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2- and 3-[(Aryl)(azolyl)methyl]indoles as Potential Non-steroidal Aromatase Inhibitors

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The present study was designed to follow our pharmacomodulation work in the field of non-steroidal aromatase inhibitors. All target compounds 12a–h and 28a–h were tested *in vitro* for human placental aromatase inhibition, using testosterone or androstenedione as the substrate for the aromatase enzyme and the IC_{50} and relative potency to aminoglutethimide data are included. A SAR study indicated that 3-[(4-fluorophenyl)(1*H*-imidazol-1-yl)methyl]-1-ethyl-2-methyl-1*H*-indole (28 g) was a highly potent and selective aromatase inhibitor with IC_{50} value of 0.025 μM. 28 g was also a weak inhibitor of androstenedione synthesis.

Keywords: Aromatase; 17-α-Hydroxylase/17,20-lyase; Azoles; Breast cancer

INTRODUCTION

In Europe, every year breast cancer brings about 70,000 deaths[1,2] and is consequently the most frequent form of cancer among women. Two thirds of breast cancer in postmenopausal women is sensitive to hormones, so hormonotherapy is an effective treatment option.

Estrogens support the growth of breast cancer cells. The ovaries are the primary source of estrogens in the fertile woman but after menopause, estrogens are produced in peripheral tissue (e.g. muscle, adipose tissue) *via* aromatisation of androgens to estrogens requiring a microsomal enzyme complex, called aromatase. This complex is formed by cytochrome P450 hemoprotein, aromatase (CYP19, P450arom) and a redox partner, NADPH P450 oxidoreductase, which contains FAD and FMN cofactors. Consequently, CYP19 belongs to the class II of eukaryotic microsomal P450s[3] and catalyses the last step of the biotransformation of androstenedione and testosterone to estrone (E_1) and estradiol (E_2), respectively. E_2 is the biologically active estrogen (Figure 2).

Endocrine therapy in breast cancer is directed either to block estrogen action by antiestrogens at the receptor level in the tumor or to reduce the synthesis of estrogens. Antiestrogens, belonging to the concept of selective estrogen receptor modulators, SERMs,[4] such as tamoxifen and toremifen, have proved to be a significant advance in the treatment of hormone-dependent breast cancer and are presently used as a standard first-line therapy. However, tamoxifen has a partial agonist activity and some patients become resistant to this drug. Consequently, the reduction or blockade of E_2 biosynthesis becomes an important strategy for treatment. Two principal pathways are implicated in biosynthesis of E_2, (i) the sulfatase pathway which converts estrone sulfate (E_1S) into E_1 by the estrone-sulfatase, E_1 is then transformed into E_2 by the action of a reductive 17β-hydroxysteroid dehydrogenase type I; (ii) the aromatase pathway which transforms androgens to E_1 and E_2. Our group chose to target CYP19, leading to the design and synthesis of non-steroidal aromatase inhibitors (NSAIs).

NSAIs are competitive inhibitors which bind to the heme iron of the enzyme. The first drug of this type launched on the market was aminoglutethimide (AG) but its use has been limited due to a lack of specificity and intrinsic toxicity. Further investigations by many research teams have

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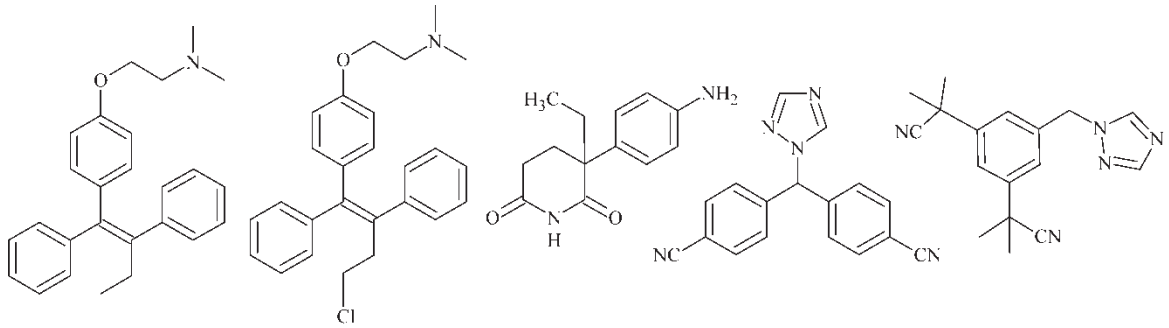


FIGURE 1 Structures of some SERMs and NSAIs.

notably resulted in the discovery of several azole derivatives, such as letrozole and anastrozole.[5–7] These two compounds have become the established second-line treatment for metastatic hormone-dependent breast cancer after SERMs and have recently been authorized as first-line therapy in several countries (Figure 1).

Previously, we described potent NSAIs based on an indole skeleton with azolylbenzyl as side chain;[8] the choice of the main heterocycle was focused on indole by analogy with the structure of zindoxifen, a formerly-studied antiestrogen.[9] Two compounds, 5-bromo-1-ethyl-2-[(4-fluorophenyl)(1*H*-imidazol-1-yl)methyl]-3-methyl-1*H*-indole **1** and 5-bromo-1-ethyl-3-[(4-fluorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole **2**, showed IC_{50} values of 0.238 and 0.0518 μM, respectively. These encouraging results prompted us to extend pharmacomodulation in the previously studied series, 2- and 3-(α-azolylbenzyl)indoles, in order to establish structure-activity relationships (Figure 3).

