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New Imidazole Compounds Derived from Pyrrolidonic and Piperidonic Acids as Non-Steroidic Aromatase Inhibitors

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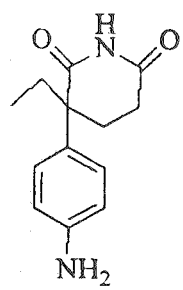
Imidazole and indole compounds derived from pyrrolidine and piperidine carboxylic acids are synthesized and characterized. The inhibition activity of those compounds was evaluated *in-vitro* on aromatase extracted from human placenta, compared to the first representative non-steroid agent (aminoglutethimide). Imidazoles derivatives showed significant inhibition of aromatase (Ki < 78.0 mM).

Key Words: Pyrrolidonic, Piperidonic acids, Aromatase inhibitors.

INTRODUCTION

Aromatase, a cytochrome P450 enzyme, is recognized as the principal responsible for breast cancer[1–3]. This enzyme catalyses the bioconversion of androgen to estrogen and has not been characterized. The hormone dependence of this carcinoma is proved in 35% of patients[4]. That is why some researchers were incited to look for more efficient and selective new molecules not having secondary effects[5–8]. The first representative non-steroid aromatase inhibitor, the aminoglutethimide 1 (AG) (K_i = 78.0 μM) was described by Banting *et al.*[9]



1: 3-(4-aminophenyl)-3-ethyl piperidine-2,6-dione

Imidazole or triazole compounds such as fadrazole, letrozole and Org 33201 are effective azole-type aromatase inhibitors and some of them are in clinical trial phase. Antifungal derivatives such as thioconazole, econazole or miconazole are also potent inhibitors of aromatase.

Baroudi *et al.*[10] described the five synthetic and physical properties of imidazolides of piperidone carboxylic acids[11]. These derivatives gave interesting results on aromatase inhibition[12].

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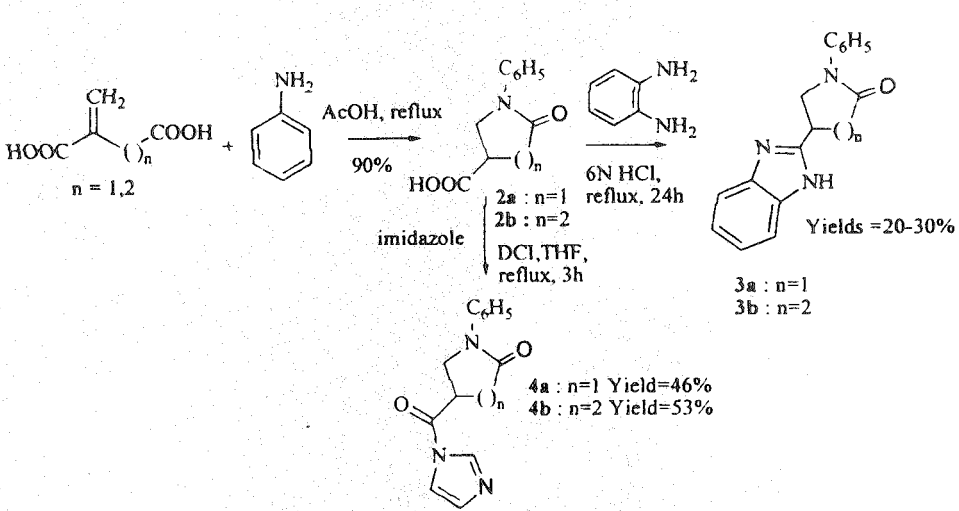
In this paper, the synthesis of a new series of non-steroidic inhibitors derived from pyrrolidone and piperidone carboxylic acids as reported, tested and evaluated their efficiency and anti-aromatase activity (*in-vitro*) on aromatase extracted from human placenta, compared to the standard compound aminoglutethimide **1**. Imidazole derivatives showed a significant activity towards the extracted enzyme ($K_i < 78.0$ μM).

RESULTS AND DISCUSSION

Both strategies applied to obtain polyfunctionnal compounds containing imidazole and benzimidazole ring are described below:

