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THE SYNTHESIS OF *N*-ARYL ANDROSTERONE PYRAZOLES AS AROMATASE INHIBITORS

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Abstract: *N*-aryl androsterone pyrazoles, which showed a good inhibitory activity against aromatase, were synthesized.

Aromatase is the enzyme responsible for catalyzing the conversion of androgens to estrogens in the last step of estrogen biosynthesis and is important in estrogen metabolic and reproductive processes. The inhibition of aromatase is an important and specific route for control of estrogen levels and estrogen-dependent diseases.[1,2] Since the discovery of 4-hydroxyandrost-4-en-3,17-dione (4-OH-A) as an effective aromatase inhibitor,[3] many 4-substituted 4-OH-A analogues were synthesized and tested as aromatase inhibitors.[4-7] Very recently Giudici et al. reported that androst-3,17-dione-4-eno[4,5,6-*b*,*c*]pyrole was effective as an aromatase inhibitor.[8] The recentl work from our group shows androst-3,17-dione-4-eno[6,5,4-*c*,*d*]isoxazole is also very effective against aromatase.[9]

The prevailing explanation for the inhibitory activity of these type of compounds is that the nearby nucleophile attacks at the C-4 position, and the following elimination of C-4 leaving group results in permanent binding of the inhibitor to the enzyme.[10] This hypothesis is based on the assumption that these inhibitors take the same orientation as the substrate in the active site of the enzyme. We synthesized three pairs of *N*-aryl substituted androsterone pyrazole derivatives as aromatase inhibitors. If these inhibitors take the same orientation as the substrate, the aryl groups on the C-6 nitrogen could project to the 7α-hydrophobic pocket and increase the affinity according to the molecular modelling.[11] The comparison of the low energy conformers, generated by the means of the SPARTAN molecular modelling program using the MM2 force field, of *N*-benzylandrost-3,17-dione-5-eno[4,5,6-*c*,*d*]1*H*-pyrazole **1** **2** and 7α-(phenylthio)androst-4-ene-3,17-dione indicated the phenyl groups on these two molecules could be partially overlapped (Figure 1). However, the increased inhibitory activity was not observed for these *N*-substituted compounds in comparison with androst-3,17-dione-5-eno[4,5,6-*c*,*d*]1*H*-pyrazole **9**. The results imply these inhibitors may not take the same orientation as the substrate in the enzyme active site.The C-4 heteroatom could orient toward the heme in the enzyme active site, which resulted from the molecular rotation around axis from C-3 to C-17, and just act as heme binders.

The synthesis started with 3β,17β-diacetoxyandrost-5-ene **1**, which was oxidized with selenium dioxide to yield 3β,17β-diacetoxy-4β-hydroxyandrost-5-ene **2** in 39% yield with a 40% recovery of the starting material

