Steroidal C-19 Sulphur and Nitrogen Derivatives designed as Aromatase Inhibitors

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The discovery that in the steroidal compounds (**6**) and (**15**) iodine in the 19-position is smoothly displaced by reactive nucleophiles (CN^-, $MeSO_2S^-$, N_3^-) without rearrangement was exploited in the synthesis of 19-methylthio-4-androstene-3,17-dione (**14**) which was shown to inhibit aromatase by co-ordination of the steroidal sulphur atom to the haem-iron of cytochrome P-450.

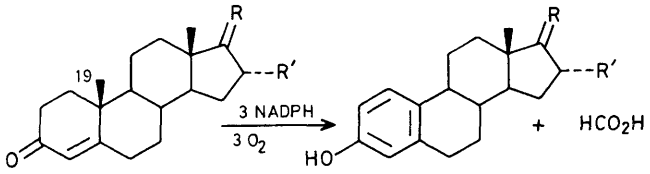
The conversion of androgens (**1a, b, c**) into oestrogens (**2a, b, c**) occurs through the participation of three sequential reactions at the C-19 carbon, each requiring 1 mol of NADPH and 1 mol of O_2. This process culminates in the release of the target C-19 atom as formic acid and the aromatisation of ring A (Scheme 1). The overall transformation is catalysed by aromatase, which consists of one or more P-450 type cytochromes,† in conjunction with NADPH–cytochrome P-450 reductase, an enzyme responsible for the transfer, in two steps, of a hydride equivalent from NADPH to the haem-iron of the cytochrome.[1]

The mechanistic details of the three constituent reactions of the sequence have been extensively studied in our laboratory and we have postulated that all these reactions involve a common hexa-co-ordinated species (**3**) produced from the substrate, O_2, and the reduced form of the cytochrome. The fate of this species (**3**) depends upon the type of reaction required by the sequence.[1]

This conjecture suggested that, by the introduction of sulphur or nitrogen containing groups at C-19, it might be possible to produce compounds which not only interact with the steroid-binding site of the enzyme but also provide the sixth ligand to its haem-iron. We reasoned that such compounds should be strong and specific aromatase inhibitors. From a clinical viewpoint, such inhibitors may be of value as contraceptives and also for treating oestrogen-dependent diseases, in particular, breast cancer.[2]

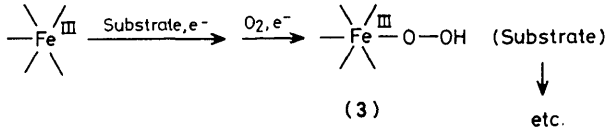
It is known from earlier work[3] that the tosyl or mesyl derivatives of Δ^5-19-hydroxy compounds undergo rapid displacement with I^-, Br^-, and Cl^- to produce the corresponding 19-halogenated steroids of the type (**6**), (**7**), and (**8**). In contrast to this displacement, attempts to use other nucleophiles such as H^-, CN^-, and N_3^- have only produced

† P-450 type cytochromes contain haem b as the prosthetic group and derive their name from the difference spectrum obtained by the addition of CO to the reduced form when a peak at 450 nm is produced. The cytochromes of this class catalyse a variety of oxidative transformations but are particularly involved in hydroxylation reactions.



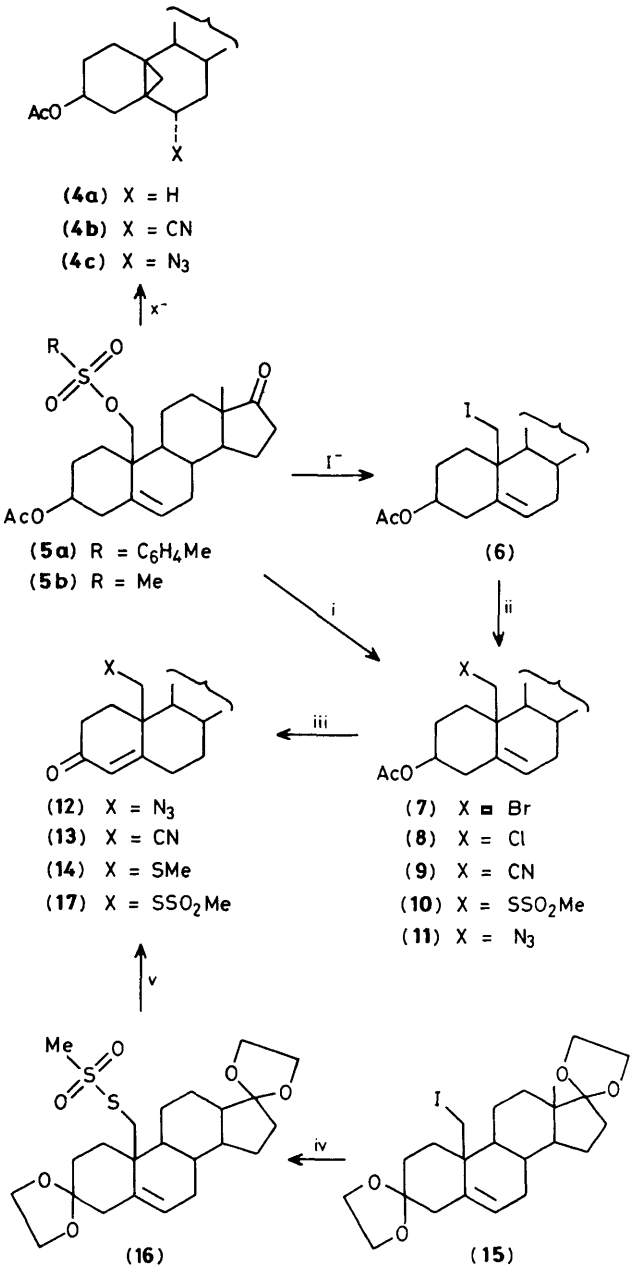
(1a) R = O, R' = H (2a) R = O, R' = H
(1b) R = α-H(β-OH), R' = H (2b) R = α-H(β-OH), R' = H
(1c) R = α-H(β-OH), R' = OH (2c) R = α-H(β-OH), R' = OH

