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Synthesis of Co-Drugs of Alkylating Agents and Steroidal Anti-Inflammatories

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ABSTRACT

Co-drugs of the anti-inflammatory agents dexamethasone and prednisone, and anti-neoplastic agents melphalan and chlorambucil, were synthesized using an esterification reaction. The carboxylic acids were activated using dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) to give esters (11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione 21-{4′-[bis(2″-chloroethyl)amino]-L-phenylalaninate} hydrochloride, 17,21-dihydroxypregna-1.4-diene-3,11,20-trione 21-{4′-[bis(2″-chloroethyl)amino]-L-phenylalaninate} hydrochloride, (11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methyl-pregna-1,4-diene-3,20-dione 21-{4′-[p-[bis(2″chloroethyl)amino]phenyl]-butyrate} and 17,21-dihydroxypregna-1.4-diene-3,11,20-trione 21-{4′-[p-[bis(2″-chloroethyl)-amino]phenyl]-butyrate}. Preliminary investigations have shown that these esters are hydrolyzed to their component drugs and show comparable in vitro activity to the parent drugs.

INTRODUCTION

Chemical modification of drug molecules to improve drug delivery and hence effectiveness, as well as to minimize toxicity, has been investigated over the last 25 years (1). These so-called soft or pro-drugs are converted in the body to the well-established drugs by enzymes or pH. Drugs are frequently given together in single or separate dosage forms. The drugs may be chemically unrelated and the bioavailability may be very different. There may be several advantages in giving the coadministered drugs in the form of a single chemical entity or a co-drug.

Esters make attractive co-drug candidates, especially if the drug is absorbed intact and hydrolyzed by esterases back to the component drugs. The drugs then have similar bioavailability and may reach the site of action together. Ideal candidates for co-drugs should have similar doses on a molar basis and similar half-lives. Possible candidates are alkylating agents such as melphalan and steroids coadministered as anti-inflammatories and antiemetics such as dexamethasone. The alcoholic group of the steroids can be esterified with the carboxyl group of the alkylating agent to give the ester, a co-drug. In addition to dexamethasone ((11β,16α)-9-fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-

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3,20-dione), prednisone (17,21-dihydroxypregna-1.4-diene-3,11,20-trione) was chosen as a candidate for co-drug synthesis based on the fact that a melphalan-prednisone regimen (2) has been used for treating myeloma patients. The synthesis of co-drugs of the alkylating agents melphalan (4-[bis(2-chloroethyl)amino]-L-phenylalanine) and chlorambucil (4-[p-[bis(2-chloroethyl)amino]-phenyl]butyric acid) with steroids dexamethsasone and prednisone, respectively, is reported below. For simplicity these esters are referred to as dexamethasone 21-melphalanate.HCl (DM.HCl), prednisone 21-melphalanate.HCl (PM.HCl), dexamethasone 21-chlorambucilate (DC), and prednisone 21-chlorambucilate (PC), respectively.

MATERIALS AND METHODS

Dexamethasone, prednisone, and chlorambucil were purchased from Sigma Chemical, St. Louis, MO, and melphalan was a gift from Burroughs Wellcome, Research Triangle Park, NC. Di-tert-butyl dicarbonate, dicyclohexyl carbodiimide, and 4-dimethylaminopyridine were purchased from Aldrich Chemical Co, Milwaukee, WI. Proton nuclear magnetic resonance Spectra were obtained on a General Electric QE-300 and Varian VXR-300 instruments. Chemical shifts are reported in parts per million downfield from the internal standard tetramethylsilane. Coupling constants (*J*) are in Hertz. Elemental analyses were performed by Atlantic Microlab Inc. Norcross, GA.

Synthesis of Dexamethasone 21-Chlorambucilate (DC)

