International Journal of Pharmaceutics, 68 (1991) 191–198

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ADONIS 037851739100088V

IJP 02304

Lipidic peptides. II. Synthesis, activity and transport of anti-inflammatory benzoquinolizine-lipidic peptide conjugates

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(Received 11 July 1990)
(Modified version received 12 September 1990)
(Accepted 24 September 1990)

Key words: Lipidic amino acid; Lipidic peptide; Transmembrane drug delivery; Anti-inflammatory drug; Oral absorption; Histamine release

Summary

Two series of lipidic peptide conjugates **1c–1i** and **2c–2e** of the anti-inflammatory benzoquinolizine alkaloids **1b** and **2b** were synthesised. The biological activities of the conjugates **1c**, **1d** and **1f** were assessed by determining their ability to inhibit the release of histamine from rat mast cells in vitro. Conjugates **1d** and **1f**, their parent alkaloid **1b** and its methyl ester **1a**, gave 40–50% inhibition, whereas conjugate **1c** showed 2-fold greater activity. The radiolabelled compounds **2a*** and **2c*** were produced by reduction of **1a** and **1f** with NaB^3H_4. Following their oral administration to rats, peak serum levels of the conjugate **2c*** were 5 times greater than that observed for the parent compound **2a***. The results suggest that conjugation to lipidic amino acids and peptides can be a useful approach to improving the oral absorption of poorly absorbed drugs.

Introduction

The α-amino acids with long hydrocarbon side chains, the so-called lipidic amino acids and their homo-oligomers, the lipidic peptides, represent a class of compounds which combine stuctural features of lipids with those of amino acids (Gibbons et al., 1990). Several uses of lipidic amino acids and peptides have been proposed (Gibbons, 1990). Of particular interest is their potential use as a drug delivery system (Toth et al., 1990). The lipidic amino acids and peptides could be covalently conjugated to or incorporated into poorly absorbed peptides and drugs, to enhance the passage of the pharmacologically active compounds across biological membranes.

Because of their bifunctional nature, the lipidic amino acids and peptides have the capacity to be chemically conjugated to drugs with a wide variety of functional groups. The linkage between drug and lipidic unit may either be biologically stable (i.e. a new drug is formed) or possess biological or chemical instability (i.e. the conjugate is a pro-drug). In either case, the resulting conjugates would be expected to possess a high degree of membrane-like character, which may be sufficient to facilitate their passage across membranes. The

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long alkyl side chains may also have the additional effect of protecting a labile parent drug from enzymatic attack, thereby enhancing metabolic stability.

The benzoquinolizine acids (**1b** and **2b**) and their methyl esters (**1a** and **2a**) possess anti-inflammatory activity (Szabo et al., 1979; Szantay et al., Pat. (a), (b)). The free acid function of **1b** and **2b** can be conjugated to the amino group of a lipidic carrier forming a relatively stable amide bond. These compounds thus provide a suitable model system to compare the absorption of parent anti-inflammatory drug with its lipidic conjugate.

Materials and Methods

Infrared spectra were recorded with a Perkin Elmer 841 spectrophotometer. Melting points are not given for diastereomeric mixtures. ^{1}H-NMR spectra were obtained on 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 on a VG Analytical ZAB-SE instrument, using fast atom bombardment (FAB) techniques. Reaction progress was monitored by thin layer chromatography (TLC) on Kieselgel PF_{254} using dichloromethane : methanol 10 : 1 as the mobile phase. Purification was achieved by TLC using Kieselgel $PF_{254+366}$ (Merck) on 20 × 20 cm plates of thickness 1.5 mm, or column or flash chromatography through Kieselgel G (system: dichloromethane : methanol 10 : 1). Solvents were evaporated under reduced pressure with a rotary evaporator. Infrared spectra gave characteristic NH, carbonyl and aromatic absorbances. Purity of the compounds was determined by thin-layer and high-pressure liquid chromatography (HPLC). HPLC separation was carried out on a Whatman Partisil 5 RAC silica column. HPLC grade dichloromethane (Aldrich) and methanol (Rathburn) were filtered through a 25 μm membrane filter and degassed with helium flow prior to use. Separation was achieved with a solvent gradient beginning with 0% methanol, increasing constantly to 50% methanol at 15 min and decreasing steadily to 0% methanol from 17 to 20 min at a constant flow of 3 ml min^{-1}. The gradient was effected by two microprocessor-controlled Gilson 302 single piston pumps. Compounds were detected with a Holochrome UV-Vis detector at 254 nm. Chromatographs were recorded with an LKB 2210 single-channel chart recorder. Fluorimetric histamine assays were determined on a Perkin-Elmer 3000 spectrofluorimeter at an excitation wavelength of 360 nm and emission wavelength of 440 nm.