Scheme 1



rearranged products of the type (**4a, b, c**).[4] We have now discovered that clean displacement at C-19, with a variety of nucleophiles, may be achieved by using the 19-iodo compound (**6**) rather than the toluene-*p*-sulphonates or methanesulphonates (**5a, b**).[5] Treatment of the 19-iodide (**6**) with NaCN, NH_4SSO_2Me,[6] or NaN_3, in hexamethylphosphoramide (HMPA) at 80 °C for 5 h, gave the corresponding 19-substituted compounds (**9**), (**10**), and (**11**) in high yields (60—80%). The 19-azide (**11**) and 19-cyanide (**9**) were converted into the biologically more important Δ^4-3-keto compounds, (**12**)‡ and the known (**13**)[7] by treatment with methanolic KOH followed by Jones oxidation and isomerization with oxalic acid in refluxing ethanol. The 19-methanesulphonylsulphide (**10**) was reduced to the thiol using $LiAlH_4$ in tetrahydrofuran (THF). Methylation with MeI in methanolic K_2CO_3 followed by Oppenauer oxidation gave 19-methylthio-4-androstene-3,17-dione (**14**).‡ Displacement of the 19-iodide was also achieved in the 3,17-bis-ethyleneacetal series (**15**)§ and enabled the synthesis of the 19-methanesulphonylsulphide (**16**) which, after deprotection with toluenesulphonic acid in acetone, gave 19-methanesulphonylthio-4-androstene-3,17-dione (**17**).‡

19-Azido-4-androstene-3,17-dione (**12**) and 19-methylthio-4-androstene-3,17-dione (**14**) were competitive inhibitors of human placental aromatase with K_i values of 5 nM and 1 nM respectively (K_m for androstenedione is 25 nM) which makes them amongst the highest affinity inhibitors for this enzyme.



Scheme 2. *Reagents:* i, LiBr [(**5a, b**) → (**7**)] or LiCl [(**5a, b**) → (**8**)]; ii, NaCN [(**6**) → (**9**)], or NH_4SSO_2Me [(**6**) → (**10**)], or NaN_3 [(**6**) → (**11**)] in HMPA; iii, KOH–MeOH then Jones reagent followed by oxalic acid–ethanol [(**9**) → (**13**) and (**11**) → (**12**)] or $LiAlH_4$–THF then MeI in K_2CO_3–MeOH, followed by Oppenauer oxidation [(**10**) → (**14**)]; iv, NH_4SSO_2Me in HMPA; v, $MeC_6H_4SO_2OH$–acetone [(**16**) → (**17**)].

19-Methanesulphonylthio-4-androstene-3,17-dione (**17**) had a more complex interaction with the enzyme and in the presence of NADPH and O_2 resulted in the inactivation of aromatase.