In respect to liarozole and vorozole, the substitution of on a phenyl group with a chlorine atom seems to be interesting, so we followed our pharmacomodulations in this way and we report here the pharmacological evaluation of some new indole derivatives as aromatase and 17-α-hydroxylase/17,20-lyase (CYP17) inhibitors (Figure 4).

MATERIALS AND METHODS

Chemistry

All common chemicals and solvents utilized were reagent grade and purchased from Sigma-Aldrich (Saint-Quentin, France). Melting points were determined on a Electrothermal IA9300 melting point digital apparatus and are reported uncorrected. Infrared (IR) spectra were obtained in KBr pellets or neat liquid films with a Perkin-Elmer Paragon FTIR 1000 PC spectrometer. ^{1}H-NMR spectra were obtained using a Bruker AC 250 apparatus operating at 250 MHz with d_6-DMSO as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard. EI-MS spectra were obtained on

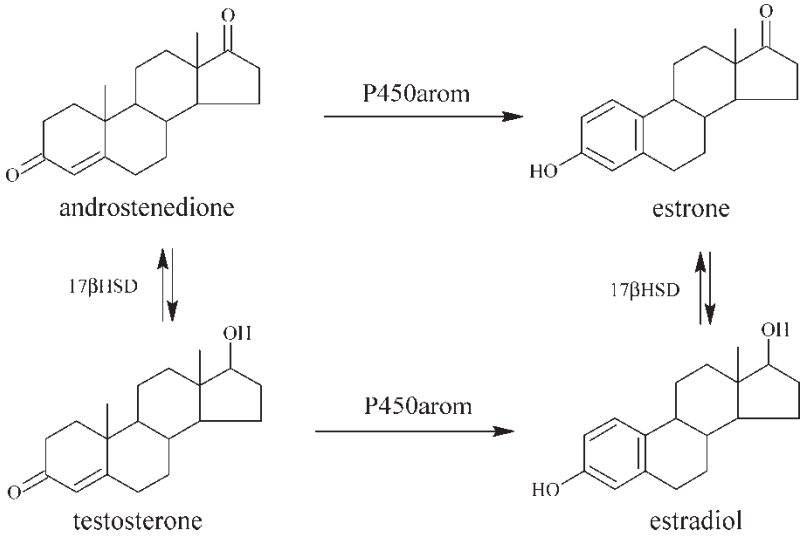


FIGURE 2 Conversion of androgens to estrogens.

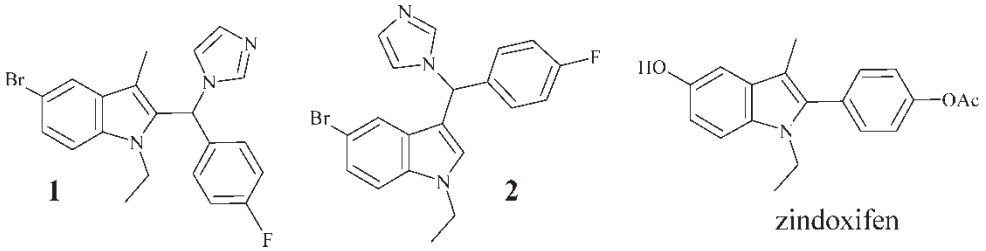


FIGURE 3 Structures of **1**, **2** and zindoxifen.

a Hewlett-Packard HP 5989A spectrometer (250°C, 70 eV). All reactions were monitored by TLC, using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with dichloromethane (DCM) or dichloromethane/ethanol gradients. Compounds were purified by column chromatography (CC) using silica gel 60 as stationary phase and eluted with dichloromethane or dichloromethane/ethanol gradients. 3-Methyl-1*H*-indole **3** was purchased from Sigma-Aldrich (Saint-Quentin, France). The synthesis of 5-bromo-3-methyl-1*H*-indole **4** has been previously described.[10] 5-Bromo-1*H*-indole **9**, 5-chloro-1*H*-indole **10**, 5-fluoro-1*H*-indole **11** and 2-methyl-1*H*-indole **13** were purchased from Sigma-Aldrich (Saint-Quentin, France). 5-Cyano-1*H*-indole **12** was prepared as described in reference.[11]

Synthesis

5-Bromo-1-ethyl-3-methyl-1*H*-indole (**6**)

To anhydrous DMF (30 mL), at 25°C, was added NaH (1.22 g, 30.6 mmol) as a suspension (60%) in mineral oil. The mixture was gently heated at 40°C and 5-bromo-3-methyl-1*H*-indole **4** (2.14 g, 10.2 mmol) was added gradually. When evolution of H_2 had ceased, the mixture was cooled to 25°C, and ethyl iodide (1.23 mL, 15.3 mmol) was added. The reaction mixture was heated for 6 h. Most of the DMF was removed under reduced pressure, and the residue was dissolved in water and DCM. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue was purified by CC using DCM as eluent and appropriate fractions gave **6**. Yield: 92%, yellow liquid. IR (NaCl) cm^{-1}: 2974, 2923, 2855 (νCH alkane). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 1.33 (t, 3H, $^3J = 7.20$, CH_3), 2.25 (s, 3H, CH_3), 4.15 (q, 2H, $^3J = 7.20$, CH_2), 7.22 (s, 1H, H^2), 7.25 (dd, 1H, $^3J = 8.70$, $^4J = 1.60$, H^6), 7.42 (d, 1H, $^3J = 8.70$, H^7), 7.69 (d, 1H, $^4J = 1.60$, H^4).