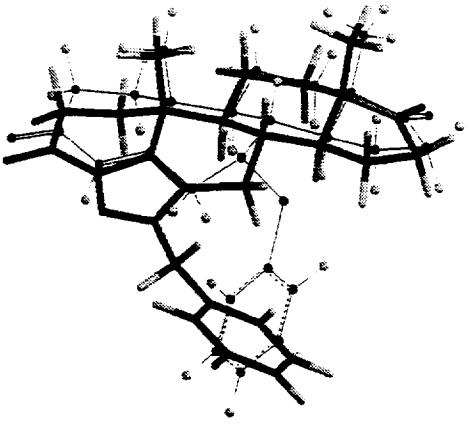
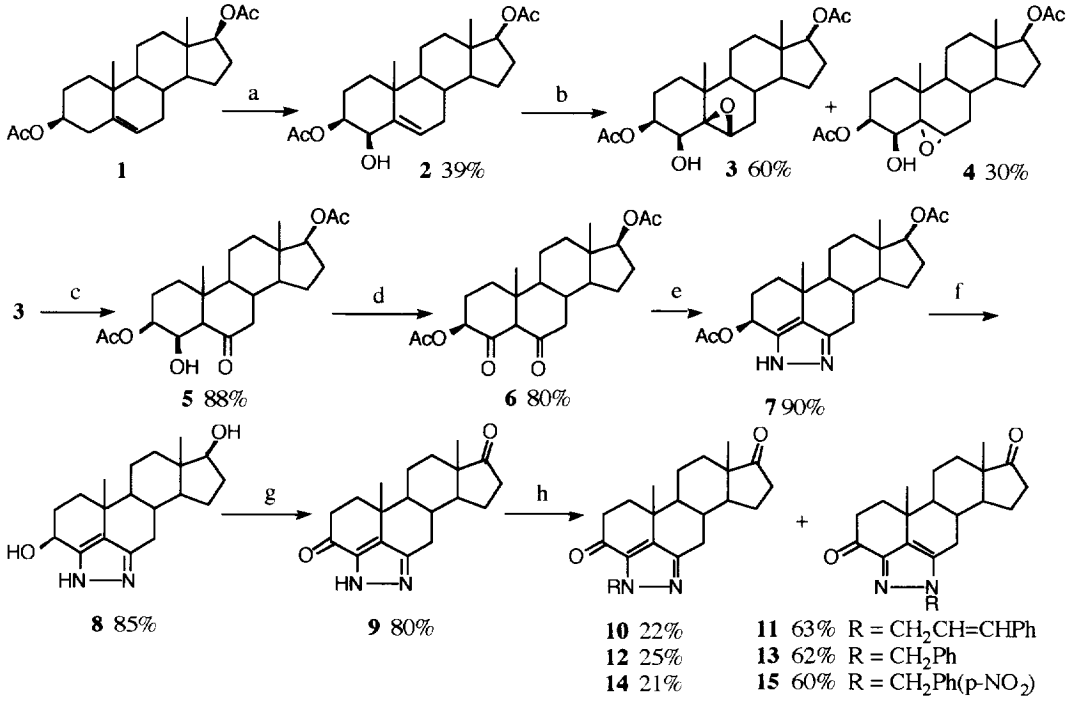


Figure 1. The overlap of 7α-(phenylthio)androst-4-ene-3,17-dione and compound **13**.



Scheme 1. The synthesis of androst-3,17-dione-4-eno[6,5,4-*c,d*]1*H*-pyrazole and its *N*-substituted derivatives. (a) SeO_2, KH_2PO_4, toluene, reflux, 4 h. (b)CH_2Cl_2, MCPBA, rt, 36 h. (c) BF_3Et_2O, Et_2O, 0 °C, 12 h. (d) Trifluroacetyl anhydride, DMSO, CH_2Cl_2, Et_3N, -78 °C. (e) $CHCl_3$, NH_2NH_2, reflux. (f) $LiAlH_4$, Et_2O. (g) Acetone, Jones reagent, 0 °C. (h) RBr, ethanol, NaOEt, reflux.

(Scheme 1). The epoxidation of compound **2** with MCPBA in methylene chloride gave 3β,17β-diacetoxy-4β-hydroxy-5β,6β-epoxyandrostane **3** as the major product (60% yield) due to the directing effect of the 4β-hydroxy group. The structure assignment of compound **3** was confirmed by the X-ray structure of its isomer 3β,17β-diacetoxy-4β-hydroxy-5α,6α-epoxyandrostane **4** (Figure 2), which was obtained as a minor product in 30% yield.

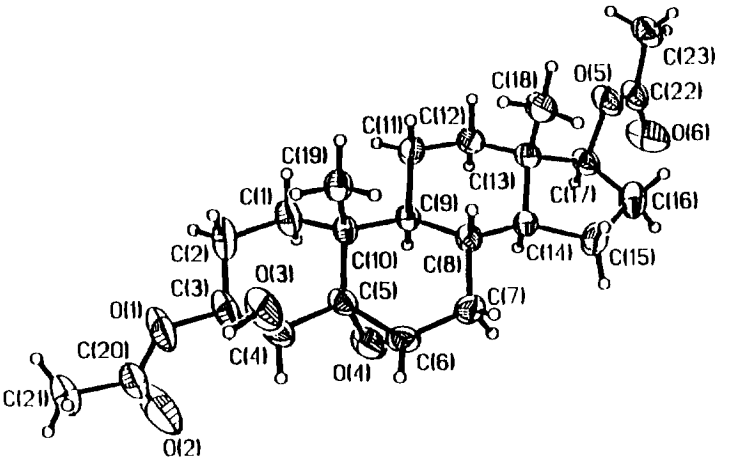


Figure 2. The X-ray struture of 3β,17β-diacetoxy-4β-hydroxy-5α,6α-epoxyandrostane **4.**

