



Synthesis and Biological Screening of 1,3-Dialkyl Derivatives of 4-(2',5-Dioxopyrrolidine-3-yl) Phenyl Sulphinic Acid as Inhibitors of Oestrone Sulphatase

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Abstract

1,3-dialkyl 3-phenylpyrrolidine-2, 5-diones were modified to produce potential steroid sulphatase inhibitors. These modifications were aimed at producing compounds, which could be expected to bind reasonably well to the active site of the steroid sulphatase enzyme but could not be hydrolyzed readily by the enzyme due to the covalent S-C bond present. In this regard the sulphinic acid derivatives of di-substituted 3-phenylpyrrolidine-2, 5-diones were prepared. On biological testing, only compound 4-(2,5-dioxo-1, 3-dipentylpyrrolidine-3-yl) phenylsulphinic acid (F5) was found to be an inhibitor of the steroid sulphatase enzyme from human placenta and was about twice as potent as the known inhibitor danazol.

Keywords: Breast cancer; Oestrone sulphatase; Phenylpyrrolidine-2,5-dione; Steroid sulphatase.

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1. Introduction

Oestrogen concentrations in plasma are similar in oestrogen receptor positive and oestrogen receptor negative cancer patients [1], thus *in situ* formation of oestrogen is thought to make a major contribution to the high concentration of oestrogens in breast tumors [2]. The presence of the aromatase enzyme complex in 50-60% of breast tumors [3] may play an important role in local

oestrogen synthesis [2, 4]. Oestrone sulphatase on the other hand is present in most breast tumors and is about a million-fold more active than aromatase [5-6]. At the physiological concentrations of oestrone sulphate and androstenedione the amount of oestrone produced via the sulphatase pathway is ten times higher than through the aromatase pathway, suggesting that in breast tumors the sulphatase pathway is the main route for the *in situ* production of oestrogens [7].

Steroid sulphates were thought to be the end products of steroid metabolism for a long time but it is now clear that sulphate conjugates

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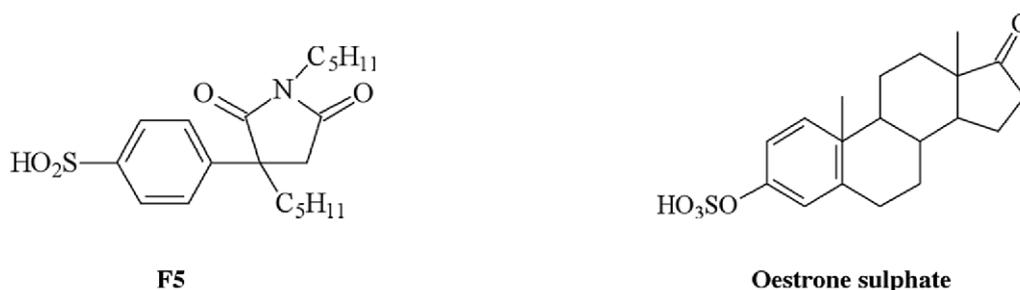


Figure 1. Two dimensional structures of 4-(2,5-dioxo-1, 3-dipentylpyrrolidine-3-yl) phenyl sulphonic acid F5 and oestrone sulphate

of steroids are important intermediates in the synthesis, transport and action of steroid hormones [8-10].

Among a number of existing steroid sulphates, oestrone sulphate (E1S) and dehydroepiandrosterone sulphate (DHEA-S) have the highest supporting role in breast tumor growth. Serum oestrone sulphate levels are 10 times higher than those of unconjugated oestrone and oestradiol [11], and the half-life of oestrone sulphate is much longer than that of free oestrone and oestradiol [12].

Oestrone sulphate is important as an oestrogen precursor, which can be converted to free oestrone and oestradiol via oestrone sulphatase and 17 β -hydroxysteroid dehydrogenase (17 β -HSD). The fact that oestrogen sulphatase inhibitors could provide a means to block oestrogen biosynthesis has led to the synthesis and evaluation of a number of potential steroid sulphatase inhibitors for use, either alone or in conjugation with an aromatase inhibitor, as potential therapeutic agents [10, 13-14].

