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STRUCTURE-ACTIVITY RELATIONSHIPS FOR NON-STEROIDAL INHIBITORS OF AROMATASE

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A model is described for the binding of non-steroidal inhibitors of aromatase to the enzyme with interaction of a ligand with the Fe^{3+}-haem of the cytochrome. The model suggests that the inhibitors utilise a common binding site with ring A of the steroidal substrates.

KEY WORDS: Aromatase, structure-activity, aminoglutethimide, reversible inhibitors, androstendedione.

INTRODUCTION

The aromatase inhibitor, aminogluthethimide (AG) is currently used for the treatment of estrogen-receptor positive breast cancer in post menopausal women.[1] It is co-administered with a glucocorticoid to supress the resulting reflex rise in ACTH level due to inhibition of cholesterol side chain cleavage enzyme (CSSC).[2] Use of AG has been associated with side-effects of hypercholesterolaemia[3] mineralocorticoid deficiency,[4] as well as the somnolent effects of the drug alone.[5] Attempts to develop specific inhibitors of aromatase (AR) without action on the CSSC enzyme and the removal of the somnolent effect have led to the discovery of the more specific inhibitors 3-(4^1-pyridyl)-3-ethylpiperidine-2,6-dione,[6] and the 3-substituted-(unsubstituted)-3-(4^1-aminophenyl)pyrrolidine-2,5-diones[7] (WSP). Other non-steroidal inhibitors include 4-cyclohexylaniline,[8] ketoconazole,[9], miconazole,[9] clotrimazole(BAYb5097),[9] 4-(5,6,7,8-tetrahydroimidazolo-[1,5-α]-pyridin-5-yl)benzonitrile monohydrochloride, CG16949,[10] FCE24328,[10] 3-(4^1-aminophenyl)-1-azabicyclo (3,0,1)-2,6-dioxohexane,[11] LY113174,[12] LY56110[12] and Fenarimol.[12]

In this paper we have examined the structural requirements for inhibitory action against AR in analogues of WSP in which, (a) the heterocyclic ring is altered, and (b) the rotation of the phenyl ring is restricted by fusion with the 3-alkyl side chain to give a spiro compound. These results together with molecular graphics studies of the active and inactive inhibitors have provided a basis for the rationalisation of the essential structural requirements for inhibitory activity in analogues of AG and some of the other nitrogen containing heterocyclic inhibitors.

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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 napthalene,xylene and 1,4-dioxane (BDH, Poole), all being of "Scintran" grade

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Biochemical Studies

Preparation of the enzymes Aromatase and CSSC (cholesterol side-chain cleavage enzyme) were prepared from human term placental tissue and bovine adrenal gland following the general method of Thompson and Siiteri[13] and Rabe *et al.*[14] respectively.

Assays Aromatase activity was determined by the measurement of $^{3}H_2O$ released from [1β, 2β-^{3}H] testosterone and [1β, 2β-^{3}H] androstenedione by the general method described previously[15] except that the substrate concentrations used were 500 nM and 400 nM respectively. CSSC activity was determined by measurement of ^{14}C-4-methylpentanal released from [26(27)-^{14}C] cholesterol by the method described previously.[15]

Inhibition Studies (1) Aromatase: Assays were performed at an inhibitor concentration of 150 μM with either substrate. The results were expressed as a percentage inhibition of the enzyme compared with a control value determined in the absence of inhibitor. Under these conditions, aminoglutethimide gave 90% (androstenedione) and 85% (testosterone) inhibition.

(2) CSSC: Assays were performed at an inhibitor concentration of 7 μM. The results were expressed in a similar manner to that used for the aromatase studies. Aminoglutethimide gave 85% inhibition under these conditions.

Results The analogues **(12)** and **(13)** were inactive or showed weak inhibitory activity respectively *in vitro* against aromatase using testosterone or androstenedione as substrates. (see Table III) Neither compound showed inhibitory properties for CSSC.

Modelling Studies

Computer generated molecular models of the compounds prepared in this paper were obtained from a search of the Cambridge Crystallographic Data Base (via the Daresbury Chemical Data Bank Service) for the x-ray co-ordinates of suitable starting fragments. Final models were generated by modification of the starting fragments followed by the geometry optimisation and energy minimisation procedures available within the Chem-X (Chemgraf) Molecular Mechanics Suite. The geometry parameters were obtained from the minimum energy content conformers in each case, unless otherwise stated.

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Synthesis

^{1}Hn.m.r. spectra were determined on solutions in Me_2SOd_6 (internal Me_4Si) unless otherwise stated with a Perkin-Elmer R32 instrument (90 MHz) or a Bruker WM360 (360 MHz) instrument. Mass spectra were determined at PCMU, Harwell, UK using a VG Analytical ZAB 1F spectrometer. Melting points were determined with an Electrothermal capillary instrument, and are corrected. Infra-red spectra were determined in KBr discs, using a Perkin-Elmer 681 spectrophotometer. Elemental analyses were determined at the school of Pharmacy, University of London.

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6-Aminoindanespiro [1', 3] pyrrolidine-2,5-dione (12)

A mixture of ethyl 2-cyano-2-indan-1-ylidene-ethanoate (16 g, 0.07 M)[16] and potassium cyanide (9.1 g, 0.14 M) in ethanol–water (1:1, 200 ml) was stirred at room temperature for 12 h, filtered and the ethanol was evaporated. A solution of the residue in water was extracted with chloroform and the mother liquor concentrated to dryness. The residue was heated under reflux with hydrochloric acid (100 ml, 10M) for 30 h. On cooling, white crystals (12.5 g) were deposited which on recrystallisation from ethanol gave *indane spiro [1'3] pyrrolidine-2,5-dione* m.p. 151–3°. (Found: C, 71.56; H, 5.51; N, 6.75. $C_{12}H_{11}NO_2$ requires C, 71.62; H, 5.51; N, 6.96%).

