# Analogues of 3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione as Selective Inhibitors of Aromatase: Derivatives with Variable 1-Alkyl and 3-Alkyl Substituents

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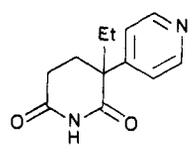
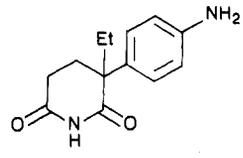
3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione (1) is a strong competitive inhibitor of human placental aromatase ($K_i$ = 1.1 $\mu$M; testosterone as substrate) that, unlike the structurally related aromatase inhibitor aminoglutethimide (2), is not also an inhibitor of the cholesterol side-chain cleavage enzyme desmolase. An improved synthesis of 1 is described, which was readily adapted to the preparation of homologues in a series of 3-alkyl-3-(4-pyridyl)-piperidine-2,6-diones (6–13). Alkylation of 1 afforded a second series, comprising 1-alkyl-3-ethyl-3-(4-pyridyl)-piperidine-2,6-diones (14–23). Inhibitory activity toward aromatase was maximal in both series for the octyl derivatives. Respective $K_i$ values for the competitive inhibition exerted by the 3-octyl (12) and the 1-octyl (21) analogues with testosterone as substrate were 0.09 and 0.12 $\mu$M. The compounds 1, 2, 12, and 21 differed in their relative potencies as inhibitors of the aromatization of testosterone and androstenedione. Respective $K_i$ values were as follows: for 1, 1.1 and 14 $\mu$M (ratio 12.7); for 2, 0.6 and 1.8 $\mu$M (3); for 12, 0.09 and 0.20 $\mu$M (2.2); and for 21, 0.12 and 0.48 $\mu$M (4).

3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione (1)[1] is an analogue of aminoglutethimide [2, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione], an agent widely used for the treatment of breast cancer in postmenopausal women.[2]




1




2

Inhibition of the conversion of the androgens androst-4-ene-3,17-dione (androstenedione) and testosterone into the estrogens estrone and estradiol, respectively, mediated by the enzyme complex aromatase, is believed to be the principal mode of action of 2. Both 1 ($K_i$ = 1.1 $\mu$M) and 2 ($K_i$ = 0.60 $\mu$M) are comparably potent inhibitors, but 1 has the potential advantage over 2 of being noninhibitory toward the cholesterol side-chain cleavage enzyme desmolase. Inhibition of this early step in steroidogenesis is mainly responsible, in patients treated with 2, for depleting corticosteroids, necessitating replacement therapy. Inhibitors of the aminoglutethimide type that are selective for aromatase have also been found among analogues of 2 in which the glutarimide (piperidine-2,6-dione) residue is replaced by succinimide.[3] However, considerations of metabolism point to a potential advantage of 1 over such succinimido derivatives, namely, the lesser propensity of 1 compared with 2 for metabolic inactivation. Thus both N-acetylation[4] and N-hydroxylation[5] of the amine functions are major inactivation pathways for 2, which would presumably apply to its succinimido analogues, whereas preliminary studies[6] of the metabolism of 1 indicate a lesser extent of metabolic inactivation to the pyridine *N*-oxide analogue. Hence we preferred to explore further the lead provided by 1 rather than the succinimide derivatives.

Selective inhibition of aromatase has also been achieved in steroidal analogues, particularly 4-hydroxyandrost-4-ene-3,17-dione (4-hydroxyandrostenedione), which has recently been introduced into the clinic[7] and is a more powerful aromatase inhibitor ($K_i$ = 0.16 $\mu$M) than 1 or 2. An equally potent inhibitor of the same structural type as 1 or 2 would therefore be of great interest for comparative studies, and the present paper concerns the search for such an inhibitor among analogues of 1 possessing alkyl substituents other than ethyl (*C*-alkyl series) or *N*-alkyl substituents in the piperidinedione ring (*N*-alkyl series). We also report an improved synthesis of 1, which also has general applicability to members of the *C*-alkyl series.

Very recently, a comparable study[8] of analogues of 2 in which the *C*-alkyl substituent was varied showed markedly enhanced inhibitory activity toward aromatase in some homologues. In the *n*-alkyl series the most active analogue was the *n*-pentyl derivative, which had an $IC_{50}$ value 29-fold lower than that of 2. Inhibition of desmolase did not increase with these higher homologues, which were therefore more selective than 2 as aromatase inhibitors. However, neither was it diminished, so the goal of eliminating this activity remains.