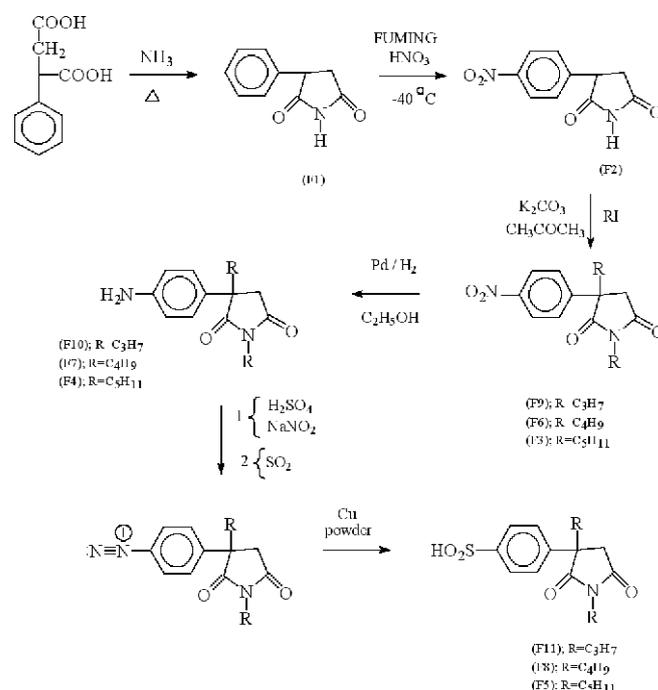
Oestrone sulphate, the natural substrate for oestrone sulphatase has a flat phenolic ring, and an extended hydrophobic structure, so that logically in the development of potential inhibitors of oestrone sulphatase this backbone should be represented. 1,3-Dialkyl-3(4'-aminophenyl)pyrrolidine-2,5-diones "potent aromatase inhibitors" [15] resemble this requirement in having a flat aryl ring with a pitched flat heterocyclic ring with two extended hydrophobic alkyl chains.

The chains can freely rotate and are capable of interaction with an existing hydrophobic binding site on the enzyme active site. Since the backbone of this structure is reasonably fitted in the aromatase active site, it could be similarly fitted in the sulphatase active site. Introduction of a suitably positioned sulphonate or similar moieties (sulphinate, etc) into the aromatic ring of this structure could make it capable of interaction with the enzyme active site but could not be hydrolyzed readily by the enzyme due to the covalent S-C bond present (Figure 1). This could result in inhibition of oestrone sulphatase enzyme.

2. Materials and methods

2.1. Chemistry

All reagents and solvents employed were of general purpose grade. Melting points were determined on a Gallenkamp hot stage apparatus and are uncorrected. Infra-red spectra were obtained as solid via a diffuse reflectance accessory using KBr matrix, or between NaCl plates using a Perkin Elmer 1600 series FTIR. ¹H-NMR spectra were recorded on either a Perkin-Elmer R32 instrument (90 MHz), a Bruker DPX 300 (300 MHz) spectrometer or a Bruker WM 360 instrument (360 MHz) as dilute solutions in CDCl₃ or DMSO-d₆ with tetramethylsilane as internal standard. Mass spectra were determined at the SERC Mass spectrometry Service Center, University College of Swansea, UK. Elemental analyses were

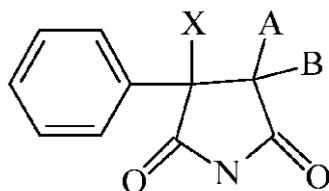


Scheme 1: Synthesis of 1,3-dialkyl derivatives of 4-(2,5-dioxopyrrolidine-3-yl) phenyl sulphinic acid.

determined at the School of Pharmacy, University of London.

Since preparation of the 1,3-dialkyl derivatives of 4-(2,5-dioxopyrrolidine-3-yl) phenyl sulphate or sulphonic acid were unsuccessful [16], the synthesis of the corresponding sulphinic acid was undertaken by the method outlined in Scheme 1.

