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SOME 1-, AND 3-SUBSTITUTED 3-(4′-AMINOPHENYL)PYRROLIDINE-2,5-DIONES AS SELECTIVE INHIBITORS OF AROMATASE

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1-Alkyl-3-(4′aminophenyl)pyrrolidine-2,5-diones (**7**,R = C_3H_7-C_7H_{15}) are potent inhibitors of aromatase *in vitro*, the 1-hexyl (K_i = 62nM) being about 100-fold more potent than aminoglutethimide (AG), and more selective in their ratio of aromatase:CSCC inhibitory potency. The 1-pentyl, 1-hexyl and 1-heptyl derivatives are more stable to liver microsomal metabolism *in vitro* than AG possibly due to inhibition of the liver cytochrome P450s.

1,3-Dialkyl-3(4′-aminophenyl)pyrrolidine-2,5-diones (**9**) have been synthesised by a novel method. Although the higher homologues (di-pentyl and di-hexyl) are more potent *in vitro* as inhibitors of aromatase than AG, they are less active than their 1-alkyl counterparts with the same alkyl substituent.

KEY WORDS: Aromatase, inhibition, 1-, and 3-substituted 3-(4′-aminophenyl)pyrrolidine-2,5-diones, aminoglutethimide, pyridoglutethimide.

INTRODUCTION

Oestrogens are considered to play an important role in the incidence and promotion of breast cancer growth.[1] In postmenopausal women with breast cancer, removal of the tumour mass is followed by radiotherapy and anti-endocrine chemotherapy to prevent further tumour growth in the breast and metastases. The anti-oestrogen tamoxifen is used as a first line drug which reduces the effects of circulating oestrogens and oestrogens produced in breast tissue by preventing combination with the oestrogen receptor.[2] Patients which become refactory to tamoxifen after continued use can be successfully treated in some cases with a second line drug, an aromatase inhibitor.[3] In postmenopausal women this decreases oestrogen synthesis from androstenedione, produced by the adrenals, by aromatase in peripheral fatty tissue sites.

Aminoglutethimide (AG;**1**) is a competitive, reversible inhibitor of aromatase and is the drug currently in use in the clinic.[4] However, AG has several undesirable clinical features:[4,5] (1) it needs to be administered with hydrocortisone due to depletion of

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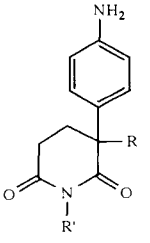
corticosteroids through inhibition of the cholesterol side chain cleavage enzyme (CSCC) in the adrenal steroidogenic pathway; (2) it has CNS-depressant activity which, although temporary on persistent administration of the drug, can lead to patient non-compliance due to drowsiness and ataxia; (3) it is a liver cytochrome P-450 enzyme inducing agent, an action affecting its own metabolism and the metabolism of other drugs; (4) its use may be associated with skin rashes and blood dyscrasias (c. 1%) in a few patients. The undesirable side effects possessed by AG have led to a search for potent, more selective aromatase inhibitors which do not possess CNS activity and although several have been described and are now in clinical trials (e.g., CGS16949A,[6] R76713,[7] PED,[8] and 4-hydroxyandrostenedione[9]) new inhibitors are being reported and the search for "clean" inhibitors still has momentum.

Extensive modification of AG has been carried out to improve its clinical profile. Rogletimide (**2**),[10] now in clinical trials, is a much weaker inhibitor of aromatase than AG when tested with the main androgen substrate androstenedione but is almost equipotent with testosterone as a substrate. It is devoid of CSCC inhibitory activity.

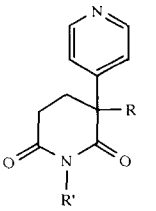
1-Alkyl derivatives of pyridoglutethimide (**2**; R' = CH_3-$C_{10}H_{23}$) have been described[11] with increased potency for *n*-pentyl through to *n*-decyl with a maximum for the *n*-octyl (10 times potency of AG with testosterone as substrate). The *n*-octyl derivative had about one third the CSCC potency of AG. In the 3-alkyl series (**3**) a similar maximum activity was seen with a *n*-octyl (24 times potency of AG). It was found[12] for both the 1- and the 3-alkyl series that they were highly susceptible to oxidative metabolism in the alkyl residue in *in vitro* liver microsome studies, thus negating the potential *in vivo* advantage of much greater inhibitory potency towards aromatase compared with the parent compound.



(1); R = Et, R' = H

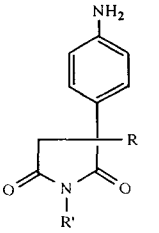
(4); R = alkyl, R' = H

(5); R = Et, R' = alkyl



(2); R = Et, R' = H

(3); R = alkyl, R' = H



(6); R = R' = H

(7); R = H, R' = alkyl or aryl

(8); R = alkyl, benzyl, propargyl or aryl, R' = H

(9); R = R' = alkyl

In a similar manner 3-alkyl derivatives of AG (**4**) exhibit an increase in potency with chain length (C_3H_7-C_7H_{15}) although the effect is not linear and is maximal with the *iso*-pentyl derivative (93 times potency of AG).[13] The pentyl derivative has about half the potency of AG towards the CSCC enzyme.

The 1-alkyl derivatives of AG (**5**; R = C_3H_7-C_8H_{19}) do not show a striking increase in potency with increased chain length, the hexyl and octyl being the most potent (about 4 times potency of AG).[14] These derivatives have about two thirds the potency of AG towards CSCC enzyme.

In a series of 3-alkyl-1-phenyl-3-azabicyclo[3.1.0] hexane-2,4-diones the 3-butyl and 3-pentyl compounds are approximately 100 times more potent than AG in inhibiting aromatase with no significant activity towards the CSCC enzyme.[15]

We have reported[16] that 3-(4′-aminophenyl)pyrrolidine-2,5-dione (WSP-3;**6**), a ring contracted analogue of AG, had a comparable activity to AG, *in vitro* and *in vivo* and lacked the undesirable CSCC inhibitory[17] and CNS side effects of AG.[18] This paper describes certain 1- and 3-monosubstituted and 1,3-disubstituted analogues of WSP-3, (**7**, **8** and **9** respectively), as aromatase inhibitors in the continued search for potent, selective inhibitors of the enzyme free from the undesirable clinical features of AG.