A crystal of **4** suitable for X-ray diffraction was obtained by evaporation of a toluene solution, and results of an X-ray crystal structure determination are presented in Figure 2.[12] The chemical shift of H-6α in compound **3** was slightly downfield (δ_H = 3.23 ppm) compared to the 5α,6α-epoxy isomer ($\delta_{H\text{-}6\beta}$ = 3.02 ppm), which was in agreement with the other steroidal epoxy isomer pairs.[12] Compound **3** smoothly rearranged to 3β,17β-diacetoxy-4β-hydroxyandrost-6-one **5** with boron trifluoride etherate in ethyl ether. However, the corresponding 5α, 6α-epoxy isomer did not rearrange under the same conditions. The failure of the 5α,6α-epoxy isomer to rearrange may be explained based on the following; the 6β-hydrogen shift was difficult due to (1) the steric hindrance of the 19-methyl group and (2) the rearrangement would result in a high energy cis-A/B ring junction product. The oxidation of compound **5** was first attempted with chromium oxidants (Jones' reagent, PCC and PFC), but the product could not be isolated due to the strong coordination to the metal ion. The oxidation was accomplished by Swern oxidation with trifluoroacetic anhydride as the activating reagent. 3β, 17β-Diacetoxyandrost-4,6-dione **6** was obtained in 90% yield. The condensation of compound **6** with hydrazine resulted in 3β,17β-diacetoxyandrost-4-eno[6,5,4-*c,d*]1*H*-pyrazole **7** in 90% yield (Scheme 1). Reductive deprotection with lithium aluminum hydride was used to obtain 3β,17β-dihydroxyandrost-4-eno[6,5,4-*c,d*]1*H*-pyrazole **8**, which was oxidized to androst-3, 17-dione-4-eno[6,5,4-*c,d*]1*H*-pyrazole **9** with Jones' reagent. The *N*-alkylation of androst-3,17-dione-4-eno[6,5,4-*c,d*]1*H*-pyrazole was successful with benzylic (benzyl bromide and para-nitrobenzyl bromide) and allylic (cinnamyl bromide) bromides in the presence of sodium ethoxide. The

nitrogen on C-6 was preferentially alkylated under these conditions. The structure assignment of those two *N*-cinnamyl isomers was made by examining the proton NMR spectra of both isomers with a lanthanide NMR shift reagent. When the stoichiometric ratio of Resolve-Al and substrate is less than one,the lanthanide ion from the shift reagent Resolve-Al ($Eu(fod)_3$) mainly coordinated on the 3-ketone group of the steroids due to the steric hindrance. Apparently the larger shift should be observed for *N*-cinnamyl androst-3,17-dione-4-eno[6,5,4-*c*,*d*]pyrazole **10** since the allyl CH_2 is closer to the lanthanide metal than another isomer **11**. When the lanthanide-substrate ratio is 1:4 the observed proton NMR signal shift for the allyl CH_2 was +0.087 ppm for compound **10** in contrast to +0.001 ppm for **11**. When the ratio was raised to 1:2, the shift value was +0.205 ppm for compound **10**, and +0.010 ppm for compound **11**. The structure assignments of *N*-benzyl and *N*-(p-nitro)benzyl analogues were done by comparing their ^1H and ^{13}C NMR spectra with the corresponding *N*-cinnamyl isomers.[13]

The inhibitory activity of compounds **9-15** against aromatase is summarized in Table 1. Microsomes were prepared from term human placentas and stored at -70 °C until used 1-3 days later in the aromatase assays. Time-dependent aromatase assays were performed as described previously.[14] The clinically used drug 4-OH-A was tested in the same system for comparison. The androst-3,17-dione-4-eno[6,5,4-*c*,*d*]1*H*-pyrazole and its *N*-benzyl derivatives display relatively good inhibitory activity and appear to be about equiactive as 4-OH-A. Interestingly, substitution of a 4'-nitro group in these compounds as in **14** and **15** appears to double the IC_{50} values (or decrease inhibitory activity by about 50%), suggesting that this substitution is detrimental toward activity. Replacing the *N*-benzyl substituents with a 3-phenylallyl moiety as in **10** and **11** appears to result in a further decrease in inhibitory activity.