The spiro compound (1.5 g, 7.5 mmol) was nitrated in fuming nitric acid (7 ml) at −40° to give a mixture (1.73 g) of 4- and 6-nitroindanespiro [1', 3] pyrrolidine-2,5-dione (Found: C, 58.82; H, 4.19; N, 11.12. $C_{12}H_{10}N_2O_4$ requires C, 58.53; H, 4.09; N, 11.38%).

The mixture of nitro compounds was recrystallised from methanol to give *6-nitroindanespiro [1', 3] pyrrolidine-2,5-dione* m.p. 191–193°; ν max 3208, 1754, 1693 (C=0), 1598 (Ph), 1530 (NO_2) 1350 ($C-NO_2$) cm^{-1}; ^{1}Hn.m.r. (360 MHz) δ 11.49 (1H, s,N-H), 8.20 (1H, s, CH), 8.07 (1H, d, J 8 Hz, CH), 7.33 (1H, d, J 8 Hz, CH), 3.58–3.57 (2H, m, CH_2), 3.03 (2H, dd, J 18 Hz, CH_2), 2.75–2.65 (1H, m, CH), 2.42–2.33 (1H, m, CH).

The mother liquors from the recrystallisation of the 6-nitro compound were concentrated to dryness and the residue was recrystallised five times from methanol to afford *4-nitroindanespiro [1', 3] pyrrolidine-2,5-dione,* m.p. 183–184°; ν_{max} 3210 (N-H), 1788, 1715 (C=0), 1600 (Ph), 1530 (NO_2), 1355 ($C-NO_2$) cm^{-1}; ^{1}Hn.m.r. (360 MHz) 11.45 (1H, s, N-H), 8.09 (1H, d, J 8 Hz, CH), 7.72 (1H, d, J 8 Hz, CH), 7.52 (1H, t, J 8 Hz, CH), 3.49–3.29 (2H, m, CH_2), 3.01 (2H, dd, J 18 Hz, CH_2), 2.64–2.54 (1H, m, CH), 2.32–2.22 (1H, m, CH).

Application of the n.o.e. difference spectroscopy technique confirmed the structure as the 4-isomer (rather than the 7-isomer) since (i) irradiation at δ 7.72 caused an enhancement of δ 7.52 by 20% and δ 3.01 by 3% (ii) irradiation of δ 8.09 caused an enhancement of δ 7.52 by 15% with no effect on aliphatic protons (see Figure 1).

A mixture of the 6-nitro compound (1 g, 4 mmol) in ethanol (60 ml) was shaken with 10% Pd/C (0.1 g) under hydrogen until the uptake of hydrogen was complete (300 ml). The suspension was filtered and concentrated to leave a brown solid (0.8 g) which was recrystallised, with decolourisation, from ethanol to give the *6-amino compound* (0.8 g) mp 150–2°. (Found: C, 66.74; H, 5.61, N, 13.02. $C_{12}H_{12}N_2O_2$ requires C, 66.65; H, 5.59; N, 12.96%). ν_{max}, 3160, 3030 (NH), 1743 and 1682 (C=0) cm^{-1}, ^{1}Hn.m.r. (360 MHz), δ 2.01–2.12 (1H, m, H_3), 2.42–2.53 (1H, m, H_4), 2.66 (1H, d, H_2, J18 Hz), 2.69–2.79 (1H, s, H_5), 2.79–2.91 (1H, m, H_6), 2.96 (1H, d, H_1, J 18 Hz), 4.97 (2H, s,

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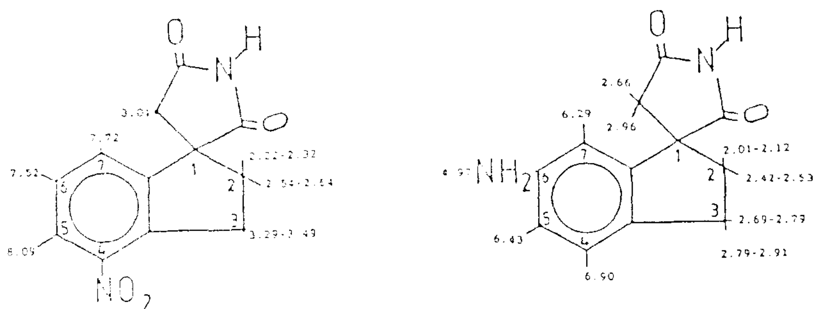

FIGURE 1 Structure and chemical shift data for the 4-nitro and 6-amino derivatives of indanespiro[1',3]pyrrolidine-2,5-dione.

NH_2), 6.29 (1H, d, H_9, J_{H8H9} 1 Hz), 6.43 (1H, dd, H_8, J_{H7H8} 8 Hz, J_{H9H8} 1 Hz) 6.90 (1H, d, H_7, J_{H8H7} 8 Hz), 11.29 (2H, s, NH_2). Application of the n.o.e difference spectroscopy technique confirmed the structure as the 6-isomer (rather than the 5-isomer) since (i) irradiation of δ 6.29 caused a 3% enhancement of 4.97 (NH_2) and 3% enhancement of the protons at δ 2.66, (ii) irradiation at δ 6.90 caused a 14% enhancement of the proton at δ 6.43 and 2% enhancement of protons at δ 2.69–2.79 and 2.79–2.91 (see Figure 1).