MATERIALS AND METHODS

Materials

D-Glucose-6-phosphate (mono-phosphate salt). NADP (mono-sodium salt) were purchased from Sigma Chemicals Co., and D-Glucose-6-phosphate dehydrogenase from Boehringer-Mannheim, (1β, 2β-^{3}H] testosterone (41.6 Ci/mmol) and [1β, 2β-^{3}H] androstenedione (48.6 Ci/mmol) were purchased from New England Nuclear, Boston, Mass and [26, 27)-^{14}C] cholesterol (52 Ci/mmol) from Amersham International. Radioactivity was determined on a LKB Wallac 1217 Rackbeta liquid scintillation counter. Scintillation fluid for the aromatase assay was Instagel (Packard Instrument Co., Illinois) and for the CSCC assay, a mixture of 2,5-diphenoxazole (Sigma) and naphthalene, xylene and 1,4-dioxane (BDH, Poole), all being of "Scintran" grade.

Biochemical Studies

Preparation of the enzymes

Aromatase and CSCC (cholesterol side-chain cleavage enzyme) were prepared from human term placental tissue and bovine adrenal gland following the general method of Thompson and Siiteri[19] and Hochberg *et al.*[20] respectively.

Assays

Aromatase activity was determined by the measurement of ^3H_2O released from [1β, 2β-^{3}H] testosterone and [1β, 2β-^{3}H] androstenedione by the general method of Graves and Salhanick.[21] IC_{50} values were determined from (% inhibition) *vs* (log[I]) plots at the substrate concentrations given in the Tables. K_i values were determined by Dixon plots.[22] Regression lines were calculated by least-squares analysis. Aminoglutethimide (AG) was included in the tests for comparative purposes. The results are given in Table 1–4.

CSCC actvity was determined by measurement of ^{14}C-methylpentanal released from [26(27)-^{14}C] cholesterol by the method of Hochberg *et al.*[20] The substrate concentration and inhibitor concentration used are given in the Tables. The results are expressed as a percentage inhibition of the enzyme compared with a control value determined in the absence of inhibitor. Aminoglutethimide (AG) was included in the tests for comparative purposes.

Preparation of liver microsomes

Freshly excised livers from six rats, pretreated with sodium phenobarbitone (lg/litre) in their drinking water for five days, were pooled, washed with potassium chloride (1.15%) and homogenised in sucrose (0.25M)-Tris buffer (0.1 M, pH 7.4). The homogenate was centrifuged (800 g) for 15 min and the supernatant spun (11,000 g) for 20 min. The pellet obtained from the supernatant spun (105,000 g) for 1 h was resuspended in buffer and re-centrifuged (108,000 g) for 1 h. The microsomal pellet was resuspended in buffer to give a final protein concentration, as determined by the Pierce Protein Assay Kit, of c. 20 mg/ml.

Metabolism of 1-alkyl-3-(4'-aminophenyl)pyrrolidine-2,5-diones and AG by liver microsomes

Liver microsomes (300 μl), phosphate buffer (1.2 ml, 50 mM, pH 7.4), cofactor (500 μl) and test compound (25, 50, 100 and 200 μM) were separately incubated at 37°C in a shaking water bath. After 30 min further cofactor (100 μl) was added and oxygen bubbled through for 15 s. The reaction was terminated after 1 h by placing the tubes in a dry ice-acetone mixture. In the control experiment the microsomal preparation was added just prior to the termination step.

The incubation mixtures were shaken with dichloromethane (5 ml) for 30 min after addition of the internal standard. The tubes were then spun (880 g) for 15 min and the organic layer separated from the supernatant, filtered and evaporated. The residue was suspended in acetonitrile (1 ml) and analysed by HPLC using a Spherisorb 50DS column (25 × 4.6 mm) with a flow rate of 1.5 ml/min and detection at 243–5 nm.

The conditions for each compound were as follows: 1-Pentyl: mobile phase, water-acetonitrile (60:40); internal standard (80 μg), 1,3-dipropyl-3-(4'-aminophenyl)-pyrrolidine-2,5-dione. 1-Hexyl: water-acetonitrile (50:50); 1-heptyl-3-(4'-aminophenyl)-pyrrolidine-2,5-dione. 1-Heptyl: water-acetonitrile (50:50); 1-hexyl-3-(4'-aminophenyl)-pyrrolidine-2,5-dione. AG: water-methanol (50:50); 1-propyl aminoglutethimide.

The results are shown in Table 5.

Inhibition of benzphetamine metabolism

Liver microsomes (4.3 mg/mvol protein 0.5 ml) were separately incubated at 37°C for 25 min in Tris buffer (pH 7.4) containing benzphetamine (2.5 mM), cofactor generating system (0.5 ml) and 1-alykl-3-(4'-aminophenyl)pyrrolidine-2,5-dione or AG (100, 200 and 400 μM). The reaction was terminated by the addition of zinc sulphate solution (0.25 ml, 25%) and saturated barium hydroxide (0.25 ml). The suspension was spun (2,900 g) for 10 min and the formaldehyde content of the supernatant determined using Nash's reagent. The results are shown in Table 6.

Chemistry

M.p.s. were determined on an Electrothermal instrument and are uncorrected. I.r. spectra on KBr discs were recorded on a Perkin-Elmer 681 spectrophotometer. ^{1}H n.m.r. spectra in ($^2H_6$)DMSO unless otherwise stated were recorded on a Perkin-Elmer R32 (90 MHz) spectrometer and are quoted in p.p.m. relative to tetramethylsilane as internal reference. ^{13}C n.m.r. spectra in ($^2H_6$)DMSO were recorded on a Jeol-FX90Q (90 MHz) spectrometer using either tetramethylsilane or ($^2H_6$) dimethylsulphoxide as internal standard. Mass spectra were determined by the SERC Mass Spectrometry

Centre as Swansea. Elemental analyses were determined at the School of Pharmacy, London.

Representative spectral data is given although all compounds had satisfacrory data.

Synthesis

We have found that 3-phenylpyrrolidine-2,5-dione is readily alkylated with the appropriate alkyl halide in the 1-position using potassium carbonate in acetone. Furthermore, novel 1,3-disubstitution occurs with 3-(4′-nitrophenyl)pyrrolidine-2,5-dione and 3-insertion of a reactive halide can be achieved with a 1-substituted-3-phenylpyrrolidine-2,5-dione under these conditions. These methods have been used here for the synthesis of 1,3-dialkyl- and 3-alkyl-(4′-aminophenyl)pyrrolidine-2,5-diones as an alternative to the general method[25] from the appropriate aralkyl ketone (see later).