## Results and Discussion

**Synthesis of 1 and Analogues.** In the published synthesis[1] of 1, 4-pyridylacetonitrile (3) was converted into 2-(4-pyridyl)butanenitrile (4; R = $C_2H_5$) by reaction with ethyl iodide in the presence of sodium hydride. A drawback of this method was the simultaneous and unavoidable formation of a diethylated product, 2-ethyl-2-(4-pyridyl)butanenitrile, which was not readily separated from the desired intermediate nor from the subsequent intermediate 4-cyano-4-(4-pyridyl)hexanenitrile (5; R = $C_2H_5$). This problem of dialkylation was circumvented by using a method previously described[9] for the monoalkylation of phenylacetonitrile, namely, reaction with a primary alcohol, a trivalent rhodium salt, and triphenylphosphine under mildly alkaline conditions. The reaction proved general for the synthesis from 3 and the appropriate alkanol (Scheme I) of 2-alkyl-2-(4-pyridyl)aceto-

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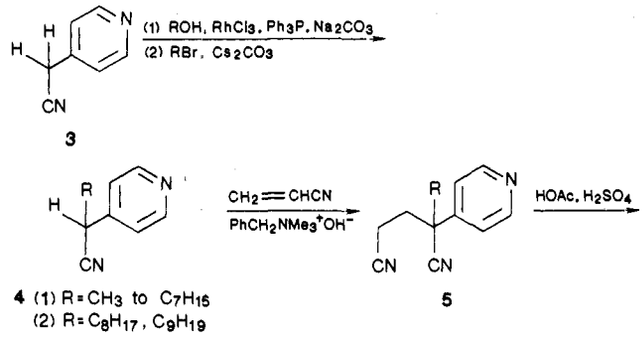
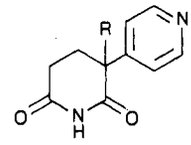
**Scheme I**

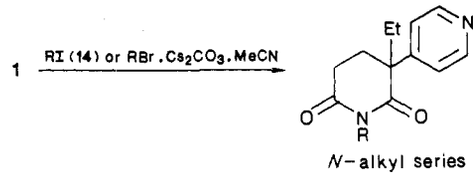


*C*-alkyl series

| no. | R | no. | R |
|---|---|---|---|
| 6 | $CH_3$ | 10 | $n$-$C_6H_{13}$ |
| 7 | $n$-$C_3H_7$ | 11 | $n$-$C_7H_{15}$ |
| 8 | $n$-$C_4H_9$ | 12 | $n$-$C_8H_{17}$ |
| 9 | $n$-$C_5H_{11}$ | 13 | $n$-$C_9H_{19}$ |

**Scheme II**




*N*-alkyl series

| no. | R | no. | R |
|---|---|---|---|
| 14 | $CH_3$ | 19 | $n$-$C_6H_{13}$ |
| 15 | $C_2H_5$ | 20 | $n$-$C_7H_{15}$ |
| 16 | $n$-$C_3H_7$ | 21 | $n$-$C_8H_{17}$ |
| 17 | $n$-$C_4H_9$ | 22 | $n$-$C_9H_{19}$ |
| 18 | $n$-$C_5H_{11}$ | 23 | $n$-$C_{10}H_{21}$ |

nitriles (4) up to the *n*-heptyl derivative (R = $C_7H_{15}$). For higher homologues success was hampered by the poor solubility of the rhodium salt in the alcohol. However, the octyl (R = $C_8H_{17}$) and the nonyl (R = $C_9H_{19}$) derivatives were conveniently prepared by using the alkyl halide in refluxing acetonitrile with cesium carbonate as base. No dialkylated products were detected: presumably the more stringent steric requirements of these longer chain alkyl substituents hindered their formation. Whereas the intermediates 4 and 5 were routinely purified by column chromatography on silica gel, this has proved unnecessary in the synthesis of 1 itself, thus further simplifying its preparation. Compounds in the *N*-alkyl series were in general made (Scheme II) by reacting 1 with the appropriate alkyl bromide in acetonitrile, again using cesium carbonate, which gave a much more rapid reaction than did potassium carbonate. For the synthesis of the methyl derivative 6, methyl iodide was used. Variations in the standard procedures (see Experimental Section) used to prepare the analogues of 1, together with yields and physical characteristics, are collected in Tables I and II. Chemical ionization spectra were used to confirm the molecular weights of the analogues. Since their NMR spectra contain no details of interest additional to those already reported[1] for 1 itself and its precursors, these are not recorded here. Members of the *C*-alkyl series were crystalline, but most members of the *N*-alkyl series were oils, and these were analyzed as their crystalline hydrochlorides.