Table 1. The aromatase inhibitory data (IC_{50} values) of compounds **9-15**.

Compound	**4-OH-A**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
IC_{50}, nM	370	236	800-1000	950	342	245	658	490

References and notes

1. Brodie, A. H. M. *J. Steroid Biochem. Molec. Biol.* **1994**, *49*, 281.
2. Schwarzel, W. C.; Kruggel, W. G.; Brodie, H. J. *Endocrinol.* **1973**, *92*, 866.
3. Marsh, D. A.; Brodie, H. J.; Garrett, W.; Tsai-Morris, C.H.; Brodie, A. M. H. *J. Med. Chem.* **1985**, *28*, 788.
4. Brodie, A. M. H.; Garrett, N.; Hendrickson, J. R. *Steroids* **1981**,*3 8*, 693.
5. Lombardi, P. *The Seventh Conference on Hormonal Steroids*, Spain, 1986.
6. Abuj-Haij, Y. J. *J. Med. Chem.* **1986**, *29*, 582.
7. Brueggemeier, R. W. *J. Enzyme Inhib.* **1990**, *4*, 101.
8. Giudici D.; Ornati, G.; Briatico, G.; Buzzetti, F.; Lombardi,D.; Di Salle, E. *J. Steroid Biochem Molec. Biol.* **1994**, *49*, 289.
9. Li, S; Parish, E. J. *Bioorg. Med. Chem.* **1997** in press.

10. Brueggemeier, R. W.; Li, P. K. *J. Steroid Biochem. Molec. Biol.* **1993,** *44*, 357.

11. Bruggemeier, R. W. *J. Med. Chem.* **1995,** *38*, 378.

12. Crystal structure of **4**. Slow evaporation of a toluene solution of **4** afforded colorless needles. A crystal, 0.12 x 0.30 x 0.60 mm, was chosen, orthorhombic, space group $P2_12_12_1$, a = 7.269(2) Å, b = 14.028(3) Å, c = 21.018(7) Å, V = 2143.3(10) Å^3, D = 1.260 Mg/M^3, Z = 4, Nicolet R3m/v diffractometer, Mo Kα (λ = 0.71073 Å), $\theta/2\theta$ scans, 4.0°<2θ<50.0°, 2 standard reflections measured every 100 reflections. 2846 reflections collected (2823 independent of which 1846 were considered observed (F>4.0σ(F)). the structure was solved by direct methods using the SHELXTL PLUS system of programs. Data-to-parameter ratio 7:1, goodness of fit 1.06, largest Δ/σ = 0.018, largest difference peak = -0.21 e/Å^3, R(F) = 5.27%, R_w(F) = 7.13%.

13. Spectral data of **3**: mp 204-207 °C; IR 3510, 2941, 1724, 1450, 1290, 1118 cm^{-1}; MS(CI, NH_3) m/z 424 (M+NH_4, 100), 407(M+H, 88), 389 (M+H-H_2O, 18); ^{1}H-NMR ($CDCl_3$) δ 0.76 (s, 3H, H-18), 1.13 (s, 3H, H-19), 2.06(s, acetate), 2.14 (s, acetate), 3.45 (d, J = 2.7 Hz, 1H, H-4), 4.55 (dd, 1H, H-17α), 4.75 (m, 1H, H-3α), 3.23 (s, 1H, H-6). Elemental Analysis: $C_{23}H_{34}O_6$ calc.: C 67.96%, H 8.43%, O 23.61%; found C 67.81%, H 8.47%, O 23.72%.

