Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic Chemistry 32 (2004) 494–503
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Synthesis and biological evaluation of 4-imidazolylflavans as nonsteroidal aromatase inhibitors

Christelle Pouget, Samir Yahiaoui, Catherine Fagnere, Gerard Habrioux, Albert Jose Chulia*

UPRES EA 1085, Biomolécules et Cibles Cellulaires Tumorales, Faculté de Pharmacie, 2 rue du Docteur Marcland, 87025 LIMOGES cedex, France

Received 17 December 2003
Available online 6 August 2004

Abstract

A series of 4-imidazolylflavans having a variety of substituents on the 2-phenyl ring was synthesized and investigated for their inhibitory effect against aromatase. Structure–activity relationships of these compounds were determined. An additional chlorine atom or a cyano group at the 4′ position did not enhance aromatase inhibition as well as a 3′-hydroxyl group. The influence of an additional 4′-hydroxyl group depends on the substitution pattern of A ring. Among these molecules, 4′-hydroxy-4-imidazolyl-7-methoxyflavan is only 2.2-fold less active than the letrozole (as assessed by IC_{50} values). Letrozole is used as the first-line therapy for metastatic breast cancer.

Keywords: 4-Imidazolylflavans; Breast cancer; Anti-aromatase effect; Structure–activity relationships

* Corresponding author. Fax: +33-555-435-910.
E-mail address: jose.chulia@unilim.fr (A.J. Chulia).

0045-2068/$ - see front matter
doi:10.1016/j.bioorg.2004.06.008

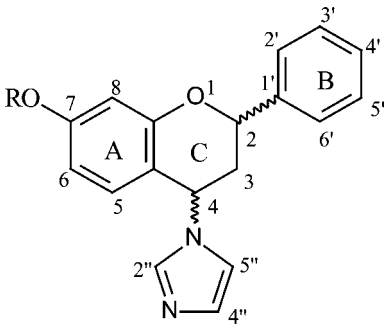
1. Introduction

Estrogens are known to be important in the growth of breast cancer in both pre- and post-menopausal women. Therefore, two effective strategies have been devised to antagonize the action of these hormones and to treat endocrine-related breast cancers. One pharmacological approach is the inhibition of estrogen action by anti-estrogens (e.g., tamoxifen) that interact with estrogen receptors [1]. The second approach is to block estrogen synthesis by inhibiting a key enzyme of the process. Aromatase, which catalyses the final step in the biosynthesis of estrogens, has been the target for the design of inhibitors which have become the established second-line treatment for metastatic breast cancer in post-menopausal women [2].

Aromatase inhibitors can be both steroidal and nonsteroidal compounds. The first nonsteroidal inhibitor was aminoglutethimide. This compound has now been replaced by other more selective and potent nonsteroidal inhibitors like fadrozole, letrozole, or anastrozole. These compounds are currently being compared with tamoxifen in first-line metastatic, adjuvant, and neoadjuvant settings. Thus, based primarily on a superior side effect profile, letrozole has recently been approved as a first-line therapy of metastatic breast cancer in several countries [3]. The structure of these nonsteroidal aromatase inhibitors can be considered to consist of two parts. One part is the azole part with a nitrogen atom which interacts with the heme iron atom of the cytochrome P450 of aromatase. The second part is the bulky aryl part, which mimics the steroid ring of the substrate.

Our previous studies focused mainly on the flavonoids, which are natural compounds inhibiting aromatase with about the same activity as aminoglutethimide [4,5]. Structure–activity relationship studies enabled us to determine the binding characteristics and the structural requirements for flavonoids to inhibit aromatase. First, these compounds bind to the active site of aromatase in an orientation in which A and C rings mimic rings D and C of the androgen substrate, respectively [6]. It also appears that the C-4 keto group of flavonoids is essential for aromatase inhibition because it interacts with the heme of the enzyme [6]. With respect to substitutions in the A ring, a 7-hydroxyl group is essential for enhanced aromatase inhibitory activity while a 7-methoxy group is also responsible for an anti-aromatase effect [4]. Finally, we demonstrated that additional hydroxyl groups at position 3′ and/or 4′ on the 7-methoxyflavanone skeleton led to an increase in aromatase inhibition [5].