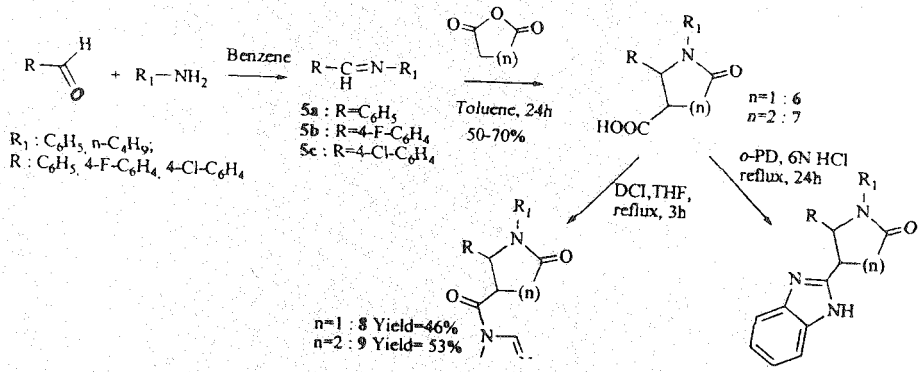
(1) The first strategy is a reaction between α-methylene succinic acid (n = 1) or α-methylene glutaric acid (n = 2) and a primary amine (aniline), leading to 1-N-phenyl-5-oxopyrrolidine-3-carboxylic acids (**2a**) and 1-(N-phenyl)-6-oxo-piperidine-3-carboxylic acids (**2b**) with an excellent yield of 90%. Compounds **2a** and **2b** are starting products of condensation with *o*-phenylenediamine (*o*-PD) in HCl leading to benzimidazolyl compounds **3a** and **3b** or with imidazole leading to imidazolyl derivatives **4a** and **4b (Scheme-1: Way A).**

(2) The second strategy is based on condensation between imines **5** and succinic or glutaric anhydrides followed by condensation with imidazole or *o*-PD **(Scheme-2: Way B)** leading to imidazolyl **8** and **9** or benzimidazolyl **10** and **11** compounds.



Scheme-1: Way A

N-aryl or N-butylidine-phenylimines (**5a–c**) were prepared by amine condensation (aniline or butylamine) with an aromatic aldehyde into benzene as solvent. The Dean Stark separator was used to remove the water evaporated by azeotropic distillation under low pressure. The characteristics of the compounds **5a–c** are given in Table-1.



Scheme-2: Way B

TABLE-1
SOME CHARACTERISTICS OF PREPARED IMINES (5a–c)

Compound	R	R_1	b.p. (°C)	Yield (%)	IR (cm^{-1})	Colour/State
5a	$-C_6H_5$	$-C_6H_5$	58	90	1640	Colourless oily
5b	4-F—C_6H_4—	$-C_6H_5$	—	75	1625	Yellow solid*
5c	4-Cl—C_6H_4—	$-C_6H_5$	—	75	1625	White solid*

*Solid product, m.p. 40°C.

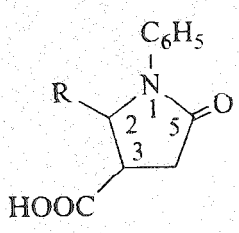
The best yields were obtained with the benzaldehyde (**5a**). Therfore, with 4-(Cl or F) substituted benzaldehydes the solid products **5b** and **5c** were obtained with lower yields because of aldehyde inactivation. Acid derivatives were prepared by imine (**5a–c**) condensation with succinic anhydride (n = 1) or glutaric anhydride (n = 2): 1-N-phenyl-2-aryl-5-oxopyrrolidine-3-carboxylic acids **6a–c** and 1-N-phenyl-2-aryl-6-oxopiperidine-3-carboxylic acids **7a–c**. Theses condensations had to be done under nitrogen atmosphere by heating at reflux within 24 h into anhydrous toluene.

If R = butyl or phenyl substituent, the yields and physical characteristics of the different 5-oxopyrrolidine-3-carboxylic acids (**6a–c**) and 6-oxopiperidine-3-carboxylic acids (**7a–c**) are given respectively in Table-2 (**6a–c**) and Table-3 (**7a–c**).

The condensation yields were better in the case of pyrrolidonic cycles (n = 1) than for piperidonic cycles (n = 2).

Intermediates **2a, 2b (Scheme-1), 6** and **7 (Scheme-2)** were starting products of following transformations leading to final compounds **3a, 3b, 4a, 4b, 8, 9, 10, 11.** In order to achieve the synthesis of product **9**, two methods have been carried out starting from the 5-oxopyrrolidine (**6**) and 6-oxopiperidine-3-carboxylic acids (**7**). After trying the way of thionyl chloride and coupling reaction with imidazole, the low yields incited us to realize an activation of 3-carboxyl group with 1,1′-carbonyldiimidazole (DCI) before coupling reaction with imidazole. The experimental results are given in Tables 4 and 5.