Compound **5** was prepared in a similar way as described for **6**.

5-Bromo-2-(3-chlorobenzoyl)-1-ethyl-3-methyl-1*H*-indole (**9**)

To a magnetically stirred suspension of $AlCl_3$ (1.33 g, 10 mmol) in DCM (25 mL) at 25°C was added 3-chlorobenzoyl chloride (1.28 mL, 10 mmol) and the mixture was stirred for 1 h. A solution of 5-bromo-1-ethyl-3-methyl-1*H*-indole **6** (1.07 g, 4.5 mmol) in DCM (10 ml) was added dropwise. The reaction mixture was stirred at room temperature for 12 h. The solution was poured onto crushed ice and ethyl acetate. After extraction with ethyl acetate (2 × 50 mL), the combined organic layers were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuum to give the corresponding 2-benzoylindole **9**. Yield: 80%, yellow oil. IR (NaCl) cm^{-1}: 2972, 2931, 2872 (νCH alkane), 1640 (νC=O). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 1.28 (t, 3H, $^3J = 7.0$, CH_3), 2.02 (s, 3H, CH_3), 4.36 (q, 2H, $^3J = 7.0$, CH_2), 7.52 (dd, 1H, $^3J = 8.75$, $^4J = 1.80$, H^6), 7.64 (d, 1H, $^3J = 8.75$, H^7), 7.65–7.68 (m, 1H, $H^{5'}$), 7.75–7.84 (m, 3H, $H^{2'}$, $H^{4'}$, $H^{6'}$), 7.93 (d, 1H, $^4J = 1.80$, H^4).

Compounds **7**, **8** and **10**, **11** were prepared in a similar way as described for **9**.

5-Bromo-2-[(3-chlorophenyl)(1*H*-imidazol-1-yl) methyl]-1-ethyl-3-methyl-1*H*-indole (**12d**)

A solution of sodium borohydride (21 mmol) in methanol (10 mL) was added dropwise to a solution of 5-bromo-2-(3-chlorobenzoyl)-1-ethyl-3-methyl-1*H*-indole **9** (2.64 g, 7 mmol) in methanol (10 mL) and the reaction mixture was stirred at room temperature for 1 h. Water (30 mL) was added and the solution was extracted three times with diethyl ether. The combined organic layers were dried (Na_2SO_4) and the solvent was carefully evaporated leaving a light yellow oil. The corresponding carbinol (2.31 g, 6.1 mmol) and CDI (0.99 g, 6.1 mmol) in dry THF (20 mL) were stirred at room temperature for 3 h. The reaction mixture was

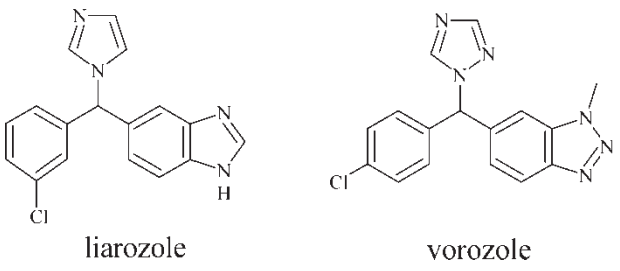


FIGURE 4 Structures of liarozole and vorozole.

partitioned between water and diethyl ether and extracted three times with diethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by CC (DCM/absolute ethanol: 19/1) and recrystallized from ethyl acetate to give the corresponding imidazole derivative **12d**. Yield: 57%, white crytals; m.p.: 143–144°C (ethyl acetate). IR (KBr) cm^{-1}: 2985, 2924, 2906 (νCH alkane), 1596, 1505, 1474 (νC=C, νC=N). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 0.91 (t, $^3J = 6.60$, CH_3), 1.70 (s, 3H, CH_3), 4.18–4.39 (m, 2H, CH_2), 6.95–6.99 (m, 2H, $H^{5'}$, $H^{6'}$), 7.08 (s, 1H, CH), 7.15 (s, 1H, Himid), 7.33 (d, 1H, $^3J = 8.60$, H^6), 7.42–7.49 (m, 4H, H^7, $H^{2'}$, $H^{4'}$, Himid), 7.74 (s, 2H, H^4, Himid). EI-MS: $m/z(\%) = 428(35)[M^+]$, 362 (100), 361 (20, $[M^+ - 67$, imidazole]) 360 (74).

Compounds **12b, 12f** and **12h** were prepared in a similar way as described for **12d**.

5-Bromo-2-[(3-chlorophenyl)(1*H*-1,2,4-triazol-1-yl)methyl]-1-ethyl-3-methyl-1*H*-indole (**12e**)