In the search of new lead compounds that inhibit aromatase, we have recently described the synthesis and biological evaluation of two new 4-imidazolylflavans, the 2,4-*trans*-7-hydroxy-4-imidazolylflavan $\mathbf{A_1}$ and the 2,4-*trans*-4-imidazolyl-7-methoxyflavan $\mathbf{A_2}$ (Scheme 1) [7]. Replacement of the carbonyl function of flavanones by an imidazolyl group led to a marked increase of inhibitory potency as these two compounds were found to have IC_{50} values of 0.041 and 0.091 μM, respectively, while the IC_{50} value of aminoglutethimide was 5.2 μM. These encouraging results prompted us to modify these lead compounds. In particular, we examined the effects of substitutions on the 2-phenyl group (B ring in $\mathbf{A_1}$ and $\mathbf{A_2}$).



$\mathbf{A_1}$: R = H
$\mathbf{A_2}$: R = Me

Scheme 1. Structure of 4-imidazolylflavans $\mathbf{A_1}$ and $\mathbf{A_2}$.

In the present paper, we report the synthesis of the 4-imidazolylflavans **3a–3e** and their biological evaluation against aromatase. Our previous studies concerning the B ring structural requirements for enhanced aromatase inhibitory effect of flavonoids allowed us to identify some active flavanones as molecular scaffolds [5]. Thus, 3′ and 4′-hydroxy groups, which were found to increase aromatase inhibition, were introduced onto the 2-phenyl of the 4-imidazolylflavan skeleton. A chlorine atom and a cyano group were also introduced at the 4′ position of the B ring since it is known that in the azole family of aromatase inhibitors, the presence of such a *para*-substituted phenyl group reinforces the interaction between the azole nitrogen atom of these inhibitors and the heme iron atom of the enzyme [8,9]. The results show that an additional chlorine atom or a cyano group at the 4′ position did not enhance aromatase inhibition as well as a 3′-hydroxyl group. The influence of an additional 4′-hydroxyl group was found to depend on the substitution pattern of A ring.

2. Materials and methods

2.1. Chemistry

2.1.1. General experimental procedures

NMR spectra were recorded on a Bruker 400 MHz spectrometer with Me_4Si as the internal standard. ESI-MS spectra were performed on a Waters Alliance system equipped with an API–MS interface. Compounds were purified by preparative thin layer chromatography on Macherey–Nagel silica gel. Preparative HPLC was performed on a Merck prep Sep Tech system.

2.1.2. Synthesis and characterization of the 4-imidazolylflavans

2.1.2.1. General procedure. To a solution of flavanone in ethanol was added sodium borohydride (5 eq). The mixture was kept under a nitrogen atmosphere at a temper-

ature of 6 °C for 24–72 h (except flavanone **1a** for which the reaction occurred at room temperature for 4 h). The mixture was evaporated under reduced pressure and water was added. The pH of the mixture was adjusted to 6 using 1M HCl and the resulting solution was extracted with chloroform. The organic layers were washed, dried over Na_2SO_4 and evaporated. The resulting flavan-4-ols were purified via preparative TLC on silica gel using toluene/Et_2O (75/25) as eluent.

To a solution of flavan-4-ol in anhydrous THF was added 1,1′-carbonyldiimidazole (4 eq). The mixture was kept under a nitrogen atmosphere at room temperature for 4 h. Subsequently, it was evaporated under reduced pressure. The residue was dissolved into chloroform and the organic layer was washed, dried over Na_2SO_4 and evaporated. Preparative TLC on silica gel using $CHCl_3$/MeOH (97/3) as eluent afforded pure 4-imidazolylflavans.