2.1.1. Synthesis of 3-Phenylpyrrolidine-2,5-dione (F1)

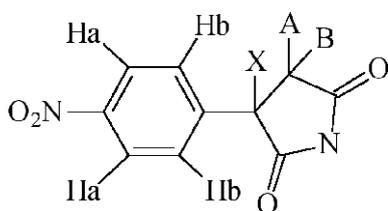


Phenylsuccinic acid (40 g, 20.6 mmol) was dissolved portionwise in ammonia solution (20 ml, 88%) with the temperature maintained under 100 °C and then the mixture was heated on a metal bath (180 °C) until a white solid was formed. Further heating to 210 °C resulted in formation of a brown viscous

liquid. Completion of the reaction was confirmed using a moisted universal paper where the neutral color indicated the termination of NH₃ evolution. The resulting liquid solidified on trituration in ether and was recrystallized from ethanol to give the dione (F1) as a white powder (20.27 g, 81.6%), m.p. 87.5-88.2 °C (Lit., [17]), 90-91.5 °C); ν_{\max} 3200 (N-H), 1770, 1700 (C=O, imide), 1600 (C=C, Ar) cm⁻¹; δ_{H} (90MHz, CDCl₃) 7.5 - 7.1 (5H, m, Ph-H), 4.12 (1H, dd, $J_{\text{AX}} = 9\text{Hz}$ and $J_{\text{BX}} = 5\text{Hz}$, $\text{CH}_X\text{-CH}_A\text{H}_B$), 3.19 (1H, dd, $J_{\text{BA}} = 18\text{Hz}$ and $J_{\text{XA}} = 9\text{Hz}$, $\text{CH}_X\text{-CH}_A\text{H}_B$), 2.71 (1H, dd, $J_{\text{AB}} = 18\text{Hz}$ and $J_{\text{XB}} = 5\text{Hz}$, $\text{CH}_X\text{-CH}_A\text{H}_B$).

2.1.2. Synthesis of 3-(4-Nitrophenyl) pyrrolidine-2,5-dione (F2)

The 3-phenylpyrrolidine dione (F1) (12 g, 68.6 mmol) was added portionwise to the stirred fuming nitric acid (72 ml) maintained at a temperature of -39 °C to -40 °C using a dry ice / petroleum ether bath. Each portion was added when the previous one had totally dissolved. The solution was then poured with vigorous stirring into ice/water (500 ml) and



allowed to stand at 5 °C overnight when 3-(4-nitrophenyl)-pyrrolidine-2, 5-dione (F2) precipitated as a white solid (6.4 g, 39.5%). An analytical sample was recrystallised from ethanol, m.p. 137.5-141.5 °C (Lit., [17]), 134 – 137 °C]. ν_{\max} 3300 (N-H), 1770, 1710 (C=O, imide), 1600 (C=C, Ar), 1510, 1345 (NO₂) cm⁻¹; δ_{H} (90MHz; CDCl₃), 8.23 (2H, d, J = 9Hz, 3',5'-PhH), 7.65 (2H, d, J = 9Hz, 2',6'-PhH), 4.38 (1H, dd, J_{AX} = 8Hz and J_{BX} = 5Hz, CH_X-CH_AH_B), 3.22 (1H, dd, J_{BA} = 18Hz and J_{XA} = 9Hz, CH_X-CH_AH_B), 2.84 (1H, dd, J_{AB} = 18Hz and J_{XB} = 5Hz, CH_X-CH_AH_B).

2.1.3. Synthesis of 1,3-dipentyl-3-(4-nitrophenyl) pyrrolidine-2,5-dione (F3)

A mixture of the 3-(4-nitrophenyl) pyrrolidine-2,5-dione (F2) (2.2 g, 9.3 mmol), iodopentane (8 g, 40.2 mmol) and potassium carbonate (5 g) in acetone (50 ml) was stirred under reflux for 6 h. The mixture was filtered and the filtrate evaporated. The resulting brown oil (3.6 g) was dissolved in ethyl acetate, washed with water (2×10 ml) and dried (MgSO₄). The solution was then clarified with decolorizing charcoal and concentrated to give a yellow oil (3.2 g), which was fractionated down a column of dry silica (200 g) with light petroleum - ethyl acetate (5 : 1) as eluent. Evaporation of the main fraction gave a yellow oil which was kept under vacuum at room temperature overnight to yield the dipentyl nitro dione (F3) (1.4 g, 41.9%) as colorless crystals, m.p. 65.3-66.0 °C (Lit., [15]), 67.5-68.2 °C], ν_{\max} 3120-2860 (C-H), 1768-1690 (C=O, imide), 1600 (C=C, Ar), 1515-1345 (NO₂) cm⁻¹, δ_{H} (90 MHz; CDCl₃), 8.25 (2H, d, J = 9Hz,