*Methyl N-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminodecanoate (**1c**)*

In a 100 ml round-bottom flask acid **1b** (1.00 g, 3.16 mmol), methyl α-aminodecanoate hydrochloride **3a** (0.749 g, 3.16 mmol), triethylamine (0.637 g, 6.31 mmol), 1-hydroxybenzotriazole hydrate (0.426 g, 3.15 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (0.665 g, 3.47 mmol) were dissolved in dichloromethane (50 ml) at 0°C. The solution was stirred for 2 h in an ice bath and then overnight at room temperature. The reaction mixture was washed with water (3 × 50 ml), dried ($MgSO_4$) and the solvent removed in vacuo. The crude product was purified on silica gel (system I). The yellow oil obtained was triturated with cold acetonitrile, yielding a pale yellow solid which was dried in a desiccator under vacuum. Yield: 1.13 g (72%) yellow solid.

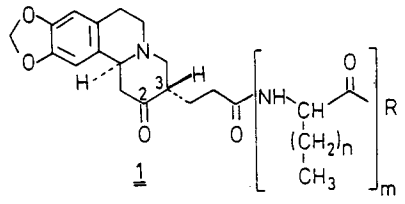
NMR ($CDCl_3$): 6.58 (1H, s, C_{11}-H), 6.53 (1H, s, C_8-H), 6.00 (1H, m, CONH), 5.91 (2H, s, OCH_2O), 4.57 (1H, m, α-CH), 3.75, 3.73 (3H, 2s, $COOCH_3$), 1.27 (12H, m, 6 × CH_2), 0.87 (3H, m, CH_3).

MS *m/z* (%): $C_{28}H_{42}N_2O_6$ (500.62); 501 (79) $[M + H]^+$, 298 (100), 256 (66), 242 (55), 228 (17), 214 (26), 202 (18), 189 (49), 176 (86), 142 (45).

*Methyl N^1-[N^2-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminodecanoyl]-α-aminodecanoate (**1d**)*

Compounds **1b** (0.803 g, 2.53 mmol) and **3b** (1.030 g, 2.53 mmol) were reacted as described in the preceding section. Yield: 1.36 g (80%) pale yellow solid.

NMR ($CDCl_3$): 6.57 (1H, s, C_{11}-H), 6.52 (1H, s, C_8-H), 6.40, 6.12 (2H, m, 2 × CONH), 5.89 (2H,



1	n	m	R
a	–	0	OCH_3
b	–	0	OH
c	7	1	OCH_3
d	7	2	"
e	7	3	"
f	11	1	"
g	11	2	"
h	11	3	"
i	17	1	"

Scheme 1.

s, OCH_2O), 4.54, 4.39 (2H, m, 2 × α-CH), 3.72 (3H, m, $COOCH_3$), 1.27 (24H, m, 12 × CH_2), 0.87 (6H, m, 2 × CH_3).

MS *m/z* (%): $C_{38}H_{59}N_3O_7$ (669.88); 670 (62) $[M + H]^+$, 501 (23), 298 (86), 270 (16), 256 (36), 242 (45), 214 (17), 202 (23), 189 (38), 176 (77), 142 (100), 55 (18).