2,4-cis-3′-hydroxy-7-methoxyflavan-4-ol (**2a**) was obtained in 70% yield. ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.11(1H, ddd, J = 10.0, 11.2, and 13.2 Hz, H-3$_{ax}$), 2.50 (1H, ddd, J = 2.0, 6.4, and 13.2 Hz, H-3$_{eq}$), 3.78 (3H, s, OCH_3), 5.04 (1H, br s, H-4), 5.13 (1H, dd, J = 2.0 and 11.2 Hz, H-2), 6.45 (1H, d, J = 2.4 Hz, H-8), 6.58 (1H, dd, J = 2.4 and 8.8 Hz, H-6), 6.80 (1H, br dd, J = 2.0 and 8.0 Hz, H-4′), 6.94 (1H, t, J = 2.0 Hz, H-2′), 6.99 (1H, br d, J = 8.0 Hz, H-6′), 7.26 (1H, t, J = 8.0 Hz, H-5′), 7.40 (1H, d, J = 8.8 Hz, H-5); ^{13}C NMR (100 MHz; $CDCl_3$): δ 39.9 (C-3), 55.4 (OCH_3), 65.5 (C-4), 76.8 (C-2), 101.3 (C-8), 108.2 (C-6), 112.9 (C-2′), 115.4 (C-4′), 118.0 (C-4a), 118.2 (C-6′), 128.1 (C-5), 130.0 (C-5′), 142.1 (C-1′), 155.4 (C-3′), 156.2 (C-8a), and 160.3 (C-7); ESP-MS (−40 V): m/z 271 $[M-H]^-$.

2,4-trans-3′-hydroxy-4-imidazolyl-7-methoxyflavan (**3a**) was obtained in 40% yield. ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.31(1H, br dt, J = 2.2 and 14.5 Hz, H-3$_{eq}$), 2.46 (1H, ddd, J = 4.4, 11.2, and 14.5 Hz, H-3$_{ax}$), 3.79 (3H, s, OCH_3), 4.75 (1H, dd, J = 1.4 and 11.2 Hz, H-2), 5.33 (1H, br t, J = 3.2 Hz, H-4), 6.55 (1H, br s, H-8), 6.56 (1H, dd, J = 2.5 and 8.2 Hz, H-6), 6.75 (1H, br s, H-2′), 6.85 (1H, br d, J = 8.1 Hz, H-4′), 6.92 (1H, br d, J = 7.7 Hz, H-6′), 6.97 (1H, s, H-4″), 7.01 (1H, d, J = 8.2 Hz, H-5), 7.11 (1H, s, H-5″), 7.26 (1H, t, J = 7.9 Hz, H-5′), 7.45 (1H, s, H-2″); ^{13}C NMR (100 MHz; $CDCl_3$): δ 38.1 (C-3), 51.5 (C-4), 55.4 (OCH_3), 73.3 (C-2), 101.8 (C-8), 109.0 (C-4a), 109.3 (C-6), 118.6 (C-4″), 113.6 (C-2′), 116.2 (C-4′), 116.4 (C-6′), 128.6 (C-5″), 130.2 (C-5′), 131.2 (C-5), 136.4 (C-2″), 140.6 (C-1′), 156.6 (C-3′), 158.0 (C-8a), and 161.6 (C-7); ESP-MS (+40 V): m/z 323 $[M + H]^+$.

2,4-cis-4′-chloro-7-methoxyflavan-4-ol (**2b**) was obtained in 61% yield. ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.06 (1H, ddd, J = 10.3, 11.4, and 13.1 Hz, H-3$_{ax}$), 2.48 (1H, ddd, J = 1.9, 6.2, and 13.2 Hz, H-3$_{eq}$), 3.77 (3H, s, OCH_3), 5.04 (1H, dd, J = 6.3 and 10.1 Hz, H-4), 5.13 (1H, dd, J = 1.9 and 11.5 Hz, H-2), 6.43 (1H, d, J = 2.5 Hz, H-8), 6.58 (1H, dd, J = 2.5 and 8.6 Hz, H-6), 7.38 (4H, m, H-2′,H-3′,H-5′, and H-6′), 7.39 (1H, d, J = 8.6 Hz, H-5); ^{13}C NMR (100 MHz; $CDCl_3$): δ 40.3 (C-3), 55.4 (OCH_3), 65.4 (C-4), 76.4 (C-2), 101.2 (C-8), 108.3 (C-6), 118.0 (C-4a), 127.5 (C-3′ and C-5′), 127.9 (C-5), 128.9 (C-2′ and C-6′), 134.0 (C-1′), 139.0 (C-4′), 155.3 (C-8a), and 160.5 (C-7); ESP-MS (+40 V): m/z 273 and m/z 275 $[M + H]^+$.