Thionyl chloride (0.61 mL, 8.4 mmol) was dropped onto an ice-cooled solution of 1*H*-1,2,4-triazole (2.32 g, 33.6 mmol) in dry acetonitrile (30 mL). The mixture was stirred at room temperature for 1 h, then filtered. This solution was added dropwise to a solution of the corresponding carbinol (0.90 g, 2.1 mmol, obtained as described for **12d**) in dry acetonitrile (10 mL). After addition, the mixture was stirred at room temperature for 2–4 h, then filtered and concentrated. The residue was dissolved in DCM. The organic solution was washed with brine, dried over Na_2SO_4, filtered, and evaporated to provide the crude product mixture, which was purified by CC (DCM/absolute ethanol: 19/1) and recrystallized from acetonitrile. Yield: 40%, white crystals; m.p.: 73–74°C (acetonitrile). IR (KBr) cm^{-1}: 2980, 2929, 2874 (νCH alkane), 1602, 1580, 1499, 1478 (νC=C, νC=N). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 0.90 (t, 3H, $^3J = 6.90$, CH_3), 1.88 (s, 3H, CH_3), 4.20–4.43 (m, 2H, CH_2), 6.97–7.04 (m, 2H, $H^{5'}$, $H^{6'}$), 7.34 (dd, 1H, $^3J = 8.80$, $^4J = 1.45$, H^6), 7.45–7.50 (m, 2H, $H^{2'}$, $H^{4'}$), 7.46 (d, 1H, $^3J = 8.80$, H^7), 7.63 (s, 1H, CH), 7.75 (d, 1H, $^4J = 1.45$, H^4), 8.21 (s, 1H, Htriaz), 8.74 (s, 1H, Htriaz). EI-MS: m/z (%) = 429 (8) $[M^+]$, 362 (40), 361 (100,$[M^+ - 68$, triazole]), 360 (55), 347 (6), 346 (19, −15) [methyl], 345 (8).

Compounds **12a, 12c** and **12g** were prepared in a similar way as described for **12e**.

5-Bromo-1-n-propyl-1*H*-indole (**18**)

To anhydrous DMF (30 mL), at 25°C, was added NaH (1.22 g, 30.6 mmol) as a suspension (60%) in mineral oil. The mixture was gently heated at 40°C and 5-bromo-1*H*-indole **9** (2.0 g, 10.2 mmol) was added gradually. When evolution of H_2 had ceased, the mixture was cooled to 25°C, and n-propyl iodide (1.49 mL, 15.3 mmol) was added. The reaction mixture was heated for 2 h. Most of the DMF was removed under reduced pressure, and the residue was dissolved in water and DCM. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue was purified by CC using DCM as eluent and appropriate fractions gave **18**. Yield: 95%, yellow oil. IR (KBr) cm^{-1}: 2972, 2940, 2920 (νCH alkane). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 0.84 (t, 3H, $^3J = 7.40$, CH_3), 1.72–1.85 (m, 2H, CH_2), 4.16 (t, 2H, $^3J = 7.00$, CH_2), 6.45 (d, 1H, $^3J = 3.10$, H^3), 7.26 (dd, 1H, $^3J = 8.70$, $^4J = 1.90$, H^6), 7.46 (d, 1H, $^3J = 3.10$, H^2), 7.54 (d, 1H, $^3J = 8.70$, H^7), 7.76 (d, 1H, $^4J = 1.90$, H^4).

Compounds **14**–**17** and **19**, **20** were prepared in a similar way as described for **18**.

5-Bromo-3-(4-fluorobenzoyl)-1-n-propyl-1*H*-indole (**25**)

To a magnetically stirred suspension of $AlCl_3$ (0.97 g, 7.3 mmol) in DCM (25 mL) at 25°C was added 4-fluorobenzoyl chloride (1.16 g, 7.3 mmol) and the mixture was stirred for 1 h. A solution of 5-bromo-1-n-propyl-1*H*-indole **18** (1.46 g, 6.1 mmol) in DCM (10 ml) was added dropwise. The reaction mixture was heated at reflux for 8 h. The solution was poured onto crushed ice and ethyl acetate. After extraction with ethyl acetate (2 × 50 mL), the combined organic layers were washed with water, dried over Na_2SO_4, and concentrated in vacuum to give the corresponding 3-benzoylindole **25**. Yield: 42%, brown crystals; m.p.: 142–144°C (diisopropyl ether/ethanol: 80/20). IR (KBr) cm^{-1}: 2960, 2930, 2876 (νCH alkane), 1618 (νC=O). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 0.87 (t, 3H, $^3J = 7.05$, CH_3), 1.78–1.87 (m, 2H, CH_2), 4.28 (t, 2H, $^3J = 7.40$, CH_2), 7.41 (dd, 2H, $^3J_{HH} = {}^3J_{HF} = 8.80$, $H^{3'}$, $H^{5'}$), 7.48 (dd, 1H, $^3J = 8.75$, $^4J = 1.65$, H^6), 7.70 (d, 1H, $^3J = 8.75$, H^7), 7.91 (dd, 2H, $^3J_{HH} = 8.45$, $^4J_{HF} = 5.65$, $H^{2'}$, $H^{6'}$), 8.18 (s, 1H, H^2), 8.45 (d, 1H, $^4J = 1.65$, H^4).

Compounds **21**–**24** and **26**, **27** were prepared in a similar way as described for **25**.

5-Bromo-1-ethyl-3-[(4-fluorophenyl)(2-methyl-1*H*-imidazol-1-yl)methyl]-1*H*-indole (**28a**)

Thionyl chloride (0.58 mL, 8.0 mmol) was dropped onto an ice-cooled solution of 2-methyl-1*H*-imidazole (2.63 g, 32.0 mmol) in dry acetonitrile (20 mL). The mixture was stirred at room temperature for 1 h, then filtered. This solution was added dropwise to a solution of the corresponding carbinol (0.70 g, 2.0 mmol, obtained as described for **12d**) in dry acetonitrile (5 mL). After addition, the mixture was stirred at room temperature for 1 h, then filtered and concentrated. The residue was dissolved in DCM. The organic solution was washed with brine, dried over Na_2SO_4, filtered, and evaporated to provide the crude product mixture, which was purified by CC (DCM/absolute ethanol: 19/1). Yield: 45%, yellow crystals; m.p.: 147–149°C (diisopropyl ether/DCM: 90/10). IR (KBr) cm^{-1}: 2976, 2931, 2886 (νCH alkane),