1-Propyl-3-phenylpyrrolidine-2,5-dione. 1-Iodopropane (9 g, 0.053 mole) was added to a solution of 3-phenylpyrrolidine-2,5-dione[16] (4.5 g, 0.0257 mole) in acetone with potassium carbonate (4.5 g). The mixture was stored and refluxed for 4 h when t.l.c. showed the absence of starting material. The mixture was filtered and excess solvent removed to leave a brown oil which solidified on ether trituration to give the 3-propyl derivative (3.2 g, 57%) m.p. 61.5–62°C (ethanol). (Found: C, 71.69; H, 6.97; N, 6.37. $C_{13}H_{15}NO_2$ requires C, 71.86; H, 6.96; N, 6.45%).ν_{max} 3030, 3010 (aryl C-H) 2970, 2950, 2880 (alkyl C-H) 1770, 1700 (imide C=O), 1605 (phenyl ring) cm^{-1}. ^{1}H.n.m.r. δ 7.5–7.1 (5H, s, Ph), 4.01 (1H, dd, $J_{AX}=8$ Hz, $J_{BX}=5$ Hz, $\underline{CH_X}CH_AH_B$), 3.55 (2H, t, J=7 Hz, N-$\underline{CH_2}$), 3.23 (1H, dd, $J_{XA}=8$ Hz, $J_{BA}=18$ Hz, $CH_X\underline{CH_A}H_B$), 2.80 (1H, dd, $J_{XB}=5$ Hz, $J_{AB}=18$ Hz, $\underline{CH_X}CH_A\underline{H_B}$). 1.65 (sextet J=7 Hz, $CH\underline{CH_2}$), 0.95 (3H, t, J=7 Hz, $\underline{CH_3}$).

1-Butyl-3-phenylpyrrolidine-2,5-dione was obtained as a brown oil (4.8 g, 90%). (Found: C, 71.25; H, 7.18; N, 6.04. $C_{14}H_{17}NO_2$ requires C, 72.7; H, 7.41; N, 6.06%). 1-Pentyl-3-phenylpyrrolidine-2,5-dione was obtained as a brown oil (6.2 g, 87%). (Found: C, 73.44; H, 7.81; N, 5.71. $C_{15}H_{19}NO_2$ requires C, 73.03; H, 7.97; N, 5.71%). 1-Hexyl-3-phenylpyrrolidine-2,5-dione was obtained as a yellow oil (70.4%). (Found: C, 72.34; H, 7.72; N, 5.35. $C_{16}H_{21}NO_2$ requires C, 74.10; H, 8.16; N, 5.40%). 1-Heptyl-3-phenylpyrrolidine-2,5-dione was obtained as a yellow oil (74.2%). (Found: C, 73.37; H, 8.48; N, 5.12. $C_{17}H_{23}NO_2$ requires C, 74.69; H, 8.14; N, 4.98%).

1-Propyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione. 1-Propyl-3-phenylpyrrolidine-2,5-dione (3 g, 0.0133 mole) was added to fuming nitric acid (25 ml) maintained at a temperature of −40°C by a dry CO_2/acetone bath. The mixture was stirred until all the solid was dissolved and the solution then poured into ice-water to give the *p*-nitro derivative as a white solid (1.7 g, 47%) m.p. 95–97°C (ethanol). (Found: C, 59.76; H, 5.60; N, 10.45. $C_{13}H_{14}N_2O_2$ requires C, 59.53; H, 5.38; N, 10.68%. ν_{max} 3110, 3080 (aryl C-H), 2970, 2940–, 2880 (alkyl C-H), 1770, 1700 (imide C=O), 1605 (phenyl ring), 1520, 1350 (NO_2) cm^{-1}. ^{1}H.n.m.r. δ 8.28 (2H, d, J=9 Hz, Ph-$\underline{H}$), 7.48 (2H, d, J=9 Hz, Ph-$\underline{H}$); 4.23 (1H, dd, $J_{AX}=8$ Hz, $J_{BX}=5$ Hz. $\underline{CH_X}CH_AH_B$), 3.58 (2H, t, J=7 Hz, N-$\underline{CH_2}$) 3.33 (1H, dd, $J_{XA}=8$ Hz, $J_{BA}=18$ Hz, $CH_X\underline{CH_A}H_B$), 2.83 (1H, dd, $J_{XB}=5$ Hz, $J_{AB}=18$ Hz, $CH_X\underline{CH_A}\underline{H_B}$), 1.68 (2H, sextet, J=7 Hz, $CH_2\underline{CH_2}CH_3$), 0.95 (3H, t, J=7.5 Hz, $\underline{CH_3}$).

1-Butyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained as a yellow oil (87%). (Found: C, 60.29; H, 5.90; N, 9.86. $C_{14}H_{16}N_2O_4$ requires C, 60.86; H, 5.84; N, 10.14%) 1-Pentyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was otained as a yellow oil (80.9%). (Found: C, 61.84; H, 6.34; N, 9.71. $C_{15}H_{18}N_2O_4$ requires C, 62.05; H, 6.25; N, 9.65%). 1-Hexyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained as a yellow oil (83%). (Found: C, 62.91; H, 6.48; N, 8.98. $C_{16}H_{20}N_2O_4$ requires C, 63.14; H, 6.62; N, 9.21%). 1-Heptyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained as a yellow oil (85.9%). (Found: C, 63.93; H, 6.87; N, 8.66. $C_{17}H_{22}N_2O_4$ requires C, 64.13; H, 6.97; N, 8.80%).

1-Propyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione. (7, $R'=C_3H_7$). The nitro compound (2.1 g, 0.009 mole) was shaken under hydrogen gas (20°C/1 atm.) in the presence of palladium/charcoal (10%, 0.2 g) as catalyst until hydrogen uptake was complete. The charcoal was removed and

the ethanol removed under reduced pressure. The brown oil which remained was dissolved in ethyl acetate and shaken with dilute hydrochloric acid. The aqueous layer was separated, made alkaline, extracted with ethyl acetate and the extract evaporated to leave a yellow oil. The oil solidified on trituration with ether to give the amino compound as white crystals (0.4 g, 21.5%) m.p. 88.5–89.7°C (ethanol). (Found: C, 67.35; H, 7.01;, N, 11.76. $C_{13}H_{16}N_2O_2$ requires C, 67.22; H, 6.94; N, 12.06%). ν_{max} 3450, 3350, 3210 (NH_2), 3040 (aryl C-H), 2950, 2880 (alkyl C-H), 1770, 1690 (imide C=O), 1635 (NH_2 def), 1605 (phenyl ring) cm^{-1}.