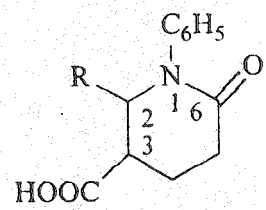
TABLE-2
CHARACTERISTICS OF 1-N-PHENYL-2-ARYL-5-OXO-PYRROLIDINE-3-CARBOXYLIC ACIDS (6a–c)



R=C_6H_5, 4-F-C_6H_4, 4-Cl-C_6H_4

Compd.	R	Recrystallization solvent	m.p. (°C)	Yield (%)	Colour/ State	IR (KBr, cm^{-1})
6a	—C_6H_5	Ethanol (45%)	280	50	White crystals	ν(CO amide) 1725 ν(CO lactam) 1650
6b	4-F—C_6H_4—	Ether : Ethanol (9 : 1)	200	45	White crystals	ν(CO amide) 1725 ν(CO lactam) 1580
6c	4-Cl—C_6H_4—	Ethanol (45%)	220	40	White crystals	ν(CO amide) 1725 ν(CO lactam) 1650

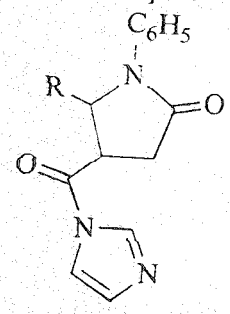
TABLE-3
CHARACTERISTICS OF 1-N-PHENYL-2-ARYL-6-OXO-PIPERIDINE-3-CARBOXYLIC ACIDS (7a–c)



R=C_6H_5, 4-F-C_6H_4, 4-Cl-C_6H_4

Compound	R	Recrystallization solvent	m.p. (°C)	Yield (%)	Colour/ State	IR (KBr, cm^{-1})
7a	—C_6H_5	Acetone	200	40	White crystals	ν(CO amide) 1710 ν(CO lactam) 1575
7b	4-F—C_6H_4—	Ethanol (45%)	220	20	White crystals	ν(CO amide) 1710 ν(CO lactam) 1610
7c	4-Cl—C_6H_4—	Ethanol (60%)	240	30	White crystals	ν(CO amide) 1710 ν(CO lactam) 1610

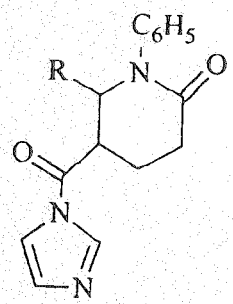
TABLE-4
CHARACTERISTICS AND BIOLOGICAL RESULTS (K_i) OF 1-(N-PHENYL-2-ARYL-3-[(1H-IMIDAZOLYL)CARBOXYL]-5-OXO-PYRROLIDINE (8a–c)



Compd.*	R	m.p. (°C)	Yield (%)	K_i (μM)	Colour/State	IR (KBr, cm^{-1})
8a	—C_6H_5	155	40	41.6	White solid	ν(CO amide) 1735 ν(CO lactam) 1680 ν(Imidazole) 3050
8b	4-F—C_6H_4—	170	50	45.5	White solid	ν(CO amide) 1725 ν(CO lactam) 1700 ν(Imidazole) 3110; 1470
8c	4-Cl—C_6H_4—	168	40	36.5	Yellow solid	ν(CO amide) 1725 ν(CO lactam) 1625 ν(Imidazole) 3100; 1470

Recrystallisation solvent: Petroleum ether.

TABLE-5
CHARACTERISTICS AND BIOLOGICAL RESULTS (Ki) OF 1-(N-PHENYL)-2-ARYL-3-[(1-H-IMIDAZOL-1-YL)CARBOXYL]-6-OXO-PIPERIDINE (9a–c)

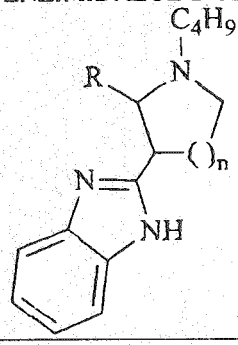


Compd.*	R	m.p. (°C)	Yield (%)	K_i (μM)	Colour/State	IR (KBr, cm^{-1})
9a	—C_6H_5	165	53	64.5	White solid	ν(CO amide) 1725 ν(CO lactam) 1625 ν(Imidazole) 3100; 1470
9b	4-F—C_6H_4—	191	51	48.5	White solid	ν(CO amide) 1740 ν(CO lactam) 1640 ν(Imidazole) 3100; 1470
9c	4-Cl—C_6H_4—	188	54	31.7	White solid	ν(CO amide) 1730 ν(CO lactam) 1645 ν(Imidazole) 3100; 1470

Recrystallisation solvent: Ethoxy ethane

The synthesis of benzimidazolyl derivatives **10** and **11** were performed first by condensation of 3-carboxylic acid on *o*-phenylene diamine with concentrated HCl by heating at reflux during 6 h. Yields were close to 10%. In order to increase yields, the condensations were carried out in dark room using 12 N HCl and maintained at 120°C during 48 h in an oil heated bath. After purification benzimidazole derivatives **10** and **11** with N-butyl substitution were obtained in 35–49% yield. These methods were used to prepare five new substituted compounds as shown in Table-6.