*Methyl N^1-[N^2-[N^3-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminodecanoyl]-α-aminodecanoyl]-α-aminodecanoate (**1e**)*

Compounds **1b** (0.610 g, 1.93 mmol) and **3c** (0.716 g, 1.93 mmol) were reacted as described for **1c**. Yield: 0.36 g (45%) pale yellow solid.

NMR ($CDCl_3$): 6.58 (1H, s, C_{11}-H), 6.52 (1H, s, C_8-H), 6.46, 6.42, 6.04 (3H, m, 3 × CONH), 5.90 (2H, s, OCH_2O), 4.53, 4.35 (3H, m, 3 × α-CH), 3.73 (3H, m, $COOCH_3$), 1.25 (36H, m, 18 × CH_2), 0.88 (9H, t, 3 × CH_3).

MS *m/z* (%): $C_{48}H_{78}N_4O_8$ (839.14); 839 (19) $[M + H]^+$, 298 (27), 256 (22), 242 (19), 198 (20), 176 (38), 142 (100), 55 (24).

*Methyl N-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoate (**1f**)*

Compounds **1b** (1.080 g, 3.41 mmol) and **3d** (1.000 g, 3.41 mmol) were reacted as described for **1c**. Yield: 0.890 g (47%) yellow solid.

NMR ($CDCl_3$): 6.59 (1H, s, C_{11}-H), 6.53 (1H, s, C_8-H), 5.98 (1H, m, CONH), 5.90 (2H, s, OCH_2O), 4.59 (1H, m, α-CH), 3.73 (3H, d, $COOCH_3$), 1.28 (20H, m, 10 × CH_2), 0.89 (3H, t, CH_3).

MS *m/z* (%): $C_{32}H_{48}N_2O_6$ (556.72); 557 (100) $[M + H]^+$, 298 (56), 256 (22), 242 (19), 198 (20), 198 (25), 176 (48).

*Methyl N^1-[N^2-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoyl]-α-aminotetradecanoate (**1g**)*

Compounds **1b** (0.682 g, 1.93 mmol) and **3e** (1.000 g, 1.93 mmol) were reacted as described for **1c**. Yield: 0.980 g (65%) pale yellow solid.

NMR ($CDCl_3$): 6.58 (1H, s, C_{11}-H), 6.52 (1H, s, C_8-H), 6.38, 6.09 (2H, m, 2 × CONH), 5.88 (2H, s, OCH_2O), 4.53, 4.41 (2H, m, 2 × α-CH), 3.71 (3H, m, $COOCH_3$), 1.24 (40H, m, 20 × CH_2), 0.88 (6H, t, 2 × CH_3).

MS *m/z* (%): $C_{46}H_{75}N_3O_7$ (782.09); 782 (76) $[M + H]^+$, 298 (100), 270 (16), 256 (35), 242 (34), 198 (61), 198 (24), 176 (50).

*Methyl N^1-[N^2-[N^3-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoyl]-α-aminotetradecanoyl]-α-aminotetradecanoate (**1h**)*

Compounds **1b** (0.123 g, 0.349 mmol) and **3f** (0.260 g, 0.349 mmol) were reacted as described for **1c**. Yield: 0.235 g (67%) pale yellow solid.

NMR ($CDCl_3$): 6.59 (1H, s, C_{11}-H), 6.54 (1H, s, C_8-H), 6.47, 6.09 (3H, m, 3 × CONH), 5.92 (2H, s, OCH_2O), 4.53, 4.41, 4.37 (3H, m, 3 × α-CH),

3.73 (3H, m, $COOCH_3$), 1.26 (60H, m, $30 \times CH_2$), 0.88 (9H, m, $3 \times CH_3$).

MS *m/z* (%): $C_{60}H_{102}N_4O_8$ (1007.46); 1008 (100), $[M + H]^+$, 497 (9), 387 (15), 371 (29), 344 (20), 288 (67), 242 (26), 198 (68), 121 (46).