*5-(4'-aminophenyl) imidazolidine-2,4-dione (**13**)*

A solution of 4-aminobenzaldehyde (2.5 g), ammonium carbonate (7.92) and potassium cyanide (2.6 g) in ethanol-water (1:1, 50 ml) was kept at 70° for 1.5 h, then filtered, and concentrated to dryness. The yellow residue was extracted with ethanol (50 ml) and the extract was concentrated to dryness. The resulting yellow solid was washed with water and recrystallised from ethanol (95%) to give *crystals* m.p. 241–9° (d), (Found: C, 56.49; H, 4.87; N, 22.80. $C_9H_9N_3O_2$ requires C, 56.54; H, 4.75, N, 21.98%). ν_{max} 3400 and 3365 (NH_2), 3290 (NH), 1760, 1740, 1720 and 1715 (C=0) cm^{-1}. ^{1}Hn.m.r., δ, 4.89 (1H, s, CH), 5.07 (2H, s, NH_2), 6.58 (2H, d, J 9 Hz, phenyl), 6.96 (2H, d, J 9 Hz, phenyl), 8.17 (1H, s, NHCH) 10.56 (1H, s, CONHCO).

DISCUSSION

A Working Model for the Aromatase Enzyme Active Site

Due to lack of any 3-D structural information on the cytochrome P-450 enzyme aromatase, (AR), a schematic representation of the active site was derived from information available on structure-activity relationships within steroidal substrates and inhibitors.

Since both androstenedione (**1**) and testosterone (**2**) are known substrates of AR, being effectively aromatised to give estrone and estradiol respectively[13] the active site of the enzyme must be capable of recognising both steroids. The structural features of (**1**) and (**2**) are identical apart from the oxidation state at the C(17), a ketone in (**1**) and a β-alcohol in (**2**). These features are important since the affinity for AR is reflected in the K_m values for (**1**) and (**2**). Since other related 17-substituted steroids

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TABLE I
Inhibition of Aromatase by 4-(Alkyl)-, 4-(Aralkyl)-, 4-thioandrostenedione.[19]

Substituent	% Inhibition of Aromatase*		
	0.25 μm	0.75 μm	1.5 μm
(**3**) Methyl	39	61	74
(**4**) Ethyl	26	45	52
(**5**) Propyl	25	42	49
(**6**) Butyl	8	12	18
(**7**) Pentyl	2	4	8
(**8**) Phenyl	36	55	68
(**9**) Benzyl	2	5	8

*values as average for five experiments

are not known to be aromatised by AR, the interaction of the C(17) oxygen function with it's recognition site[17] is specific.

Recent X-ray data on a cytochrome P450 hydroxylase, $P450_{CAM}$, shows that a specific tyrosine residue (TYR 96)[18] acts as a H-bonding donor to the carbonyl group of the substrate camphor molecule. In addition to this interaction other non-polar residues act as hydrophobic contacts in securing the position of camphor for a highly stereospecific and selective oxidation.

Similar interactions occuring in the AR active site with (**1**) or (**2**) may be postulated since they can be involved in hydrophobic binding and which may play an important role in securing them to the AR active site with positioning dictated by the oxygen functions at C(3) and C(17) which brings the C(19) – methyl in close proximity to the haem iron in readiness for the oxidation steps leading to eventual aromatisation.

It is reasonable to suggest that binding takes place on the β-face of the androgen substrate since the α-face, at least in the region of the A ring, is required to be free for access to the reactive haem site.

In addition, all known steroidal inhibitors of AR contain the α,β-unsaturated C(3)0 carbonyl group and this is thought to be an essential feature for imparting inhibitory activity; this functional group must be providing an anchoring point for the steroidal substrate/inhibitor.

Therefore the AR active site could consist of a cleft in which (**1**) or (**2**) is supported by hydrophobic interaction and secured by two specific H-bonding interactions which recognise the A and D ring polar groups of the steroid. The model can be developed further by considering data on androstenedione derivatives. Within a series of 4-thioalkyl/aralkyl androstenediones synthesised (**3**)–(**9**),[19] enhanced binding occurred with the methyl, ethyl, propyl and phenyl derivatives, whereas the butyl, pentyl and benzyl, along with the p-substituted phenyl derivatives, were considerably less active (see Table 1). It was concluded that a "hydrophobic pocket" exists in a region extending from C(4) with definite steric requirements *ie.* it can accomodate steric bulk up to *ca.* 5.5 A, or else conformational restriction with loss of entropy is imposed at *ca.* 5.5 Å. Additional information provided by the 7-α thioalkyl/aralkyl substituted derivatives of (**1**)[20] suggests that an area is accessible for these apolar substituents which provides additional hydrophobic binding since enhanced affinity for the enzyme was observed (see Table II). The AR enzyme does not bind 1-α thioalkyl substituted androstenediones well as these are poor competitive inhibitors.[21] This situation suggests that the C(1)–C(2) "edge" of the substrate must be free for good approach to the active site, a view which is supported by other work suggesting that

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TABLE II
Inhibition of Aromatase by 7-α substituted androstenedione derivatives[20,21]

7-α Substituent	% Inhibition of Aromatase*
$-S(CH_2)_3CH_3$	74.1
$-SC_6H_5$	49.7
$-SCH_2C_6H_5$	84.1
$-SCH_2CH_2C_6H_5$	82.8
$-SCH_2C_6H_4$-*p*-OCH_3	63.0
$-SC_6H_4$-*p*-NH_2	95.0

*at test concentration 12.5 μm, maximum of ± 10%

the enzyme does not bind substrates with β-substituents along the C(1) – C(2)/C(11)-C(12) edge[22,23]