TABLE-6
CHARACTERISTICS AND BIOLOGICAL RESULTS (Ki) OF 1-(N-BUTYL-2-ARYL-3-(BENZIMIDAZOL-2 -YL)-5-OXO-PYRROLIDINE (10) AND 1-(N-BUTYL-2-ARYL-3-(BENZIMIDAZOL-2-YL)-6-OXO-PIPERIDINE (11)



Compound	R	Purification method	m.p. (°C)	Yield (%)	K_i (μM)	Colour/ State	IR (KBr cm^{-1})	UV (λ_{max} nm)
10a	—C_6H_5	a	143	45	—	White solid	ν(CO) 1680 ν(CN) 1665	275
10b	—C_6H_5	b	140	40	—	White solid	ν(CO) 1580 ν(CN) 1600	274
10c	4-F—C_6H_4—	a	169	40	86	White solid	ν(CO) 1640 ν(CN) 1600	274
11c	4-Cl—C_6H_4—	c	162	49	80	Yellow solid	ν(CO) 1600 ν(CN) 1620	276
11b	4-F—C_6H_4—	a	160	35	—	Yellow solid	ν(CO) 1600 ν(CN) 1620	275

a: Silica gel column (eluant: chloroform/ethanol 8/2, recrystallization in (CH_3CN).
b: Washing with ethyl acetate, recrystallization in CH_3CN.
c: Silica gel column (eluant toluene : methanol 3 : 1, recrystallization in alcohol at 45°C).

The biological assays were performed on human placental aromatase in comparison with aminogluthetimide **1** inhibitory activity ($K_i = 78.0$ μM). K_i values for the competitive inhibition exerted by the more potent inhibitors with androstenedione as substrate were in the range of 20 μM as already described by the authors[11]. The most potent inhibitors obtained in this new series were in the range of 40 μM (Tables 4 and 5) with a significant influence when the compounds have the 4-Cl—C_6H_4 rings, an N-butyl substitution and an imidazole cycle. The presence of an imidazole on C-3 of the lactame ring was absolutely necessary for

inhibitory activity. None of the 3-carboxylic compounds gave an inhibitory effect[12]. The presence of benzimidazole ring gave no inhibitory effect (**10a**, **11a**, **11b**) or a slight effect in the range of AG **1** (**11c** and **10b**).

EXPERIMENTAL

Melting points were obtained using a Buchi micromelting-point capillary apparatus and were corrected. ^{1}H NMR spectra are recorded on a Bruker AC 200 MHz spectrophotometer using tetramethylsilane as an internal standard, chemical shifts are quoted in parts per million. Coupling constants were reported in hertz (Hz). Infrared spectra were recorded for KBr discs using a Perkin-Elmer 1320 spectrometer. Thin layer chromatography is carried out on aluminum plates pre-coated with Merck silica gel 60F254, which was visualized by quenching of UV fluorescence or iodide. [1,2,6,7 ^{3}H]-androst-4-ene-3,17-dione (98 Ci/mmol) was purchased from Amersham, d,l-aminoglutethimide from Ciba-Geigi, 4-androstene-3-17-dione and NADPH from Sigma Chemical. Radioactivity was measured on a Packard liquid scintillation spectrophotometer. Elementary analyses were performed on a Flash EA 1112 Thermofinnigan analyzer.

Synthesis of 1-phenyl-5-oxo-pyrrolidine-3-carboxylic acid (2a) and 1-phenyl 6-oxo-piperidine-3-carboxylic acid (2b)

A mixture of α-methylene succinic acid (n = 1) or α-methylene glutaric acid (n = 2) (1 mmol), aniline (1 mmol, 91 μL) and water was refluxed from 45 min to 1 h or until the amine odour was faint; then the mixture was chilled in an ice-bath. The product was filtered and acidified with 1 N HCl. The precipitated pyrrolidone **2a** was recrystallized from water, diluted alcohol, diluted acetic acid or diluted HCl as white crystals.

2a: Yield: 90%; m.p.: 189–190°C. IR ν_{max} (KBr, cm^{-1}): 3200–2500, 1725, 1650. ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.1 (5H, C_6H_5), 4.15 (m, 2H-2), 3.3 (m, H-3), 2.95 (m, 2H-4). Anal. (%) Calcd. for $C_{11}H_{11}O_3N$ (205): C, 64.39; H, 5.36; N, 6.83; Found: C, 64.32; H, 5.34; N, 6.85.