1-Butyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione (**7**, R′ = C_4H_9) was obtained as a brown oil (73.43%). (Found: C, 67.81; H, 7.43;, N, 11.24. $C_{14}H_{18}N_2O_2$ requires C, 68.27; H, 7.37; N, 11.37%). 1-Pentyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione (**7**, R = C_5H_{11}) (54.1%) m.p. 99.7–101.1°C (ethanol). (Found: C, 69.19; H, 7.77; N, 10.89. $C_{15}H_{20}N_2O_2$ requires C, 69.20; H, 7.74; N, 10.76%). 1-Hexyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione (**7**, R′ = C_6H_{13}) was obtained as the hydrochloride salt as white crystals (0.4 g, 17.9%) m.p. 187.4–188.6°C (ethanol). (Found: C, 61.98; H, 7.46; N, 8.98; $C_{16}H_{23}N_2O_2$ Cl requires C, 61.77; H, 7.40; N, 9.01%). 1-Heptyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione (**7**, R′ = C_7H_{15}) was obtained as the hydrochloride salt as white crystals (1.8 g, 38.3%) m.p. 187.4–188.6°C (ethanol). (Found: C, 62.66; H, 7.68; N, 8.56. $C_{17}H_{25}N_2O_2$ Cl requires C, 62.85; H, 7.76; N, 8.26%).

1-Aryl-3-(4′-aminophenyl)pyrrolidine-2,5-diones.

1-Phenyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained by heating 2-(4′-nitrophenyl)-succinic acid[16] with excess aniline for 1 h at 180°C, as a solid (31%) m.p. 145–152°C (ethanol). (Found: C, 64.77; H, 4.13; N, 9.47. $C_{16}H_{12}N_2O_4$ requires C, 64.86; H, 4.08; N, 9.46%); ν_{max} 1798, 1722 (C=O), 1610 (Ph-H), 1530, 1360 (NO_2) cm^{-1}; δ 8.25 (2H, d, J = 9 Hz, Ph-H), 7.52 (2H, d, J = 9 Hz, Ph-H), 7.24 (5H, s, N-Ph-H), 4.31 (1H, dd, J_{AX} = 6 Hz and J_{BX} = 9 Hz, $CH_XCH_AH_B$), 3.42 (1H, dd, J_{AB} = 18 Hz and J_{XB} = 9 Hz, $CH_XCH_AH_B$), 2.98 (1H, dd, J_{BA} = 18 Hz and J_{XA} = 6 Hz. $CH_XCH_AH_B$).

1-(4′-nitrophenyl)-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained by nitration from 1-(4′-nitrophenyl)-3-phenylpyrrolidine-2,5-dione as a white solid (61%), m.p. 188–192°C (dichloromethane). (Found: C, 56.40; H, 3.27; N, 12.25. $C_{16}H_{11}N_3O_6$ requires C, 56.31; H, 3.25; N, 12.31%).

1-Phenyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione. (**7**, R′ = C_6H_5) was obtained by reduction of the nitro compound with Pd/C and hydrogen as a solid (90%), m.p. 118–122°C (ethanol). (Found: C, 72.16; H, 5.30; N, 10.52. $C_{16}H_{14}N_2O_2$ requires C, 71.88; H, 5.31; N, 10.21%; ν_{max} 3440, 3360 (NH_2), 1770, 1700 (C=O), 1620 (Ph-H), 1340 (CH_2), 840 (1:4 disub. Ph-H) cm^{-1}; δ 7.49 (5H, m, N-Ph-H), 7.03 (2H, d, J = 9 Hz, Ph-H), 6.61 (2H, d, J = 9 Hz, Ph-H), 5.00 (2H, s, NH_2), 4.09 (1H, dd, J_{AX} = 6 Hz and J_{BX} = 9 Hz, $CH_XCH_AH_B$), 3.31 (1H, dd, J_{AB} = 18 Hz and J_{XB} = 9 Hz, $CH_XCH_AH_B$), 2.78 (1H, dd, J_{BA} = 18 Hz and J_{XA} = 9 Hz, $CH_XCH_AH_B$).

1-(4′-Aminophenyl)-3-(4′-aminophenyl)pyrrolidine-2,5-dione. (**7**, R′ = pNH_2-C_6H_4) was obtained by reduction of the dinitro compound as a solid (81%) m.p. 150°C (decomp.) (ethyl acetate). (Found: C, 66.95; H, 5.37; N, 14.22. $C_{16}H_{15}N_3O_2$ requires C, 68.31; H, 5.38; N, 14.98%).

1,3-Dipropyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione. 3-(4′-Nitrophenyl)pyrrolidine-2,5-dione[16] (3 g, 0.014 mole), 1-iodopropane (14 g, 0.082 mole) and potassium carbonate (7.6 g) were refluxed in acetone (50 ml) until t.l.c. showed absence of starting material. The mixture was then filtered and the acetone and excess 1-iodopropane evaporated. The products were separated using column chromatography (65 × 2.2 cm column, silica gel 60, petroleum ether:ethyl acetate (2:1)) to give the dipropyl derivative (first fraction) as pale brown crystals (0.7 g, 18.2%) m.p. 63.0–63.2°C (ethanol). (Found: C, 62.87; H, 6.50; N, 9.24. $C_{16}H_{20}N_2O_4$ requires C, 63.14; H, 6.62; N, 9.21%). ν_{max} 3100, 2960 (alkyl C-H), 1770, 1700 (imide C=O), 1525, 1320 (NO_2) cm^{-1}. δ 8.27 (2H, d, J = 9 Hz, Ph-H), 7.71 (2H, d, J = 9 Hz, Ph-H), 3.56 (2H, t, J = 7 Hz, N-CH_2), 3.20 (1H, d, J = 18 Hz, CH_AH_B), 2.94 (1H, d, J = 18 Hz, CH_AH_B), 2.05 (2H, t, J = 8 Hz, C-CH_2), 1.8–0.8 (10H, m, CH_2CH_3, CH_2CH_3).

1,3-Dibutyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained as buff coloured crystals (2.5 g, 32.7%) m.p. 81.1–81.6°C (ethanol). (Found: C, 65.11; H, 7.26; N, 8.59. $C_{18}H_{24}N_2O_4$ requires C, 65.04; H, 7.28; N, 8.43%). 1,3-Dipentyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was

obtained as buff coloured crystals (0.6 g, 7.3%) m.p. 67.5–68.2°C. (Found: C, 66.89; H, 7.66; N, 8.01. $C_{20}H_{28}N_2O_4$ requires C, 66.63; H, 7.83; N, 7.78%). 1,3-Dihexyl-3-(4′-nitrophenyl)-pyrrolidine-2,5-dione was obtained as buff coloured crystals (1.1 g, 14.7%) m.p. 62.8–63.3°C. (Found: C, 67.92; H, 8.29; N, 7.23. $C_{22}H_{32}N_2O_4$ requires C, 68.01; H, 8.30; N, 7.21%). 1,3-Diheptyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained as buff coloured crystals (0.3 g, 3.5%) m.p. 62.4–63.4°C (ethanol). (Found: C, 69.39; H, 8.72; N, 6.91. $C_{24}H_{36}N_2O_4$ requires C, 69.21; H, 8.71; N, 6.73%).