Molecular Modelling Analysis of known AR inhibitors

Positioning of the Co-ordinate Nitrogen atoms of the known inhibitors of AR. D(+)-Aminoglutethimide (**10**) (R enantiomer) is considerably more active than its' antipode[24,25] which indicates a high degree of stereoselectivity in the way D(+) – AG (**10**) interacts with the active site of the AR enzyme. It is generally accepted[26] that the aryl amino group interacts with the iron atom in the haem preventing the binding of oxygen and the subsequent oxidation of the steroid at C(19) since absence of this function leads to loss of inhibitory potency. On the basis that the shape of the steroid nucleus would reflect the shape of the binding area of the enzyme, a preliminary study was made using Dreiding models of the superimposition of D(+)-AG (**10**) on the steroid nucleus, recognising the requirements to locate the amino group in the region of the C(19)-methyl of (**1**) and to superimpose the C(6) or C(2) carbonyl function of the inhibitor on the C(3) or C(17) carbonyl groups on (**1**). Androstenedione (**1**) was chosen for the comparison since it is the primary substrate of AR. The most productive comparison was of the (C3) carbonyl of the A ring in (**1**) with the C(6) carbonyl of D(+)-AG (see Figure 2). A strong correlation of the C(5), C(6) and N(1) atoms in (**10**) with the C(4), C(3) and C(2) atoms in (**1**) was found which placed the amino group in a reasonable location near the C(19) of (**1**) (see Table III). Examination of Figure 3 shows that the axial* C(3)-alkyl chain of (**10**) extends along the steroid backbone thus providing additional binding. This mode of interaction is supported by recent work with C(3)-alkyl substituted analogues of (**10**) which indicate that an increase in chain length to pentyl results in enhanced activity by *ca.* 30 fold over that of (**10**) presumably due to increased hydrophobic binding.[27]

Once a satisfactory superimposition of D(+)-AG had been achieved the known analogue inhibitors of (**10**) namely (**11**) and (**14**) were compared as well as the lesser active (**12**) and (**13**) (see Table III). The correlated positions of their p-aminophenyl groups are shown in Figure 4 and are defined in Table III. The further three diverse structural types (**15–17**) representing known inhibitors were also subjected to this procedure

The interaction of 4-cyclohexylaniline (**16**)[8] with the substrate binding site was found to be most striking when the cyclohexyl ring carbons of (**16**) were superimposed

*The equatorial 3-(p-aminophenyl) conformation was chosen for comparison; with the axial conformer comparisons proved fruitless.

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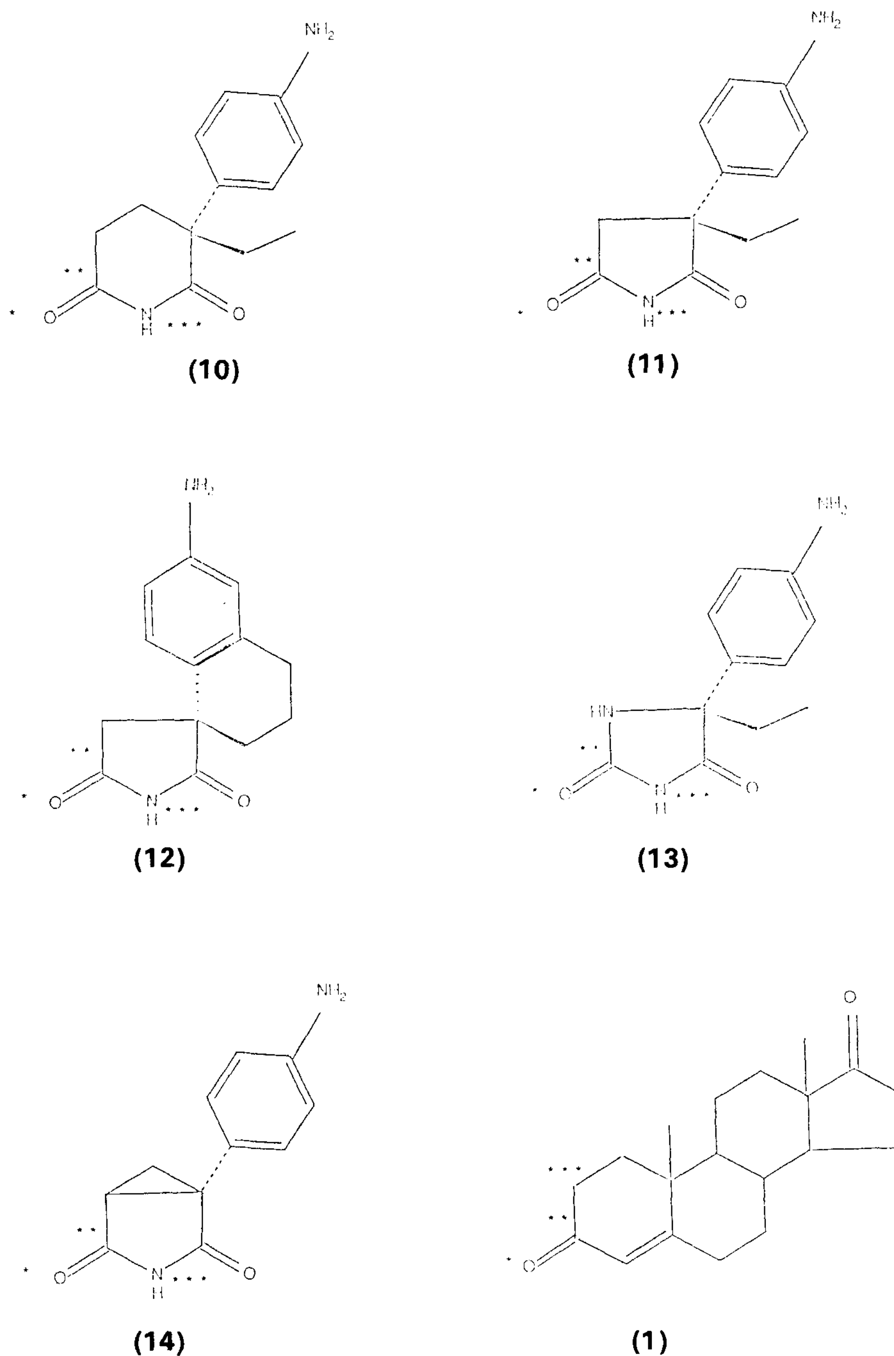

FIGURE 2 Comparison of known AR non-steroidal inhibitors, analogues of AG with androstenedione (1) (*,**,*** indicates atoms correlated in matching procedure).