3',5'-PhH), 7.7 (2H, d, J = 9Hz, 2',6'-PhH), 3.57 (2H, t, J = 7Hz, N-CH₂-CH₂-), 3.15 (1H, d, J_{BA} = 18Hz, CH_AH_B-(C=O)), 2.95 (1H, d, J_{AB} = 18Hz, CH_AH_B-(C=O)), 2.2 - 0.75 (20H, m, C-(CH₂)₄-CH₃, N-CH₂-(CH₂)₃-CH₃).

2.1.4. Synthesis of 3-(4-aminophenyl)-1,3-dipentylpyrrolidine-2,5-dione (F4)

A mixture of the nitro imide (F3) (1.4 g, 3.88 mmol) and 10% palladium on charcoal (0.14 g) in absolute ethanol was placed in a hydrogenation flask, the flask evacuated, filled with hydrogen and shaken until 261 ml gas was absorbed (theory 261 ml). The catalyst was removed by filtration through a bed of celite and the filtrate concentrated under reduced pressure to afford the amine (F4) as a yellow oil (1.1 g, 86.8%) (Lit., [15]). ν_{\max} (neat) 3470-3380 (NH₂, str), 3100-2880 (C-H), 1772-1700 (C=O, imide), 1630 (C=C, Ar) cm⁻¹, δ_{H} (90MHz; CDCl₃), 7.15 (2H, d, J = 9Hz, 2',6'-PH), 6.6 (2H, d, J = 9Hz, 3',5'-PhH), 3.6 (2H, s, NH₂), 3.4 (2H, t, J = 7Hz, N-CH₂-CH₂-), 3.03 (1H, d, J_{BA} = 18Hz, CH_AH_B-(C=O)), 2.8 (1H, d, J_{AB} = 18Hz, CH_AH_B-(C=O)), 2.2-0.6 (20H, m, C-(CH₂)₄-CH₃, N-CH₂-(CH₂)₃-CH₃).

2.1.5. Synthesis of 4-(2,5-dioxo-1,3-dipentylpyrrolidine-3-yl) phenyl sulphinic acid (F5)

Sodium nitrite solution (20%, 1 ml) was added dropwise to a stirred solution of 3-(4-aminophenyl)-1,3-dipentylpyrrolidine-2,5-dione (F4) (0.5 g, 1.5 mmol) in 25% sulphuric acid (10 ml). On completion of the reaction (starch paper) acetone was added as a co-solvent and SO₂ gas was bubbled into the solution until no further gas uptake was observed (constant weight of the reaction mixture). The mixture was transferred to a beaker containing copper powder (2 g), stirred at room temperature for 2h and finally filtered through a bed of celite. The residue was washed with acetone (20 ml) and glacial

acetic acid (20 ml) and the combined filtrates diluted with water (100 ml). Refrigeration of the mixture overnight, furnished a white precipitate which was collected, washed with water (10 ml) and dried at 40 °C under vacuum to give the sulphinic acid F5 as pale yellow crystals (0.29 g, 50.7 %) m.p. 86.5-88.1 °C. (Found: C, 63.15; H, 7.65; N, 3.72. $C_{20}H_{29}NO_4S$ requires; C, 63.30; H, 7.70; N, 3.69%). ν_{\max} 2959-2860 (C-H), 1773, 1701 (C=O, imide), 1595 (C=C, Ph), 1039-1010 (S=O, str) cm^{-1} . δ_H (300 MHz; $CDCl_3$) 10.3 (1H, br s, O-H), 7.9 (2H, br s, 3',5'-PhH), 7.6 (2H, d, J = 6.6Hz, 2',6'-PhH), 3.58 (2H, t, J = 7.3Hz, N-CH₂), 3.15 (1H, d, J_{BA} = 18.3Hz, CH_AH_B-(C=O)), 3 (1H, d, J_{AB} = 18.3Hz, CH_AH_B-(C=O)), 2.03 (2H, m, C-CH₂-CH₂-), 1.62 (2H, quintet, J = 7.5Hz, N-CH₂-CH₂-), 1.4-1.1 (10H, m, N-CH₂-CH₂-(CH₂)₂-CH₃, C-(CH₂)₃-CH₃), 0.95 - 0.83 (6H, m, (CH₃)₂).