A mixture of chlorambucil (1.53 g, 5.03 mmol), DCC (1.14 g, 5.53 mmol), dexamethasone (2.17 g, 5.53 mmol), and a catalytic amount of 4-DMAP (25 mg) in dry CH_2Cl_2 (80 mL) was stirred for 24 h at ambient temperature. After removal of dicyclohexylurea by filtration, and evaporation of the filtrate, the crude product obtained was chromatographed on silica gel using gradient elution with EtOAc /Hexane (1:4 to 1:1), to give 3.20 g (94%) of a white oily solid. This, on trituration with EtOAc/hexane, gave a white powder (mp: 95–97°C [dec]). ^{1}H NMR ($CDCl_3$) d 0.91 (d, 3H, J = 8), 1.05 (s, 3H), 1.24 (m, 2H), 1.55 (s, 3H), 1.60–1.87 (m, 4H), 1.95 (p, 2H, J = 6), 2,14 (m, 1H), 2.20–2.40 (m, 4H), 2.45 (dt, 2H, J = 8, 4), 2.60 (t, 2H, J = 6), 2.62 (m, 1H), 3.10 (m, 1H), 3.60–3.75 (m, 8H), 4.30 (m, 1H), 4.89 (two d, 2H, J = 20), 6.10 (m, 1H), 6.30 (dd, 1H, J = 10, 3), 6.63 (d, 2H, J = 8), 7.09 (d, 2H, J = 8). Anal. for $C_{36}H_{46}Cl_2FNO_6$ Calcd: C 63.76, H 6.83, N 2.06. Found: C 63.82, H 6.38, N 2.09.

Synthesis of Prednisone 21-Chlorambucilate (PC)

A mixture of chlorambucil (1.53 g, 5.03 mmol), DCC (1.14 g, 5.53 mmol), prednisone (1.98 g, 5.53 mmol), and a catalytic amount of 4-DMAP (25 mg) in dry CH_2Cl_2 (80 mL) was stirred for 24 h at ambient temperature. After removal of dicyclohexylurea by filtration, and evaporation of the filtrate, the crude product obtained was chromatographed on silica gel using gradient elution with EtOAc/hexane (1:4 to 1:1) to give 1.41 g (44%) of a white oily solid. This, on trituration with EtOAc/hexane, gave a white powder (mp: 193–195°C). 1H NMR (dmso-d_6) δ 0.50 (s, 3H), 1.18 (s, 3H), 1.35 (s, 3H), 1.67 (m, 2H), 1.78 (p, 2H, J = 6), 2.0 (m , 2H), 2.18(two d, 2H, J = 10), 2.34 (t, 4H, J = 6), 2.52 (m, 2H), 2.89 (d, 1H, J = 12), 3.68 (s, 8H), 4.85 (two d, 2H, J = 18), 5.80 (s, 1H), 6.00 (br. s, 1H), 6.10 (dd, 1H, J = 10, 2), 6.56 (d, 2H, J = 8), 7.03 (d, 2H, J = 8), 7.60 (d, 2H, J = 10). Anal. for $C_{35}H_{43}Cl_2NO_6$ Calcd: C 65.21, H 6.72 N 2.17. Found: C 65.05, H 6.76, N 2.12.

Synthesis of Boc-Melphalan

Triethylamine (0.76 g, 7.52 mmol) was added to melphalan (1.53 g, 5.01 mmol) dissolved in 50% aqueous THF (20 mL) and cooled to 0°C. Di-*tert*-butyl dicarbonate (1.20 g, 4.72 mmol) dissolved in THF (10 mL) was added dropwise to the cooled mixture over a period of 10 min, stirred for 30 min at 0°C, and at room temperature for 4 h. After removal of THF, 10% citric acid solution was added until the solution had a pH 5–6, extracted with EtOAc (3 × 60 mL), dried (anhydrous Na_2SO_4), filtered and evaporated to give a brown oil, which on chromatography using silica gel, gave 1.79 g (88%) of Boc-Melphalan as a viscous oil. ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 3.66 (two dd, 2H, J = 14, 6), 3.62, 3.71(two t, 8H, J = 6), 4.50 (m, 1H), 6.00 (br. s, 1H), 4.96 (d, 1H, J = 6), 6.65 (dd, 1H, J = 10, 2), 7.08 (d, 2H, J = 8).

Synthesis of Dexamethasone 21-Boc-Melphalanate

A mixture of Boc-Melphalan (2.00 g, 4.93 mmol), DCC (1.12g, 5.42 mmol), dexamethasone (1.94 g, 5.42 mmol), and a catalytic amount of 4-DMAP (25 mg) in dry CH_2Cl_2 (100 mL) was stirred for 24 h at ambient temperature. After removal of dicyclohexylurea by filtration, and evaporation of the filtrate, the crude prod-