*Methyl N-[9,10-(methylenedioxy)-2-oxo-1,3β,4,6,7,11bα-hexahydrobenzo[a]quinolizine-3-yl propionic]-α-aminoeicosanoate (**1i**)*

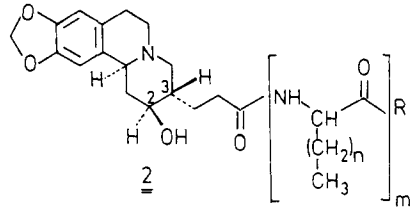
Compounds **1b** (0.936 g, 2.65 mmol) and **3g** (1.000 g, 2.65 mmol) were reacted as described for **1c**. Yield: 1.24 g (73%) pale yellow solid.

NMR ($CDCl_3$): 6.56 (1H, s, C_{11}-H), 6.52 (1H, s, C_8-H), 5.98 (1H, m, CONH), 5.90 (2H, s, OCH_2O), 4.59 (1H, m, α-CH), 3.73 (3H, d, OCH_3), 1.28 (32H, m, $16 \times CH_2$), 0.88 (3H, t, CH_3).

MS *m/z* (%): $C_{38}H_{60}N_2O_6$ (640.88); 641 (98) $[M + H]^+$, 298 (100), 282 (33), 270 (18), 256 (42), 242 (37), 189 (29), 176 (58).

*Methyl N-[9,10-(methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoate (**2c**)*

Keto-ester **1f** (20 mg, 0.036 mmol) was dissolved in dichloromethane (4 ml) and sodium borohydride (3.4 mg, 0.087 mmol) added to the reaction mixture at 0 °C. The reaction mixture was stirred for 1 h at 0 °C, methanol (2 drops) was added and stirred for a further 1 h. The pH of the solution was adjusted to neutral with acetic acid (1/2 drop), the solvent was evaporated and the residue treated with 2% sodium hydrogen carbonate (5 ml) and extracted with dichloromethane (3×2 ml). The organic phase was dried ($MgSO_4$), evaporated and the residue purified by TLC. Yield: 16 mg (80%). R_f **2c** < R_f **1f**.



2	n	m	R
a	–	0	OCH_3
b	–	0	OH
c	11	1	OCH_3
d	11	2	OCH_3
e	11	3	OCH_3

Scheme 2.

NMR ($CDCl_3$): 6.66 (1H, d, C_{11}-H), 6.53 (1H, s, C_8-H), 6.22 (1H, d, NH), 5.87 (2H, s, OCH_2O), 4.60 (1H, m, α-CH), 3.73 (3H, s, $COOCH_3$), 3.44 (1H, bm, C_2-H), 1.24 (20H, m, $10 \times CH_2$), 0.86 (6H, t, CH_3).

MS *m/z* (%): $C_{32}H_{50}N_2O_6$ (558.74); 581 (79) $[M + Na]^+$, 559 (32), 537 (100), 402 (10), 252 (27), 226 (19), 198 (23), 176 (50), 147 (22), 133 (48), 95 (23), 73 (66).

*Methyl N^1-[N^2-[9,10-(methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoyl]-α-aminotetradecanoate (**2d**)*

Keto-ester **1g** (20 mg, 0.256 mmol) was reduced as described for **2c**. Yield: 15 mg (75%). R_f **2d** < R_f **1g**.

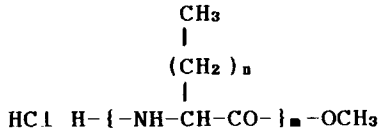
NMR ($CDCl_3$): 6.68 (1H, d, C_{11}-H), 6.54 (1H, s, C_8-H), 6.80, 6.44 (2H, m, $2 \times$ NH), 5.90 (2H, s, OCH_2O), 4.56, 4.43 (2H, m, $2 \times$ αCH), 3.74 (3H, 3s, $COOCH_3$), 3.43 (1H, bm, C_2-H), 1.24 (40H, m, $20 \times CH_2$), 0.85 (3H, t, CH_3).

MS *m/z* (%): $C_{46}H_{77}N_3O_7$ (784.10); 784 (76) $[M + H]^+$, 302 (22), 242 (30), 198 (100), 174 (55), 147 (17), 73 (28).