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TABLE III
Distance of the Co-ordinating Nitrogen atom of known inhibitors of AR from C(3)0, C(17)0 and C(19)-methyl of androstenedione after matching proceedure and and their relative potency.

Compound	Distance to Co-ordinating N (Å)			Relative Potency
	C(3)0	C(17)0	C(19)	
(**10**)	9.2	7.6	4.5	100
(**11**)	8.5	8.3	4.2	101
(**12**)	8.4	8.7	4.3	< 5
(**13**)	8.0	9.4	4.4	19
(**14**)	8.8	7.3	4.6	88
(**15**)	7.5	7.7	2.9	400
(**16**)	9.3	6.7	4.6	214
(**17**)	7.4	7.3	2.7	1195

on the C(3), C(4), and C(.10) carbons of (**1**); the position of the aniline nitrogen is recorded in Table III.

Two recently disclosed AR inhibitors, FCE24328 (**17**) and CG16949 (**15**)[10] were also considered in the model. The likeness of FCE24328 to D(+)-AG led to an attempt to mimic the positioning of (**17**) with that of D(+)-AG but this approach failed. A match was achieved, however, by the superimposition illustrated in Figure 5. This placed the cyclohexyl group of (**17**) in the region of the active site previously defined as capable of providing a hydrophobic binding site, as described earlier. The position of the aniline group of (**17**) by rotation around the carbonyl-benzyl bond was arbitrary with respect to the internal energy of (**17**) but was so disposed to place it in close proximity to the nitrogen of D(+)-Ag (see Table III).

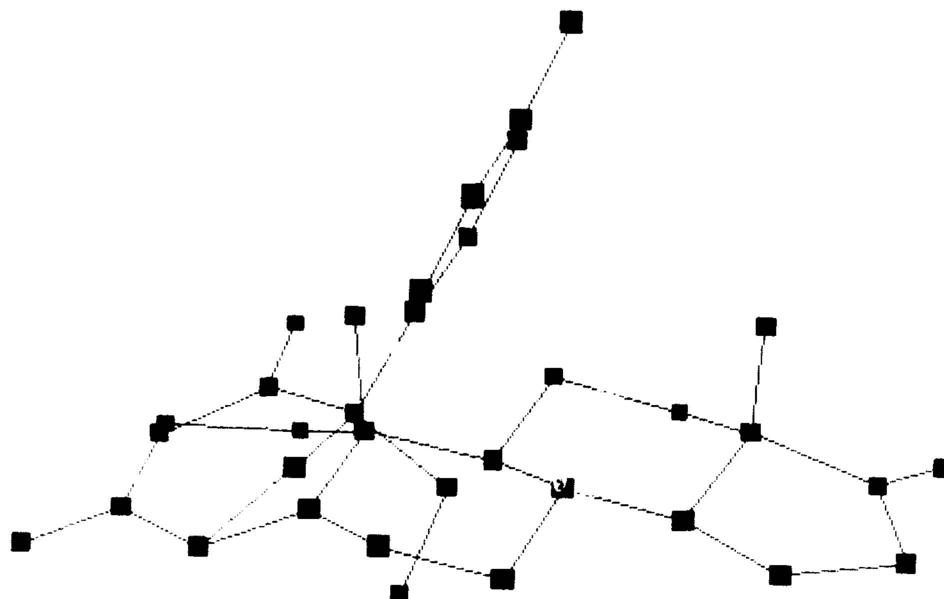

FIGURE 3 (See colour plate XI at the back of the issue). Correlation of the 3-(*p*-aminophenyl)-equatorial conformation of AG with (**1**).

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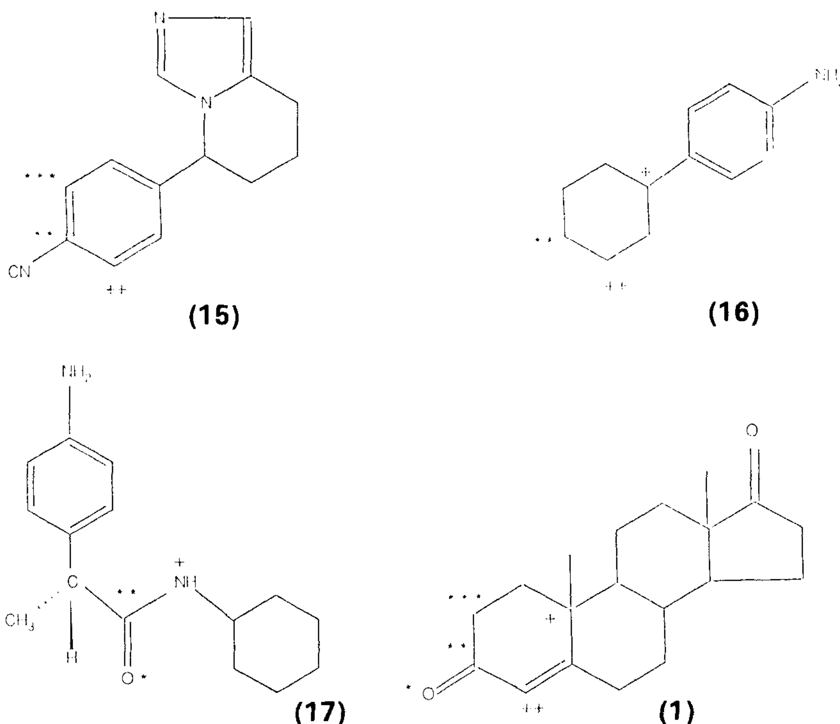

FIGURE 4 Comparison of other known non-steroidal inhibitors of AR with androstenedione (**1**) (*,** etc. show atoms correlated in matching procedure).

