, NH), 4.63 (H, m, α-CH), 3.75 (3H, s, $COOCH_3$), 3.68 (4H, t.CH_2N), 3.62 (4H, t, CH_2Cl), 2.58 (2H, t, bz-CH_2), 2.28 (2H, t, CH_2CO), 1.94 (2H, t, CH_2), 1.83–1.65 (2H, m, β-CH_2), 1.26 (12H, m, $6 \times CH_2$), 0.88 (3H, t, CH_3).

MS m/z (%) = 491, 489, 487$[M + H]^+$ (9, 60, 82), 454(52), 437(56), 290, 288, 286 $[CLB\text{-}OH]^+$ (10, 60, 100), 250(25), 230(38), 194(48), 142(75), 118(35), 55(45).

*α-{4-[4-Bis-(2-chloroethyl)aminophenyl]butanoyl}-ω-methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (**3b**)* Yield: 43.2%.

^{1}H-NMR ($CDCl_3$): $\delta = 7.08$ (2H, d, aro.H), 6.63 (2H, d, aro.H), 5.85 (H, d, NH), 4.62 (H, m, α-CH), 3.74 (3H, s, $COOCH_3$), 3.68 (4H, t, CH_2N), 3.62 (4H, t, CH_2Cl), 2.58 (2H, t.bz-CH_2), 2.40, 2, 21 (2H, $2 \times$ t, CH_2CO), 1.94 (2H, t, CH_2), 1.83–1.60 (4H, m, $2 \times \beta$-CH_2, $2 \times \gamma$-CH_2), 1.25 (22H, m, $11 \times CH_2$), 0.879 (3H, t, CH_3).

MS m/z (%) = 570, 568, 556 $[M + Na + H]^+$ (3, 24, 35), 569, 567, 565 $[M + Na]^+$ (12, 73, 100), 532(12), 507(15), 291(1), 289(6), 286(9), 250(13).

*α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}-ω-methoxy[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (**3c**)* Yield = 66.3%.

^{1}H-NMR ($CDCl_3$): $\delta = 7.05$ (2H, d, aro.H), 6.65 (2H, d, aro.H), 5.85 (1H, d, NH), 4.6 (1H, m, α-CH), 3.75 (3H, s, $COOCH_3$), 3.70 (4H, t, $2 \times CH_2N$), 3.62 (4H, t, $2 \times CH_2Cl$), 2.55 (2H, m, bz-CH_2), 2.2 (2H, m, CH_2CO), 1.8–1.55 (4H, m, $4 \times CH$), 1.25 (24H, m, $12 \times CH_2$), 0.875 (3H, t, CH_3).

MS m/z (%) = 598, 596, 594 $[M + Na + H]^+$ (4, 23, 37), 597, 595, 593 [M + Na] (10, 65, 100), 290(5), 288(22), 286(35), 250(26), 194(26), 188(22), 69(30).

*α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoyl}-ω-methoxybis[imino-(1-tetradecyl-2-oxo-1,2-ethanediyl)] (**3d**)* Yield 92.3%.

^{1}H-NMR ($CDCl_3$): $\delta = 7.05$ (2H, d, aro.H), 6.65 (2H, d, aro.H), 6.55–5.96 (H, m, NH), 5.98 (H, m, NH), 4.55–4.90 (2H, m, $2 \times \alpha$-CH), 3.75 (3H, s, $COOCH_3$), 3.70 (4H, m, $2 \times CH_2N$), 3.60 (4H, m, $2 \times CH_2Cl$), 2.55 (2H, m, bz-CH_2), 2.85–2.15 (2H, m, CH_2CO), 1.95 (2H, m, CH_2), 1.85–1.50 (4H, m, $2 \times CH_2$), 1.5 (48H, m, $24 \times CH_2$), 0.85 (6H, t, $2 \times CH_3$).

MS m/z (%) = 850, 848, 846 $[M + Na]^+$ 15, 74, 100, 809(25), 744(7), 748(7), 733(10), 613(13), 290(3), 288(10), 286(19), 250(61), 226(70), 173(72), 73(100).