1605, 1523, 1508, 1473 (νC=C, νC=N). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 1.32 (t, 3H, $^3J = 7.10$, CH_3), 2.32 (s, 3H, CH_3), 4.21 (q, 2H, $^3J = 7.10$, CH_2), 6.73 (d, 1H, $^3J = 1.20$, Himid), 6.75 (d, 1H, $^3J = 1.20$, Himid), 6.98–7.01 (m, 2H, H^2, CH), 7.25–7.33 (m, 4H, $H^{3'}$, $H^{5'}$, H^4, H^6), 7.33–7.35 (m, 2H, $H^{2'}$, $H^{6'}$), 7.39 (d, 1H, $^3J = 8.50$, H^7). EI-MS: *m/z* (%) = 412 (1) [M^+], 332 (100), 331 (20, [$M^+ - 81$, 2-methylimidazole]), 330 (99), 223 (11), 222 (30, [$M^+ - 109$, 4-F-C_6H_4]), 221 (7).

5-Bromo-3-[(4-fluorophenyl)(1*H*-imidazol-1-yl)-methyl]-1-n-propyl-1*H*-indole (**28e**)

Using the same procedure as for **12d**, compound **28e** was obtained, from 5-bromo-3-(4-fluorobenzoyl)-1-n-propyl-1*H*-indole **25** (0.80 g, 2.2 mmol). Yield: 75%, white powder; m.p.: 120°C (diethyl ether). IR (KBr) cm^{-1}: 2984, 2934, 2864 (νCH alkane). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 0.81 (t, 3H, $^3J = 7.35$, CH_3), 1.69–1.78 (m, 2H, CH_2), 4.14 (t, 2H, $J = 6.90$, CH_2), 6.99 (s, 1H, Himid), 7.12–7.36 (m, 8H, H^2, H^4, H^6, $H^{2'}$, $H^{3'}$, $H^{5'}$, $H^{6'}$, CH), 7.20 (s, 1H, Himid), 7.54 (d, 1H, $^3J = 8.70$, H^7), 7,77 (s, 1H, Himid). EI-MS: *m/z* (%) = 413 (7) [M^+], 347 (24), 346 (100, [$M^+ - 67$, imidazole]), 345 (23), 304 (16), 303 (6, [$M^+ - 43$, n-propyl]), 302 (17).

Compounds **28b–d** and **28f–g** were prepared in a similar way as described for **28e**.

1-ethyl-3-[(4-Fluorophenyl)(1*H*-1,2,4-triazol-1-yl)methyl]-2-methyl-1*H*-indole (**28 h**)

A solution of sodium borohydride (0.52 g, 14 mmol) in methanol (10 mL) was added dropwise to a solution of 1-ethyl-3-(4-fluorobenzoyl)-2-methyl-1*H*-indole **27** (1.96 g, 7 mmol) in methanol (10 mL). The reaction mixture was stirred at room temperature for 2 h. Water (30 mL) was added and the solution was extracted three times with diethyl ether. The combined organic layers were dried (Na_2SO_4) and the solvent was carefully evaporated leaving a light yellow oil. Dry toluene (10 mL) was added to the carbinol. 1-(Trimethylsilyl)-1*H*-1,2,4-triazole (2.1 g, 15.4 mmol) was placed in a flask under nitrogen atmosphere and thionyl chloride (0.6 mL, 8.4 mmol) was added dropwise. The mixture was stirred at room temperature for fifteen minutes. Dry toluene (10 mL) was added, followed by the addition of the carbinol. The reaction mixture was stirred overnight at room temperature. The residue was purified by CC using DCM/ethanol: 19/1 as eluent and appropriate fractions gave **28 h**. Yield: 63%, white powder; m.p.: 122–123°C (DCM/ethanol: 80/20). IR (KBr) cm^{-1}: 2969 (νCH alkane), 1608, 1561, 1508 (νC=C, νC=N). ^{1}H-NMR (d_6-DMSO), δ (ppm), J (Hz): 1.25 (t, 3H, $^3J = 7.16$, CH_3), 2.40 (s, 3H, CH_3), 4.19 (q, 2H, $^3J = 7.16$, NCH_2), 6.88 (dd, 1H, $^3J = {}^3J = 7.60$, H^5), 7.01 (dd, 2H, $^3J_{HH} = 8.80$, $^4J_{HF} = 5.60$, $H^{2'}$, $H^{6'}$), 7.06 (dd, 1H, $^3J = {}^3J = 7.60$, H^6), 7.14 (d, 1H, $^3J = 7.60$, H^7), 7.17 (dd, 2H, $^3J_{HH} = {}^3J_{HF} = 8.80$, $H^{3'}$, $H^{5'}$), 7.32 (s, 1H, CH), 7.46 (d, 1H, $^3J = 7.60$, H^4), 8.05 (s, 1H, Htriaz), 8.56 (s, 1H, Htriaz). EI-MS: *m/z* (%) = 334 (16) [M^+], 266 (100, [$M^+ - 68$, triazole]), 237 (18, −29) [ethyl].

Pharmacology

Inhibition of Aromatase In Vitro

Preparation of the Enzyme

The enzyme was obtained from the microsomal fraction of freshly delivered human term placental tissue according to the method previously described[12] and stored at −70°C.