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The comparison of CG16949 (**15**) with (**1**) is as illustrated in Figure 3 where the hydrophobic interaction with the aromatic ring is of great importance. An initial positioning of the molecule (see Figure 5) was determined by the CN group, which was placed so that the nitrogen of the cyano group was superimposed with the C(3)-0 of (**1**). A H-bonding donor group can effectively interact either directly with the nitrogen lone-pair or with the Π-electron system, the former being highly directional, the latter presenting a less restrictive mode of interaction and is also the mode expected to occur (Figure 6). The orthogonal conformation of the 4-cyanophenyl substituent with respect to the plane of the imidazole ring of (**15**) is as a result of the lowest energy conformation calculations but also coincides with the features present in the hypothetical model of the AR model site. The mode of interaction envisaged for CG16949 also applies to the known AR inhibitors, LY113174(**18**), LY56110(**19**), Fenarimol (LY, **20**) and Clotrimazole (**21**)[9,12] where the appropriate haloaryl ring superimposes on ring A of (**1**).

Directional nature of the Co-ordinate Nitrogen/Haem Interaction of known inhibitors of AR

It is assumed that all the inhibitors described here act by co-ordination of the nitrogen lone-pair of electrons with a vacant site of the haem iron to form a covalent bond.

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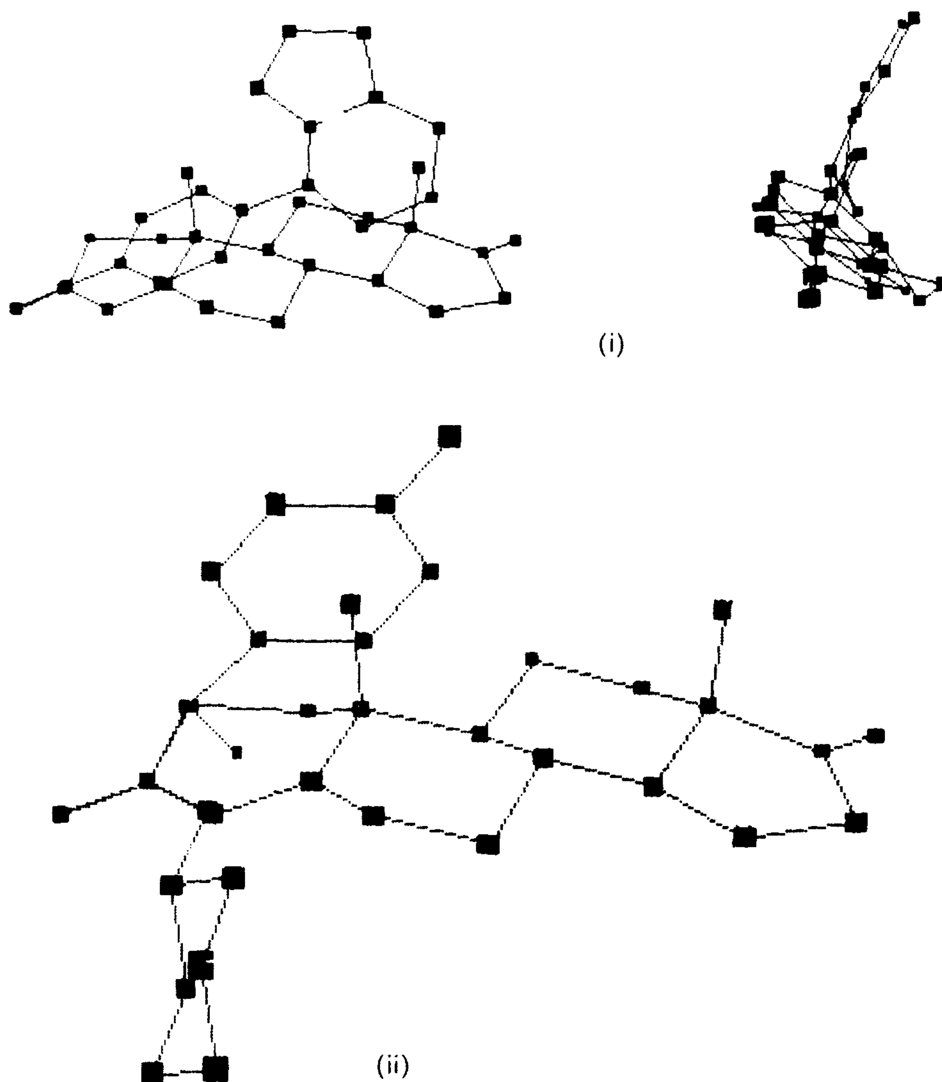

FIGURE 5 (see colour plate XIII at the back of the issue). Comparison of CG16949 (initial), (i) and FCE24328 (ii) with androstenedione.

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Successful bonding of an inhibitor then depends on a reasonably strict directional requirement of the nitrogen for bonding in the vicinity of the haem iron atom.[28]

The energy barrier to the rotation of the phenyl group in the AG-analogues carrying a 3-ethyl substituent was calcuated using a combination of the torsion angles of the phenyl substituent (C_2-C_3-$C_{1'}$-$C_{2'}$) and the ethyl group (C_2-C_3-C_α-C_β) with the heterocyclic ring. Representative Figures 7 and 8 for compounds **(10)** and **(11)** show that appreciable rotation (*ca.* 90°) of the phenyl ring can occur within an accepted 3 to 4 kcal mol^{-1} energy barrier[29] thus allowing the amino nitrogen atom to take up a successful bonding position. However, **(12)**, the designed rigid congener of **(11)**, shows

