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STRUCTURE-ACTIVITY STUDIES OF NON-STEROIDAL AROMATASE INHIBITORS: THE CRYSTAL AND MOLECULAR STRUCTURES OF CGS 16949A AND CGS 18320B

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The crystal and molecular structures of 4-(5,6,7,8-tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile hydrochloride (CGS 16949A) and bis(p-cyanophenyl)imidazo-1-yl methane hemisuccinate (CGS 18320B) have been determined as part of structure-activity relationship studies of non-steroidal aromatase inhibitors. CGS 18320B crystallizes with two inhibitor molecules in the asymmetric unit that are similar in conformation. The cyanophenyl groups and the imidazole moieties in the CGS 18320B molecules display a propellor-like arrangement. The orientation of the imidazole ring in CGS 16949A, which is constrained by the piperidine ring, differs by about 80° from the orientations in both CGS 18320B molecules. The conformations of both compounds are consistent with the proposed model (Banting *et al.* (1988) *J. Enz. Inhibit.*, **2**, 216) for inhibitor binding by positioning of the cyanophenyl group in the steroid A-ring binding site and interaction of the imidazole nitrogen with the iron of the haem.

KEY WORDS: Aromatase inhibitors, Crystal structure, CGS 16949A, CGS 18320B.

INTRODUCTION

Hormone deprivation by surgical or pharmacological means has been used in the treatment of a variety of hormone-dependent cancers.[1-3] Aromatase, a complex of a cytochrome P450 and a NADPH-cytochrome P450 reductase, has been shown to be an attractive target for chemotherapeutic treatment. Aromatase catalyzes the terminal androgen 19-desmolase step in the conversion of androgen to estrogen.[4] Specific inhibitors of aromatase have shown promising results.[5-7] Schematic diagrams of the non-steroidal inhibitors discussed in this paper are shown in Figure 1. 4-(5,6,7,8-Tetrahydroimidazo[1,5-a]pyridin-5-yl)benzonitrile (CGS 16949A) and bis(p-cyanophenyl)imidazo-1-yl methane hemisuccinate (CGS 18320B) are among the most

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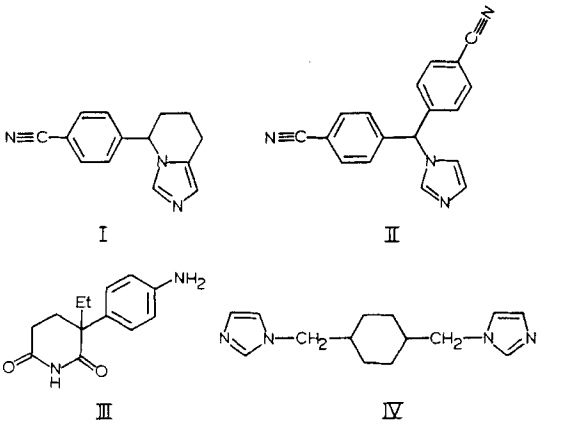

FIGURE 1 Schematic diagrams showing the structures of: (I) CGS 16949A; (II) CGS 18320B; (III) aminoglutethimide; and, (IV) CGS 14796C.

potent non-steroidal aromatase inhibitors in *in vitro* experiments.[8,9] Both are more than 100-fold more active than the clinically used non-steroidal inhibitor, aminoglutethimide. CGS 18320B exhibits a two-fold lower K_i than CGS 16949A towards androstenedione aromatization by human placental microsomal aromatase.[9]

The nature of the mechanism of action of non-steroidal aromatase inhibitors is not clearly understood. Many inhibitors lack any structural or chemical similarity with the steroid substrates and related inhibitors (for a recent review, see Cole and Robinson.[10]) Banting *et al.*[11] have proposed that the non-steroidal aromatase inhibitors, including aminoglutethimide and CGS 16949A, act by interaction with the haem iron of aromatase and that they bind in the active site in the same location as the A-ring of the steroidal substrates. This hypothesis forms the basis for recent structure-activity studies.[12]

In this paper we report the crystal structures of CGS 16949A and CGS 18320B as part of on-going studies aimed at determing structure-activity relationships of non-steroidal aromatase inhibitors. CGS 16949A contains an imidazole ring in a constrained conformation and a cyanophenyl moiety. Two cyanophenyl substituents and an unconstrained imidazole group are present in CGS 18320B. The goal of this analysis is to determine similarities in the overall molecular conformations and the location of the functional groups in particular and thus determine how the compounds may bind to the active site of the enzyme.

MATERIALS AND METHODS

Crystals of CGS 16969A and CGS 18320B were obtained by slow evaporation of aqueous methanol solutions at 37°C. X-ray diffraction intensities were measured on a Siemens (formely Nicolet) P3 diffractometer, using Nb-filtered Mo$K\alpha$ radiation (λ = 0.710 69 Å). The intensities of six reference reflections were monitored throughout the data collection but did not change significantly. Lorentz and polarization corrections were applied, but absorption corrections were not considered necessary.