2,4-trans-4′-chloro-4-imidazolyl-7-methoxyflavan (**3b**) was obtained in 54% yield. ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.34 (1H, br dt, J = 2.8 and 14.4 Hz, H-3$_{eq}$), 2.43

(1H, ddd, $J = 4.4$, 11.1, and 14.4 Hz, H-3_{ax}), 3.82 (3H, s, OCH_3), 4.94 (1H, dd, $J = 2.2$ and 11.1 Hz, H-2), 5.34 (1H, br t, $J = 3.5$ Hz, H-4), 6.56 (1H, d, $J = 2.4$ Hz, H-8), 6.59 (1H, dd, $J = 2.4$ and 8.5 Hz, H-6), 6.93 (1H, br s, H-4″), 7.04 (1H, d, $J = 8.5$ Hz, H-5), 7.12 (1H, br s, H-5″), 7.29 (2H, d, $J = 8.5$ Hz, H-3′ and H-5′), 7.36 (2H, d, $J = 8.5$ Hz, H-2′ and H-6′), 7.43 (1H, s, H-2″); ^{13}C NMR (100 MHz; $CDCl_3$): δ 38.4 (C-3), 50.9 (C-4), 55.4 (OCH_3), 72.5 (C-2), 101.7 (C-8), 109.4 (C-6), 109.5 (C-4a), 118.3 (C-4″), 127.5 (C-3′ and C-5′), 128.9 (C-2′ and C-6′), 129.8 (C-5″), 131.2 (C-5), 134.2 (C-1′), 136.8 (C-2″), 138.2 (C-4′), 156.2 (C-8a), and 161.5 (C-7); ESP-MS (+40 V): *m/z* 341 $[M + H]^+$.

2,4-cis-4′-cyano-7-methoxyflavan-4-ol (**2c**) was obtained in 54% yield. ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.05 (1H, ddd, $J = 10.2$, 11.4, and 13.2 Hz, H-3_{ax}), 2.51 (1H, ddd, $J = 2.1$, 6.2, and 13.2 Hz, H-3_{eq}), 3.79 (3H, s, OCH_3), 5.07 (1H, m, H-4), 5.23 (1H, dd, $J = 1.8$ and 11.4 Hz, H-2), 6.45 (1H, d, $J = 2.5$ Hz, H-8), 6.61 (1H, dd, $J = 2.5$ and 8.6 Hz, H-6), 7.40 (1H, d, $J = 8.5$ Hz, H-5), 7.57 (2H, d, $J = 8.2$ Hz, H-2′ and H-6′), 7.70 (2H, d, $J = 8.3$ Hz, H-3′ and H-5′); ^{13}C NMR (100 MHz; $CDCl_3$): δ 40.2 (C-3), 55.4 (OCH_3), 65.2 (C-4), 76.1(C-2), 101.3 (C-8), 108.5 (C-6), 112.0 (C-4′), 117.9 (C-4a), 118.6 (CN), 126.6 (C-2′ and C-6′), 128.0 (C-5), 132.5 (C-3′ and C-5′), 145.9 (C-1′), 154.9 (C-8a), and 160.6 (C-7); ESP-MS (−40 V): *m/z* 280 $[M-H]^-$.