**Table I.** Preparation of 3-Alkyl-3-(4-pyridyl)piperidine-2,6-diones 6–13

| compd | nitrile 3, mmol | acrylonitrile, mmol | yield,[a] % | mp, °C | formula[b] |
|---|---|---|---|---|---|
| 6 | 4.24 | 2.28 | 39 | 156–157 | $C_{11}H_{12}N_2O_2$ |
| 7 | 3.73 | 2.50 | 66 | 150–151 | $C_{13}H_{16}N_2O_2$ |
| 8 | 3.64 | 2.50 | 65 | 130–131 | $C_{14}H_{18}N_2O_2$ |
| 9 | 7.63 | 13.2 | 51 | 115–116 | $C_{15}H_{20}N_2O_2$ |
| 10 | 4.24 | 7.60 | 53 | 106.5–107.5 | $C_{16}H_{22}N_2O_2$ |
| 11 | 5.42 | 7.60 | 47 | 46–48 | $C_{17}H_{24}N_2O_2$ |
| 12 | 5.00 | 7.60 | 67 | 60–62 | $C_{18}H_{26}N_2O_2$ |
| 13 | 5.00 | 8.36 | 60 | 63–65 | $C_{19}H_{28}N_2O_2$ |

[a] Based on 3. [b] All compounds gave C, H, N analysis accurate to ±0.4% except for 13 (C: calcd, 72.1; found, 70.7; solvent?), which, however, correctly analyzed as its picrate.

**Table II.** Preparation of 1-Alkyl-3-(4-pyridyl)piperidine-2,6-diones 15–23

| compd | yield, % (free base) | mp, °C | formula[a] (free base or hydrochloride) | anal. |
|---|---|---|---|---|
| 15 | 95 | oil | $C_{14}H_{19}N_2O_2Cl$ | C, H, N, Cl |
| 16 | 93 | 57–58 | $C_{15}H_{20}N_2O_2$ | C, H, N |
| 17 | 94 | oil | $C_{16}H_{23}N_2O_2Cl$ | C, H, N, Cl |
| 18 | 90 | oil | $C_{17}H_{25}N_2O_2Cl$ | C, H, N, Cl |
| 19 | 92 | oil | $C_{18}H_{27}N_2O_2Cl$ | C, H, N, Cl |
| 20 | 90 | oil | $C_{19}H_{29}N_2O_2Cl$ | C, H, N, Cl |
| 21 | 90 | oil | $C_{20}H_{31}N_2O_2Cl$ | C, H, N, Cl |
| 22 | 89 | oil | $C_{21}H_{33}N_2O_2Cl$ | C, H, N, Cl |
| 23 | 90 | oil | $C_{22}H_{35}N_2O_2Cl$ | C, H, N, Cl |

[a] Compounds obtained as oils gave crystalline hydrochlorides. All analyses were accurate to ±0.4%, except for the following: 19 (C: calcd, 63.8; found, 63.25. Cl: calcd, 10.5; found, 11.2); 22 (Cl: calcd, 9.3; found, 9.9).

**Table III.** Inhibition of Aromatase and Desmolase by 1, 2, and Analogues of 1: Concentration Giving 50% Inhibition of Enzyme Activity ($IC_{50}$)

| compd | variable substit | aromatase $IC_{50}$, μM | desmolase $IC_{50}$, μM |
|---|---|---|---|
| 1 | ethyl | 10 | none |
| 2 | – | 8 | 30 |
| *C*-alkyl series | | | |
| 6 | methyl | 245 | none |
| 7 | *n*-propyl | 6 | none |
| 8 | *n*-butyl | 4 | none |
| 9 | *n*-pentyl | 2.5 | none |
| 10 | *n*-hexyl | 3.6 | none |
| 11 | *n*-heptyl | 3.2 | none |
| 12 | *n*-octyl | 0.33 | none |
| 13 | *n*-nonyl | 2.4 | none |
| *N*-alkyl series | | | |
| 14 | methyl | 30 | none |
| 15 | ethyl | 385 | none |
| 16 | *n*-propyl | 31 | none |
| 17 | *n*-butyl | 40 | none |
| 18 | *n*-pentyl | 21 | 209 |
| 19 | *n*-hexyl | 6.6 | none |
| 20 | *n*-heptyl | 1.5 | 210 |
| 21 | *n*-octyl | 0.8 | 110 |
| 22 | *n*-nonyl | 3.0 | 175 |
| 23 | *n*-decyl | 10 | 340 |