Enzyme Inhibition Tests

Method (a) The assay was performed according to previously described methods.[13,14] In brief, the reaction mixture, containing, [1β,2β-^{3}H]testosterone (0.225 μCi), unlabeled testosterone (5 μM), the NADPH-generating system, the inhibitor (0–250 μM) and phosphate buffer (0.05 M, pH 7.4) was preincubated for 5 min at 30°C. Microsomal protein (0.5 mg, from human placental tissue) was added to start the reaction. The reaction was terminated by withdrawing 100-μL aliquots at 0, 7, 14, 21 min and pipetting them into 200 μL of a cold $HgCl_2$ solution (1 mM). After addition of 200 μL of an aqueous dextran-coated charcoal (DCC) suspension (2%), the vials were shaken then centrifugated to separate the charcoal-absorbed steroids. Aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture in a Beckman liquid scintillation spectrometer (LS8000).

Method (b) The assay was performed according to previously described methods.[13,14] In brief, the reaction mixture, containing, [1β-^{3}H]androstenedione (0.08 μCi, 15 nM), unlabeled androstenedione (485 nM), the NADPH-generating system, the inhibitor (0–100 μM) and phosphate buffer (0.05 M, pH 7.4) was preincubated for 5 min at 30°C. Microsomal protein (0.1 mg, from human placental tissue) was added to start the reaction. After incubation for 14 min at 30°C, the reaction was stopped by adding 200 μL of a cold $HgCl_2$ solution (1 mM). After addition of 200 μL of an aqueous dextran-coated charcoal (DCC) suspension (2%), the vials were shaken then centrifugated to separate the charcoal-absorbed steroids. Aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture using a LKB-Wallac β-counter.

Determination of the IC_{50} Value

The calculation of the IC_{50} values was performed by plotting the percent inhibition *vs.* the concentration of inhibitor on a semilog plot. From this the molar concentration causing 50% inhibition was calculated.

Inhibition of 17 α-hydroxylase/17,20-lyase In Vitro

Preparation of the Enzyme

The enzyme was obtained from rat testes by the method previously described[15] and stored at −70°C.

Enzyme Inhibition Test

The assay was performed similar to described methods.[15] In brief, the reaction mixture containing, progesterone (1.25 mM), NADPH (125 nmol), the inhibitor and phosphate buffer (pH 7.4) was preincubated for 5 min at 32°C. Microsomal protein was added to start the reaction. After incubation of 20 min at 32°C, the reaction was stopped by adding 50 μL of 1 M HCl solution. After addition of 1 mL ethyl acetate, the vials were shaken then centrifuged. The organic phase was removed, vortexed with phosphate buffer (250 μL), 1 M HCl (50 μL) and then dried. Aliquots of 25 μL methanol, containing 250 pmol of fluorocortisol acetate as internal standard, were added to the extracts. The samples (20 μL) were submitted to HPLC (RP-8 column, methanol:water 1:1, v/v) and measured using UV (240 nm).

For compounds causing an inhibition over 80%, IC_{50} values were determined.

RESULTS AND DISCUSSION

Chemistry

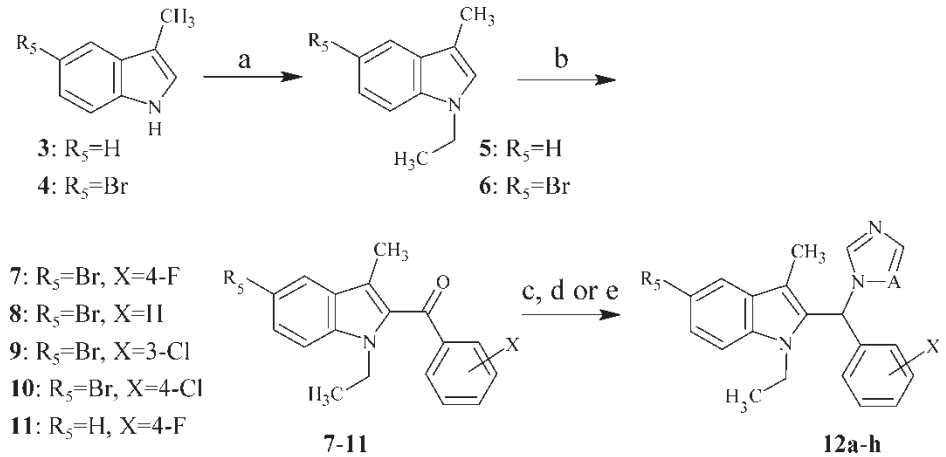
All ketones **7**–**11** were classically prepared by a Friedel-Crafts procedure involving 1,3-disubstituted indoles **5,6** with halogenobenzoyl chlorides (Scheme 1).[16,17] Reduction of the ketones with sodium borohydride gave the secondary alcohols in quantitative yields, and sufficiently pure after an aqueous extraction to be used in the following reactions. The alcohols were treated with *N*,*N*′-carbonyldiimidazole (CDI)[18] to give the desired imidazole derivatives **12b**, **12d**, **12f** and **12h**. The introduction of a triazole moiety was also accomplished, using thionyl chloride and 1*H*-1,2,4-triazole in acetonitrile to obtain the intermediate sulfinylditriazole (SDT)[19] which reacted with the corresponding carbinols, to afford the triazol-1-yl isomers **12a**, **12c**, **12e** and **12g**. After purification by CC, the structures of all azole compounds were confirmed by ^{1}H NMR and mass spectral data.