1,3-Dipropyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione. (**9**, $R = R' = C_3H_7$) 1,3-Dipropyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione (1.5 g, 0.006 mole) was catalytically reduced as described previously to give the corresponding aminophenyl compound as white crystals (0.7 g, 51.8%) m.p. 96.7–96.4°C (ethanol). (Found: C, 69.96; H, 8.09; N, 10.11. $C_{16}H_{22}N_2O_2$ requires C, 70.04; H, 8.08; N, 10.21%). ν_{max} 3450, 3380 (NH_2), 3040 (aryl C-H), 2970 (alkyl C-H), 1770, 1690 (imide C=O), 1635 (NH_2 def.), 1610 (phenyl ring) cm^{-1}. δ 7.17 (2H, d, J=9 Hz, Ph-H), 6.62 (2H, d, J=9 Hz, Ph-H), 3.45 (2H, t, J=7 Hz, N-CH_2), 3.04 (1H, d, J=18 Hz, CH_AH_B), 2.77 (1H, d, J=18 Hz, CH_AH_B), 2.1–0.7 (12H, m, $CH_2CH_2CH_3$, CH_2CH_3).

1,3-Dibutyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione was obtained as a yellow-brown oil (92.7%). (Found: C, 70.50; H, 8.72; N, 8.90. $C_{18}H_{26}N_2O_2$ requires C, 71.49; H, 8.67; N, 9.26%). 1,3-Dipentyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione was obtained as a brown oil (89%). (Found: C, 72.45; H, 9.18; N, 8.39. $C_{20}H_{30}N_2O_2$ requires C, 72.69; H, 9.15; N, 8.48%). 1,3-Dihexyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione was obtained as a brown oil (97%). (Found: C, 73.69; H, 9.29; N, 7.81. $C_{22}H_{34}N_2O_2$ requires C, 73.70; H, 9.56; N, 7.81%). 1,3-Diheptyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione was obtained as a brown oil (48.5%). (Found: C, 74.04; H, 9.92; N, 7.04. $C_{24}H_{38}N_2O_2$ requires C, 74.57; H, 9.91; N, 7.25%).

3-Substituted-3-(4′-aminophenyl)pyrrolidine-2,5-diones.

Method A. The ethyl 3-substituted-2,3-dicyano-3-phenylalkanoate was obtained by cyanide treatment of the corresponding ethyl 3-substituted-2-cyano-3-phenylalkenoate formed by reaction of the aralkyl ketone with ethylcyanoacetate using ammonium acetate as catalyst.[25]

3-Isopropyl-3-phenylpyrrolidine-2,5-dione. Ethyl 2,3-dicyano-4-methyl-3-phenylpentanoate (4.5 g, 0.0166 mol) was dissolved in acetic acid (40 ml) and concentrated sulphuric acid (16 ml) was added portionwise, with gentle shaking. The mixture was then heated under reflux in an oil bath at 130°C for 1 h. The dark brown solution was poured onto the crushed ice with vigorous agitation, left at room temperature for 3 h, basified (pH 8–9) and extracted with dichloromethane. The extracts were washed with water, dried ($MgSO_4$) and the solvent removed to leave a brown oil which crystallized on trituration with ether. Recrystallization (ethanol) gave pale yellow crystals of 3-isopropyl-3-phenylpyrrolidine-2,5-dione (3.2 g, 90% yield) m.p. = 105–7°C. (Found: C, 71.31; H, 7.01; N, 6.46. $C_{13}H_{15}O_2N$ requires C, 71.86; H, 6.96; N, 6.45%). ν_{max} (KBr); 3220 (N-H); 1770, 1700 (C=O), 1600 (Ph) cm^{-1}. $\delta(CDCl_3)$ 8.94 (1H, s, N-H), 7.4 (5H, m, Ph), 3.04 (2H, dd, J=7 and 19 Hz, CH_2); 2.51 (1H, m, CH); 0.98 (3H, d, J=7 Hz, CH_3); 0.79 (3H, d, J=7 Hz, CH_3).

3-Benzyl-3-(4′-nitrophenyl)pyrrolidine was obtained from ethyl 2,3-dicyano-3-benzyl-3(4′-nitrophenyl)propanoate as creamy crystals (10%) m.p. 194–6°C (ethanol). (Found: C, 65.72; H, 4.75; N, 8.94. $C_{17}H_{15}O_4N_2$ requires C, 65.58; H, 4.85; N, 9.00%). 3-Propyl-3-phenylpyrrolidine-2,5-dione was obtained from ethyl 2,3-dicyano-3-phenylhexanoate as a yellow oil (71%). 3-Isopropyl-3-(4′-nitrophenyl)pyrrolidine-2,5-dione was obtained from 3-isopropyl-3-phenylpyrrolidine-2,5-dione by nitration as white crystals (86%) m.p. = 133–135° (ethanol). (Found: C, 59.59; H, 5.43; N, 10.47. $C_{13}H_{14}O_4N_2$ requires C, 59.53; H, 5.38; N, 10.68%). ν_{max} (KBr) 3200 (NH), 1770, 1715, 1690 (C=O), 1590 (Ph), 1510, 1350 (NO_2) cm^{-1}. $\delta(CDCl_3)$ 8.65 (1H, s, N-H); 8.21 (2H, d, J=9 Hz, Ph); 7.72 (2H, d, J=9 Hz, Ph); 3.22 and 2.90 (2H, dd, J=18 Hz, CH_2); 1.1 and 0.8 (6H, d, J=7 Hz, 2 × CH_3). 3-Propyl-3-(4′-nitrophenyl)-pyrrolidine-2,5-dione was obtained from 3-propyl-3-phenylpyrrolidine-2,5-dione as a pale yellow solid (78%) m.p. 146–148°C (Lit.[27] m.p. 143–5°C) (ethanol).

3-Isopropyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione. (**8**, R = iso-C_3H_7) was obtained from the nitro-compound as white crystals (92%) m.p. 167–169°C (ethanol). (Found: C, 66.77; H, 6.92; N, 11.81. $C_{13}H_{16}O_2N_2$ requires C, 67.22; H, 6.94; N, 12.06%). ν_{max}; 3400, 3300 (NH_2), 1770,

1705 (C=O), 1615 (Ph) cm^{-1}. δ(DMSO) 11.1 (1H, s, N-H), 7.24 (2H, d, J=9 Hz, Ph), 6.61 (2Hz, d, J=9 Hz, Ph), 5.10 (2H, s, NH_2), 3.06 (1H, d, J=18, CH_AH_B), 2.87 (1H, d, J=18 Hz, CH_ACH_B), 2.45 (1H, m, J=7 Hz, CH), 0.98 (3H, d, J=7 H, CH_3), 0.73 (3H, d, J=7 Hz, CH_3).