**Enzyme-Inhibitory Activity of Analogues.** Table III expresses the inhibitory potency toward aromatase and desmolase of the analogues compared with 1 and 2, expressed as the concentration producing 50% inhibition of enzyme activity. In both the *C*-alkyl series (1, 6–13) and the *N*-alkyl series (14–23), there was a trend toward an increasing inhibitory activity with alkyl chain length, which was optimal for the respective octyl derivatives 12 and 21 but which decreased thereafter. This progression was largely uninterrupted in the *C*-alkyl series, but in the

**Table IV.** Inhibition of Aromatase: A Comparison with either Testosterone or Androstenedione as Substrate of the 4-Pyridyl Derivative (1) with Aminoglutethimide (2) and the *C*-Octyl (12) and *N*-Octyl (21) Analogues of 1

| | $K_i$, μM ($IC_{50}$ values in μM in parentheses) | |
|---|---|---|
| compd[a] | testosterone | androstenedione |
| 1 | 1.1 (10) | 14 (45) |
| 2 | 0.6 (8) | 1.8 (14) |
| 12 | 0.09 (0.33) | 0.20 (0.6) |
| 21 | 0.12 (0.8) | 0.48 (5) |

[a] For comparison, a value of 14 μM was obtained for half-maximal inhibition by 2 with testosterone as substrate.[25]

*N*-alkyl series, there was an initial decrease, and the activity of 1 was only exceeded in this series for homologues beyond the *n*-pentyl derivative 18. All the compounds were also tested as inhibitors of desmolase. None in the *C*-alkyl series was inhibitory, but there were examples of modest inhibitors in the *N*-alkyl series. Selective inhibition of aromatase was therefore a general feature of the 4-pyridyl derivatives.

The Lineweaver–Burk plots (not shown) for the most powerful inhibitors, namely, the octyl derivatives 12 and 21, were of a competitive nature, which is similar to the type of inhibition exerted by 1 and 2. The $K_i$ values for 12 and 21 are compared with the values for 1 and 2 in Table IV. The values with testosterone as substrate confirm that these octyl derivatives are much more powerful inhibitors, and indeed both have $K_i$ values lower than that reported[10] (0.165 μM) for 4-hydroxyandrostenedione. Hitherto, we have used testosterone in the evaluation of potential inhibitors of aromatase, considering that the *relative* (as opposed to the absolute) values for inhibitory potency would be unaffected by the choice of substrate. However, in the present study we attempted to confirm this assumption, initially using 1 and 2, but found it to be invalid. The pyridine analogue 1 was, relative to 2, a much worse inhibitor of aromatization of androstenedione than of testosterone (Table IV). The ratio $K_i$(androstenedione):$K_i$(testosterone) was 12.7 for 1 but 3.0 for 2. Likewise, the relevant $IC_{50}$ values (Tables III and IV) reflect this difference, the ratio $IC_{50}$(testosterone) being 4.5 for 1 and 1.75 for 2. Interestingly, the octyl analogues (12 and 21) differ from each other in this respect. Analogue 12 was similar to 2 in relative inhibitory potency toward aromatization of the two substrates, in terms of both $K_i$ and $IC_{50}$ values, whereas 21 was relatively a much worse inhibitor of androstenedione aromatization. The significance of these findings is unclear at present. Their possible clinical relevance is complicated by the interconvertibility through 17β-hydroxysteroid dehydrogenases of both the substrates of aromatase (testosterone and androstenedione) and the products (estradiol and estrone). Estradiol has a much higher binding affinity for the estrogen receptor than estrone,[11] which would tend to suggest effective inhibition of the aromatization of testosterone as more important. However, during treatment with 2, there is some evidence that estrone levels rise in relapsing patients[12] and that estrone levels during treatment are higher in nonresponders than in responders[13] whereas estradiol remains suppressed. Finally, it is reassuring that the observed markedly differential inhibition of the aromatization of testosterone and androstenedione is not an intrinsic property of 4-pyridyl derivatives, since the *C*-octyl derivative (12) is a good inhibitor of the aromatization of both substrates.

**Table V.** Lipophilicity Data for the 3-Alkyl-3-(4-pyridyl)piperidine-2,6-diones (*C*-Alkyl Series)

| | | | | log *P* | |
|---|---|---|---|---|---|
| no. | alkyl group | $m$[a] | Rm[b] | obsd[c] | calcd[d] |
| 6 | methyl | 0 | −0.666 | | 0.072 |
| 1 | ethyl | 1 | −0.424 | 0.58 | 0.577 |
| 7 | propyl | 2 | −0.159 | 1.13 | 1.13 |
| 8 | butyl | 3 | 0.103 | | 1.68 |
| 9 | pentyl | 4 | 0.379 | 2.26 | 2.26 |
| 10 | hexyl | 5 | 0.659 | 2.94 | 2.85 |
| 11 | heptyl | 6 | 0.943 | | 3.44 |
| 12 | octyl | 7 | 1.230 | | 4.04 |
| 13 | nonyl | 8 | 1.517 | | 4.64 |

[a] Number of methylene groups in the alkyl chain Z = $(CH_2)_mCH_3$. [b] Elution conditions: 1.5 mL/min; MeCN–0.01 M phosphate (pH 6.9), 2:3 (v/v). [c] Determined by the shake-flask technique. [d] Calculated from eq 7.

