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Synthesis and biological evaluation of 5-[(aryl)(1*H*-imidazol-1-yl)methyl]-1*H*-indoles: Potent and selective aromatase inhibitors

Marie-Pierre Lézé,[a,*] Marc Le Borgne,[a] Patricia Pinson,[a] Anja Palusczak,[b] Muriel Duflos,[a] Guillaume Le Baut[a] and Rolf W. Hartmann[b]

[a]*Department of Pharmacochemistry, BioCiT UPRES EA1155, Faculty of Pharmacy, Nantes University, 1 rue Gaston Veil, F-44035 Nantes Cedex, France*
[b]*Fachrichtung 8.5 Pharmaceutische und Medizinische Chemie, Universität des Saarlandes, PO Box 151150, D-66041 Saarbrücken, Germany*

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Abstract—The synthesis and the aromatase (CYP19) inhibitory activity of 5-[(aryl)(imidazol-1-yl)methyl]-1*H*-indoles were reported. Among the tested racemate compounds, 5-[(4-chlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole **8b** emerged as a potent CYP19 inhibitor (IC_{50} = 15.3 nM). Chiral chromatography allowed isolation of the (+) enantiomer **8b2**, which was about twice as active as the racemate (IC_{50} = 9 nM).

Breast cancer is the most common cancer in women and it causes 70,000 deaths per year in Europe.[1]

Estrogens are known to play a pivotal role in the proliferation of cancer cells.[2] In endocrine therapy, the two major approaches consist in: (i) blocking estrogen receptors using selective estrogen receptor modulators such as tamoxifen,[3] (ii) blocking estrogen biosynthesis using aromatase (CYP19) inhibitors such as aminoglutethimide (Fig. 1). Among the nonsteroidal aromatase inhibitors (NSAIs), more specific compounds than the well-established inhibitor aminoglutethimide have been marketed for the treatment of hormone-dependent breast cancer in postmenopausal women; letrozole[4] and anastrozole[5] are potent inhibitors exerting less severe side effects and are used in second-line therapy. However, several reports[6] showed advantages of NSAIs over tamoxifen in adjuvant treatment. Therefore, aromatase inhibitors represent an interesting alternative in the first-line therapy.

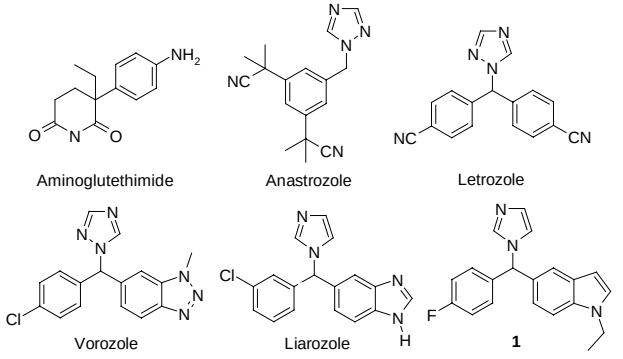

Figure 1. P450 enzyme inhibitors.

In our effort to design more potent and selective aromatase inhibitors, we have developed a novel class of NSAI-based 2-, 3-, 5- or 7-[(aryl)(azolyl)methyl]-1*H*-indoles.[7,8]

Considering the structure of reference drugs such as vorozole and liarozole (Fig. 1), we carried out pharmacomodulations in the imidazole series starting from compound **1** which exhibits a promising activity (IC_{50} = 0.040 μM). We present here the first data resulting from modifications at the level of indolic nitrogen

Keywords: Breast cancer; Aromatase inhibitors; 5-[(Aryl)-(imidazolyl)methyl]indoles.
* Corresponding author. Tel.: +33 2 40 41 11 14; fax: +33 2 40 41 28 76; e-mail: mpl_leze@yahoo.fr