2.1.6. Synthesis of 1,3-dipropyl-3-(4-nitrophenyl) pyrrolidine-2,5-dione (F6)

A mixture of the 3-(4-nitrophenyl) pyrrolidine-2,5 dione (F2) (6 g, 27 mmol), 1-iodopropane (18.5 g, 110 mmol) and potassium carbonate (15 g, 109 mmol) in acetone was stirred at 60 °C under reflux for 5 h. The mixture was filtered and the filtrate evaporated. The residue was dissolved in ether (40 ml), washed with water (2×20 ml), dried (MgSO₄) and clarified with activated charcoal. The clear solution was concentrated and fractionated on a column of dry silica using petroleum ether (60-80)-ethyl acetate (4:1, 100 ml) to initially remove the excess of iodopropane. Separation was then continued with petroleum ether (60-80)-ethyl acetate (2:1) as eluent. Evaporation of the main fraction gave an orange oil, which solidified on trituration with petroleum ether (60 - 80) to give the dione (F6) as a white powder (4 g, 48.1%). An analytical sample was recrystallised from ethanol, m.p. 61.9-62.8

°C, (Lit., [15]), 63.0-63.2°C] ; ν_{\max} 3100-2860 (C-H), 1777, 1690 (C=O, imide), 1600 (C=C, Ar), 1520, 1345 (NO₂) cm^{-1} , δ_H (90 MHz ; $CDCl_3$) 8.27 (2H, d, J = 9Hz, 3',5'-PhH), 7.7 (2H, d, J = 9Hz, 2',6'-PhH), 3.55 (2H, t, J = 7Hz, N-CH₂-CH₂-CH₃), 3.18 (1H, d, J_{BA} = 18Hz, CH_AH_B-CO), 2.95 (1H, d, J_{AB} = 18Hz, CH_AH_B-CO), 2.02 (2H, t, J = 8Hz, C-CH₂-CH₂-CH₃), 1.9-0.8 (10H, m, N-CH₂-CH₂-CH₃, C-CH₂-CH₂-CH₃).

2.1.7. Synthesis of 3-(4-aminophenyl)-1,3-dipropylpyrrolidine-2,5-dione (F7)

A solution of the 1,3-dipropyl-3-(4-nitrophenyl)-pyrrolidine-2,5-dione (F6) (3 g, 12.7 mmol) in absolute ethanol (50 ml) was placed in a hydrogenation flask. Palladium 10% on charcoal (0.3 g) was added and the flask evacuated, filled with hydrogen and shaken until no further hydrogen uptake was observed. The catalyst was removed by filtration through a bed of celite and the filtrate concentrated under reduced pressure to afford a white solid which was recrystallized from ethanol to give the amine (F7) (1.9 g, 54.3%) as a white powder, m.p. 95.5-96.4 °C (Lit., [15]), 95.7-96.4 °C]; ν_{\max} 3450-3360 (NH₂), 3040-2870 (C-H), 1770, 1690 (C=O, imide), 1610 (C=C, Ar) cm^{-1} ; δ_H (90MHz ; $CDCl_3$), 7.17 (2H, d, J = 9Hz, Ph-H), 6.62 (2H, d, J = 9Hz, Ph-H), 3.47 (2H, t, J = 7Hz, N-CH₂-CH₂-CH₃), 3.05 (1H, d, J_{BA} = 18Hz, CH_AH_B-C=O), 2.77 (1H, d, J_{AB} = 18Hz, CH_AH_B-C=O), 2.1 - 0.6 (12H, m, CH₂-CH₂-CH₃, N-CH₂-CH₂-CH₃).