Method B

*α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoate}-ω-methoxy(1-dodecyl-2-oxo-1,2-ethanediyl) (**5b**)* Chlorambucil **1** (199.8 mg, 0.66 mM) and 18-crown-6 (173.7 mg, 0.66 mM) were added to an ethanolic potassium hydroxide solution 5 mg ml^{-1} (22.3 ml), and stirred at 0°C for 3 h. The solvent was evaporated in vacuo then the residue lyophilised. The resulting chlorambucil crown ether complex and methyl 2-bromotetradecanoate (211 mg, 0.66 mM) were dissolved in 5 ml dimethylformamide and stirred for 12 h. The dimethylformamide was evaporated off under high vacuo and the crude product was purified by TLC using solvent A then solvent B. Yield = 63.7%.

^{1}H-NMR ($CDCl_3$): $\delta = 7.05$ (2H, d, aro.H), 6.62 (2H, d, aro.H), 4.23 (H, m, α-CH), 3.60 (3H, s, $COOCH_3$), 3.67 (4H, m, $2 \times CH_2N$), 3.61 (4H, m, $2 \times CH_2Cl$), 2.58 (2H, m, bz-CH_2), 2.48 (2H, m, CH_2CO), 1.98 (2H, m, CH_2), 1.96–1.80 (4H, m, $2CH_2$), 1.25 (18H, m, $9 \times CH_2$), 0.85 (3H, t, CH_3).

MS m/z (%) = 570, 568, 566 $[M + Na]^+$ (6)(22)(35), 547, 545, 543 $[M]^+$(7)(65)(80), 517(46), 496(47), 494(69), 290(10), 288(58), 286(100), 259(22), 230(40), 194(28), 168(22), 188(38).

*α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoate}-ω-methoxy(1-hexyl-2-oxo-1,2-ethanediyl) (**5a**)* Yield = 69.2%.

^{1}H-NMR ($CDCl_3$): $\delta = 7.06$ (2H, d, aro.H), 6.62 (2H, d, aro.H), 4.24 (H, m, α-CH), 3.80 (3H, s, $COOCH_3$), 3.65 (4H, m, $2 \times CH_2N$), 3.61 (4H, m, $2 \times CH_2Cl$), 2.58 (2H, m, bz-CH_2), 2.48 (2H, m, CH_2CO), 1.98 (2H, m, CH_2), 1.90–1.80 (2H,

m, β-CH_2), 1.26 (8H, m, $4 \times CH_2$), 0.85 (3H, t, CH_3).

MS m/z (%) = 524, 522, 520 [M + Na](11)(59)(100), 501, 449, 447 $[M]^+$(3)(17)(28), 290(4), 288(22), 286(37), 250(19), 230(42).

α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoate}-ω-methoxy(1-tetradecyl-2-oxo-1,2-ethanediyl) (5c) Yield = 67%.

^{1}H-NMR ($CDCl_3$): δ = 7.06 (2H, d, aro.H), 6.62 (2H, d, aro.H), 4.25 (H, m, α-CH), 3.82 (3H, s, $COOCH_3$), 3.65 (4H, m, $2 \times CH_2N$), 3.61 (4H, m, $2 \times CH_2Cl$), 2.57 (2H, m, bz-CH_2), 2.47 (2H, m, CH_2CO), 1.98 (2H, m, CH_2), 1.9–1.81 (4H, m, $2 \times CH_2$), 1.27 (22H, m, $11 \times CH_2$), 0.58 (3H, t, CH_3).

MS m/z (%) = 575, 573, 571 $[M]^+$ (20–100), 536(35), 522(46), 290(10), 288(60), 286(87), 230(30), 194(20), 118(17).

α-Bromoethanoyl-ω-methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (6) Bromoacetic acid (1 g, 7.2 mM), methyl 2-aminotetradecanoate hydrochloride (2.1 g, 7.2 mM) and dicyclohexylcarbodiimide (1.48 g, 7.2 mM) were added to 5 ml dichloromethane and allowed to react at 0°C for 1 h then at 20°C for 12 h. The mixture was filtered, the solvent evaporated in vacuo and ethyl acetate added to the residue. The precipitate formed was filtered and the filtrate evaporated in vacuo. The crude material was purified by flash chromatography using solvent B. Yield = 2.04 g (75%).

^{1}H-NMR ($CDCl_3$): δ = 7.00, 6.65 (H, $2 \times$ d, NH), 4.59 (1H, m, α-CH), 4.07, 3.89 (2H, $2 \times$ S, $1 \times CH_2Cl$, $1 \times CH_2Br$), 3.75 (3H, s, $COOCH_3$), 1.9–1.65 (4H, m, $2 \times CH_2$), 1.26 (18H, m, $9 \times CH_2$), 1.85 (3H, t, CH_3).