**Lipophilicity Determinations.** Lipophilicity has been shown during studies of structure–activity relationships to play an important role in determining drug activity.[14,15] As part of this structure–activity study we investigated this parameter in the two homologous *C*-alkyl and *N*-alkyl series. The lipophilicity of the compounds was determined by a combination of reverse-phase high-performance liquid chromatography (HPLC) and the shake-flask technique. Partition coefficients, *P*, are generally determined by this traditional shake-flask method[16] using 1-octanol and aqueous buffer, but this method can be tedious. HPLC has been found useful and is now routinely used to determine log *P* values.[17–20] In the reverse-phase HPLC system, Rm was determined by measuring the retention time, *t*, of the compound. This measured parameter, *t*, is related to the partition coefficient by the following equations:

$$\mathrm{Rm} = \log [1/R_f - 1] \quad (1)$$

where $R_f$ is the retention in the system,[21]

$$\mathrm{Rm} = \log k' \quad (2)$$

where $k'$ is the capacity factor in HPLC, and

$$k' = (V_R - V_0)/V_0 = (t_R - t_0)/t_0 \text{ at constant flow rate} \quad (3)$$

where $V_R$ and $V_0$ are the elution volumes of retained compound and unretained compound, respectively, and $t_R$ and $t_0$ are the retention times of retained compound and unretained compound, respectively.

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**Table VI.** Lipophilicity Data for the 1-Alkyl-3-ethyl-3-(4-pyridyl)piperidine-2,6-diones (*N*-Alkyl Series)[a]

| no. | alkyl group | $m$[a] | Rm[b] | log $P$ obsd[c] | log $P$ calcd[d] |
|---|---|---|---|---|---|
| **14** | methyl | 0 | −0.415 | | 1.09 |
| **15** | ethyl | 1 | −0.242 | 1.64 | 1.64 |
| **16** | propyl | 2 | −0.066 | 2.18 | 2.19 |
| **17** | butyl | 3 | 0.115 | 2.76 | 2.76 |
| **18** | pentyl | 4 | 0.300 | | 3.34 |
| **19** | hexyl | 5 | 0.493 | | 3.94 |
| **20** | heptyl | 6 | 0.690 | | 4.56 |
| **21** | octyl | 7 | 0.890 | | 5.19 |
| **22** | nonyl | 8 | 1.092 | | 5.82 |
| **23** | decyl | 9 | 1.294 | | 6.45 |

[a] Number of methylene groups in the alkyl chain Z = $(CH_2)_mCH_3$. [b] Elution conditions: 1.5 mL/min; MeCN–0.01 M phosphate (pH 6.9), 3:2 (v/v). [c] Determined by the shake-flask technique. [d] Calculated from eq 9.

Finally, Rm is related to the partition coefficient, $P$, in the linear equation

$$\log P = a\mathrm{Rm} + b \tag{4}$$

where $a$ and $b$ are constants.

Reverse-phase HPLC was used to measure $t$ and thus obtain Rm. The shake-flask method was used to determine the $P$ value of selected compounds in order to find the values of $a$ and $b$ in each series. $P$ was calculated from the following equation:

$$P = V_{aq} \times C_{org}/V_{org}(C_{org}' - C_{org}) \tag{5}$$

where $V_{aq}$ and $V_{org}$ are the volumes of aqueous and organic solution used, respectively, and $C_{org}'$ and $C_{org}$ are the initial and final concentrations of the organic layer. It was impracticable to use the shake-flask method for every compound in each homologous series, owing to the very high lipophilicity of some members. Those that were not determined by this method were calculated from HPLC data using eq 4.

Table V shows the Rm values for members of the *C*-alkyl series obtained by HPLC. There is a very good correlation between the number of methylene groups and the Rm value, as shown in the following equation:

$$\mathrm{Rm} = 0.274\ (\pm 0.003)m - 0.699 \tag{6}$$

$$n = 9,\ r = 1.000,\ s = 0.02$$

From the experimental values of Rm and log $P$, obtained by the shake-flask method, $a$ and $b$ were calculated according to eq 4.