2.1.8. Synthesis of 4-(2,5-dioxo-1,3-dipropylpyrrolidine-3-yl) phenyl sulphinic acid (F8)

3-(4-Aminophenyl)-1,3-dipropylpyrrolidine-2,5-dione (F7) (0.3 g, 1.1mmol) was dissolved in 25% sulphuric acid (5 ml) and to the stirred solution was added 20% sodium nitrite in water (0.5 ml) dropwise. When the

reaction was completed (starch paper) 50% sulphuric acid (4 ml) was added and SO₂ was introduced into the reaction mixture until a constant weight was obtained. The mixture was then transferred to a beaker containing copper powder (1 g), and the contents stirred mechanically for 1 h, and then filtered through a bed of celite. The residue was washed with acetone (3×10 ml) and the filtrate was evaporated and the residue extracted with ether (2×20 ml). The combined ethereal extracts were washed with water (10 ml), dried (MgSO₄) and concentrated to give a yellow viscous oil (0.3 g), which was fractionated using preparative TLC plates (20×20×0.1 cm) with ethyl acetate-methanol (3:1). The main band was separated and washed with acetone (4×5 ml) through a small sintered funnel. The filtrate was evaporated to give the sulphinic acid (F8) as a yellow oil (0.13g, 36.4%). [Found: C, 57.85; H, 6.40; N, 4.24. (M+NH₄)⁺ 341.1535 C₁₆H₂₁NO₄S (1/2 H₂O) requires C, 57.80; H, 6.67; N, 4.22%. (M+NH₄)⁺ 341.1535]. ν_{\max} (neat) 2963 - 2875 (C-H), 1773, 1698 (C=O, imide), 1595 (C=C, Ph), 1044, 1012 (S=O) cm⁻¹. δ_{H} (360 MHz; DMSO) 7.58 (2H, d, J = 8.1Hz, 3',5'-PhH), 7.4 (2H, d, J = 8Hz, 2',6'-PhH), 3.4 (2H, overlap with water in DMSO, N-CH₂) 3.08 (1H, d, J_{BA} = 18.4Hz, CH_AH_B-(C=O)), 3.05 (1H, d, J_{AB} = 18.4Hz, CH_AH_B-(C=O)), 2.07 - 1.8 (2H, m, C-CH₂-CH₂-CH₃), 1.48 (2H, sextet, J = 7.2Hz, N-CH₂-CH₂-CH₃), 1.3 - 0.95 (2H, m, C-CH₂-CH₂-CH₃), 0.87 (3H, t, J = 7.1Hz, C-(CH₂)₂-CH₃), 0.78 (3H, t, J = 7.3Hz, N-(CH₂)₂-CH₃).

2.1.9. Synthesis of 1,3-dibutyl-3-(4-nitrophenyl) pyrrolidine-2,5-dione (F9)

A mixture of the 3-(4-nitrophenyl) pyrrolidine-2,5-dione (F2) (6 g, 25.4 mmol), iodobutane (24 g, 131 mmol) and potassium carbonate (11 g) in acetone (50 ml) was stirred at 60 °C under reflux for 6 h. The powder bed was collected by filtration and filtrate was evaporated to dryness. The residue was dissolved in ether (50 ml), washed with water