Spectral data of **4**: mp 248-250 °C; IR: 3489, 2947, 2874, 1373, 1250 cm^{-1}; MS(CI, NH_3) m/z 424 (M+NH_4, 100), 407 (M+H, 99), 389 (M+H-H_2O, 16); ^{1}H-NMR ($CDCl_3$) δ 0.74 (s, 3H, H-18), 1.18 (s, 3H, H-19), 2.10 (s, acetate), 2.13 (s, acetate), 3.31 (d, J = 3.1 Hz, 1H, H-4), 4.54 (m, 1H, H-17α), 4.92 (m, 1H, H-3α), 3.02 (d, 1H, J = 3.7 Hz, H-6). Elemental Analysis: $C_{23}H_{34}O_6$ calc.: C 67.96%, H 8.43%, O 23.61%; found C 67.74%, H 8.49%, O 23.77%.

Spectral data of **9**: mp 240-241 °C; IR 3445, 2943, 1734, 1684, 1522, 1456, 1375, 1016 cm^{-1}; MS(EI) m/z 312 (M, 40), 313 (M+1, 10), 297 (M-CH_3, 30); HR-MS: $C_{19}H_{24}O_2N_2$, required 312.1839, found 312.1846. ^{1}H-NMR ($CDCl_3$) δ 0.98 (s, 3H, H-18), 1.30 (s, 3H, H-19), 12.4 (s, br, 1H, NH). ^{13}C-NMR($CDCl_3$) δ 220.3, 190.1, 144.1, 136.2, 135.3, 51.8, 49.4, 47.0, 38.3, 36.5, 35.6, 32.8, 32.7, 31.0, 26.6, 22.1, 20.3, 20.1, 13.7.

Spectral data of **10**: mp 273-274 °C (white crystal); IR 2943, 2863, 1736, 1688, 1452, 1390, 1095 cm^{-1}; MS(EI) m/z 428 (M, 21), 429 (M+1, 6.5), 311 (M-CH_2CH=CHPh, 3), 117 (CH_2CH=CHPh, 100); HR-MS: $C_{28}H_{32}O_2N_2$, required 428.2466, found 428.2439. ^{1}H-NMR($CDCl_3$) δ 0.95 (s, 3H, H-18), 1.28 (s, 3H, H-19), 5.19 (m, 2H, allyl CH_2), 6.35 (dt, 1H, alkene), 6.64 (d, 1H, alkene), 7.30 (m, 5H, aromatic). ^{13}C-NMR($CDCl_3$) δ 220.1, 188.4, 144.9, 136.6, 136.3, 133.4, 131.2, 128.4, 127.7, 126.5, 124.3, 53.0, 52.9, 49.2, 47.8, 38.0, 36.8, 35.6, 33.0, 32.8, 30.9, 27.0, 22.0, 20.2, 20.1, 13.6.

Spectral data of **11**: IR 2941, 2859, 1732, 1681, 1459, 1391, 1098 cm^{-1}; MS(EI) m/z 428 (M, 14), 429 (M+1, 4), 311 (M-CH_2CH=CHPh, 3), 117 (CH_2CH=CHPh, 100); HR-MS: $C_{28}H_{32}O_2N_2$, required 428.2466, found 428.2449. ^{1}H-NMR($CDCl_3$) δ 0.96 (s, 3H, H-18), 1.27 (s, 3H, H-19), 4.89 (m, 2H, allyl CH_2), 6.33 (dt, 1H, alkene), 6.53 (d, J = 16.0 Hz, 1H, alkene), 7.30 (m, 5H, aromatic). ^{13}C-NMR($CDCl_3$) δ 219.7, 192.9, 142.5, 136.3, 135.7, 134.2, 133.7, 128.6, 128.2, 126.5, 123.0, 52.6, 51.5, 49.5, 48.0, 37.8, 36.8, 35.5, 33.0, 32.7, 30.9, 25.9, 22.0, 20.4, 20.3, 13.6.