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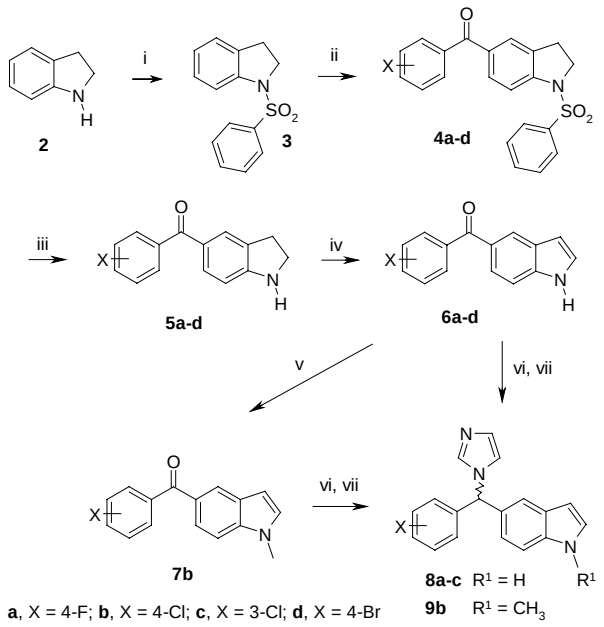
(R^1 = H, CH_3) and phenyl substitution (X = halogens, cyano).

Taking into account that the biosynthesis of androgens (androstenedione, testosterone) is also regulated by monooxygenase belonging to the cytochrome P450 enzyme family, 17α-hydroxylase-C17,20-lyase (CYP17), the studied compounds were also considered in order to determine their selectivity.

The synthetic pathway to provide 5-[(aryl)(1*H*-imidazolyl)methyl]-1*H*-indoles **8a–c**, **9b** is described in Scheme 1. It required three main steps: acylation of indoline precursor **3**, followed by oxidation into the indole counterparts and fixation of imidazole moiety.

1-Benzenesulfonyl-1*H*-indoline **3** was synthesized by sulfonation of the commercially available indoline **2** in the presence of triethylamine. The *N*-protected halogenobenzoylindolines **4a–d** were prepared by a Friedel–Crafts acylation with aluminum chloride as Lewis acid.[9,10] Compounds **5a–d** were obtained by deprotection of indolic nitrogen with sulfuric acid under microwave dielectric heating.[11] The key intermediates, aroylindoles **6a–d**, were prepared by oxidation using activated manganese oxide.[12,13] Alkylation of compound **6b** was achieved using methyl iodide in the presence of sodium hydride in dimethylsulfoxide to provide **7b**. The synthesis of target compounds **8a–c**, **9b**, and **11** (Scheme 1) was carried out by reduction of the ketone in the presence of sodium borohydride in methanol, followed by the fixation of imidazole moiety using 1,1′-carbonyldiimidazole (CDI) in dry acetonitrile.[14,15]



Scheme 1. Reagents and conditions: (i) $PhSO_2Cl$, Et_3N, $ClCH_2CH_2Cl$, 0 °C to rt, 1 h, quantitative; (ii) X-C_6H_4COCl, $AlCl_3$, CH_2Cl_2, reflux, rt, 4 h 1-week; (iii) H_2SO_4 80%, microwave, 40 W, 90 °C, 5 min, 85%-quantitative; (iv) MnO_2, CH_2Cl_2, reflux, 16 h, 73% quantitative; (v) NaH, CH_3I, DMSO, rt, 1 h, 95%; (vi) $NaBH_4$, CH_3OH, rt; (vii) CDI, CH_3CN, rt, 21–96 h, 30–53%.

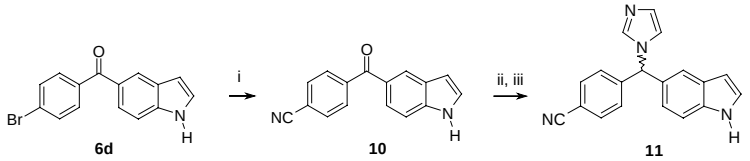
The compound **6d** was used as a precursor to afford the nitrile derivative **10** by a bromine/cyano exchange reaction in the presence of zinc cyanide and $Pd(PPh_3)_4$ as catalyst under microwave irradiation (Scheme 2).[16,17]