(2×10 ml), dried (MgSO₄) and concentrated to give a yellow oil (8.9 g), which was fractionated on a column of dry silica with light petroleum ether-ethyl acetate (5:1) as eluent. Evaporation of the main fraction gave the nitro dione (5.5 g, 65.3 mmol) as a white solid. An analytical sample was recrystallised from ethanol to give the nitro dione (F9) as white crystals, m.p. 78.9-80.1 °C (Lit., [15]), 81.1-81.6 °C; ν_{\max} 3100-2840 (C-H), 1770-1690 (C=O, imide), 1520-1350 (NO₂) cm⁻¹; δ_{H} (90MHz; CDCl₃), 8.2 (2H, d, J = 9Hz, 3',5'-PhH), 7.68 (2H, d, J = 9Hz, 2',6'-PhH), 3.55 (2H, t, J = 7Hz, N-CH₂-CH₂-CH₃), 3.15 (1H, d, J_{BA} = 18Hz, CH_AH_B-(C=O)), 2.90 (1H, d, J_{AB} = 18Hz, CH_AH_B-(C=O)), 2.0 (2H, t, J = 8Hz, C-CH₂-CH₂-), 1.9 - 0.7 (14H, m, C-CH₂-CH₂-CH₃, N-CH₂-CH₂-CH₃).

2.1.10. Synthesis of 3-(4-aminophenyl)-1,3-dibutyl-pyrrolidine-2,5-dione (F10)

A mixture of the 1,3-dibutyl-3-(4-nitrophenyl)-pyrrolidine-2,5-dione (F9) (5.3g, 6 mmol) and 10% palladium on charcoal (0.53 g) in absolute ethanol (40 ml) was placed in a hydrogenation flask. The flask was then evacuated, filled with hydrogen and shaken until no further hydrogen uptake was observed. The catalyst was removed by filtration through a bed of celite and the filtrate concentrated under reduced pressure to give the amino derivative as a white solid (4.5 g, 93%) (Lit., [15]). An analytical sample was recrystallised from ethanol to give the amine (F10) as a white crystalline powder, m.p. 164.5-166 °C ν_{\max} 3450-3350 (NH₂, str), 3150-2885 (C-H), 1770, 1690 (C=O, imide) cm⁻¹; δ_{H} (90MHz; CDCl₃), 7.18 (2H, d, J = 9Hz, 2',6'-PhH), 6.3 (2H, d, J = 9Hz, 3',5'-PhH), 3.8 - 3.3 (4H, m, N-CH₂-CH₂-CH₂-CH₃, NH₂), 3.06 (1H, d, J_{BA} = 18Hz, CH_AH_B-(C=O)), 2.79 (1H, d, J_{AB} = 18Hz, CH_AH_B-(C=O)), 2.08 - 0.7 (16H,

m, C- $\underline{\text{CH}_2}$ - $\underline{\text{CH}_2}$ - $\underline{\text{CH}_2}$ - $\underline{\text{CH}_3}$, N- CH_2 - $\underline{\text{CH}_2}$ - $\underline{\text{CH}_2}$ - $\underline{\text{CH}_3}$)

2.1.11. Synthesis of 4-(2,5-dioxo-1,3-dibutylpyrroline-3-yl) phenylsulphinic acid (F11)

Sodium nitrite 20% (1 ml) was added to a stirred mixture of the 3-(4-Aminophenyl)-1,3-dibutyl-pyrrolidine-2,5-dione (F10) (0.5g, 1.6 mmol) in 20% sulphuric acid (7 ml) at 5 °C. After completion of the reaction (starch paper) 50% sulphuric acid (5 ml) was added and SO₂ gas introduced until no further SO₂ absorption was observed (constant weight). The mixture was then transferred to a beaker contain copper powder (1 g), stirred for 1 h at room temperature and filtered through a bed of celite. The residue was washed with acetone (20 ml) and glacial acetic acid (20 ml). The filtrate was diluted with cold water (150 ml) and refrigerated overnight to give a white solid which was collected, washed with water (10 ml) and dried to yield the sulphinic derivative (F11) as a white fine powder (0.2g, 35.6%) m.p. 93-94.3 °C. [Found: C, 61.29; H, 7.18; N, 3.93%. (M+NH₄)⁺ 369.1848. C₁₈H₂₅NO₄S requires C, 61.52; H, 7.17; N, 3.99%. (M+NH₄)⁺ 369.1848]. v_{max} 2960-2860 (C-H), 1765, 1690 (C=O, imide), 1595 (C=C, Ph), 1060-1010 (S=O, str) cm⁻¹. δ_{H} (300MHz; CDCl₃) 7.8-7.55 (4H, m, Ph-H), 3.43 (2H, t, J = 7Hz, N-CH₂), 3.15 (1H, d, J_{BA} = 18.3Hz, CH_AH_B-(C=O)), 3.08 (1H, d, J_{AB} = 18.3Hz, CH_AH_B-(C=O)), 2.1-1.85 (2H, m, C- $\underline{\text{CH}_2}$ -CH₂), 1.45 (2H, quintet, J = 7Hz, N-CH₂- $\underline{\text{CH}_2}$ -CH₂-CH₃), 1.3-0.95 (6H, m, N-CH₂-CH₂- $\underline{\text{CH}_2}$ -CH₃, C-CH₂- $\underline{\text{CH}_2}$ - $\underline{\text{CH}_2}$ -CH₃), 0.855 (3H, t, J = 7.2Hz, N-(CH₂)₃- $\underline{\text{CH}_3}$), 0.83 (3H, t, J = 7.3Hz, C-(CH₂)₃- $\underline{\text{CH}_3}$).