The data with $F > 3\sigma(F)$ were considered observed and used in the refinement. The sructures were determined by direct methods, using the program MULTAN,[13] and refined by full-matrix least-squares. Coordinates for the hydrogen atoms were determined from difference maps and included in the refinement after the anisotropic refinement of the non-hydrogen atoms had converged. Programs used include Blessing's data reduction package,[14] locally modified versions of the refinement program contained in the Enraf-Nonius SDP package,[15] the plotting program ORTEP-II[16] and the least-squares molecular fitting program FITMOL.[17]

CGS 16949A

$C_{14}H_{13}N_3$. HCl, $M_r = 258.73$, orthorhombic, Fdd2, $a = 15.982(2)$, $b = 25.777(3)$, $c = 13.156(2)$Å, $V = 5421.7(7)$Å^3, $Z = 16$, $D_x = 1.27\,\mathrm{Mgm}^{-3}$, $\mu = 2.649\,\mathrm{mm}^{-1}$, $F(000) = 2160$. The sample was recrystallized from an aqueous methanol solution. The crystal was shaped as a triangular plate and had approximate dimensions of $0.30 \times 0.40 \times 0.60$ mm. In total, 2640 intensity data with $4 < 2\theta < 55°$, $-2 < h < 20$, $-2 < k < 31$, $0 < l < 16$, were measured. Of the 1634 unique data, 1456 had $F > \sigma(F)$ and were considered observed. $\sigma(F)$ calculated according to Stout & Jensen:[18] $\sigma^2(F) = (k/4LpI)[\sigma^2(I) + (0.01I)^2]$. The maximum value of the shift/e.s.d. during the last cycle of refinement was 0.03. Final R values are $R = 0.036$ and $R_w = 0.041$ for the observed data, and $R_{all} = 0.042$ for all 1634 data. The final value of the standard deviation of an observation of unit weight was $s = 1.874$. The final difference map has maximum and minimum densities of 0.057 and $-0.056\,\mathrm{eA}^{-3}$.

CGS 18320B

$C_{18}H_{12}N_4$. $1/2[CH_2\text{–}COOH]_2$, $M_r = 343.37$, triclinic, $P1$, $a = 9.753(1)$, $b = 10.982(1)$, $c = 17.443(2)$Å, $\alpha = 100.51(1)$, $\beta = 93.15(1)$, $\gamma = 99.32(1)°$, $V = 1808.1(4)$Å^3, $Z = 4$, $D_x = 1.26\,\mathrm{Mgm}^{-3}$, $\lambda(\mathrm{Mo}K\alpha) = 0.71069$Å, $\mu = 7.90\,\mathrm{mm}^{71}$, $F(000) = 716$. The sample was recrystallized from an aqueous methanol solution. The crystal used had approximate dimensions of $0.10 \times 0.40 \times 0.60$ mm. In total, 8160 total data with $4 < 2\theta < 52°$, $0 < h < 13$, $-14 < k < 14$, $-22 < l < 22$ were measured. Of the 6817 unique data, 4864 had $F > 3\sigma(F)$ and were considered observed $(\sigma^2(F) = (k/4LpI)[\sigma^2(I) + (0.01I)^2]$. The maximum value of shifts/e.s.d. during the last cycle of refinement was 0.02. Final R values are $R = 0.064$, $wR = 0.057$ for 4864 observed data and $R_{all} = 0.091$ for all 6817 data; $s = 2.204$. The final difference map has maximum and minimum densities of 0.339 and $-0.380\,\mathrm{eA}^{-3}$ respectively.

RESULTS

Atomic coordinates and isotropic thermal parameters for both compounds are listed in Table I.† Selected torsion angles that are important in describing the conformations are listed in Table II. Figure 2 shows the observed molecular conformations of both compounds and the numbering scheme used in the text and tables. Table III lists the geometries of the least-squares planes of the conjugated systems in both compounds.

†Tables of coordinates and thermal parameters have been deposited with the Cambridge Crystallographic Database. Tables of anisotropic thermal parameters, bond lengths and angles and observed and calculated structure factors are available from the authors (PVR) upon request.

TABLE I

Atomic coordinates ($\times 10^4$, $\times 10^5$ for Cl) and isotropic thermal parameters ($\times 10^2$) for CGS 16949A and CGS 18320B. The e.s.d.'s are given in parentheses. B_{eq} shown is the equivalent isotropic thermal parameter calculated from the refined anisotropic thermal parameters by means of the equation: $B_{eq} = 4/3\Sigma_i\Sigma_j B_{ij}a_ia_j$.