2,4-trans-4′-cyano-4-imidazolyl-7-methoxyflavan (**3c**) was obtained in 42% yield. ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.37 (1H, br dt, $J = 3.0$ and 14.2 Hz, H-3_{eq}), 2.41 (1H, ddd, $J = 4.3$, 9.8, and 14.2 Hz, H-3_{ax}), 3.83 (3H, s, OCH_3), 5.01 (1H, dd, $J = 4.2$ and 9.6 Hz, H-2), 5.36 (1H, br t, $J = 3.5$ Hz, H-4), 6.58 (1H, d, $J = 2.5$ Hz, H-8), 6.62 (1H, dd, $J = 2.5$ and 8.5 Hz, H-6), 6.94 (1H, br s, H-4″), 7.06 (1H, d, $J = 8.5$ Hz, H-5), 7.14 (1H, br s, H-5″), 7.47 (1H, s, H-2″), 7.48 (2H, d, $J = 8.2$ Hz, H-2′ and H-6′), 7.69 (2H, d, $J = 8.2$ Hz, H-3′ and H-5′); ^{13}C NMR (100 MHz; $CDCl_3$): δ 38.6 (C-3), 50.7 (C-4), 55.5 (OCH_3), 72.3 (C-2), 101.7 (C-8), 109.3 (C-4a), 109.6 (C-6), 112.3 (C-4′), 118.3 (C-4″), 118.5 (CN), 126.7 (C-2′ and C-6′), 129.9 (C-5″), 131.3 (C-5), 132.6 (C-3′ and C-5′), 136.8 (C-2″), 144.9 (C-1′), 155.8 (C-8a), and 161.6 (C-7); ESP-MS (+40 V): *m/z* 332 $[M + H]^+$.

2,4-cis-4′-hydroxy-7-methoxyflavan-4-ol (**2d**) was obtained in 14% yield. ^{1}H NMR (400 MHz; CD_3OD): δ 2.02 (1H, ddd, $J = 10.9$, 12.0, and 12.9 Hz, H-3_{ax}), 2.33 (1H, ddd, $J = 1.6$, 6.2, and 12.9 Hz, H-3_{eq}), 3.73 (3H, s, OCH_3), 4.99 (1H, dd, $J = 6.2$ and 10.7 Hz, H-4), 5.03 (1H, dd, $J = 1.0$ and 12.0 Hz, H-2), 6.34 (1H, d, $J = 2.5$ Hz, H-8), 6.52 (1H, dd, $J = 2.5$ and 8.6 Hz, H-6), 6.80 (2H, d, $J = 8.6$ Hz, H-3′ and H-5′), 7.27 (2H, d, $J = 8.6$ Hz, H-2′ and H-6′), 7.36 (1H, d, $J = 8.6$ Hz, H-5); ^{13}C NMR (100 MHz; CD_3OD): δ 41.2 (C-3), 55.7 (OCH_3), 66.3 (C-4), 78.6 (C-2), 102.0 (C-8), 108.4 (C-6), 116.2 (C-3′ and C-5′), 120.0 (C-4a), 128.7 (C-2′ and C-6′), 129.1 (C-5), 133.3 (C-1′), 157.2 (C-4′), 158.5 (C-8a), and 161.6 (C-7); ESP-MS (−40 V): *m/z* 271 $[M-H]^-$.

2,4-trans-4′-hydroxy-7-methoxyflavan-4-ol (**2d′**) was obtained in 17% yield. ^{1}H NMR (400 MHz; CD_3OD): δ 2.07 (1H, br dt, $J = 3.3$ and 14.2 Hz, H-3_{eq}), 2.11 (1H, ddd, $J = 2.5$, 9.6, and 14.2 Hz, H-3_{ax}), 3.75 (3H, s, OCH_3), 4.71 (1H, br t, $J = 2.9$ Hz, H-4), 5.12 (1H, dd, $J = 4.5$ and 9.6 Hz, H-2), 6.40 (1H, d, $J = 2.5$ Hz, H-8), 6.52 (1H, dd, $J = 2.5$ and 8.5 Hz, H-6), 6.80 (2H, d, $J = 8.6$ Hz, H-3′ and H-5′), 7.22 (1H, d, $J = 8.5$ Hz, H-5), 7.27 (2H, d, $J = 8.6$ Hz, H-2′ and H-6′); ^{13}C

NMR (100 MHz; CD_3OD): δ 40.0 (C-3), 55.7 (OCH_3), 64.3 (C-4), 74.3 (C-2), 102.2 (C-8), 108.8 (C-6), 116.2 (C-3′ and C-5′), 117.5 (C-4a), 128.8 (C-2′ and C-6′), 132.5 (C-5), 133.5 (C-1′), 157.5 (C-4′), 158.4 (C-8a), 162.3 (C-7); ESP-MS (−40 V): *m*/*z* 271 $[M-H]^-$.