uct obtained was chromatographed on silica gel using gradient elution with EtOAc/hexane (1:3 to 1:1) to give 2.36 g (61%) of a pale yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 3H, J = 8)), 1.05 (s, 3H), 1.25 (m, 3H), 1.40 (s, 9H), 1.50 (s, 3H, J = 6), 1.6–2.0 (m , 3H), 2.15 (m, 1H), 2.48 (m, 4H), 2.60 (m, 1H), 3.00–3.33 (m, 2H), 3.62, 3.71 (two t, 8H, J = 6), 4.38 (m, 1H), 4.60 (m, 1H), 4.87 (d, 1H, J = 6), 4.88 (d, 1H, J = 18), 5.05 (d, 1H, J = 18), 6.11 (m, 1H), 6.10 (dd, 1H, J = 10, 3), 6.63 (d, 2H, J = 8), 7.13 (d, 2H, J = 8), 7.21 (d, 1H, J = 10). Anal. for $C_{40}H_{53}Cl_2FN_2O_8$ Calcd: C 61.61, H 6.85 N 3.59. Found: C 61.34, H 6.91, N 3.52.

Synthesis of Prednisone 21-Boc-Melphalanate

A mixture of Boc-Melphalan (2.26 g, 5.58 mmol), DCC (1.27 g, 6.14 mmol), prednisone (2.20 g, 6.14 mmol), and a catalytic amount of 4-DMAP (25 mg) in dry CH_2Cl_2 (120 mL) was stirred for 24 h at ambient temperature. After removal of dicyclohexylurea by filtration, and evaporation of the filtrate, the crude product obtained was chromatographed on silica gel using gradient elution with EtOAc /hexane (1:3 to 1:1) to give 2.78 g (69%) of a white foamlike solid. ^{1}H NMR (dmso-d_6) δ 0.70 (s, 3H), 1.40 (s, 9H), 1.45 (s, 3H), 1.70 (m, 3H), 1.85–2.10 (m, 2H), 2.30–2.45 (m, 4H), 2.50(m, 1H), 2.75–2.92 (m, 3H, J = 6), 3.00 (dd, 1H, J = 14,6), 3.18 (dd, 1H, J = 14,6), 3.58–3.75 (2t, 8H, J = 6), 4.60 (m, 1H), 4.94 (m, 1H), 4.95 (2d, 2H, J = 18), 6.09 (br. s, 1H), 6.22 (dd, 1H, J = 10, 2), 6.62 (d, 2H, J = 8), 7.11 (d, 2H, J = 8), 7.70 (d, 2H, J = 10). Anal. for $C_{39}H_{50}Cl_2N_2O_8$.H2O Calcd: C 61.33, H 6.86, N 3.67. Found: C 61.83, H 6.95, N 4.08.

Synthesis of Dexamethasone 21-Melphalanate.HCl (DM.HCl)

Dexamethasone 21-Boc-Melphalanate (2.28 g, 2.92 mmol) was cooled to 0°C and 3 M HCl in EtOAC (20 mL) was added and stirred for 5 min and at room temperature for 5 min. The solvent was removed in vacuo and the residue was coevaporated with EtOH to give a white solid (1.92 g, mp: 179–181°C [dec], 92%). ^{1}H NMR (DMSO-d_6) δ 0.80 (d, 3H, J = 8)), 0.90 (s, 3H), 1.48 (s, 3H), 1.55–1.80 (m, 4H), 2.25 (m, 3H), 2.35 (m, 2H), 2.60 (m, 1H), 2.90 (m, 1H) 3.02 (dd, 1H, J = 14, 6), 3.16 (dd, 1H, J = 14, 6), 3.70 (s, 8H), 4.16 (m, 1H), 4.26 (m, 1H), 4.90 (d, 1H, J = 18), 5.20 (d, 1H, J = 18), 5.97 (br s, 1H), 6.20 (dd, 1H, J = 10, 2), 6.68 (d, 2H, J = 8), 7.18 (d, 2H, J = 8), 7.40 (d, 1H, J = 10), 8.50 (br s 3H). Anal. for $C_{40}H_{53}Cl_2FN_2O_8.3H_2O$ Calcd: C 54.58, H 6.81 N 3.64. Found: C 54.87, H 6.61, N 3.88.