	x	*y*	*z*	B_{eq}
CGS 16949A				
N(1)	3324(2)	1663(1)	557	494(6)
C(2)	2628(2)	1400(1)	578(3)	446(7)
N(3)	2807(1)	900(1)	621(3)	390(5)
C(4)	3662(2)	846(1)	616(3)	465(7)
C(5)	3977(2)	1328(1)	586(3)	516(8)
C(6)	4064(2)	326(2)	643(4)	624(11)
C(7)	3464(3)	−92(1)	323(5)	691(12)
C(8)	2632(2)	−34(1)	875(4)	565(10)
C(9)	2196(2)	465(1)	555(3)	441(7)
C(10)	1421(2)	586(1)	1151(3)	431(7)
C(11)	1437(2)	683(1)	2185(3)	517(8)
C(12)	710(2)	781(2)	2710(4)	556(9)
C(13)	−47(2)	782(1)	2207(3)	459(7)
C(14)	−75(2)	682(1)	1177(3)	537(9)
C(15)	655(2)	582(1)	659(3)	519(8)
C(16)	−820(2)	881(1)	2741(3)	569(9)
N(17)	−1434(2)	958(2)	3138(3)	778(11)
CL(18)	10827(5)	22319(3)	9527(22)	584(2)
CGS 18320B				
C(2A)	6113(4)	1378(3)	3734(2)	837(14)
C(3A)	5250(3)	2156(3)	3433(2)	587(10)
C(4A)	5307(3)	2326(3)	2676(2)	614(11)
C(5A)	4542(3)	3143(3)	2400(2)	523(9)
C(6A)	3715(2)	3776(2)	2885(1)	419(7)
C(7A)	3646(3)	3574(3)	3641(2)	524(9)
C(8A)	4422(3)	2776(3)	3918(2)	594(10)
C(9A)	2963(3)	4743(2)	2593(2)	422(8)
C(10A)	1645(3)	4887(2)	2995(1)	423(8)
C(11A)	1594(3)	5862(3)	3598(2)	604(10)
C(12A)	402(3)	5915(3)	3989(2)	667(11)
C(13A)	−749(3)	4994(3)	3768(2)	518(9)
C(14A)	−723(3)	4025(3)	3150(2)	561(10)
C(15A)	469(3)	3987(3)	2766(2)	514(9)
(C16A)	−1986(3)	5051(3)	4180(2)	644(11)
C(19A)	4899(3)	6512(3)	3295(2)	518(9)
C(20A)	5461(3)	7625(3)	3137(2)	508(9)
C(22A)	3990(3)	6737(3)	2176(2)	439(8)
N(1A)	6813(4)	794(3)	3996(3)	1167(16)
N(17A)	−2938(3)	5108(3)	4524(2)	879(12)
N(18A)	3935(2)	5935(2)	2676(1)	415(6)
N(21A)	4896(2)	7772(2)	2435(1)	479(7)
C(1C)	4663(3)	4328(3)	−162(2)	470(8)
C(2C)	3376(3)	3903(3)	213(1)	432(8)
O(3C)	2846(2)	2714(2)	−30(1)	670(7)
O(4C)	2822(2)	4578(2)	663(1)	716(7)
C(2B)	2171(3)	5716(3)	8705(2)	537(9)
C(3B)	1351(3)	4475(2)	8547(1)	430(8)
C(4B)	173(3)	4200(3)	8934(2)	494(9)
C(5B)	−591(3)	3007(3)	8779(1)	470(8)
C(6B)	−217(2)	2072(2)	8229(1)	406(7)

TABLE I
Continued

	x	y	z	B_{eq}
CGS 18320B				
C(7B)	952(3)	2362(3)	7842(2)	512(9)
C(8B)	1739(3)	3552(3)	8002(2)	509(9)
C(9B)	−1126(3)	780(2)	8047(1)	446(8)
C(10B)	−1218(3)	169(2)	7192(1)	423(7)
C(11B)	−418(3)	−710(3)	6908(2)	531(9)
C(12B)	−496(3)	−1207(3)	6126(2)	536(9)
C(13B)	−1386(3)	−824(2)	5604(1)	441(8)
C(14B)	−2195(3)	51(3)	5880(2)	540(9)
C(15B)	−2112(3)	535(3)	6669(2)	533(9)
C(16B)	−1443(3)	−1349(3)	4785(2)	525(9)
C(19B)	608(3)	−195(3)	8813(2)	466(9)
C(20B)	440(3)	−1137(3)	9213(2)	526(9)
C(22B)	−1588(3)	−915(3)	8827(2)	504(9)
N(1B)	2833(3)	6694(3)	8823(2)	756(10)
N(17B)	−1467(3)	−1790(3)	4142(2)	706(9)
N(18B)	−701(2)	−43(2)	8564(1)	441(6)
N(21B)	−948(3)	−1591(2)	9223(1)	538(7)
C(1D)	5504(3)	10038(3)	348(2)	507(9)
C(2D)	4946(3)	9306(2)	939(2)	448(8)
O(3D)	5832(2)	9381(2)	1545(1)	599(6)
O(4D)	3785(2)	8691(2)	873(1)	685(7)

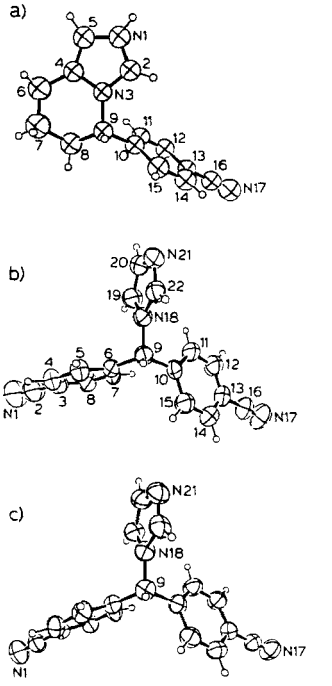

FIGURE 2 Molecular conformations of (a) CGS 16949A; (b) CGS 18320B, molecule A; and (c) CGS 18320B, molecule B showing the numbering scheme used in the text.