From eq 7, the value of log $P$ was calculated for all the members in this *C*-alkyl series (Table V). Analogous data were obtained for the *N*-alkyl series (Table VI). Equations 8 and 9 were derived from these data in a similar manner.

$$\log P_{cal} = 2.093\ (\pm 0.006)\mathrm{Rm} + 1.466 \tag{7}$$

$$n = 3,\ r = 1.000,\ s = 0.003$$

$$\mathrm{Rm} = 0.190\ (\pm 0.002)m - 0.442 \tag{8}$$

$$n = 10,\ r = 1.000,\ s = 0.017$$

$$\log P_{cal} = 3.137\ (\pm 0.039)\mathrm{Rm} + 2.395 \tag{9}$$

$$n = 3,\ r = 1.000,\ s = 0.010$$

The values of Rm, log $P_{obsd}$ and log $P_{cal}$ are shown in Table VI. Qualitative comparison of the aromatase inhibition data in Table III with the calculated log $P$ values of Tables V and VI for the *C*-alkyl and *N*-alkyl homologous series, respectively, suggests the existence of a parabolic relationship between these biological and physicochemical properties, the maximum obtainable activity being in the region of the octyl substituent in each case. This is borne out by quantitative comparison. Equation 10, an inverse parabola, was derived for compounds **1** and **6–13**. The

$$\log (1/IC_{50}) = 1.053\ (\pm 0.371)\log P - 0.145\ (\pm 0.076)(\log P)^2 - 2.019 \tag{10}$$

$$n = 9,\ r = 0.817,\ s = 0.437$$

omission of compounds **10** and **11** from this data set results in only a marginal improvement in the fit (eq 11) while

$$\log (1/IC_{50}) = 1.391\ (\pm 0.389)\log P - 0.205\ (\pm 0.078)(\log P)^2 - 2.199 \tag{11}$$

$$n = 7,\ r = 0.889,\ s = 0.398$$

compounds **6–13** alone are poorly correlated ($r = 0.593$). Equation 10 predicts that maximal aromatase inhibitory activity will be found when the log $P$ is 3.63.

In the *N*-alkyl series a somewhat better correlation is obtained. Equation 12 is based on the complete data set compounds **14–23**. The first three compounds in this

$$\log (1/IC_{50}) = 0.877\ (\pm 0.526)\log P - 0.074\ (\pm 0.069)(\log P)^2 - 3.099 \tag{12}$$

$$n = 10,\ r = 0.704,\ s = 0.559$$

series obviously do not lie on the parabola as do compounds **17–23**, which produce equation 13. The predicted

$$\log (1/IC_{50}) = 3.257\ (\pm 0.720)\log P - 0.327\ (\pm 0.078)(\log P)^2 - 8.325 \tag{13}$$

$$n = 7,\ r = 0.898,\ s = 0.269$$

value of log $P$ for maximal activity in this series is 4.98.

## Experimental Section

Aminoglutethimide (**2**) was a gift from Ciba-Geigy Ltd, Horsham, U.K. Chemical-ionization mass spectra ($[M + H]^+$ ions) were determined with a VG 7070H spectrometer and VG 2235 data system using the direct insertion method and an ionizing voltage of 100 eV. For flash column chromatography, Merck Kieselgel 60, Art. No. 9385, was used. Melting points were determined on a Reichert micro hot stage apparatus and are uncorrected. Elemental analyses were obtained from Butterworth Laboratories Ltd, Teddington, Middlesex, U.K. The following applies to the lipophilicity studies. Ultraviolet spectra were recorded in 0.02 M phosphate buffer, pH 6.85, or 1-octanol solution on a Pye SP8-150 spectrometer. Acetonitrile was of HPLC grade obtained from Rathburn Chemical Co. Ltd., Walkerburn, Peebleshire, Scotland, U.K. 1-Octanol was of spectroscopic grade and obtained from Fisons plc, Loughborough, Leicestershire, U.K. Buffers and salts were of analytical grade. Double-distilled water was used throughout. Aqueous and octanolic phases were mutually saturated and kept at constant temperature.