MS m/z (%) = 402, 400 $[M + Na]^+$ (98, 100), 380, 378 $[M + H]^+$ (55, 60), 245(96), 198(45), 176(16), 137(26).

α-{4-[4-Bis(2-chloroethyl)aminophenyl]butanoate}-ethanoyl-ω-methoxy[imino-(1-dodecyl-2-oxo-1,2-ethanediyl)] (7) [method B] Yield = 53.4%.

^{1}H-NMR ($CDCl_3$): δ = 7.06 (2H, m, aro.H), 6.62 (2H, d, aro.H), 4.65 (H, m, α-CH), 4.55 (2H, d, $COOCH_2CO$), 3.75 (3H, s, $COOCH_3$), 3.70 (4H, t, $2 \times CH_2N$), 3.60 (4H, t, $2 \times CH_2$), 2.60 (2H, t, bz-CH_2), 2.45 (2H, t, CH_2CO), 2.00 (2H, m, CH_2), 1.85–1.75 (2H, m, CH_2), 1.30 (20H, m, $10 \times CH_2$), 0.85 (3H, t, CH_3).

MS m/z (%) = 605, 603, 601 $[M + H]^+$ (4)(28)(35), 551(15), 433(10), 274(100), 290(2), 288(12), 286(17), 198(41).

Results and Discussion

Chemistry

The chlorambucil conjugates **3a–d**, **5a–c** and **7** are novel derivatives whose stability and toxicity in biological systems were unknown. Conjugates with amido linkage **3a–d** were prepared by coupling the appropriate methyl α-aminoalkanoates **2a–d** (Gibbons et al., 1990) to chlorambucil using standard solution phase peptide synthetic methods. The chlorambucil conjugates linked via ester bonds **5a–c** were synthesized by coupling the appropriate methyl-2-bromoalkanoates **4a–c** to the potassium salt of chlorambucil **1**, using a crown ether assisted coupling method (Hughes et al., 1992). Firstly, a complex of the potassium salt of chlorambucil **1** with the macrocyclic ether, 18-crown-6 was prepared, then reacted with the appropriate methyl-2-bromoalkanoates **4a–c**, furnishing the ester conjugates **5a–c**. Conjugate **7**, with ester and amide bonds was synthesized by coupling methyl-2-bromoacetylalkanoate **6**, with the crown ether complex of **1**, using a method similar to that for synthesising conjugates **5a–c**.

In vitro toxicity

The in vitro toxicity of chlorambucil conjugates **3a–d**, **5a–c** and **7** towards ADJ/PC6 plasmacytoma cells was probably due to the release of free chlorambucil in the cell, since the conjugates themselves did not react with naked DNA. The chlorambucil conjugates **3a–d** showed varying degrees of toxicity (Fig. 1) as assessed by [^{3}H]-thymidine incorporation into DNA. However, the biological activity was not in linear correlation with the lipophilicity (i.e., length of alkyl side chain) of the conjugates. A re-bound DNA synthesis may explain the increased [^{3}H]thymidine incorporation observed for low concentrations of conjugate **3d**. Conjugates **5a–c** exhibited a decrease in toxicity with increase in the alkyl chain length (Fig. 2). Although the long alkyl chain may increase the uptake of the compounds, it may also cause steric hindrance of the conjugating

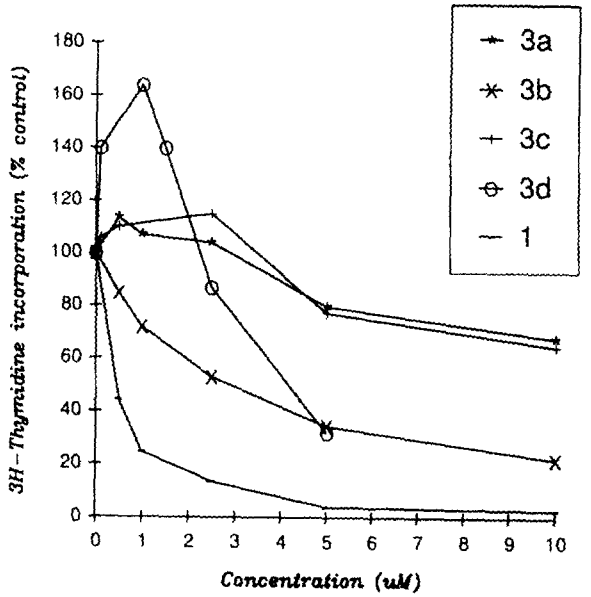


Fig. 1. In vitro toxicity of compounds **1** and **3a–d** to ADJ/PC6 plasmacytoma cells.