TABLE II
Selected torsion angles (°) for CGS 16949A and CGS 18320B.

CGS 16949A									
N3	C2	N1	C5	0.2(4)	C10	C9	N3	C2	−41.2(4)
N1	C2	N3	C4	−0.8(4)	C10	C9	N3	C4	147.1(3)
N1	C2	N3	C9	−173.6(3)	N3	C9	C10	C11	−59.8(4)
C5	C4	N3	C2	1.1(4)	N3	C9	C10	C15	122.6(3)
C5	C4	N3	C9	174.0(3)	C8	C9	C10	C11	63.3(4)
C6	C4	N3	C2	−179.1(3)	C8	C9	C10	C15	−114.4(4)
C6	C4	N3	C9	−6.2(5)	N3	C4	C5	N1	−1.0(4)
C6	C4	C5	N1	179.2(4)	N3	C4	C6	C7	19.3(5)
C5	C4	C6	C7	−160.9(4)	C4	C5	N1	C2	0.5(4)
C4	C6	C7	C8	−48.0(5)	C6	C7	C8	C9	65.7(4)
C7	C8	C9	N3	−48.9(4)	C7	C8	C9	C10	−173.1(3)
C8	C9	N3	C2	−167.4(3)	C8	C9	N3	C4	21.0(4)
CGS 18320B									
C10A	C9A	N18A	C19A	83.0(3)	C10B	C9B	N18B	C19B	86.3(3)
C10A	C9A	N18A	C22A	−91.8(3)	C10B	C9B	N18B	C22B	−86.7(3)
C5A	C6A	C9A	C10A	153.0(2)	C5B	C6B	C9B	C10B	143.3(2)
C5A	C6A	C9A	N18A	−81.5(3)	C5B	C6B	C9B	N18B	−89.3(3)
C7A	C6A	C9A	C10A	−30.7(3)	C7B	C6B	C9B	C10B	−34.5(3)
C7A	C6A	C9A	N18A	94.8(3)	C7B	C6B	C9B	N18B	92.9(3)
C20A	C19A	N18A	C9A	−174.9(2)	C20B	C19B	N18B	C9B	−174.2(3)
C6A	C9A	C10A	C11A	100.3(3)	C6B	C9B	C10B	C11B	98.6(3)
C6A	C9A	C10A	C15A	−77.5(3)	C6B	C9B	C10B	C15B	−79.4(3)
N18A	C9A	C10A	C11A	−23.4(4)	N18B	C9B	C10B	C11B	−28.5(3)
N21A	C22A	N18A	C9A	175.1(2)	N21B	C22B	N18B	C9B	174.7(2)
N18A	C9A	C10A	C15A	158.9(2)	N18B	C9B	C10B	C15B	153.4(2)
C6A	C9A	N18A	C19A	−42.2(3)	C6B	C9B	N18B	C19B	−41.7(4)
C6A	C9A	N18A	C22A	142.9(2)	C6B	C9B	N18B	C22B	145.4(3)

CGS 16949A

N1 of the inhibitor molecule is protonated and forms a salt bridge with the chloride anion (N1–HN1 ... Cl18 : N ... Cl, 3.048(3) Å; N–H, 0.87(4) Å; H ... Cl, 2.22(4) Å, N–H ... Cl, 160(3)°). The only other significant intermolecular contact is a moderately close contact between C4 and N17 (3.226 Å).

The bond lengths and angles of the imidazole ring indicate only limited contribution of resonance forms in which N1–C5 (1.354(4) Å), N3–C2 (1.323(3) Å) and N3–C4 (1.375(3) Å) have double bond characteristics. The N1–C2 bond (1.302(4) Å) is much shorter than the N1–C5 bond and the C4–C5 bond (1.341(5) Å) is only slightly longer than a standard double bond. But the ring is planar. Atoms C9 and C6 are on the same side of the plane. The angle between the least-squares planes of the imidazole ring and the p-cyanophenyl group is 100.0°. The absolute configuration this compound has not been determind. The configuration used is that also used by Banting *et al.*[11] for their modeling studies.

CGS 18320B

The asymmetric unit contains two CGS 18320B molecules and two half succinic acid molecules. The two half succinic acid molecules are independent and located on

TABLE III

Least-squares planes geometry, deviations from the planes and interplanar angles of the conjugated systems in CGS 16949A (I) and CGS 18320B (II).

CGS 16949A (I).