2b: Yield: 90%; m..p: 130°C. IR ν_{max} (KBr, cm^{-1}): 3100–2500, 1730, 1630; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.1 (5H, C_6H_5), 4.2 (m, 2H-2), 3.4 (m, H-3), 2.9 (m, 2H-5), 2.6 (m, 2H-4). Anal. (%) Calcd. for $C_{12}H_{13}O_3N$ (219): C, 65.75; H, 5.93; N, 6.39; Found: C, 65.71; H, 5.92; N, 6.41.

Synthesis of 1-phenyl-3-[(1′-N-imidazolyl)carboxyl]-5-oxo-pyrrolidine (4a)

2a (10 mmol, 2.05 g) and 1,1′-carbonyldiimodazole (13 mmol, 2.11 g) were dissolved into anhydrous THF (30 mL). The mixture was stirred and refluxed for 3 h in an oil bath. The solvent was evaporated and the residue was treated with $NaHCO_3$ solution (20%, 25 mL) then extracted with chloroform (75 mL). The organic layer was washed with distilled water, then dried with Na_2SO_4. The solid obtained after solvent evaporation was washed with anhydrous diethyl ether and purified by two recrystallizations into a mixture of anhydrous ether and ethyl

acetate (1 : 1). **4a**: Yield 30%, white crystals; m.p.: 155°C. IR (KBr, cm^{-1}): 3050 and 1470 ν(imidazole), 1735, 1680. Anal. (%) Calcd. for $C_{14}H_{13}O_2N_3$ (255): C, 65.88; H, 5.10; N, 16.47; Found: C, 65.84; H, 5.09; N, 16.49.

General procedure of imines synthesis: N-arylidine-alkylamine (5a–c)

Arylaldehyde (0.2 mol) and *N*-alkyl amine or aniline (0.2 mol) were dissolved in anhydrous benzene (100 mL). The mixture was stirred in a round bottom flask equipped with a Dean Stark separator and a refrigerant. The heating was stopped when the quantity of water separated reached the theoretical value (36 mL). The mixture was cooled to room temperature, then the solvent was evaporated and the oily product **5a** was purified by distillation under 0.1 mm Hg; yield: 75–92%, (Table-1).

5a: Anal. (%) Calcd. for $C_{13}H_{11}N$ (181): C, 86.19; H, 6.07; N, 7.73; Found: C, 86.13; H, 6.06; N, 7.75.

5b: Anal. Calcd for $C_{13}H_{10}NF$ (199): C, 78.39; H, 5.02; N, 7.03; F, 9.55. Found: C = 78.35; H = 5.00; N = 7.06; F = 9.54.

5c: Anal. (%) Calcd. for $C_{13}H_{10}NCl$ (215.45): C, 72.40; H, 4.64; N, 6.50; Cl, 16.45; Found: C, 72.38; H, 4.63; N, 6.52; Cl, 16.43.

General procedure: Synthesis of 1-N-phenyl-2-aryl-5-oxopyrrolidine-3-carboxylic acid (6) and 1-N-phenyl-2-aryl-6-oxopiperidine-3-carboxylic acid (7)

N-benzylidene-N-phenylamine **5** (0.135 mol) and succinic anhydride (0.15 mol, 15 g) or glutaric anhydride (0.15 mol, 17.11 g) were dissolved in anhydrous toluene (150 mL) and stirred under nitrogen during 24 h. The mixture was cooled to room temperature and the solid was separated by filtration and purified by extraction with ethyl acetate after addition of sodium carbonate. The aqueous layer was treated with phosphoric acid. The white solid product separated by filtration was recrystallized in 45% ethanol to obtain white crystals. TLC on silica gel shows two isomers *cis* (< 10%) and *trans* (> 90%). The *trans*-isomer was the only one isolated after purification. R_f: **6a:** 0.70; **6b:** 0.73; **6c:** 0.75; **7a:** 0.69; **7b:** 0.72; **7c:** 0.74; solvent: partridge mixture (4 : 1 : 5) butanol : acetic acid : water.

6a: ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.32 (m, 10H), 4.95 (d, H-2), 3.10 (m, H-3), 2.85 (m, 2H-4). Anal. (%) Calcd. for $C_{17}H_{15}O_3N$ (281): C, 72.60; H, 5.34; N, 4.98; Found: C, 72.55; H, 5.33; N, 4.99.

6b: ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.36 (dd, 2H), 7.11 (t, 2H), 7.00 (m, 5H), 4.97 (d, H-2), 3.14 (m, H-3), 2.90 (m, 2H-4). Anal. (%) Calcd. for $C_{17}H_{14}O_3NCl$ (299): C, 68.23; H, 4.68; N, 4.68; F, 6.35; Found: C, 68.18; H, 4.67; N, 4.69; F, 6.33.