bond, reducing the rate of release of the parent chlorambucil. The cytotoxicities of conjugates **3b**, **5b** and **7** were compared (Fig. 3) and we found that the conjugating linkage had a great influence on the toxicity of the chlorambucil conjugates.

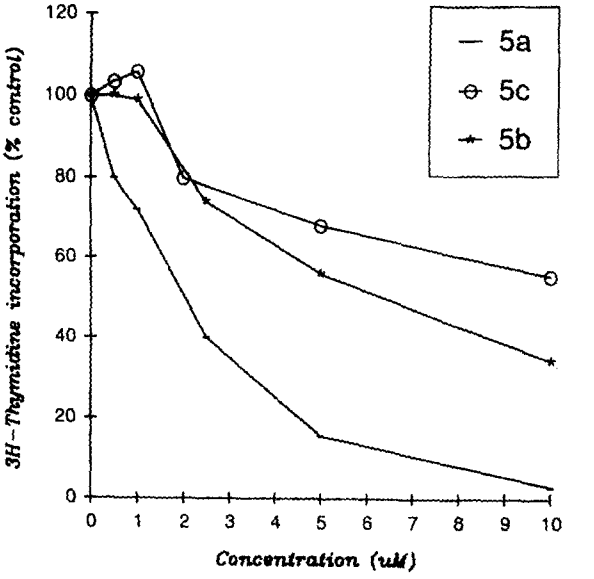


Fig. 2. In vitro toxicity of compounds **5a–c** to ADJ/PC6 plasmacytoma cells.

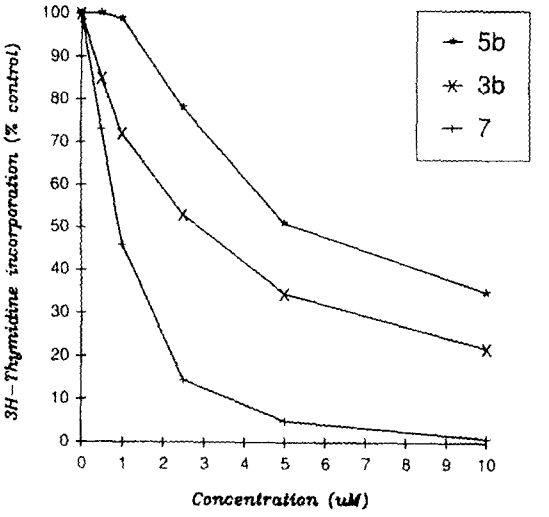


Fig. 3. In vitro toxicity of compounds **3b**, **5b** and **7** to ADJ/PC6 plasmacytoma cells.

The most toxic compound was conjugate **7** with an ester linkage and a small spacer between active parent and conjugate moiety; the least toxic compound, as expected, was conjugate **5b** with an amido linkage.

Conclusion

A series of chlorambucil conjugates **3a–d**, **5a–c** and **7** of varying lipophilicities were synthesized by conjugating chlorambucil **1** with compounds **2a–d**, **4a–c** and **6**, respectively. They showed varying degrees of toxicity to ADJ/PC6 plasmacytoma cells, and we suggest that the conjugates are hydrolysed, releasing the parent chlorambucil. The most important observation in the case of compounds **5a–c** was that increasing the length of the alkyl side chain brought about a decrease in toxicity. The longer alkyl side chains may decrease the rate of hydrolysis due to increase in steric hindrance. The linkage in the chlorambucil conjugates also influences their toxicity. Conjugates **3b**, **5b** and **7** had the same length of alkyl side chain, conjugate **7** with an ester and amide linkage showing the highest toxicity followed by **3b** with an ester linkage, then compound **5b** which had an amide linkage. By varying the lipophilicity and linkage of the chlorambucil conjugates we

were able to modify chlorambucil toxicity towards ADJ/PC6 plasmacytoma cells. Thus, the lipidic amino acid based delivery system may be used as a tool in the development of alkylating agents, such as chlorambucil, with increased selectivity.