2.2. Screening of compounds as inhibitors of oestrone sulphatase

Tubes (in triplicate) with an assay volume

of 0.5 ml containing substrate [6,7-³H]-oestrone sulphate ammonium salt (10 µl of 400 nM stock in propylene glycol) and oestrone sulphate potassium salt (10 µl of 60 µM in ethanol), inhibitors (20 µl of 5 mM solution in absolute ethanol), NADPH generating system (50 µl, NADP, 0.0082 g/ml; G-6P, 0.0228 g/ml; G-6PD, 15 µl/ml (4IU)) and phosphate buffer pH 7.4 (5mM, 400 µl) were prepared. The content of the tubes were mixed and pre-heated in a water bath for 4 min. at 37 °C. The final substrate concentration was 1.2 µM.

The enzyme reaction was started by addition of 10 µl of 25 mg/ml human placental microsomes. The tubes were incubated for 35 min. at 37 °C and the reaction was terminated by addition of mercuric chloride (300 µl, 1 mM). To each tube ethyl acetate (3 ml) was added and the tube then vortexed for 10 sec. The tubes were left at room temperature for 10 min. and the organic layer was then transferred to another set of tubes where the ethyl acetate was evaporated to dryness as previously described and the residue was reconstituted in 100 µl of absolute ethanol. A 50 µl of aliquot was injected into the HPLC system equipped with a 10 µm C18 µBonda pack column (3.9×300 mm, Millipore) and an on line radioactive detector using a mixture of 50:50 HiSafe 3 and methanol as scintillation fluid. The results were analyzed using an available data base system.

The inhibitory potency of the compounds was calculated by comparing the rate of reaction to that of a control as determined by measuring the area under the curve of the metabolite peak in comparison to that of the control [16].

2.3. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Tukey test. $p < 0.05$ was considered significant.

The biological assay described here for

sulphatase inhibition was kindly carried out by Dr M. Ahmadi at the Welsh School of Pharmacy, university of Wales, college of Cardiff, England.

3. Results

At 200µM concentration among 3 novel 4-(2,5-Dioxo-1,3-dialkylpyrrolidine-3-yl)phenylsulphinic acids tested here (F5, F8, F11) only F5 showed detectable inhibition (30.06%). In the assay danazol (200 µM), a known inhibitor gave 20% inhibition.

4. Discussion

The most likely cause of low activity in this series of compounds as sulphatase inhibitors is due to, (1) the higher polarity of the pyrrolidine dione ring compared to that of the C-ring of the steroid backbone and, presumably, consequent rejection by an existing hydrophobic region in the enzyme active site, since F5 with lower polarity due to longer side chains on pyrrolidine dione ring showed better inhibitory effect. (2) the lack of binding by the shorter alkyl chains (propyl and butyl) which are oriented above the plane of the steroid binding site. Possibly repositioning occurs to accommodate the alkyl groups and carbonyl groups in hydrophobic and hydrophilic areas respectively with movement of the $-SO_2^-$ group away from the active site.

Since the only active compound in this series (F5) has the longest side chain and the most flexible structure, synthesis of more lipophil structures by introducing the longer side chains on similar positions may result in productions of better oestrone sulphatase inhibitors.

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