6c: ^1H NMR (200 MHz, $CDCl_3$) δ_H: 7.33 (m, 5H), 7.25 (d, 2H), 7.15 (d, 2H), 4.95 (d, H-2), 3.10 (m, H-3), 2.90 (m, 2H-4). Anal. (%) Calcd. for $C_{17}H_{14}O_3NCl$ (315.45): C, 64.67; H, 4.44; N, 4.44; Cl, 11.24; Found: C, 64.62; H, 4.43; N, 4.45; Cl, 11.21.

7a: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.32 (m, 10H), 5.16 (d, H-2), 2.91 (m, H-3), 2.65 (m, 2H-5), 2.05 (m, H-4a), 1.90 (m, H-4b). Anal. (%) Calcd. for $C_{18}H_{17}O_3N$ (295): C, 73.22; H, 5.76; N, 4.74; Found: C, 73.19; H, 5.77; N, 4.76.

7b: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.36 (dd, 2H), 7.24 (t, 2H), 7.08 (m, 5H), 5.33 (d, H-2), 2.98 (m, H-3), 2.66 (m, 2H-5), 2.09 (m, H-4a), 2.02 (m, H-4b). Anal. (%) Calcd. for $C_{28}H_{16}O_3NF$ (313): C, 69.01; H, 5.11; N, 4.47; F, 6.07. Found: C, 68.94; H, 5.09; N, 4.49; F, 6.05.

7c: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: 7.33 (m, 5H), 7.23 (d, 2H), 7.13 (d, 2H), 5.34 (d, H-2), 2.96 (m, H-3), 2.62 (m, 2H-5), 1.96 (m, H-4a), 1.90 (m, H-4b). Anal. (%) Calcd. for $C_{18}H_{16}O_3NCl$ (329.45): C, 65.56; H, 4.85; N, 4.25; Cl, 10.76; Found: C, 65.52; H, 4.84; N, 4.26; Cl, 10.73.

Synthesis of 1-(N-phenyl)-2-(aryl)-3-[(1H-imidazol-1-yl)-carbonyl]-5-oxo-pyrrolidine (8) and 1-(N-phenyl)-2-(aryl)-3-[(1H-imidazol-1-yl)-carbonyl]-6-oxo-piperidine (9)

Method 1: 1-(N-phenyl)-2-(aryl)-6-oxo-piperidine-3-carboxylic acids **7** (0.03 mole) was dissolved in anhydrous chloroform (100 mL). Freshly distilled thionyl chloride (342 mmol, 25 mL) and two drops of pyridine were added to the mixture and stirred for 4 h at 80°C. The solvent and thionyl chloride were eliminated by evaporation under reduced pressure. The residue was purified from $SOCl_2$ traces with benzene. The yellow precipitate formed was washed with anhydrous ether and dried. Acid chloride (0.03 mol) and imidazole (0.088 mol, 6 g) were dissolved in acetonitrile (150 mL). The mixture was maintained at reflux 3 h under nitrogen. The solvent was eliminated by evaporation under vacuum. The residue was treated with $NaHCO_3$ solution (20%, 30 mL), then extracted by chloroform (2 × 75 mL). The organic phase was dried with Na_2SO_4. The solid obtained after solvent evaporation was purified by recrystallization into a mixture of anhydrous ether : ethyl acetate (1 : 1) and gave yellow crystals in 53% yield.

Method 2: Same procedure with DCI as for the preparation of **4**.

8a: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: Imidazole: 8.0 (s, H-2′), 7.31 (dd, H-4′), 7.06 (dd, H-5′). Phenyl: 7.25 (m, 10H), 5.23 (d, H-2), 3.40 (m, H-3), 2.66 (m, 2H-5), 2.17 (m, 2H-4). ^{13}C NMR (200 MHz, $CDCl_3$) δ_C: CO amide: 169.3, CO lactam: 168.5, Imidazole: C-2′: 135.9, C-4′: 115.9, C-5′: 131.4. Anal. (%) Calcd. for $C_{20}H_{17}O_2N_3$ (331): C, 72.51; H, 5.13; N, 12.69; Found: C, 72.49; H, 5.11; N, 12.72.

8b: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: Imidazole: 8.1 (s, H-2′), 7.39 (dd, H-4′), 6.96 (dd, H-5′). Phenyl: 7.28 (m, 9H), 5.34 (d, H-2), 3.56 (m, H-3), 2.78 (m, 2H-5), 2.33 (m, 2H-4). ^{13}C NMR (200 MHz, $CDCl_3$) δ_C: CO amide: 169.1, CO lactam: 168.7, Imidazole: C-2′: 135.9, C-4′: 131.7, C-5′: 115.5. Anal. (%) Calcd. for $C_{20}H_{16}O_2N_3F$ (349): C, 68.76; H, 4.58; N, 12.03; F, 5.44; Found: C, 68.74; H, 4.56; N, 12.05; F, 5.42.