The in vitro antiaromatase activity of the compounds **8a–c**, **9b**, and **11** was evaluated using microsomal fraction of human placental tissue,[18] according to a previously described procedure.[19] [1β-^{3}H]Androstenedione (0.08 μCi, 15 nM), unlabeled androstenedione (485 nM), NADPH-generating system, and inhibitor (0–100 μM, DMSO as solvent) in phosphate buffer (pH 7.4) were preincubated for 5 min at 30 °C in a shaking water bath. Microsomal protein was added to start the enzymatic reaction. After incubation for 14 min at 30 °C, a cold $HgCl_2$ 1 mM solution and a Norit A 2% suspension were added. After shaking and centrifugations, aliquots of the supernatant were assayed for ^3H_2O by counting in a scintillation mixture using LKB-Wallac β-counter. The IC_{50} values were determined by plotting the percent inhibition versus the concentration of inhibitor on a semilog plot.

The CYP17 assay was performed in vitro using membrane fractions of recombinant *Escherichia coli* pJL17/OR coexpressing human CYP17/rat NADPH-P450 reductase and progesterone as a substrate.[20] Results are summarized in Table 1.

All the studied target racemate compounds showed a higher activity toward CYP19 than our previously described derivatives[7] or aminoglutethimide (AG, IC_{50} = 29.75 μM). Moreover, they were inactive on the inhibition of CYP17 (% inhibition <52).

Replacement of ethyl chain on indolic nitrogen in compound **1** by hydrogen (**8a**) increased 1.5-fold the activity (IC_{50} = 40 and 26 nM, respectively) and the decrease of activity by N-methylation of **8b**, leading to **9b** (IC_{50} = 15 and 24 nM, respectively), tend to confirm the detrimental effect of N-alkylation.



Scheme 2. Reagents and conditions: (i) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, microwave, 60 W, 153 °C, 3 min, 68%; (ii) $NaBH_4$, CH_3OH, rt; (iii) CDI, CH_3CN, rt, 23 h, 54%.

Table 1. In vitro CYP19 and CYP17 inhibitions by 5-[(aryl)(1*H*-imidazolyl)methyl]-1*H*-indoles

Compound	R[1]	X	CYP19		CYP17
			IC_{50}[a] (μM)	RP[b]	% inhibition[c]
8a	H	4-F	0.0263	1129	25
8b	H	4-Cl	0.0153	1144	28
(−)-**8b1**	H	4-Cl	0.045	661	26
(+)-**8b2**	H	4-Cl	0.009	3305	37
8c	H	3-Cl	0.0297	1000	52
9b	Me	4-Cl	0.0237	1255	24
11	H	4-CN	0.0193	1541	31
AG	—	—	29.75	1	—

[a] Values are the mean of at least two experiments performed in duplicate.
[b] Relative potency RP = IC_{50}(AG)/IC_{50} (tested compound).
[c] Progesterone (25 μM), inhibitor: 2.5 μM. Values are the mean of two experiments performed in duplicate.

The nature and position of halogen on phenyl moiety provided significant difference in aromatase inhibitory activity (compounds **8a**–**c**). Thus, a chlorine atom at the C-4 position of the phenyl ring (**8b**) showed the highest antiaromatase activity with an IC_{50} of 15 nM. Its replacement by a cyano group (**11**), present in different reference drugs, allowed a comparable level of activity. The liarozole analogue **8c**, with a chlorine atom fixed at carbon 3 of the phenyl ring, was the less active tested derivative.

To investigate the influence of stereochemistry on biological activity, the most active compound **8b** was separated into its enantiomers (−)-**8b1** and (+)-**8b2** using chiral HPLC.[21,22] The slower eluting enantiomer **8b2** was found to be highly potent, with an IC_{50} of 9 nM, in comparison with its racemate and its enantiomer which exhibited IC_{50} values of 15 and 45 nM, respectively. This result confirms that the configuration of the chiral center plays a key role in biological activity, as previously observed with the (+) enantiomer of vorozole (Fig. 1).[23]