Plane 1: Cyanophenyl moiety.
Atoms: C9, C10, C11, C12, C13, C14, C15, C16, N17
r.m.s. deviation: 0.008 Å
C8 −1.284 N3 1.160

Plane 2: Imidazole Ring
Atoms: C2, C4, C5, C6, C9, N1, N3
r.m.s. deviation: 0.027 Å
C8 0.343 C10 0.778

Plane 3: C9 substituents
Atoms: C8, C10, N3
C9 −0.457 N1 0.339 N17 1.399
Angle Plane 1-Plane 2: 100.0°
Angle Plane 1-Plane 3: 93.1°
Angle Plane 2-Plane 3: 17.3°

CGS 18320B Molecule A.

Plane 1: Cyanophenyl moiety.
Atoms: C2, C3, C4, C5, C6, C7, C8, C9, N1
r.m.s. deviation: 0.062 Å
C10 −0.509 N18 1.532

Plane 2: Cyanphenyl moiety.
Atoms: C9, C10, C11, C12, C13, C14, C15, C16, N17
r.m.s. deviation: 0.037 Å
C6 1.498 N18 −0.392

Plane 3: imidazole moiety.
Atoms: C9, C19, C20, C22, N18, N21
r.m.s. deviation: 0.024 Å
C6 0.863 C10 −1.449

Plane 4: C9 substituents
Atoms: C6, C10, N18
C9 −0.453 N1 1.859 N17 1.749 N21 0.022
Angle Plane 1-Plane 2: 93.2°
Angle Plane 1-Plane 3: 83.7°
Angle Plane 2-Plane 3: 75.2°
Angle Plane 1-Plane 4: 60.2°
Angle Plane 2-Plane 4: 54.3°
Angle Plane 3-Plane 4: 112.0°

CGS 18230B Molecule B.

Plane 1: Cyanophenyl moiety.
Atoms: C2, C3, C4, C5, C6, C7, C8, C9, N1
r.m.s. deviation: 0.013 Å
C10 −0.864 N18 1.322

Plane 2: Cyanophenyl moiety.
Atoms: C9, C10, C11, C12, C13, C14, C15, C16, N17
r.m.s. deviation: 0.017 Å
C6 1.433 N18 −0.569

Plane 3: imidazole moiety.
Atoms: C9, C19, C20, C22, N18, N21
r.m.s. deviation: 0.025 Å
C6 0.803 C10 −1.466

Plane 4: C9 substituents
Atoms: C6, C10, N18
C9, −0.428 N1 1.361 N17 1.541 N21 −0.064
Angle Plane 1-Plane 2: 92.5°
Angle Plane 1-Plane 3: 74.5°
Angle Plane 2-Plane 3: 79.6°
Angle Plane 1-Plane 4: 63.0°
Angle Plane 2-Plane 4: 56.2°
Angle Plane 3-Plane 4: 114.1°

distinct inversion centers (1/2, 1/2, 0 and 1/2, 0, 0). The bond lengths of C1 to the symmetry related atoms are C1C–C1C($1-x$, $1-y$, $-z$) 1.511(4) Å and C1D–C1D($1-x$, $2-y$, $-z$) 1.503(4) Å. Succinic acid molecules B and D form strong hydrogen bonds with CGS 18320B molecules C and A, respectively, with the following goemetries: O3D–HO3D...N21A:O...N, 2.640(3) Å; O–H, 1.10(3) Å; H...N, 1.55(3) Å; O–H...N, 178(2)° and O3C–HO3C...N21B:O...N, 2.662(3) Å; O–H, 1.06(4) Å; H...N, 1.66(4) Å; O–H...N, 155(3)°.

The two CGS 18320B molecules have nearly identical geometries and conformations. Again, both imidazole rings show limited resonance structure but are very planar. The only conjugated system that differs significantly from planarity is the first p-cyanophenyl group of molecule A. The phenyl ring appears to be bowed,

TABLE IV

Results of least-squares fitting of the molecular structures of CGS-16949 (I) and CGS-189320B, molecule A (IIA) and molecule B (IIB).

A. Fit of the Central atoms:

I: C9, C8, C10, N3
II: C9, C6, C10, N18

Molecules	Distances of fitted atoms (Å)				Other distances		
	Average	*Maximum*		*RMS*	*Atom*	*Atom*	*Distance*
IIA-IIB	0.017	0.023	C9	0.018	C8A	C8B	0.159
					C12A	C12B	0.044
					C20A	C20B	0.139
					N1A	N1B	0.867
					N17A	N17B	0.302
					N21A	N21B	0.164
IIA-I	0.016	0.022	C9	0.017	C5A	C7	0.799
					C12A	C12	0.570
					C13A	C13	0.244
					C20A	C5	0.870
					N17A	N17	0.542
					N21A	N1	0.425
IIB-I	0.21	0.031	C9	0.022	C5B	C7	1.002
					C12B	C12	0.595
					C13B	C13	0.132
					C20B	C5	0.857
					N17B	N17	0.241
					N21B	N1	0.450