3-Benzyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione (**8**, R=$CH_2C_6H_5$) was obtained from the nitro-compound as pale yellow crystals (72%) m.p. 179–80°C (ethanol). (Found: C, 72.79; H, 5.98; N, 9.76. $C_{17}H_{16}O_2N_2$ requires C, 72.84; H, 5.75; N, 9.99%). 3-Propyl-3(4′-aminophenyl)-pyrrolidine-2,5-dione (**8**, R=C_3H_7) was obtained from the nitro-compound as a brown oil (95%). (Found: C, 67.12; H, 7.18; N, 11.89. $C_{13}H_{16}O_2N_2$ requires C, 67.22; H, 6.94; N, 12.06%).

Method B.

1-Carbethoxy-3(4′-N-acetylaminophenyl)pyrrolidine-2,5-dione. 3-(4′-Acetylaminophenyl)pyrrolidine-2,5-dione[26] (4.5 g, 0.015 mole) was suspended in dry acetone (30 ml). Ethyl chloroformate (10 g, 0.09 mole) and potassium carbonate anhydrous (5.2 g, 0.04 mole) were added. The suspension was stirred and heated under reflux for 18 h. The resulting yellow suspension was filtered and evaporated to leave an oil which was triturated with ethanol to give a solid, 1-carbethoxy-3-(4′-*N*-acetylaminophenyl)pyrrolidine-2,5-dione (2.4 g, 42%) m.p. 153–155°C (ethanol). (Found: C, 59.23; H, 5.45; N, 9.10. $C_{15}H_{16}N_2O_5$ requires C, 59.20; H, 5.30; N, 9.21%); ν_{max} 3396 (NH), 1805 and 1765 and 1710 (C=O), 1680 (C=O), 1600, 1530 cm^{-1}. δ($CDCl_3$) 9.77 (1H, s, N-H amide), 7.50 (2H, d, J=9 Hz, Ph), 7.18 (2H, d, J=9 Hz, Ph), 4.37 (2H, q, J=7 HZ, CH_2CH_3), 4.18 (1H, dd, J_{AX}=6 Hz and J_{BX}=9 Hz, $CH_XCH_AH_B$), 3.40 (1H, dd, J_{AB}=18 Hz and J_{XB}=9 Hz, $CH_XCH_AH_B$), 2.85 (1H, dd, J_{BA}=18 Hz and J_{XA}=9 Hz, $CH_XCH_AH_B$), 2.09 (3H, s, $COCH_3$), 1.38 (3H, t, J=7 Hz, CH_2CH_3).

1-Carbethoxy-3-propenyl-3-(4′-N-acetylaminophenyl)pyrrolidine-2,5-dione. 1-Carbethoxy-3-(4′-*N*-acetylaminophenyl)pyrrolidine-2,5-dione (2.0 g, 0.0066 mol), 3-bromopropene (0.68 g, 0.0057 mole) and potassium carbonate anhydrous (2.5 g, 0.016 mole) in dry acetone (50 ml) were heated under reflux for 15 h. The resulting brown suspension was filtered and the solvent removed under reduced pressure to leave a brown oil, 1-carbethoxy-3-propenyl-3(4′-*N*-acetylaminophenyl)pyrrolidine-2,5-dione (1.2 g, 44%). (Found: C, 62.55; H, 6.05; N, 8.22. $C_{18}H_{20}N_2O_5$ requires C, 62.78; H, 5.85; N, 8.14%); ν_{max} 3350 (NH), 2396, 1812 and 1765 and 1728 (C=O), 1670 (C=O), 1600, 925 (C=CH) cm^{-1}; δ($CDCl_3$) 9.56 (1H, s, N-H amide), 7.53 (2H, d, J=9 Hz, Ph), 7.32 (2H, d, J=9 Hz, Ph), 5.63 (1H, m, CH_2-CH=CH_2), 5.13 (2H, dd, J=2 Hz and J=2 Hz, CH_2-CH=CH_2), 4.36 (2H, q, J=7 Hz, CH_2CH_3), 3.10 (2H, s, CH_2C=O), 2.76 (2H, d, CH_2-CH=CH_2), 2.10 (3H, s, $COCH_3$), 1.33 (3H, t, J=7 Hz, CH_2CH_3).

3-Propenyl-3-(4′-acetylaminophenyl)pyrrolidine-2,5-dione. Freshly prepared ethanolic sodium hydroxide (4%, 50 ml) was added to 1-carbethoxy-3-propenyl-3(4′-acetylaminophenyl)pyrrolidine-2,5-dione (4 g) and the solution was stirred and allowed to stand at room temperature for 24 h. Ether (50 ml) was added to the yellow suspension, and the resulting solid filtered off. The solid was dissolved in water, acidified with hydrochloric acid (10%) to pH 2, and extracted with ethyl acetate (3 × 50 ml). The combined extracts were dried and the solvent removed under reduced pressure. The resulting oil was triturated with ether and the solid removed to give 3-propenyl-3-(4′-acetylaminophenyl)pyrrolidine-2,5-dione, (1.1 g, 34%) m.p. 229–231°C (ethanol and ether). (Found: C, 65.15; H, 5.77; N, 10.43. $C_{15}H_{16}N_2O_3$ requires C, 66.16; H, 5.92; N, 10.29%); ν_{max} 3310 (NH), 3200 (NH), 1765 and 1715 (C=O), 1670 (C=O), 1634 (C=C), 1610, 928 (C=CH) cm^{-1}; δ 11.30 (1H, s, N-H ring), 9.95 (1H, s, HN-C=O), 7.58, (2H, d, J=9 Hz, Ph), 7.36 (2H, d, J=9 Hz, Ph), 5.57 (1H, m, CH_2CH=CH_2), 5.17 (2H, m, CH_2-CH=CH_2), 2.97 (2H, s, CH_2C=O), 2.72 (2H, d, CH_2CH=CH_2), 2.02 (3h, s, $COCH_3$).