In conclusion, we described some 5-[(aryl)(imidazol-1-yl)methyl]-1*H*-indoles acting as potent and selective CYP19 inhibitors. We showed the importance of a chlorine or cyano group on the C-4 position of the phenyl ring as well as a free indolic nitrogen. Moreover, we underlined the importance to separate the racemic compounds in order to enhance by far the IC_{50} values. X-ray crystallographic and molecular modeling studies of these enantiomers are now in progress. Although an imidazole is more efficient than a triazole moiety, the latter may be more selective and also more stable metabolically. So, it would be of interest to carry out future synthesis and evaluation works in the subseries of the corresponding triazole derivatives. In parallel, the inhibitory activity of 4 and 6-[(aryl)(azolyl)methyl]-1*H*-indoles will be investigated.

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10. Synthesis of (1-benzenesulfonyl-2,3-dihydro-1*H*-indol-5-yl)(4-chlorophenyl)methanone **4b**. To a suspension of aluminum chloride (3.29 g, 1.6 equiv) in 80 mL CH_2Cl_2 was added 4-chlorobenzoylchloride (4.32 mL, 1.6 equiv). After the mixture was stirred for 1 h at rt, a solution of 1-benzenesulfonyl-1*H*-indoline **3** (4 g, 15.42 mmol) in 30 mL CH_2Cl_2 was added dropwise. The reaction mixture was stirred overnight at rt prior to pouring onto crushed ice and CH_2Cl_2. The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic fractions were washed with brine and dried over Na_2SO_4. The solvent was evaporated and the residue was recrystallized from diisopropylic ether to afford compound **4b** in 95% overall yield.
Beige powder; mp 111–112 °C; IR (KBr): 3060 (CH_{arom}), 2937 (CH_{alkane}), 1648 (C=O), 1168 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.09 (t, J = 8.55 Hz, 2H, CH_2), 4.05 (t, J = 8.55 Hz, 2H, NCH_2), 7.61–7.76 (m, 10H, indolyl H-4, H-6, H-7, 4-chlorophenyl H-2, H-3, H-5, H-6, benzenesulfonyl H-3, H-4, H-5), 7.95 (d, J = 7.3 Hz, 2H, benzenesulfonyl H-2, H-6).
11. Synthesis of (2,3-dihydro-1*H*-indol-5-yl)(4-chloro-phenyl)methanone **5b**. A solution of **4b** (1 g, 2.51 mmol) in 2 mL of sulfuric acid (80%) was stirred for 5 min at 90 °C under MW irradiation (40 W). After cooling, H_2O and aqueous NaOH solution (25%) were added up to pH 9. The aqueous layer was extracted with Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was evaporated to give compound **5b** in 85% overall yield.
Ochre powder; mp 146–147 °C; IR (KBr): 3305 (NH), 3050 (CH_{arom}), 2937 (CH_{alkane}), 1579 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.03 (t, J = 8.55 Hz, 2H, CH_2), 3.61 (t, J = 8.55 Hz, 2H, NCH_2), 6.51 (d, J = 8.1 Hz, 1H, indolyl H-7), 6.73 (s, 1H, NH), 7.44 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H, indolyl H-6), 7.50 (s, 1H, indolyl H-4), 7.60 (d, J = 8.55 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.65 (d, J = 8.55 Hz, 2H, 4-chlorophenyl H-2, H-6).
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13. Synthesis of (4-chlorophenyl)(1*H*-indol-5-yl)methanone **6b**. A solution of **5b** (303 mg, 1.18 mmol), manganese

oxide (1.23 g, 12 equiv) in 20 mL CH_2Cl_2 was stirred overnight at 40 °C. After cooling, the reaction mixture was filtered through Celite. The solvent was removed to afford compound **6b** in 99% overall yield.
Gray powder; mp 159–160 °C; IR (KBr): 3397 (NH), 3060 (CH_{arom}), 1646 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.65 (d, J = 2.75 Hz, 1H, indolyl H-3), 7.54 (d, J = 2.75 Hz, 1H, indolyl H-2), 7.60 (d, J = 8.2 Hz, 1H, indolyl H-7), 7.64 (dd, J = 8.2 Hz, J = 1.8 Hz, 1H, indolyl H-6), 7.66 (d, J = 8.55 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.77 (d, J = 8.55 Hz, 2H, 4-chlorophenyl H-2, H-6), 8.03 (s, 1H, indolyl H-4), 11.60 (s, 1H, NH).