*Methyl N^1-[N^2-[N^3-[9,10-(methylenedioxy)-2β-hydroxy-1,2,3β,4,6,7,11bα-heptahydrobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoyl]-α-aminotetradecanoyl]-α-aminotetradecanoate (**2e**)*

Keto-ester **1h** (20 mg, 0.0199 mmol) was reduced as described for **2c**. Yield: 15 mg (75%). R_f **2e** < R_f **1h**.

NMR ($CDCl_3$): 7.06–6.42 (3H, m, $3 \times$ NH), 6.64 (1H, s, C_{11}-H), 6.54 (1H, s, C_8-H), 5.90 (2H, s, OCH_2O), 4.46 (3H, m, $3 \times$ α-CH), 3.76–3.70



3	n	m
a	7	1
b	7	2
c	7	3
d	11	1
e	11	2
f	11	3
g	17	1

Scheme 3.

(3H, 6s, $COOCH_3$), 3.40 (1H, bm, C_2-α-H), 1.26 (60H, m, $30 \times CH_2$), 0.90 (9H, t, $3 \times CH_3$).

MS *m/z* (%): $C_{60}H_{104}N_4O_8$ (1009.47); 1011 (65), 1010 (100) $[M + H]^+$.

*Methyl 9,10-(methylenedioxy)-2β-hydroxy-1,3β,4,6,7,11bα-hexahydro-2α-tritiobenzo[a]quinolizine-3-yl propionate (**2a***)*

Keto-ester **1a** (40 mg, 0.12 mmol) was reduced with NaB^3H_4 (5.0 mCi) using the procedure described for **2c**. Yield: quantitative (5.0 mCi).

*Methyl N-[9,10-(methylenedioxy)-2β-hydroxy-1,3β,4,6,7,11bα-hexahydro-2α-tritiobenzo[a]quinolizine-3-yl propionic]-α-aminotetradecanoate (**2c***)*

Keto-ester **1f** (34.8 mg, 0.06 mmol) was reduced, using the procedure described for **2c**. Yield: quantitative (2.5 mCi).

Preparation of mast cells (Atkinson et al., 1980)

Male Sprague-Dawley rats (200–300 g) were killed in carbon dioxide, decapitated and exsanguinated under running water. The peritoneal cavity was lavaged with 10 ml of ice-cold THG (see below) buffer containing heparin (5 U/ml). Washings were pooled and centrifuged ($200 \times g$, 5 min, 4°C). After washing the pellets twice with THG, the cells were resuspended in THG to give a final volume of cells in slight excess of that required.

THG buffer: NaCl, 137 mM; KCl, 2.7 mM; NaH_2PO_4, 0.4 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1 mM; glucose, 5.6 mM; Hepes, 10 mM; adjusted to pH 7.4 with 1 N NaOH solution.

Inhibition of histamine release from mast cells

Compounds **1a–1d** and **1f** were dissolved in dimethyl sulphoxide to a concentration of 1×10^{-1} M, and diluted in THG to twice the required final concentration of 300 μM. 0.25 ml of each compound was incubated with an equal volume of cells for 10 min at 37°C and the release process started by the addition of anti-rat IgE (50 μl, final dilution 1/200). After incubation for 10 min at 37°C, the process was terminated by the addition of 2.5 ml ice-cold THG. The tubes were centrifuged ($1000 \times g$, 5 min) and the supernatants decanted and assayed for histamine as described below.

Measurement of histamine

To each tube were added sodium hydroxide (N, 400 μl) and *o*-phthaldialdehyde (OPT, 1%, 100 μl), with thorough mixing after each addition. The reaction was stopped after 4 min by the addition of hydrochloric acid (3 N, 300 μl). The fluorescence of each tube was measured in a spectrofluorimeter, and compared to previously prepared standard solution of histamine treated with sodium hydroxide and OPT as described above.