8c: ^{1}H NMR (200 MHz, $CDCl_3$) OH: Imidazole: 8.1 (sH-2′); 7.39 (dd, H-4′); 7.08 (dd, H-5′). Phenyl: 7.25 (m, 9H), 5.34 (d, H-2), 3.50 (m, H-3), 2.80 (m, 2H-5), 2.35 (m, 2H-4). ^{13}C NMR (200 MHz, $CDCl_3$) δ_C: CO amide: 169.0, CO

lactam: 168.6, Imidazole: C-2′: 135.9, C-4′: 131.7, C-5′: 115.9. Anal. (%) Calcd. for $C_{20}H_{16}O_2N_3Cl$ (365.45): C, 65.67; H, 4.38; N, 11.49; Cl, 9.70; Found: C, 65.65; H, 4.36; N, 11.51; Cl, 9.69.

Synthesis of 1-[N-butyl-2-aryl-3-(benzimidazol-2-yl)carbonyl]-5-oxo-pyrrolidine (10) and 1-[N-butyl-2-aryl-3-(benzimidazol-2-yl)carbonyl]-6-oxo-piperidine (11)

1-(N-butyl)-2-(4′-fluoro-phenyl)-5-oxo-pyrrolidine-3-carboxylic acid (0.017 mol, 4.74 g), *o*-phenylene diamine (0.02 mol, 2.16 g) and 12 N HCl (40 mL) were mixed and the mixture was maintained at melting point in a dark room. The mixture was cooled to room temperature, treated by 2 N NaOH solution and extracted by chloroform (3 × 100 mL). The organic layer was washed with distilled water, then dried with Na_2SO_4. The pink solid product obtained after solvent evaporation was purified on silica gel column (eluant: $CHCl_3$: EtOH, 8 : 2) and purified by recrystallization into acetonitrile.

10a: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: Benzimidazole: 7.48 (m, H-4′ and H-7′); 7.21 (m, H-5′ and H-6′); Aromatic: 7.31 (m, 5H); 5.04 (d, H-2); 3.68 (m, H-3); 3.06 (m, 2-H4). ^{13}C NMR (200 MHz, $CDCl_3$) δ_C: CO lactam: 173.2; Benzimidazole: C-2′: 153; C-4′: 119.4; C-5′: 122.3; C-6′: 123.0; C-7′: 110.6; C-8′, C-9′: 138.8. Anal. (%) Calcd. for $C_{23}H_{20}ON_3$ (354): C, 77.96; H, 5.65; N, 11.86; Found: C, 77.92; H, 5.64; N, 11.89.

11a: ^{1}H NMR (200 MHz, $CDCl_3$) δ_H: Benzimidazole: 7.40 (m, H-4′ and H-7′), 7.30 (m, H-5′ and H-6′); Aromatic: 7.08 (m, 5H); 4.25 (d, H-2); 3.04 (m, H-3); 1.87 (m, H-4a); 2.16 (m, H-4b); 2.9 (m, 2H-5). ^{13}C NMR (200 MHz, $CDCl_3$) δ_C: CO lactam: 178.2; Benzimidazole: C-2′: 155.0; C-4′, C-7′: 114.7; C-5′, C-6′: 121.6; C-8′, C-9′: 138.0. Anal. (%) Calcd . for $C_{24}H_{22}ON_3$ (368): C, 78.26; H, 5.98; N, 11.41; Found: C, 78.19; H, 5.97; N, 11.43.

10b: ^{1}H NMR (200 MHz, CD_3OD) δ_H: Benzimidazole: 7.50 (m, H-4′ and H-7′), 7.33 (m, H-5′ and H-6′); Aromatic: 7.18 (dd, 2H); 7.12 (t, 2H); 5.04 (d, H-2); 3.70 (m, H-3); 3.01 (m, H-4). ^{13}C NMR (200 MHz, d_6DMSO) δ_C: CO lactam: 172.2; Benzimidazole: C-2′: 154.2; C-4′: 118.4; C-5′: 121.1; C-6′: 121.8; C-7′: 111.0; C-8′: 142.9; C-9′: 134.3. Anal. (%) Calcd. for $C_{23}H_{19}ON_3F$ (372): C, 74.19; H, 5.11; N, 11.29; F, 5.11; Found: C, 74.12; H, 5.09; N = 11.31; F, 5.10.