Spectral data of **12**: IR 2941, 1733, 1678, 1509, 1452, 1394, 1090 cm^{-1}; MS(EI) m/z 402 (M, 20), 403 (M+1, 6), 387 (M-CH_3, 13), 311 (M-CH_2Ph, 2), 91 (CH_2Ph, 100); HR-MS: $C_{26}H_{30}O_2N_2$, required 402.2309, found 402.2321. ^{1}H-NMR($CDCl_3$) δ 0.95 (s, 3H, H-18), 1.26 (s, 3H, H-19), 5.53 (d, J = 14.4 Hz, 1H, benzyl CH_2), 5.65 (d, J = 14.4 Hz, 1H, benzyl CH_2), 7.30 (m, 5H, aromatic). ^{13}C-NMR($CDCl_3$) δ 220.2, 188.5, 145.1, 137.0, 136.8, 131.2, 128.5, 128.2, 127.7, 54.4, 51.9, 49.3, 47.9, 38.1, 36.8, 35.6, 33.1, 32.8, 31.0, 27.1, 22.0, 20.3, 13.7.

Spectral data of **13**: mp 223-224 °C (white solid); IR: 2943, 1736, 1688, 1508, 1459, 1390, 1095 cm^{-1}; MS(EI) m/z 402 (M, 7), 403 (M+1, 2), 387 (M-CH_3, 18), 311 (M-CH_2Ph, 1), 91 (CH_2Ph, 100); HR-MS: $C_{26}H_{30}O_2N_2$, required 402.2309, found 402.2328. ^{1}H-NMR($CDCl_3$) δ 0.93 (s, 3H, H-18), 1.25 (s, 3H, H-19), 5.25 (d, J = 15.7 Hz, 1H, benzyl CH_2), 5.34 (d, J = 15.7 Hz, 1H, benzyl CH_2), 7.27 (m, 5H, aromatic). ^{13}C-NMR($CDCl_3$) δ 219.5, 192.9, 142.4, 136.3, 135.3, 134.2, 128.7, 128.0, 127.5, 54.2, 51.4, 49.4, 47.9, 37.7, 36.8, 35.4, 32.9, 32.7, 30.8, 25.8, 21.9, 20.3, 20.2, 13.6.

Spectral data of **14**: IR 2941, 2862, 1736, 1689, 1526, 1454, 1388, 1093 cm^{-1}; MS(CI, NH_3) mz 465 (M+NH_4, 12), 448 (M+H, 66); HR-MS: $C_{26}H_{29}O_4N_3$ required 447.2160, found 447.2151. ^{1}H-NMR ($CDCl_3$) δ 0.97 (s, 3H, H-18), 1.30 (s, 3H, H-19), 5.53 (d, J = 15.0 Hz, 1H, benzyl CH_2), 5.74 (d, J = 15.0 Hz, 1H, benzyl CH_2), 7.46 (d, J = 8.5 Hz, 2H, aromatic), 8.14 (d, J = 8.5 Hz, 2H, aromatic). ^{13}C-NMR($CDCl_3$) δ 219.8, 188.5, 147.2, 145.7, 143.9, 137.1, 131.2, 128.6, 123.5, 53.4, 51.6, 49.0, 47.7, 37.8, 36.5, 35.4, 32.9, 32.6, 30.8, 26.8, 21.8, 20.01, 19.98, 13.5.

Spectral data of **15**: IR 2943, 2862, 1734, 1676, 1526, 1466, 1346, 1108 cm^{-1}; MS(CI, NH_3) m/z 465 (M+NH_4, 4), 448 (M+H, 32); HR-MS: $C_{26}H_{29}O_4N_3$ required 447.2160, found 447.2143. ^{1}H-NMR ($CDCl_3$) δ 0.95 (s, 3H, H-18), 1.28 (s, 3H, H-19), 5.37 (d, J = 16.0 Hz, 1H, benzyl CH_2), 5.46 (d, J = 16.0 Hz, 1H, benzyl CH_2), 7.35 (d, J = 8.6 Hz, 2H, aromatic), 8.19 (d, J = 8.6 Hz, 2H, aromatic). ^{13}C-NMR($CDCl_3$) δ 219.4, 192.9, 147.6, 143.1, 142.5, 136.6, 134.5, 128.1, 124.0, 53.0, 51.4, 49.4, 47.9, 37.6, 36.8, 35.4, 32.9, 32.7, 30.8, 25.7, 22.0, 20.4, 20.2, 13.6.

14. Santen, R. J. Brodie, A. M. H. *Clin. Oncol.* **1982**, *1*, 77.

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