Synthesis of 3-(α-azolylbenzyl)indoles **28a**–**h** was carried out in four steps as depicted in Scheme 2. N_1-Substitution of the indole ring by the alkyl chain (ethyl, n-propyl, i-propyl) was performed after deprotonation of the nitrogen using sodium hydride in anhydrous dimethylformamide and afforded compounds **14**–**20**. Regioselective aroylation of *N*-substituted indoles **14**–**20** in position-3 was carried out, using $AlCl_3$ catalysis in DCM, to give the 3-acylindole derivatives **21**–**27**. After reduction of the carbonyl group with sodium borohydride in methanol, the corresponding alcohols were reacted with CDI or SDT,[20] leading to compounds **28b**–**g** and **28h**, respectively. In the case of compound **28a**, *N*,*N*′-carbonyldi-(2-methyl)imidazole was first prepared using 2-methyl-1*H*-imidazole and thionylchloride as depicted with SDT.[19]

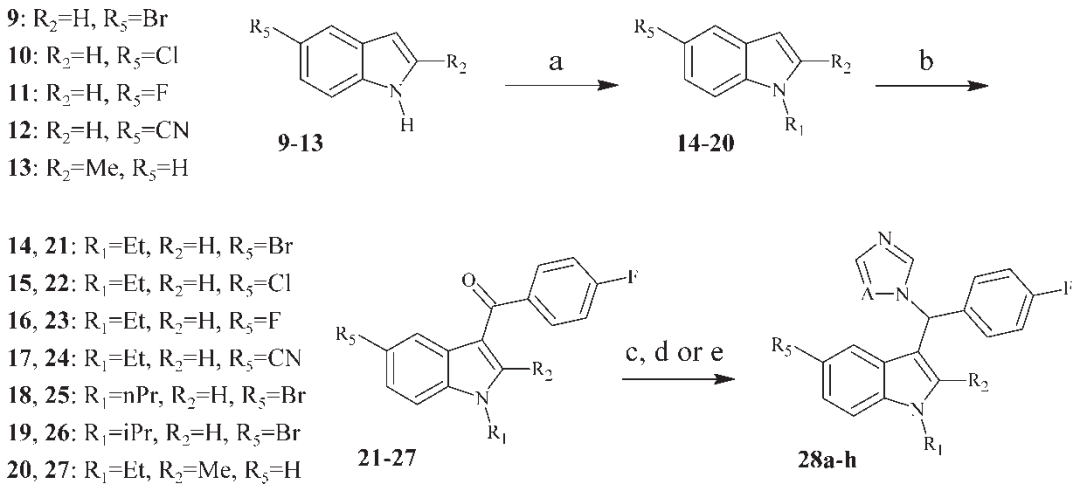
The structures of the studied indole derivatives **12a**–**h** and **28a**–**h** are shown in Figure 5.

Pharmacology

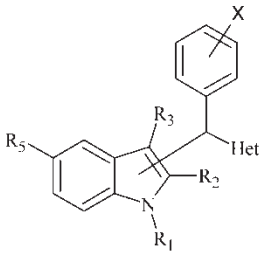
The compounds were tested for aromatase inhibitory activity according to the literature procedure.[15] The IC_{50} values and the potencies of the compounds, relative to AG, are given in Table I. Furthermore, the assay was performed using as substrate either [1β,2β-^{3}H]testosterone or [1β-^{3}H]androstenedione.



SCHEME 1 Synthesis of compounds **12a**–**h**. Reagents and conditions: (a) NaH, DMF, EtI, 40°C; (b) $AlCl_3$, DCM, benzoyl chloride, r.t.; (c) $NaBH_4$, methanol, r.t.; (d) CDI, THF, r.t.; (e) SDT, acetonitrile, r.t.



SCHEME 2 Synthesis of compounds **28a–h**. Reagents and conditions: (a) NaH, DMF, R_1I, 40°C; (b) $AlCl_3$, DCM, benzoyl chloride, reflux; (c) $NaBH_4$, methanol, r.t.; (d) CDI, THF, r.t.; (e) 1-(trimethylsilyl)-1*H*-1,2,4-triazole, $SOCl_2$, toluene, r.t.



No.	Azolylmethyl grouping position on indole nucleus	R_1	R_2	R_3	R_5	X	Het
12a	2	Et	-	CH_3	Br	4-F	1,2,4-triaz
12b	2	Et	-	CH_3	Br	H	imid
12c	2	Et	-	CH_3	Br	H	1,2,4-triaz
12d	2	Et	-	CH_3	Br	3-Cl	imid
12e	2	Et	-	CH_3	Br	3-Cl	1,2,4-triaz
12f	2	Et	-	CH_3	Br	4-Cl	imid
12g	2	Et	-	CH_3	Br	4-Cl	1,2,4-triaz
12h	2	Et	-	CH_3	H	4-F	imid
28a	3	Et	H	-	Br	4-F	2-methyl-imid
28b	3	Et	H	-	Cl	4-F	imid
28c	3	Et	H	-	F	4-F	imid
28d	3	Et	H	-	CN	4-F	imid
28e	3	nPr	H	-	Br	4-F	imid
28f	3	iPr	H	-	Br	4-F	imid
28g	3	Et	CH_3	-	H	4-F	imid
28h	3	Et	CH_3	-	H	4-F	1,2,4-triaz

FIGURE 5 Structures of the studied indole derivatives **12a–h** and **28a–h**.