B. Fit of the imidazole ring atoms:

I: C9, C4, C5, C2, N3, N1
II: C9, C19, C20, C22, N18, N21

Molecules	Distances of fitted atoms (Å)				Other distances		
	Average	*Average*		*RMS*	*Atom*	*Atom*	*Distance*
IIA-IIB	0.012	0.0187	C9	0.0127	C3A	C3B	0.568
					C8A	C8B	0.369
					C12A	C12B	0.269
					C13A	C13B	0.448
					N1A	N1B	1.190
					N17A	N17B	0.760
IIA-I	0.054	0.087	N18	0.0576	C5A	C7	2.594
					C6A	C8	1.251
					C10A	C10	1.373
					C12A	C12	3.723
					C13A	C13	3.965
IIB-I	0.058	0.099	N18	0.0627	C5B	C7	2.685
					C6B	C8	1.209
					C10B	C10	1.267
					C12B	C12	2.993
					C13B	C13	3.610
					N17B	N17	5.906

with C9 and N1 0.10 and 0.12 Å above the least-square plane. The second p-cyanophenyl group of molecule A is also somewhat distorted but the imidazole ring and all three groups of molecule B are very planar. N1 of molecule A also has a high thermal parameter, indicative of static or dynamic disorder in its position. No obvious

TABLE IV
Continued

C. Fit of the phenyl ring atoms:
I: C9, C10, C11, C12, C13, C14, C15, C16, C17
II: C9, C10, C11, C12, C13, C14, C15, C16, C17

Molecules	Distance of fitted atoms (Å)				Other distances		
	Average	*Maximum*		*RMS*	*Atom*	*Atom*	*Distance*
IIA-I	0.041	0.075	N17	0.0456	C5A	C7	1.659
					C6A	C8	0.884
					C20A	C5	2.534
					N18A	N3	0.956
					N21A	N1	2.090
IIB-I	0.037	0.081	N17	0.0417	C5B	C7	1.754
					C6B	C8	0.816
					C20B	C5	2.169
					N18B	N3	0.791
					N21B	N1	1.656

causes, such as intermolecular contacts, for the distortions in molecule A are observed. The main conformational features are to be found in the orientation of the two phenyl rings and the imidazole ring about the central atom C9. The presence of three bulky substitutents on C9 forces the molecule in a propeller-like conformation. The three bond angles between C6, C10 and N18 differ by small but significant amounts. The largest difference in the torsion angles about the bonds that link the rings to the central atom is 10°. The phenyl rings are nearly perpendicular in both molecules. The angles of the last squares planes of the imidazole ring with the phenyl rings are significantly different, but in the opposite direction in the two molecules.

Table IV summarizes the analysis of the least-squares fitting of the two molecules of CGS 18320B as well as those with the structure of CGS 16949A. Figure 3 shows the corresponding illustrations. Three different superpositions are shown. The two CGS 18320B molecules are very similar in conformation, indicating that this conformation is highly preferred. The small differences in the torsion angles do not affect the overall geometry. The largest difference in the geometry is in the positions of N1 as can best be seen at the bottom of Figure 3c. This is caused by small differences in the angles about C9 and the non-planarity of the one p-aminophenyl ring in molecule A described in the previous paragraph. This distortion is most likely not significant.

DISCUSSION

The most important differences between the conformations of CGS 16949A and CGS 18320B are in the orientations of the imidazole rings and the phenyl rings. Looking down the H9–C9 bond all three planar substituents in CGS 18320B make an angle of about 60° with the plane of the atoms C6, C10 and N18. This conformation is caused by steric interactions of the bulky substituents. In CGS 16949A, the phenyl ring is at a 93° angle with the corresponding plane (C8, C10, N3) but the plane of the imidazole ring is nearly parallel (15°) because of the constraint caused by the pyridine ringe on the orientation of the imidazole ring. Table IV summarizes the least-squares