11b: ^{1}H NMR (200 MHz, CD_3OD) δ_H: Benzimidazole: 7.48 (m, H-4′ and H-7′); 7.42 (m, H-5′ and H-6′); Aromatic: 7.1–7.2 (m, 4H); 4.27 (d, H-2); 2.70 (m, H-3); 1.8 (m, H-4a); 2.06 (m, H-4b); 3.29 (m, 2H-5). ^{13}C NMR (200 MHz, CD_3OD) δ_C: CO lactam: 179.4; Benzimidazole: C-2′: 156.4; C-4′, C-7′: 115.3; C-5′, C-6′: 123.2; C-8′, C-9′: 139.5. Anal. (%) Calcd. for $C_{24}H_{21}ON_3F$ (386): C, 74.61; H, 5.44; N, 10.88; F, 4.92; Found: C, 74.57; H, 5.42; N, 10.90; F, 4.91.

11e: ^{1}H NMR (200 MHz, CD_3OD) δ_H: Benzimidazole: 7.47 (m, H-4′ and H-7′), 7.34 (m, H-5′ and H-6′); Aromatic: 7.42 (d, 2H); 7.14 (d, 2H); 4.22 (d, H-2); 2.70 (m, H-3); 1.82 (m, H-4a); 2.05 (m, H-4b); 2.96 (m, 2H-5). ^{13}C NMR (200 MHz, CD_3OD) δ_C: CO lactam: 179.6; Benzimidazole: C-2′: 156.4; C-4′, C-7′: 115.3; C-5′, C-6′: 123.2; C-8′, C-9′: 139.4. Anal. (%) Calcd. for $C_{24}H_{21}ON_3Cl$ (402.45): C, 71.56; H, 5.22; N, 10.43; Cl, 8.81; Found: C, 71.54; H, 5.21; N, 10.46; Cl, 8.79.

Aromatase assay

Inhibitory activities of compounds towards aromatase were determined *in vitro* using human placental microsomes and [1,2,6,7-^{3}H] androst-4-ene-3,17-dione.

The microsomal fraction was prepared from human term placenta obtained immediately after delivery and carried out at –4°C. Microsomes were extracted according to Ryan procedure[13]. After isolation, microsomes were suspended in phosphate buffer 10 mM, KCl 100 mM, EDTA 1 mM and then frozen and stored in aliquots of 0.5 mL at –70°C. Protein concentration, determined by the method of Lowry *et al.*[14], was 10.6 mg of protein/mL.

The methodology for the determination of K_i values has been described previously[12, 13, 15]. Each incubation tube contained labelled and unlabelled androstenedione in buffer at varying concentrations ranging from 15–100 nM in an ethanolic solution, with and without putative inhibitors. The solvent was evaporated under nitrogen and 0.5 mL of 10 mM phosphate buffer (pH = 7.5) with EDTA 1 mM, KCl 100 mM and one drop of propylene glycol was added to the residue. The reaction was initiated with 100 µL of the microsomal suspension 0.14 mg/mL) with NADPH 0.5 mM. The incubations were carried out at 37°C in a shaking water bath during 30 min. Indeed, for a given concentration of androstenedione of 0.1 µL in microsomal suspension (0.14 mg/mL), the graph of the water released as a function of time was linear during the first 20 min and then flattens out; since the speed varies a little afterwards, the parameter incubation time was chosen as 30 min. The assays were run in duplicate and samples were removed at 2 min intervals. The reaction was stopped by the addition of chloroform (5 mL) and vortexing 30 s after centrifugation at 1 500 g, 100 µL were removed from the water phase (in duplicate) and mixed with 10 mL of the scintillation solution. The extent of aromatization was determined by measuring the amount of tritiated water released after aromatization of [1,2,6,7-^{3}H] androst-4-ene-3,17-dione as described by Rabe *et al.*[16]. The K_m and V_{max} values were determined by the Lineweaver-Burk plots. The human placental microsomes preparation used in this study showed an apparent K_m for V_{max} androstenedione of 0.08 µM. The inhibitory activity of the tested compounds was determined by ^3H_2O release from androstenedione as an index of residual aromatase activity. The inhibitors were added at various concentrations (0, 20, 50 µM) to the incubation medium prior to the addition of the microsomes. The apparent K_i values were determined by the least square analysis of secondary replots (slope *vs.* [I]] of Lineweaver-Burk plots (1/V *vs.* 1/S). The points plotted were the mean of four determinations.

Conclusion

This work allowed us to synthesize new molecules of aromatase inhibitors; also we have been able to improve the initial scheme of synthesis and to direct it towards the formation of more active compounds.

In total 10 original acids, the pyrrolidonic and piperidonic acids, basic compounds of our synthesis, were obtained with good yields by imines and acid anhydrides condensation. The results of the first series of biological tests have shown that the aromatic cycle and the nitrogen of the lactame cycle substitutions had a great influence on the biological activity ($K_i < 70$ µM). In future, the N-phenyl compounds with dichlorinated aryl substitutions would be tested in order to decrease K_i.

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