Oral absorption of C_2-[^{3}H]benzoquinolizine conjugates

Male 24 h starved mice (20–30 g) were fed the C_2-[^{3}H]benzoquinolizine alkaloid **2a** or conjugate **2c** dissolved and suspended, respectively, in dimethyl sulphoxide (2.5 mmol/100 g body weight; spec. act., 3 μCi/mmol) as an admixture with 0.15 g of powdered chow diet. Blood samples were taken by removing tail segments prior to feeding and at various time intervals following presentation of the meal. Aliquots of whole blood were

solubilised in tissue solubiliser (NCS) and counted for radioactivity in a liquid scintillation counter.

Results and Discussion

Synthesis of benzoquinolizine conjugates

A series of seven lipidic peptide conjugates (**1c**–**1i**) were prepared by reacting the appropriate lipidic amino acid and peptide methyl esters **3a**–**3g** (Gibbons et al., 1990) with the benzoquinolizine acid **1b**. The crude reaction mixtures were purified by thin layer silica chromatography, furnishing the desired conjugates in adequate yields.

Sodium borohydride reduction of the simple ester **1a** and the conjugates **1f**, **1g** and **1h** gave a series of alcohols **2a** (Szabo et al., 1979), **2c**, **2d** and **2e** as the major products. The chemical shift and axial-axial coupling constants of the proton in position 2 showed that the configuration of the hydroxyl group of **2a**, **2c**, **2d** and **2e** is equatorial. Hydrolysis of **2a** by reflux in concentrated hydrochloric acid produced the hydroxy acid **2b**.

The radiolabelled alcohols **2a*** and **2c*** were obtained by reduction of the keto function of **1a** and **1f** with sodium borotritiate in an analogous manner to that described above. Reduction of **1a** and **1f** with this reagent tritiated the C_2 position of the C-ring, since the O^3H formed during reduction was exchangeable.

Ability of benzoquinolizine conjugates to inhibit release of histamine from mast cells

The success of the various lipidic amino acid-drug conjugates was dependent on one of two factors: Either the entire conjugate possessed the required biological activity (i.e. a new drug) or the lipidic peptide delivery system is cleaved in vivo to yield the active constituent (i.e. a prodrug). The series of conjugates of the benzoquinolizine anti-inflammatory alkaloid would be expected to fall into the first category of compounds because of the relatively stable amide link between the parent alkaloid and the delivery system. Thus, the anti-inflammatory activity of the conjugates **1c**, **1d** and **1f** was compared to that of the parent alkaloids **1a** and **1b** in order to investigate the effect on biological activity of lipidic peptide conjugation in this instance.

The anti-inflammatory activities of the conjugates **1c**, **1d** and **1f** and the parent compounds **1a** and **1b** were assessed by their ability to inhibit the release of histamine from rat mast cells which had been stimulated with anti-rat IgE. Each compound was incubated with mast cells prior to stimulation of the cells with anti-rat IgE. The supernatant obtained after centrifugation of the cell preparation was assayed for histamine using a spectrofluorimetric method (Shore et al., 1959). Disodium cromoglycate (DSCG), a known inhibitor of histamine release (Ewnis et al., 1981), was included in each experiment as a positive control. DMSO-only controls, at the same final concentration as used in the test compound dilutions, allowed determination of the effect of DMSO on histamine release. Blanks, containing cells and all reagents except anti-rat IgE, were included in each experiment in order to ascertain the extent of spontaneous histamine release (typically 2–6% of total histamine release). Total histamine content of the cells was determined for each experiment by boiling cell aliquots for 10 min and assaying the supernatant for histamine as described above. Histamine release was expressed as a percentage of total histamine, calculated as follows:

$$\left\{\left(\begin{array}{c}\text{Histamine release in the}\\ \text{absence of test compound}\end{array}\right) - \left(\begin{array}{c}\text{Histamine release in the}\\ \text{presence of test compound}\end{array}\right) \times 100\%\right\} \times \left\{\begin{array}{c}\text{Histamine release in the}\\ \text{absence of test compound}\end{array}\right\}^{-1}$$

All values were corrected for spontaneous release. The results are summarised in Fig 1.