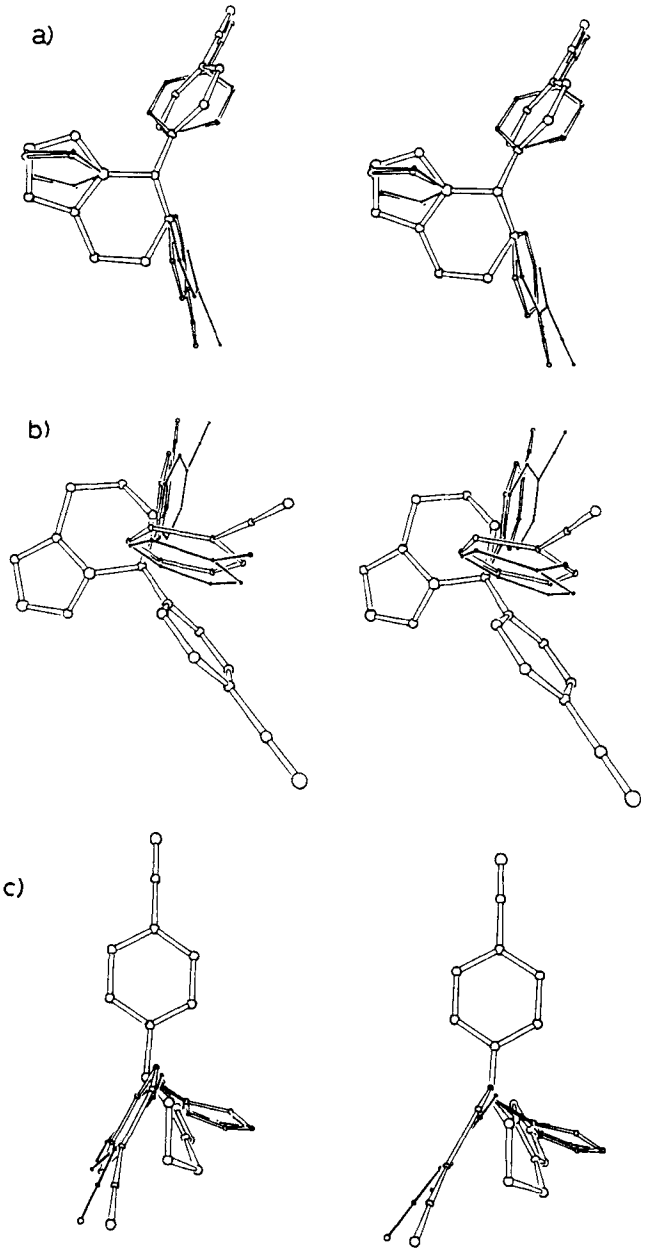

FIGURE 3 Stereodiagrams showing the overlap of CGS 16949A (largest bond radii) and CGS 18320B (molecule A, intermediate bond radii; and molecule B, smallest bond radii) after least-squares fitting of, (a) the four central atoms, (b) the imidazole rings, and (c) the cyanophenyl group. Note the similarity in the conformations of the two molecules of CGS 18320B, except for the slight change in the position of N1 and the significant differences in the orientation of the imidazole ring between the two compounds but with only small differences in the relative positions of the nitrogen atoms.

fitting of related structural parts of CGS 16949A and CGS 18320B with the accompanying illustrations shown in Figure 3. The overlap of functional groups appears to work best when the central four atoms are superimposed. Overlapping the phenyl groups or the imidazole moieties results in large distances between the positions of the other functional groups. This suggests, if the two compounds are to bind to the same binding site, that the imidazole conformation in the restrained molecule is a suitable conformation and that the more flexable compounds would have to adopt a similar conformation or, alternatively, that the location of the cyano and imidazole nitrogens are important but not the orientation of the phenyl or imidazole rings. If the imidazole ring of CGS 18320B would need to be rotated independently of the cyanophenyl rings, this would most likely result in a significantly higher energy conformation than the observed in the crystal structure. The observed two-fold higher inhibition by CGS 18320B would suggest that this higher energy conformation can be achieved upon binding to the enzyme or that the enzyme can accomodate a wider range of molecular geometries or conformations. The inhibition by CGS 14796C, aminoglutethimide and its analogues, the crystal structures of which have been reported elsewhere[20,21,12] and which differ even more significantly in structure, may provide additional evidence for this accommodation.

CGS 16949A and CGS 18320B are both active aromatase inhibitors that differ greatly in chemical structure when compared to other non-steroidal inhibitors such as aminogluetethimide. Both have the imidazole ring and the *p*-cyanophenyl group as functional groups. For the structure-activity studies we need to compare the two compounds in terms of the overall shape and size and distances between corresponding functional groups, specifically the cyano and imidazole nitrogen atoms, which according to the model of Banting *et al.*[11] form the anchor points for interaction with the binding site for the A-ring of the steroid and the interaction with the iron of the haem group. This analysis can provide information regarding the shape and properties of the binding site or active site of the target enzyme. If the two nitrogen atoms are the crucial functional groups for the effective binding and activity of the inhibitors their relative positions as well as the overall shape and geometry of the hydrophobic core are important. Both compounds have V-shaped hydrophobic cores with the nitrogen atoms at the extreme ends of essentially perpendicular axes. One of these would be oriented towards the haem iron and the other towards the protein component that interacts with the O3 of the steroid molecule. The additional cyanophenyl group of CGS 18320B may well fit within the remaining space for the steroid. Figure 3 shows that the relative positions of the nitrogen atoms do not differ greatly but that the orientation of the imidazole rings are different. For CGS 16949A the distance between the nitrogen atoms N1 and N17 is 8.525 Å. CGS 18320B has two cyano nitrogens. One, N17 is a similar distance from the imidazole nitrogen N21 : 8.931 Å in molecule A and 8.811 Å in molecule B. The distance of the other cyano nitrogen N1 to N21 is similar in molecule A, 9.004 Å, but significantly larger in molecule B, 9.409 Å.