Synthesis of Prednisone 21-Melphalanate.HCl (PM.HCl)

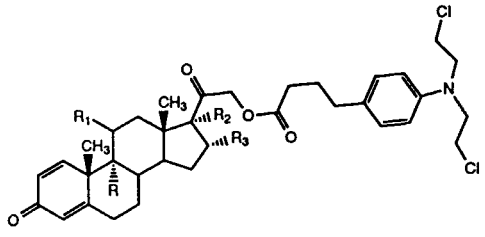
Prednisone 21-Boc-Melphalanate (2.38 g, 3.19 mmol) was cooled to 0°C and 3 M HCl in EtOAC (20 mL) was added and stirred for 5 min and at room temperature for 5 min. The solvent was removed in vacuo and the residue was coevaporated with EtOH to give a gummy residue, which on trituration with methanol and ether, afforded a white solid (1.64 g, 79%). ^{1}H NMR ($CDCl_3$) δ 0.52 (s, 3H), 1.34 (s, 3H), 1.75 (m, 2H), 2.0 (m, 2H), 2.19(m , 2H), 2.38 (m, 4H), 2.56 (m, 2H), 2.90–3.12 (m, 3H), 3.68 (m, 8H), 4.30 (m, 1H), 5.03 (two d, 2H, J = 18), 6.00 (br s, 1H), 6.10 (dd, 1H, J = 10, 2), 6.68 (d, 2H, J = 8), 7.18 (d, 2H, J = 8), 7.58 (d, 1H, J = 10), 8.47 (br s, 3H). Anal. for $C_{34}H_{43}Cl_3N_2O_6$ $2H_2O$ Calcd: C 56.87, H 6.60 N 3.90. Found: C 56.59, H 6.31, N 4.01.

RESULTS AND DISCUSSION

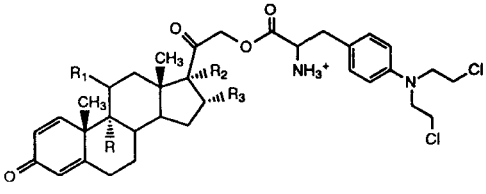
Four potential co-drugs were synthesized, the structures of which are shown in Figure 1. Structures DC, PC, DM.HCl, and PM.HCl are new compounds.

The method used to synthesize the esters involves the direct room temperature esterification of carboxylic acids under neutral conditions (3). The use of dicyclohexylcarbodiimide and dimethylformamide provides a mild and efficient method of synthesizing esters in very good yields. Dexamethasone 21-chlorambucilate ester (DC) was separated by silica gel chromatography and crystallized from ethyl acetate/hexane to give a white powder. Prednisone 21-chlorambucilate (PC) ester was synthesized in a similar manner. Identification of structures of esters was confirmed by elemental analysis and high-resolution NMR. The ester was identified by the downfield shift of the C-21 methylene protons in the NMR, compared to the corresponding protons in the alcohols, namely prednisone and dexamethasone (4).

However, synthesis of co-drugs of melphalan needed an extra step, involving the protection of the amino group with the *t*-butoxy-carbonyl (*t*-Boc) group. This proceeded smoothly using di-tert-butyl dicarbonate in tetrahydrofuran (5). Boc-melphalan was then added to DCC, 4-DMAP, and dexamethasone to obtain the Boc-protected ester. This was purified by silica gel chroma-



R = F, R_1 = OH, R_2 = OH, R_3 = CH_3
DEXAMETHASONYL 21-CHLORAMBUCILATE (DC)
R = H, R_1 = O, R_2 = OH, R_3 = H
PREDNISONYL 21-CHLORAMBUCILATE (PC)



R = F, R_1 = OH, R_2 = OH, R_3 = CH_3
DEXAMETHASONYL 21-MELPHALANATE (DM.HCl)
R = H, R_1 = O, R_2 = OH, R_3 = H
PREDNISONYL 21-MELPHALANATE (PM.HCl)

Figure 1.

tography. The protected ester on treatment with trifluoroacetic acid gave the dexamethasone 21-melphalanate (DM) ester as the trifluoroacetate salt. Storage of this ester as the trifluoroacetate salt or in the free base form showed decomposition products as indicated by thin-layer chromatography analysis (6). To minimize this decomposition, HCl/EtOAc was used as the deprotecting reagent instead of TFA, and this resulted in the isolation of a white hydrochloride salt DM (7). The procedure was repeated with prednisone to give PM.HCl. As in the case of DC and PC, the downfield shift of the C-21 methylene protons in proton NMR confirmed the formation of the ester. Structural assignments for all intermediates and product esters were confirmed by spectral data and elemental analysis.

Preliminary investigation shows that the esters are hydrolyzed to the component drugs in 0.1 M phosphate buffer and plasma at 37°C. The esters show comparable activity to the alkylating agents on L1210 leukemia cells in cell culture studies.

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