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15. Synthesis of 5-[(4-chlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole **8b**. Sodium borohydride (107 mg, 2 equiv) was added portionwise to a stirred solution of **6b** (360 mg, 1.41 mmol) in 20 mL methanol. The reaction mixture was stirred for 1 h at rt prior to quenching with H_2O. The aqueous layer was extracted with Et_2O. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to give a light yellow oil. The corresponding alcohol (363 mg, 1.41 mmol) and CDI (342 mg, 1.5 equiv) in CH_3CN were stirred for 40 h at rt. The solvent was removed and the residue was dissolved in H_2O and CH_2Cl_2. The layers were separated, the organic layer was washed with H_2O, and dried over Na_2SO_4. The solvent was evaporated and the residue was purified on silica gel (CH_2Cl_2–EtOH, 19:1 v/v) to give compound **8b** in 53% overall yield.
Beige powder; mp 172–173 °C; IR (KBr): 3449 (NH), 3120 (CH_{arom}), 1490 (C=C_{arom}) cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.44 (s, 1H, indolyl H-3), 6.96–6.98 (m, 3H, indolyl H-6, CH, imidazolyl H), 7.13 (s, 1H, imidazolyl H), 7.15 (d, J = 8.8 Hz, 2H, 4-chlorophenyl H-2, H-6), 7.32 (s, 1H, indolyl H-4), 7.43 (d, J = 8.8 Hz, 2H, 4-chlorophenyl H-3, H-5), 7.46 (dd, J = 2.9 Hz, 1H, indolyl H-2), 7.47 (d, J = 8.8 Hz, 1H, indolyl H-7), 7.65 (s, 1H, imidazolyl H), 11.20 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 63.5 (CH), 101.6 (C-3), 112.0 (C-7), 119.5 (imidazolyl C), 119.9 (C-4), 121.5 (C-6), 126.5 (C-2), 127.8 (C-4a), 128.8 (3C, imidazolyl C, 4-chlorophenyl C-3, C-5), 129.6 (2C, 4-chlorophenyl C-2, C-6), 130 (C-5), 132.4 (4-chlorophenyl C-4), 135.6 (C-7a), 137.4 (imidazolyl C), 140.3 (4-chlorophenyl C-1).
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17. Synthesis of 4-(1*H*-indol-5-ylcarbonyl)benzonitrile **10**. A solution of **6d** (500 mg, 1.66 mmol), $Zn(CN)_2$ (195 mg, 1 equiv), and $Pd(PPh_3)_4$ (57 mg, 3% equiv) in 2 mL of DMF was stirred for 4 min at 153 °C in a sealed vial under MW irradiation (60 W). After cooling, AcOEt was added. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed and the residue was purified on silica gel (CH_2Cl_2) to afford compound **10** in 68% overall yield.
Beige powder; mp 185–186 °C; IR (KBr): 3380 (NH), 2223 (C≡N), 1644 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.66 (d, J = 2.7 Hz, 1H, indolyl H-3), 7.55 (dd, J = 2.7 Hz, 1H, indolyl H-2), 7.58 (d, J = 8.6 Hz, 1H, indolyl H-7), 7.66 (dd, J = 8.6 Hz, J = 1.7 Hz, 1H, indolyl H-6), 7.88 (d, J = 8.5 Hz, 2H, 4-CN-phenyl H-3, H-5), 8.02 (s, 1H, indolyl H-4), 8.08 (d, J = 8.5 Hz, 2H, 4-CN-phenyl H-2, H-6), 11.61 (s, 1H, NH).
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22. Polarimeter Schmidt-Haensch Polartronic NH8; sample concentration: 10 mg/mL ($CHCl_3$ as solvent). **8b1**: $[\alpha]_D = -77$; **8b2**: $[\alpha]_D = +58$.
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