3-Propenyl-3-(4′-aminophenyl)pyrrolidine-2,5-dione. (**8**, R=CH_2CH=CH_2) 3-Propenyl-3-(4′-*N*-acetylaminophenyl)pyrrolidine-2,5-dione (1 g) was suspended in concentrated hydrochloric acid (25%, 30 ml), and heated under reflux until all the starting material had dissolved (2 h). The acid was removed under reduced pressure to leave a glass-like solid, which on exposure to moisture resulted in a sticky mass. The solid was dissolved in water, basified with sodium hydrogen carbonate and extracted with ethyl acetate. The extract was dried and the solvent removed under pressure, to leave a dark red oil, (0.5 g, 60%). (Found: C, 68.01; H, 6.06; N, 12.12. $C_{13}H_{14}N_2O_3$ requires C, 67.81; H, 6.13; N, 12.17%); ν_{max} (neat) 3460 and 3370 (NH_2), 3230 (NH), 1770 and 1710 (C=O), 1622, 928 (C=CH), 830 cm^{-1}; δ($CDCl_3$) 7.22 (2H, d,

J = 9 Hz, Ph), 6.66 (2H, d, J = 9 Hz, Ph), 5.64 (1H, m, $CH_2\underline{CH}=CH_2$), 5.16 (2H, dd, J = 12 Hz and 3 Hz, CH_2-CH = $\underline{CH_2}$), 3.80 (2H, s, NH_2), 2.99 (2H, s, $\underline{CH_2}C=O$), 2.74 (2H, dd, J = 8 Hz and 4 Hz, $\underline{CH_2}CH=CH_2$).

1-Carbethoxy-3-propynyl-3-(4'-N-acetylaminophenyl)pyrrolidine-2,5-dione was obtained as a dark brown viscous oil which when triturated with ether gave a solid, 1-carbethoxy-3-propynyl-3(4'-*N*-acetylaminophenyl)pyrrolidine-2,5-dione (1.8 g, 64%) m.p. 139–142°C. (Found: C, 62.87; H, 5.57; N, 7.88. $C_{18}H_{18}N_2O_5$ requires C, 63.15; H, 5.30; N, 8.18%).

3-Propynyl-3-(4'-acetylaminophenyl)pyrrolidine-2,5-dione was obtained as an oil which when triturated with ether gave a solid, 3-propynyl-3-(4'-acetylaminophenyl)pyrrolidine-2,5-dione. (0.9 g, 76%) m.p. 238–240°C (from absolute ethanol). (Found: C, 66.47; H, 5.34; N, 10.48. $C_{15}H_{14}N_2O_3$ requires C, 66.65; H, 5.22; N, 10.37%).

3-Propynyl-3-(4'-aminophenyl)pyrrolidine-2,5-dione. (**8**, R = $CH_2C\equiv CH$) 3-Propynyl-3-(4'-*N*-acetylaminophenyl)pyrrolidine-2,5-dione (0.9 g) was suspended in hydrochloric acid (50%, 30 ml), and heated under reflux until all the compound had dissolved. The solution was basified with ammonia (0.88) and extracted with ethyl acetate (2 × 50 ml). The combined extracts were dried, and the solvent removed under reduced pressure to leave a solid. (0.6 g, 79%) m.p. 179–180°C (from absolute ethanol). (Found: C, 68.18; H, 5.34; N, 12.09. $C_{13}H_{12}N_2O_2$ requires C, 68.41; H, 5.30; N, 12.27%); ν_{max} 3480 and 3375 (NH_2), 3285 ((C≡CH), 3218 (NH), 1770 and 1712 (C=O), 1621, 640 cm^{-1}; δ 11.30 (1H, s, N-H), 7.05 (2H, d, J = 9 Hz, Ph), 6.57 (2H, d, J = 9 Hz, Ph), 4.80 (2H, s, broad NH_2), 2.98 (2H, s, $\underline{CH_2}C=O$), 2.87 (2H, d, J = 2 Hz, $\underline{CH_2}C\equiv CH$), 2.64 (1H, t, J = 2 Hz, $CH_2C\equiv\underline{CH}$).

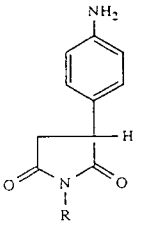
RESULTS AND DISCUSSION

In general, in *in vitro* tests, introduction of a small alkyl group on the imide nitrogen of AG (**1**),[14] rogletimide (**2**)[11] and 1-phenyl-3-azabicyclo [3.1.0] hexane-2,4-diones[15] leads to a reduction in inhibitory potency but for higher homologues an increase in potency. This pattern is followed with WSP-3 where reduced potency is seen with the 1-methyl derivative (but not *in vivo* due to demethylation) and increased potency with longer alkyl chains, the order of potency being 1-heptyl≏1-hexyl > 1-pentyl > 1-butyl > 1-propyl. The heptyl- and hexyl derivatives are 100-fold more potent than AG (see Table 1). The pyrrolidine-2,5-dione nucleus shows greater sensitivity to 1-alkylation as reflected in increased inhibitory potency than the glutarimide nucleus of AG or pyridoglutethimide by a factor of 25- and 10-fold respectively but has comparable sesitivity to the 3-azabicyclo [3.1.0] hexane-2,4-dione nucleus (*vide ultra*).

We have developed a model[23] which described the fitting of inhibitors to the steroid binding site of aromatase based on specific superimpositions of inhibitor atoms on ring A atoms present in androstenedione, the natural substrate. In our model, 1-alkyl groups in the inhibitor would not be accommodated at the sterically sensitive C-2 position of the substrate binding site. Laughton *et al.*[24] using our model have suggested that 1-alkyl groups could be accommodated in the corresponding 4-position of the binding site since this is not sensitive to steric bulk provided that alternative binding of the inhibitor occurs so that the *S*-form, rather than the *R*-form, possesses the major inhibitory activity. Evidence for this view came from the observation that *S*-1-octylrogletimide had greater potency that the *R*-form, the contrary existing for the parent compound.

Examination of the CSCC inhibitory activity of the 1-alkyl WSP-3 derivatives shows that the propyl, butyl and pentyl compounds were less potent than AG whereas the hexyl and heptyl derivatives were 1.5 and 1.9 fold more potent. In view of the

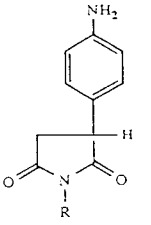
Table 1 1-Alkyl-3-(4′-aminophenyl)pyrrolidine-2,5-diones as inhibitors of aromatase and CSCC enzyme



	Aromatase			CSCC
R	IC_{50} (μM) Testosterone+*	Androstenedione+*	K_i μM (Androstenedione)	% Inhibition+*
C_3H_7	2.48	9.00	1.71	1.7
C_4H_9	0.98	2.75	0.24	15.3
C_5H_{11}	0.32	0.79	0.156	28.6
C_6H_{13}	0.17	0.40	0.062	69.2
C_7H_{15}	0.122	0.193	0.06	81.9
AG	11.75	27.54	5.76	65.9

+Substrate concentrations—1.2 μM (testosterone), 0.6 μM (androstenedione), 24 μM (cholesterol). *Inhibitor concentrations—100 μM (aromatase), 200 μM (CSCC).