Another non-steroidal aromatase inhibitor CGS 14796C has two imidazole rings at opposite ends of the molecule. The distance between the two free nitrogen atoms in the crystal structure of this compound[20] is 10.77 Å. However, the conformation observed in the crystal structure is highly extended. We have demonstrated by searching distance maps, using SYBYL[22] software, that this molecule can adopt several conformations in which the distances between the two nitrogen atoms are between 8.25 Å and 9.0 Å, without prohibitive steric interactions. One such confor-

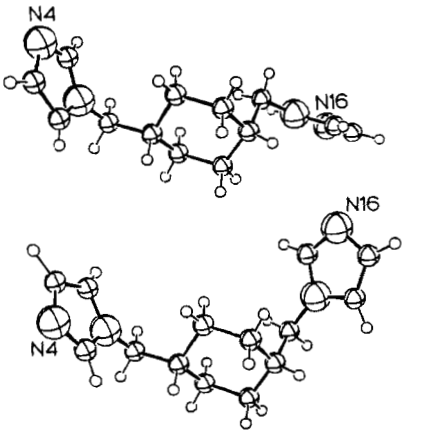

FIGURE 4 Conformation of CGS 14796C: top, extended conformation observed in the crystal structure; bottom, conformation obtained by rotating about the exocyclic bonds. This conformation has a distance of 8.95 Å between the two nitrogen atoms and does not have any prohibitive steric interactions.

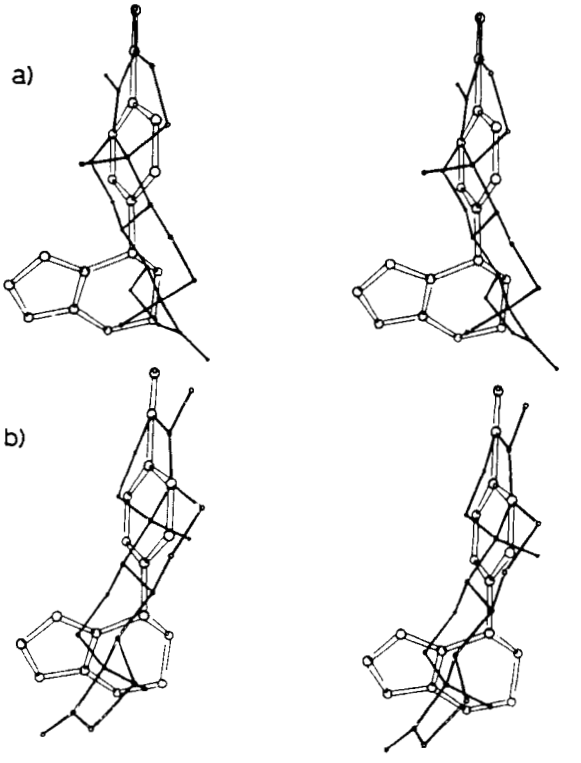

FIGURE 5 Stereodiagrams showing the superposition of CGS 16949A (largest bond radii) on the steroidal inhibitor 4-hydroxy-4-androstene-3,17-dione produced by the least squares fitting, (a) atoms N17, C16, C12 of CGS 16949A on the atoms O3, C3, C5 of the steroid, and (b) N17, C16 and C14 of CGS 16949A on O3, C3, O5 of the steroid. Fit (a) corresponds to the model of Banting *et al.*[11] where the imidazole ring is above the β-face of the steroid. Fit (b) has the imidazole ring above the α-face.

mation is shown in Figure 4. In this conformation the molecule can also be interpreted as having a V-shaped core with the free nitrogen atoms on nearly perpendicular axes. This would suggest that binding to the enzyme involving contacts with the two nitrogen atoms in a similar fashion as for CGS 16949A and CGS 18320B could be possible. Additional studies of the molecular conformations and activity of these and related compounds but especially structural data about the active site of the enzyme are required to further evaulate this hypothesis.

CGS 16949A and CGS 18320B and the other non-steroidal aromatase inhibitors do not resemble the natural substrates or steroidal inhibitors, yet are thought to function by binding in the same active site. The molecular modeling studies of Banting *et al.*[11] included the superposition of the inhibitors with the steroidal inhibitor 4-androstene-3,17-dione. Included was an overlap of CGS 16949A with the steroid based on the superposition of the cyano group of CGS 16949A on the O3 keto group of the steroid. The imidazole ring of CGS 16949A, which is supposed to interact with the haem group in the active site, was found to be placed perpendicular to the steroid and above the B-ring of the steroid. Similar superposition, using the experimentally determined structures of 4-hydroxy-4-androstene-3,17-dione[19] and CGS 16949A, is shown in Figure 5. Although similar to the modeled structure, the experimentally determined structure of CGS 16949A appears to be somewhat longer, placing the imidazole ring perpendicular to the plane of the steroid but above the D-ring rather than above the C-ring as shown by Banting *et al.*[11] This result and the similarities in the overall shape of CGS 19639A and CGS 18320B and especially the consistent distance between the two functional nitrogen atoms appears to support the proposed mechanism of action of the non-steroidal aromatase inhibitors.

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