The binding of the substrate or inhibitory ligands to the haem components of some P-450 type cytochromes has been extensively investigated using u.v.–visible difference spectroscopy.[8] This approach was extended in the present work and, as expected,[9] it was found that the addition of androstenedione (0.4 μM) to aromatase gave a 'type I' difference

‡ All new compounds have yielded satisfactory analytical and/or spectral data. Selected data: (**12**), m.p. 90—91 °C; $\nu_{max.}$ (Nujol) 2090, 1740, and 1675 cm^{-1}; ^1H n.m.r. (60 MHz, $CDCl_3$) 0.88 (3H, s, 18-Me), 3.51 (1H, d, *J* 12 Hz, 19-CH-), 3.77 (1H, d, *J* 12 Hz, 19-CH-), 5.87 (1H, s, 4-CH- vinylic); (**14**), m.p. 159 °C; $\nu_{max.}$ (Nujol) 1735, 1665 cm^{-1}; ^1H n.m.r. (60 MHz, $CDCl_3$) 0.91 (3H, s, 18-Me), 2.08 (3H, s, MeS-), 2.75 (1H, d, *J* 12 Hz, 19-CH-), 2.98 (1H, d, *J* 12 Hz, 19-CH-), 5.81 (1H, s, 4-CH- vinylic); (**17**), m.p. 110—111 °C; $\nu_{max.}$ (Nujol) 1735, 1680, 1325, and 1140 cm^{-1}; ^1H n.m.r. (60 MHz, $CDCl_3$) 0.92 (3H, s, 18-Me), 3.3 (3H, s, $MeSO_2$-), 3.42 (1H, d, *J* 12.7 Hz, 19-CH-) (partially obscured by signal from $MeSO_2$), 3.66 (1H, d, *J* 12.7 Hz, 19-CH-), 5.87 (1H, s, 4-CH- vinylic).

§ The iodide (**15**) was prepared from 19-hydroxy-4-androstene-3,17-dione[1] in three steps: i, $MeSO_2Cl$, pyridine; ii, $(CH_2OH)_2$, $(EtO)_3CH$, $MeC_6H_4SO_2OH$, THF; iii, NaI, Pr^iOH, reflux, 2 min.

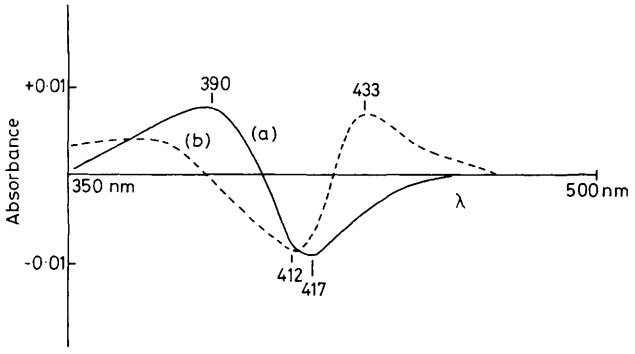


Figure 1. Difference spectra obtained by addition of steroids to aromatase. Steroids were added, in 1 to 10 μl of methanol, to 1 cm pathlength cuvettes containing solubilised aromatase (protein concentration 2 mg/ml, P-450 concentration 0.29 μM). An equivalent volume of methanol alone was added to the reference cuvette. (a) Androstenedione (**1a**) (0.4 μM) was added to the experimental cuvette (——); (b) addition of the 19-methylthio compound (**14**) from 0.13—0.67 μM to (a), only the final spectrum is shown (- - - - -).

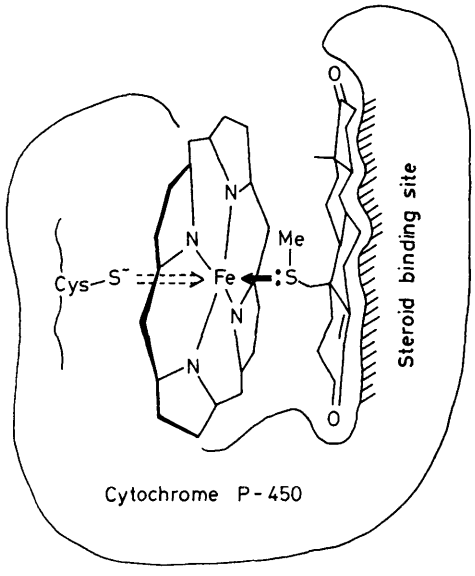


Figure 2. Proposed model for the binding of 19-SMe (**14**) to cytochrome P-450 (aromatase).

spectrum involving an increase in absorbance at 390 nm and a decrease at 417 nm (Figure 1). This type of spectrum has previously been correlated with a stabilisation of a penta-co-ordinate, high-spin form of the cytochrome.[8] Addition of the 19-methylthio compound (**14**) from 0.13 μM to 0.67 μM caused a progressive abolition of the substrate-induced difference spectrum, which demonstrated the displacement of the substrate by the inhibitor and suggested that both species interact with the same site on the cytochrome. The final difference spectrum had increased absorption at 433 nm which is characteristic of a 'type II' spectrum and indicates the formation of a low-spin form which we attribute to the co-ordination of the steroidal sulphur atom to the haem-iron of the cytochrome (Figure 2).

In conclusion, we have developed a method of introducing sulphur and nitrogen-containing groups at an angular methyl using a novel nucleophilic displacement. This enabled us to synthesise the first aromatase inhibitor to have a haem-binding ligand incorporated into the substrate at the position attacked by the enzyme.

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