TABLE I *In vitro* inhibition of human aromatase by 2-(α-azolylbenzyl)indoles **12a–h** and 3-(α-azolylbenzyl)indoles **28a–h**

Compound	% inhibition*	IC_{50} (μM)†	RP‡
1	96.5	0.2400	77.1
12a	82.0	3.4600	5.3
12b	96.0	0.4200	44.0
12c	83.0	3.2700	5.7
12d	91.0	0.9400	19.7
12e	71.0	17.5000	1.1
12f	91.0	0.4100	45.1
12g	91.0	1.8800	9.8
12h	–	0.120	154.00
2	98.0	0.052	355.80
28a	55.2	–	–
28b	–	0.150	123.30
28c	–	0.0600	308.30
28d	–	0.140	132.00
28e	–	0.3200	57.80
28f	–	0.3400	54.40
28g	–	0.0320	578.10
	–	0.025§	1192.00¶
28h	–	0.3200§	93.10¶

*Human placental microsomes, [1β,2β-^{3}H]testosterone concentration 2.5 μM, inhibitor concentration 25 μM. †IC_{50} value of aminoglutethimide under identical experimental conditions: 18.5 μM. ‡Relative potency: RP=IC_{50}(AG)/IC_{50} (tested compound). §Human placental microsomes, [1β-^{3}H]androstenedione/androstenedione concentration 0.5 μM, IC_{50} values of aminoglutethimide and fadrozole under identical experimental conditions: 29.8 and 0.03 μM, respectively. ¶Relative potency: RP=IC_{50}(AG)/IC_{50} (tested compound).

TABLE II *In vitro* inhibition of rat 17α-hydroxylase/17,20-lyase by 2-(α-azolylbenzyl)indoles **12a–h** and 3-(α-azolylbenzyl)indoles **28a–h**

Compound	% inhibition*
1	30.2
12a	10.0
12b	43.2
12c	08.0
12d	27.1
12e	23.6
12f	24.4
12g	6.7
12h	36.0
2	3.7
28a	8.3
28b	14.2
28c	28.0
28d	8.0
28e	13.0
28f	5.0
28g	60.0
28h	n.i.†

*Rat testicular microsomes, progesterone concentration 25 μM, inhibitor concentration 2.5 μM, ketoconazole: 62% inhibition. The given values are mean values of at least two experiments, the standard deviations were within ± 5%. †n.i.: no inhibition.

No significantly different results were obtained in the 2-(α-azolylbenzyl)indoles **12**. Test compounds **12a–h** exhibited moderate aromatase inhibitory activity *in vitro*. All triazole derivatives **12a**, **12c**, **12e** and **12g** were less potent than their imidazole analogues **12b**, **12d**, **12f** and **12h**. The replacement in compound **1** of the fluorine atom at the *para* position of the phenyl group by a hydrogen or chlorine atom (**12b**, **12d** and **12f**) exerted a deleterious effect. The removal of the bromine atom on indole in compound **1**, leading to **12h**, resulted in a 50% increase of activity: IC_{50} = 0.24 and 0.12 μM, respectively.

Next, we studied the inhibitory effect of 3-(α-azolylbenzyl)indoles **28** on estrogen synthesis. Compounds **28a–g** were their tested on potency to inhibit the aromatization of labeled testosterone. Furthermore, a second assay was performed for compounds **28g–h** using labeled androstenedione as substrate. The methyl substitution of the imidazole moiety of compound **2** resulted in loss of inhibitory activity. Replacement of the bromine atom on the indole ring with a chloro (**28b**) or cyano (**28d**) substituent gave a marked decrease in inhibitory activity. In the case of compound **28c** having the 5-fluoro group, the activity was maintained with an IC_{50} value of 0.06 μM. Moreover we investigated substitution of the indole nitrogen and both the n-propyl and i-propyl chains showed decreased potency. The 2-methylindole derivative **28g** exhibited inhibitory activity (IC_{50}: 0.025 μM) similar to that of CGS-16949A (fadrozole), a second generation CYP19 inhibitor, and was 1192-fold as active as AG. In both assays, **28g** was the most potent CYP19 inhibitor of this study; its triazole congener, **28h**, was 10-fold less active (IC_{50}: 0.32 μM). The imidazole compound **28g** constitutes a good candidate for an *in vivo* evaluation.

Additionally, a second pharmacological evaluation was carried out to determine the inhibitory activities of compounds **12a–h** and **28a–h** on 17α-hydroxylase/17,20-lyase (Table II). This screening was undertaken at a concentration of 2.5 μM. Ketoconazole as reference compound showed a 62% inhibition. In the 2-(α-azolylbenzyl)indoles **12a–h**, both imidazole and triazole derivatives were weak inhibitors and all inhibition values were less than 45%. In the second series of compounds **28a–h**, their androstenedione-synthesis inhibitory activity was also very weak. Only the most active CYP19 inhibitor, **28g**, exerted a significant inhibition (60%) of androstenedione biosynthesis but at a 100-fold higher concentration than that inducing 50% aromatase inhibition.

Pharmacomodulation of the previously described aromatase inhibitors **1** and **2**, leading notably to **12h** and **28g**, highlights in the two series the possibility of affording a higher level of activity with homocyclenon substituted indole derivatives and, in the second series, the favourable effect of a methyl group fixed at carbon 2. The CYP19 inhibitor **28g**, 1-ethyl-3-[(4-fluorophenyl)(1*H*-imidazol-1-yl)methyl]-2-methyl-1*H*-indole, developed in this study could be a promising lead compound to design new selective non-steroidal aromatase inhibitors. We will evaluate

in a further SAR study the effects of 4- to 7-(α-azolylbenzyl)indoles on aromatase inhibition.

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