**3-Ethyl-3-(4-pyridyl)piperidine-2,6-dione** (**1**). A solution of 4-pyridylacetonitrile[21] (**3**, 10.7 g, 90.7 mmol) in EtOH (150 mL) was heated under reflux with $RhCl_3 \cdot 3H_2O$ (1.188 g, 4.51 mmol), $Ph_3P$ (5.97 g, 22.79 mmol), and anhydrous $NaHCO_3$ (13.43 g, 0.129 mol). After 20 h, the cooled mixture was filtered and the filtrate concentrated. Vacuum distillation of the residue afforded 2-(4-pyridyl)butanenitrile (**4**; R = $C_2H_5$) (8.04 g; 61%), bp 92 °C (1 mmHg) [lit. bp 102–104 °C (1 mmHg)]. To a solution of this product (6.71 g, 45.96 mmol) in *t*-BuOH (20 mL) containing Triton B (0.216 mL) was added acrylonitrile (3.08 mL, 63.76 mmol). After 3 h, the solution was concentrated, the residue was dissolved in $CH_2Cl_2$ (200 mL), and the organic phase was washed with water (200 mL) and then concentrated. Crude 4-cyano-4-(4-pyridyl)-hexanenitrile (**5**; R = $C_2H_5$) (7.35 g) thus obtained [N.B. pure

material could be obtained by distillation, bp 160 °C (0.6 mmHg); for other physical data see ref. 1] was heated under reflux with a mixture of glacial HOAc (30 mL) and concentrated $H_2SO_4$ (6 mL) for 3 h, and then the cooled solution was poured onto ice water. The neutralized ($NaHCO_3$) solution was extracted with $CHCl_3$, the dried extract ($Na_2SO_4$) was concentrated, and the residue was crystallized from ethanol to give 1 [4.17 g, 41.6% based on 2-(4-pyridyl)butanenitrile], mp 138–139 °C.

**3-Alkyl-3-(4-pyridyl)piperidine-2,6-diones 6–11. General Method.** A solution of **3** (Table I) in the appropriate alkanol (20 mL) containing $RhCl_3 \cdot 3H_2O$ (0.05 molar equiv relative to **3**), $Ph_3P$ (0.25 molar equiv), and anhydrous $Na_2CO_3$ (1.1 molar equiv) was heated under reflux for 20 h. Flash chromatography with $CHCl_3$ afforded the appropriate 2-alkyl-2-(4-pyridyl)acetonitrile (**4**; R = $n$-$C_3H_7$ to $n$-$C_7H_{15}$) as an oil, which was then dissolved in $t$-BuOH (5–10 mL) containing acrylonitrile (Table I) and Triton B (0.025 molar equiv relative to acrylonitrile). After 2 h, flash chromatography with $CHCl_3$ afforded the appropriate 2-alkyl-2-(4-pyridyl)-4-cyanobutanenitrile (**5**) as an oil, which was then heated under reflux with a mixture (5:1, v/v) of glacial HOAc and concentrated $H_2SO_4$ for 3 h, cooled, diluted with ice water, adjusted to pH 7–7.5 ($NaHCO_3$), and extracted with $CHCl_3$. Flash chromatography with $CHCl_3$–MeOH, 19:1, afforded the appropriate 3-alkyl-3-(4-pyridyl)piperidine-2,6-dione (Table I), which crystallized from toluene (**6–10**) or on trituration with petroleum ether (bp 30–40 °C; 11).

**Preparation of 3-Alkyl-3-(4-pyridyl)piperidine-2,6-diones 12 and 13.** A solution of **3** (Table I) in MeCN (20 mL) containing the appropriate alkyl bromide (5 mmol) and anhydrous $Cs_2CO_3$ (5 g) was heated under reflux for 2 h and then filtered and the filtrate concentrated. Elution of the appropriate 2-alkyl-2-(4-pyridyl)acetonitrile (**4**; R = $n$-$C_8H_{17}$, $n$-$C_9H_{19}$) so formed from a flash column and subsequent steps to the title compounds (Table I) followed the foregoing general procedure. The products crystallized on trituration with petroleum ether (bp 30–40 °C).

**3-Ethyl-1-methyl-3-(4-pyridyl)piperidine-2,6-dione (14).** A solution of 1 (0.218 g, 1 mmol) in MeCN (5 mL) containing $Cs_2CO_3$ (0.4 g) was stirred and brought to reflux before addition of MeI (0.284 g, 0.125 mL, 2 mmol) and then further heated and stirred under reflux for 10 min. The mixture was concentrated and the residue partitioned between $Et_2O$ (20 mL) and water (20 mL). Chromatography on silica gel (10 g, 9 × 2 cm column) with $CHCl_3$–EtOH, 19:1, gave pure 14 (0.057 g, 25%), which gave colorless prisms, mp 95–96 °C, from toluene at −20 °C. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

**1-Alkyl-3-ethyl-3-(4-pyridyl)piperidine-2,6-diones 15–23. General Method.** A solution of 1 (0.218 g, 1 mmol) and the appropriate alkyl bromide (1.05 mmol) in MeCN (5 mL) containing $Cs_2CO_3$ (1 g) was heated under reflux for 30 min. Column chromatography with $CHCl_3$ afforded the appropriate 1-alkyl derivatives (89–95%) as oils [except for the crystalline $n$-propyl derivative (**16**), which solidified on standing, mp 63–65 °C. Anal. ($C_{15}H_{20}N_2O_2$) C, H, N]. Other products were analyzed as their crystalline hydrochloride salts (see Table II).