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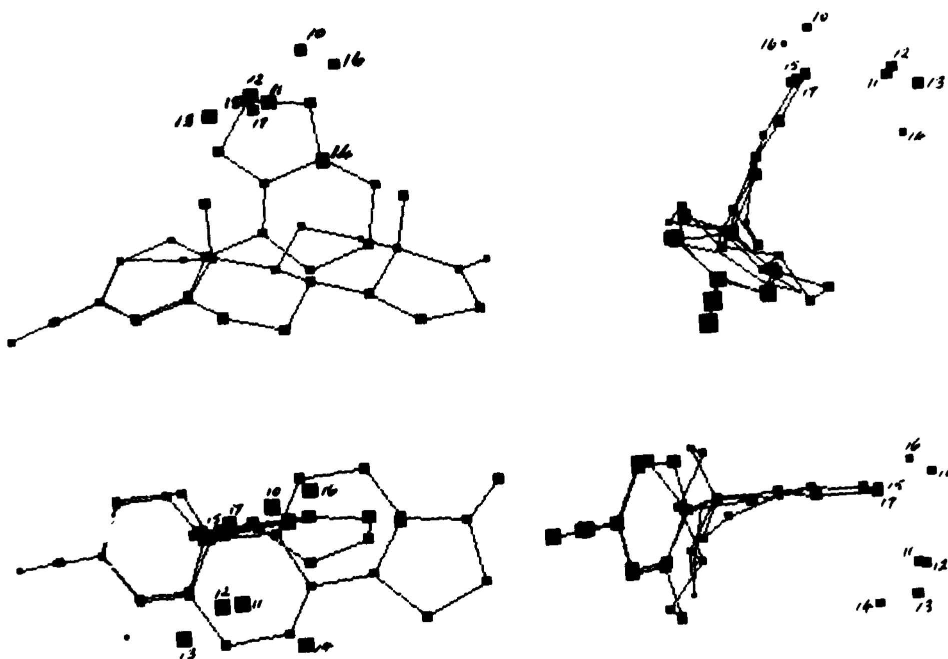

FIGURE 6 (see colour plate XIV at the back of the issue). Different views of superimpositions of (**15**) and (**1**) as well as correlated positions of all inhibitors shown in Figures 1 and 3.

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virtually no activity although it has approximately the same position of the nitrogen as (**11**) in the model (see Table III). We consider that this is due to incorrect directional positioning of the nitrogen lone pair for bonding.

The rotation of the p-aminophenyl groups in analogues of (**10**) was taken into account in the molecular modelling procedure and each matched structure, with the exception of (**17**), was the global minimum energy conformation of the compound. Rotation alters the pitch of the phenyl ring with respect to the heterocyclic ring through the series but not the relative positions of the nitrogen atoms. Compound (**15**)*, due to it's rigid structure, gives a possible further insight into the position of the haem iron since it's mode of interaction allows an arc to be constructed *via* the rotation of the p-cyanophenyl group. The strict directional restriction of the imidazole sp^2 nitrogen lone pair of electrons should define the possible *locus* of the iron atom. Recent work on 10-oxirane and 10-thiirane and 19-nor substituted androstenediones, and the stereoselective nature of their potency as AR inhibitors, suggests an active haem site centered close to C(19) as well as C(1) and C(2).[30,31]

Consideration of the interaction of the Inhibitors at the active site of AR

It is envisaged that co-ordination to the haem iron by the nitrogen centre of the inhibitors, thus displacing the natural ligand, occurs as an initial step. This primary

*Only one enantiomer is considered, although it's antipode is expected to have almost identical activity.

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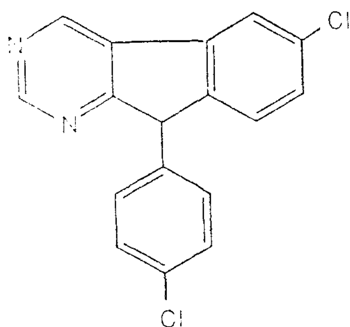
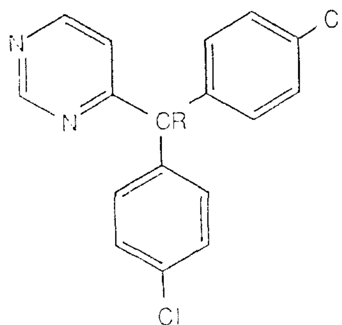
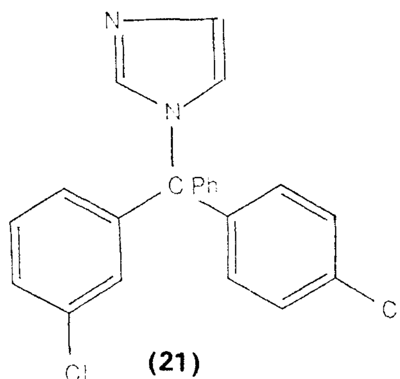


(**18**; LY113174)



(**19**; LY56110, R = H)
(**20**; Fenarimol, R = OH)



(**21**)

interaction may require the inhibitor to take up an unfavourable conformation on the steroid binding site. The additional energy required to overcome the conformational energy barriers, resulting in a productive interaction, is considered to be provided by formation of the enzyme-inhibitor complex and through further secondary interactions at the binding site. This additional binding free energy provided by the rest of the inhibitor structure is by hydrophobic interactions with the site recognising ring A of (**1**) and specific H-bonding with the site recognising the polar C(3)-0 group of (**1**).