The parent benzoquinolizine acid **1b** and its methyl ester **1a** gave approx. 40 and 50% inhibition of histamine release from mast cells and the positive control, DSCG, 80% inhibition. The lipidic peptide conjugates **1d** and **1f** both inhibited histamine release similarly to the parent compounds, while conjugate **1c** had activity comparable to that of DSCG. These results illustrated that

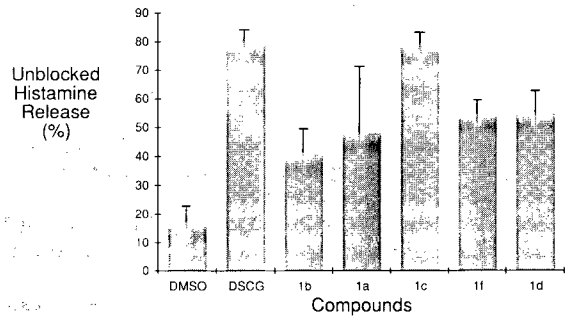


Fig. 1. Histamine release of compounds **1a–1d** and **1f**.

conjugation of the lipidic peptide delivery system to the benzoquinolizine anti-inflammatory compounds did not have a negative effect on their ability to inhibit histamine release from mast cells. Indeed, for conjugate **1c**, a positive effect was observed. If the parent compounds exert their anti-inflammatory activity in vivo via the inhibition of histamine release, the results suggest that the conjugates act through similar mechanisms.

Absorption of ^{3}H-labelled benzoquinolizine conjugates following oral administration

The effect of lipidic amino acid conjugation on the absorption of benzoquinolizine anti-inflammatories was determined directly by measuring the amount of radiolabel absorbed following oral administration. The ^{3}H-labelled benzoquinolizine methyl ester **2a*** and the benzoquinolizine methyl α-aminotetradecanoate conjugate **2c***, were dissolved and suspended, respectively, in DMSO, mixed with feed, and fed to starved mice. Blood samples were taken from the animals at regular intervals, and the amount of radioactivity present in the serum determined. Fig. 2 summarises the results of these experiments.

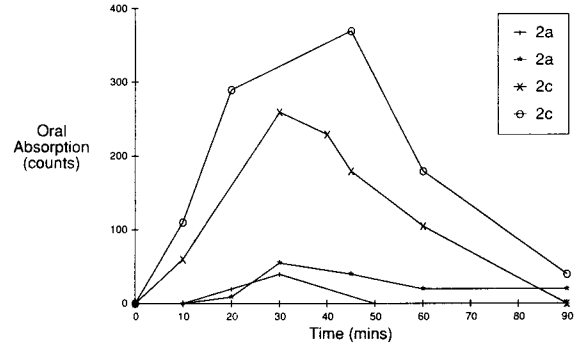


Fig. 2. Oral absorption of compounds **2a** and **2c**.

The radioactivity detectable in the blood following oral administration of the methyl ester **2a*** was no greater than background. On the other hand, the conjugate **2c*** gave peak blood levels approx. 5 times those of the methyl ester, suggesting that conjugate **2c*** was absorbed orally, although the total radiation detected for conjugate **2c*** accounted for only 1% of the total radioactivity administered to the animals.

Conclusion

The lipidic peptide conjugates **1c–1i** and **2c–2e** of the anti-inflammatory benzoquinolizine alkaloids **1b** and **2b** were synthesised. Conjugation did not reduce the biological activity of the compounds as assessed by their ability to inhibit the release of histamine from mast cells in vitro. Following oral administration to rats, peak serum levels of the radiolabelled conjugate **2c*** were 5 times greater than that observed for the parent compound **2a***. The results suggest that conjugation to lipidic amino acids and peptides is a useful approach to improving the absorption of poorly absorbed drugs.

Acknowledgments

The authors would like to acknowledge Mr S.A. Howell and Dr K.J. Welham for running the mass spectra, Dr G.J. Anderson for NMR spectra, Mr W. Baldeo for the microanalyses and Dr P.A. Peachell for advice regarding mast cell preparation and histamine determination.

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