Table 2 1-Aryl substituted-3-(4′-aminophenyl)pyrrolidine-2,5-diones as inhibitors of aromatase and CSCC enzyme



	Aromatase		CSCC
R	K_i μM (Testosterone)	K_i μM (Androstenedione)	% Inhibition+*
C_6H_5	1.65	3.8	26
p-$NH_2C_6H_5$	2.40	5.4	25
AG	0.91	5.1	65

*Inhibitor concentration 250 μM. +Substrate concentration—24 μM (cholesterol).

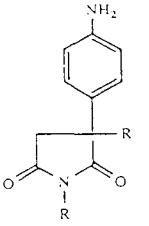
ratio of aromatase:CSCC inhibitory potency it follows that all these compounds are more selective inhibitors of aromatase than AG.

The homologous series of symmetrically 1,3-disubstituted derivatives of WSP-3, from di-propyl to di-hepyl, showed a decrease in activity for the di-propyl when compared with AG but within the series potency increased with homologation to the di-pentyl and di-hexyl (see Table 3). The di-pentyl was the most potent inhibitor within the series and was 16 times more potent than AG. Inhibitory potency towards CSCC within the series was about the same but less than for AG.

10-Propargyl androstenedione (PED) is a mechanism-based inactivator of aromatase.[8] Non-steroidal inactivators are not known and we have studied the 3-(prop-2-enyl)-, and 3-(prop-2-ynyl) derivatives of WSP-3 in this connection. These compounds were competitive reversible inhibitors of the enzyme with potency comparable with that of the 3-propyl derivative and AG (see Table 4) but did not irreversibly inhibit the enzyme on pre-incubation in the presence of NADPH and absence of substrate.

In our model[23] for the binding of inhibitors to the active site of the enzyme, AG and analogues are visualised as binding to the steroid ring A binding site with disposition of the phenylamino residue towards the Fe^{3+}-haem component of the cytochrome and the 3-alkyl substituent along the steroid backbone binding site. It would seem that for the 3-propenyl and 3-propynyl compounds liganding of the *p*-amino function to the Fe^{3+} is the main determinant for inhibitor binding so that the unsaturated groups are incorrectly placed for metabolism to active species and subsequent irreversible inhibition of the enzyme. This view is supported by the observation that 3-phenylpyrrolidine-2,5-dione is not a reversible inhibitor of the enzyme.

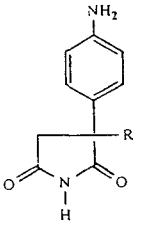
Table 3 1,3-Dialkyl-3(4′-aminophenyl)pyrrolidine-2,5-diones as inhibitors of aromatase and CSCC enzyme



	Aromatase			CSCC
R	IC_{50} (μM) Testosterone+*	Androstenedione+*	K_i μM (Androstenedione)	% Inhibition+*
C_3H_7	38.08	77.20	—	24.3
C_4H_9	1.74	3.80	0.50	7.8
C_5H_{11}	0.65	1.24	0.35	11.8
C_6H_{13}	2.19	1.72	1.33	16.0
C_7H_{15}	11.90	33.11	—	20.7
AG	12.02	21.33	5.76	65.9

*Inhibitor concentration—100 μM (aromatase), 200 μM (CSCC). +Substrate concentration—0.6 μM (androstenedione), 1.2 μM (testosterone), 24 μM (cholesterol).

Table 4 3-Substituted-3-(4′-aminophenyl)pyrrolidine-2,5-diones as *inhibitors of aromatase and CSCC enzyme*



R	Aromatase		CSCC
	K_i μM (Testosterone)	K_i μM (Androstenedione)	% Inhibition*
C_3H_7	1.5	10.1	36
i-C_3H_7	0.6	2.2	60
$C_6H_5.CH_2$	0.5	2.5	55
AG	1.8	9.1	66
CH_2-CH=CH_2	1.13	3.90	27
CH_2-C≡CH	1.11	4.50	22
AG	0.91	5.10	65

*24 μM cholesterol; 250 μM inhibitor.

The most potent inhibitors described here, the 1-pentyl, 1-hexyl and 1-heptyl derivatives of WSP-3 were selected for *in vitro* liver microsomal studies to examine their potential stability to metabolism *in vivo*.

The potent related inhibitor 1-octylrogletimide is rapidly depleted in this system due to oxidation of the 1-octyl side chain and this instability is reflected *in vivo* in the rat where it is not detectable in the plasma after 2 h (i.v.) or 15 min (i.p.).[12] This rapid metabolism thus negates its greater inhibitory potency, compared with the unsubstituted parent compound, towards aromatase.

Metabolism of 1-pentyl, 1-hexyl- and 1-heptyl-3-(4′-aminophenyl)pyrrolidine-2,5-diones by a rat liver microsomal preparation showed comparable degradation with AG at 50 μM concentration, less stability at 25 μM and greater stability at the higher

Table 5 Metabolism of some 1-alkyl-3-(4′-aminophenyl)-pyrrolidine-2,5-diones and AG by a rat liver microsomal preparation

Concentration (μM)	Metabolism (%)/h			
	1-Pentyl	1-Hexyl	1-Heptyl	AG
25	67.7	76.4	64.3	58.9
50	40.6	59.3	52.8	57.3
100	25.1	34.8	39.6	59.0
200	21.9	15.4	34.4	47.3

Table 6 Inhibition of rat liver microsomal metabolism of benzphetamine by some 1-alkyl-3-(4′-aminophenyl)pyrrolidine-2,5-diones and AG

Concentration (μM)	Inhibition (%)			
	1-Pentyl	1-Hexyl	1-Heptyl	AG
100	19	15	16	13
200	36	36	34	12
400	43	48	44	28

100 and 200 μM concentration (Table 5). The higher stability at 100 and 200 μM is probably due to inhibition by the pyrrolidine-2,5-dione of its own metabolism since using benzphetamine as substrate a greater degree of protection of benzphetamine metabolism was seen at the higher dose (Table 6). Overall, these results would indicate that the 1-alkyl derivatives have greater stability to metabolism than 1-octylrogletimide which is extensively metabolised by liver microsomes. Comparison of the 1-pentyl-, 1-hexyl- and 1-heptyl derivatives of WSP-3 with AG and its analogues (designed to improve inhibitory characteristics) shows that they compare favourably with these analogues with respect to *in vitro* inhibitory potency to aromatase and their selectivity, as measured by the ratio, aromatase:CSCC potency. Furthermore, in *in vitro* studies with liver metabolising enzymes they have acceptable metabolic profiles by comparison with AG. In a later paper the potential of these compounds as candidates for clinical studies is examined by *in vivo* studies in the rat.

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