**Enzyme Preparation and Assay Procedures.** The analogues prepared were tested for their inhibitory activity against the cholesterol side-chain cleavage and aromatase enzymes as described in ref 24. The mitochondrial fraction of bovine adrenal cortex provided the source of the cholesterol side-chain cleavage enzyme. Activity was assayed by measuring the [$^{14}$C]isocaproic acid released from the substrate, [26-$^{14}$C]cholesterol. Chromatography on alumina was used to adsorb the steroid, while the isocaproic acid filtered through the column. The radioactivity in the eluate served as a direct measure of enzyme activity. A final substrate concentration of 14 μM ($K_m$ = 4.5 μM) was employed. Aromatase was obtained from the microsomal fraction of full-term human placentas. The activity was monitored by measuring the $^3H_2O$ formed during the conversion of 1β,2β-$^3$H-labeled testosterone or androstenedione to estrogens. Separation of the $^3H_2O$ from the steroids was achieved by addition of activated charcoal followed by centrifugation. Aliquots of the resulting supernatants, containing the $^3H_2O$, were counted. The $K_m$ value for androstenedione was 0.038 μM, so a final substrate concentration of 0.38 μM was used. Similarly, the $K_m$ for testosterone was 0.13 μM; therefore, 1.5 μM was the substrate concentration. The $IC_{50}$ value is the concentration of compound required to reduce the activity of the enzyme to 50% of its control value at the stated substrate concentrations. Each analogue was examined over a range of concentrations. For the aromatase enzyme, final assay concentrations of 2, 5, 10, and 20 μg/mL were used. For the cholesterol side-chain cleavage assays, the values were 5, 10, 20, and 50 μg/mL. $IC_{50}$ values higher than the maximum concentration used were obtained by extrapolation. The term "none" in Table III refers to compounds that were noninhibitory at these maximum concentrations. The apparent $K_m$ for the substrates and the apparent $K_i$ values were determined from Lineweaver–Burk plots, with the method of least-squares analysis being used to obtain a linear fit to the data.

**Determination of Partition Coefficient by the Shake-Flask Method.** The compound was dissolved in 1-octanol and partitioned with 0.02 M phosphate buffer, pH 6.85. The volume ratio of the two phases was chosen so as to leave 60–70% of the solute in the organic phase. The two phases were placed in an appropriate volumetric flask, inverted manually for 20 min, and then allowed to separate for 1 h. The organic phase was separated from the aqueous and centrifuged to ensure complete separation. The concentrations of the solutes in the organic phase and the initial octanol solution were analyzed by measuring their UV absorptions at 258 nm. Partition coefficients were calculated from eq 5. The experiment was done at least in duplicate.

**Determination of log $k'$ by HPLC.** HPLC was performed on a Model ALC/GPC 204 liquid chromatograph (Waters Chromatography Division, Millipore, U.K.) equipped with a 25 cm × 4.5 mm column packed with 5-μm APEX ODS (Jones Chromatography Ltd., Hengoed, Mid Glamorgan, Wales, U.K.) and monitored by ultraviolet absorption at 245 nm. Compounds were dissolved in acetonitrile at a concentration of 2 mg/mL and the solutions diluted with 0.01 M phosphate buffer, pH 6.90, in the proportions 2:3 for the $C$-alkyl series and 3:2 for the $N$-alkyl series, giving final concentrations of 0.8 mg/mL and 1.2 mg/mL, respectively. Samples (4 μL) of these solutions were injected onto the column, which was eluted isocratically at 1.5 mL/min with acetonitrile–0.01 M phosphate buffer, pH 6.90, in the proportions 2:3 for the $C$-alkyl series and 3:2 for the $N$-alkyl series. The void volume of the column ($V_0$) was determined with uracil. Data were collected on a Trilab Model III computing integrator (Trivector Systems International Ltd.) and analyzed to give retention times. A mean value was determined from two injections.

**Data Processing.** All statistical correlations were performed by using a commercial multiple linear regression computer program (Abstat). The figures in parentheses are the standard errors of regression coefficients. For a given equation, $n$ is the number of compounds, $r$ is the multiple correlation coefficient, and $s$ is the standard error of the estimate.

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