Acknowledgements

The authors would like to express their thanks to Dr G.J. Anderson and Mr C. James for recording ^{1}H-NMR spectrum and the staff of University of London Intercollegiate Research Service in Mass Spectroscopy at The School of Pharmacy, Brunswick Square, for running MS.

References

Freckman, H.A., Fry, H.L., Mendez, F.L. and Maurer, E.R. Chlorambucil-prednisolone therapy for disseminated breast carcinoma. *J. Am. Med. Assoc.*, 189 (1964) 23–26.

Gibbons, W.A., Hughes, R.A., Charalambous, M., Christodoulou, M., Szeta, A., Aulabaugh, A.E., Mascagni, P. and Toth, I., Synthesis, solution and structural elucidation of lipidic amino acids and their homo- and hetero-oligomers. *Leibigs. Ann. Chem.*, (1990) 1175–1183.

Harrap, K.R. and Hill, B.T., The selectivity of alkylation agents and drug resistance. III. The uptake and degradation of alkylating drugs by Yoshide ascites sarcoma cells in vitro. *Biochem. Pharmacol.*, 19 (1970) 209–217.

Hill, B.T., Studies on the transport and cellular distribution of chlorambucil in the Yoshide ascites sarcoma. *Biochem. Pharmacol.*, 21 (1972) 495–502.

Hill, B.T. and Harrap, K.R., The uptake and utilization of chlorambucil by lymphocytes from patients with chronic lymphocytic leukaemia. *Br. J. Cancer*, 26 (1972) 439–443.

Hill, B.T., Jarmain, M. and Harrap, K.R., Selectivity of action of alkylating agents and drug resistance. IV: Resistance of tritium-labelled-chlorambucil and a study of its cellular uptake by drug-sensitive and drug resistant strains of the Yoshide ascites sarcoma in vitro. *J. Med. Chem.*, 14 (1971) 614–618.

Hughes, R.A., Toth, I., Ward, P., McColm, M. and Gibbons, W.A., Lipidic peptides. V: Penicillin and cephalosporin conjugates with increased lipophilic character. *J. Pharm. Sci.*, 81 (1992) 845–848.

Kohn, K.W., Hartley, J.A., and Mattes, W.B., Mechanisms of DNA sequence selective alkylation of guanine N-7 positions by nitrogen mustards. *Nucleic Acids Res.*, 15 (1987) 10531–10549.

Lebhertz, T.B., Huston, J.W., Austin, J.A. and Boyer, C.R., Sustained palliation in ovarian carcinoma *Obstet. Gynecol.*, 25 (1965) 475–478.

Lister, T.A., Cullen, M.H., Bearm, M.E.J., Brearley, R.L., Whitehouse, J.M.A., Wrigley, P.F.M., Standfield, A.G., Sutcliffe, S.B.J., Malpas, J.S. and Crowther, D., A comparison of combined and single agent chemotherapy in non-Hodgkin's lymphoma of favourable histological type. *Br. Med. J. (Clin. Res.)*, 1 (1978) 533–537.

McElwain, T.J., Toy, J., Smith, E., Peckham, M.J. and Austin, D.E., A combination of chlorambucil, vinblastine, procarbazine and prednisolone for treatment of Hodgkin's disease. *Br. J. Cancer*, 36 (1977) 276–280.

Savitsky, A., Rai, K.R., Flidwell, O and Silver, R.T., Comparison of daily versus intermittent chlorambucil and prednisolone therapy in the treatment of patients of chronic lymphocytic leukaemia. *Blood*, 50 (1977) 1049–1059.

Tisdale, M.J. and Phillips, B.J., Adenosine 3′,5′-monophosphate phosphodiesterase activity in experimental animal tumours which are either sensitive or resistant to bifunctional alkylating agents. *Biochem. Pharmacol.*, 19 (1975) 205–210.

Toth, I., Griffith, I.P., Fernandez, E.O., Hafeez, R.A., Holley, J.L., Ward, P. and Gibbons, W.A. Lipidic peptides. VIII: Cellular uptake studies of lipidic amino acid, its oligomers and highly lipophilic drug conjugates. *Int. J. Pharm.*, 79 (1992) 39–45.