By comparing the inhibitory activities shown in Table III. with the geometric positioning of the co-ordinating nitrogen and the other features of the inhibitor structure it is possible, broadly, to rationalise contributions important in imparting activity. Thus the comparable activities of (**11**) and (**10**) reflect a balance between the loss of the binding contribution of the extra methylene in (**10**) with the altered position of the nitrogen in (**11**) (see Table III). However the balance of these contributions in the hydantoin (**13**) has been lost, since it is less potent than the methylene analogue (**11**) probably due to a decrease in hydrophobic bonding on replacement of the CH_2 group by the more polar NH group (*ie. ca.* 2.0 kcal mol^{-1} on the log scale which is

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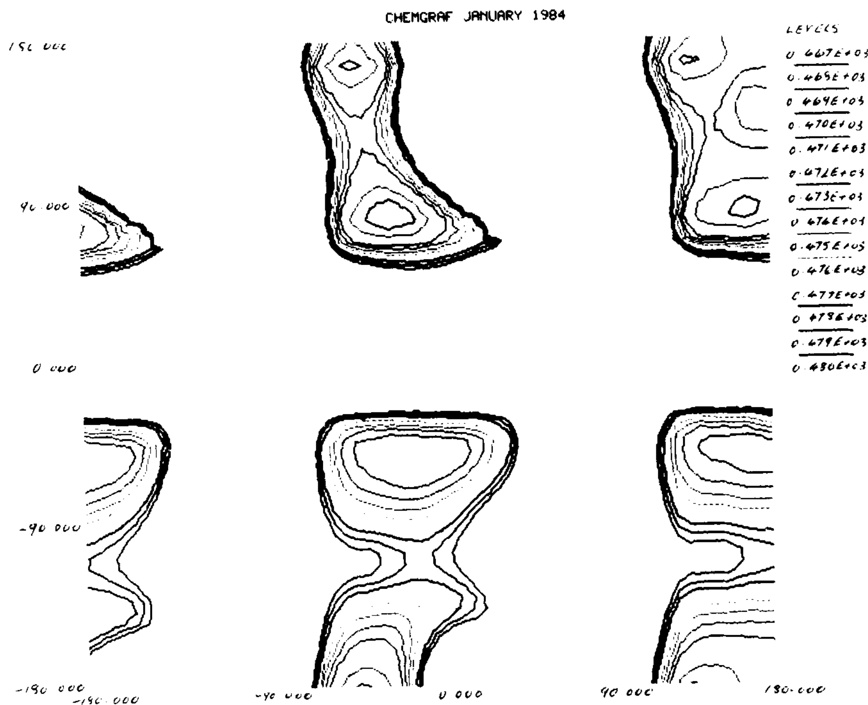

FIGURE 7 (see colour plate XV at the back of the issue). Energy contour map at intervals of 1 kcal.mol.$^{-1}$ for (**10**) generated by systematic rotation of C_1'-C_3 and C_α-C_3 bonds through 360° (Chemgraf).

equivalent to a 25-fold decrease in potency,[32]). The lack of activity of the "spiro-compound" (**12**) has been explained previously. The activities of (**14**) and (**11**) are similar with that of (**14**) being slightly lower, which probably reflects the difference in the hydrophobic binding contribution of the ethyl group in (**11**) with that of the bicyclo methylene in (**14**). Although, as with all the analogues of (**10**) possessing a reasonably freely rotating *p*-aminophenyl group, the different rotational profile may be a deciding factor.

The model developed above not only provides a reasonable basis for the design of novel reversible inhibitors of AR but also helps to explain why some of the inhibitors exhibit 11-β hydroxylase inhibitory activity[33] assuming a similar mode of binding of the substrate to that of AR. The *loci* of the nitrogens on several inhibitors come very close to C(11) of the steroid nucleus of (**1**). It may be that, in any proposed AR inhibitor, the proximity of the nitrogen to this position could be used as an indication of possible unwanted 11-β hydroxylase inhibitory activity.

More refined calculations on AR inhibitors could allow the precise positioning of the haem-iron atom to be defined with respect to the position of the bound steroid substrate. These predictions await confirmation from the X-ray crystallographic data on AR or an AR/substrate bound complex.

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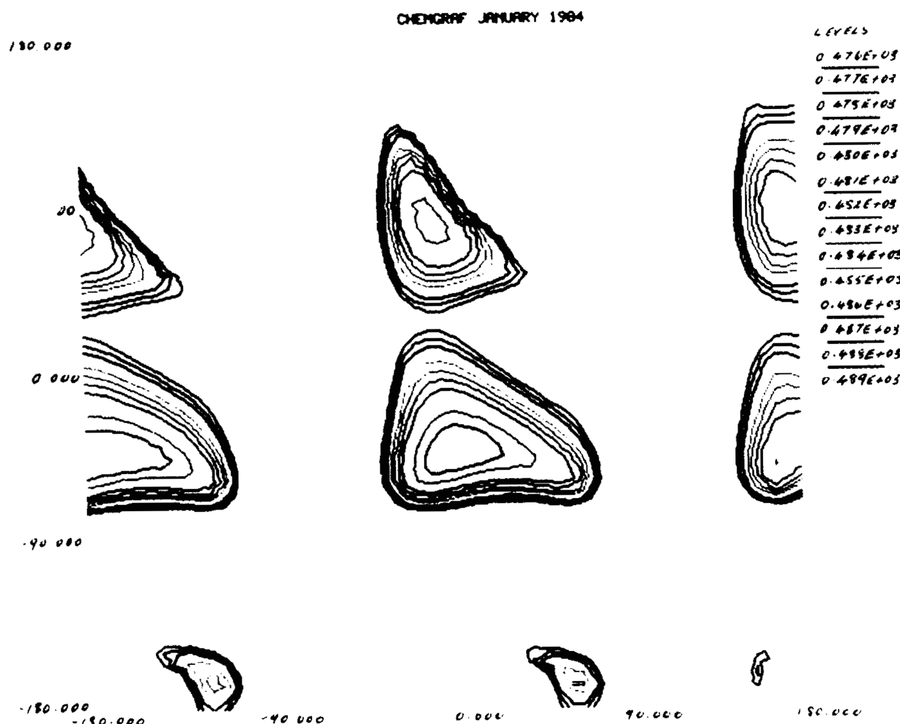

FIGURE 8 (see colour plate XVI at the back of the issue). Energy contour map at intervals of 1 kcal.mol.$^{-1}$ for (**11**) generated by systematic rotation of C_1'-C_3 and C_α-C_3 bonds through 360° (Chemgraf).

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