2,4-trans-4′-hydroxy-4-imidazolyl-7-methoxyflavan (**3d**) was obtained in 35% yield. ^1H NMR (400 MHz; CD_3OD): δ 2.34 (1H, br dt, $J = 2.5$ and 14.6 Hz, H-3_{eq}), 2.49(1H, ddd, $J = 4.5$, 11.5, and 14.6 Hz, H-3_{ax}), 3.79 (3H, s, OCH_3), 4.86 (1H, dd, $J = 2.0$ and 11.5 Hz, H-2), 5.49 (1H, br t, $J = 3.5$ Hz, H-4), 6.54 (1H, d, $J = 2.5$ Hz, H-8), 6.58(1H, dd, $J = 2.5$ and 8.5 Hz, H-6), 6.78 (2H, d, $J = 8.6$ Hz, H-3′ and H-5′), 7.03 (1H, br s, H-4″), 7.05 (1H, d, $J = 8.5$ Hz, H-5), 7.13 (1H, br s, H-5″), 7.18 (2H, d, $J = 8.6$ Hz, H-2′ and H-6′), 7.59 (1H, s, H-2″); ^{13}C NMR (100 MHz; CD_3OD): δ 39.1 (C-3), 52.7 (C-4), 55.9 (OCH_3), 74.4 (C-2), 102.7 (C-8), 109.8 (C-6), 111.4 (C-4a), 116.3 (C-3′ and C-5′), 120.3 (C-4″), 128.7 (C-2′ and C-6′), 128.9 (C-5″), 132.2 (C-1′), 132.3 (C-5), 138.0 (C-2″), 158.2 (C-4′), 158.7 (C-8a), and 163.0 (C-7); ESP-MS (+40 V): *m*/*z* 323 $[M + H]^+$.

2,4-cis-4′,7-dihydroxyflavan-4-ol (**2e**) was obtained in 10% yield. ^1H NMR (400 MHz; CD_3OD): δ 2.00 (1H, ddd, $J = 10.8$, 11.9, and 12.9 Hz, H-3_{ax}), 2.31 (1H, ddd, $J = 1.6$, 6.4, and 12.9 Hz, H-3_{eq}), 4.96 (1H, dd, $J = 6.4$ and 10.7 Hz, H-4), 5.00 (1H, dd, $J = 1.4$ and 11.9 Hz, H-2), 6.21 (1H, d, $J = 2.4$ Hz, H-8), 6.40 (1H, dd, $J = 2.4$ and 8.4 Hz, H-6), 6.80 (2H, d, $J = 8.5$ Hz, H-3′ and H-5′), 7.26 (2H, d, $J = 8.5$ Hz, H-2′ and H-6′), 7.26 (1H, d, $J = 8.4$ Hz, H-5); ^{13}C NMR (100 MHz; CD_3OD): δ 41.3 (C-3), 66.3 (C-4), 78.5 (C-2), 103.6 (C-8), 109.5 (C-6), 116.2 (C-3′ and C-5′), 119.0 (C-4a), 128.7 (C-2′ and C-6′), 129.1 (C-5), 133.4 (C-1′), 157.2 (C-4′), 158.5 (C-8a), and 158.9 (C-7); ESP-MS (−40 V): *m*/*z* 257 $[M-H]^-$.

2,4-trans-4′,7-dihydroxyflavan-4-ol (**2e′**) was obtained in 30% yield. ^1H NMR (400 MHz; CD_3OD): δ 2.04 (1H, dt, $J = 3.4$ and 14.3 Hz, H-3_{eq}), 2.08 (1H, ddd, $J = 2.6$, 9.4, and 14.3 Hz, H-3_{ax}), 4.68 (1H, t, $J = 2.8$ Hz, H-4), 5.10 (1H, dd, $J = 4.7$ and 9.4 Hz, H-2), 6.28 (1H, d, $J = 2.4$ Hz, H-8), 6.40 (1H, dd, $J = 2.4$ and 8.4 Hz, H-6), 6.80 (2H, d, $J = 8.5$ Hz, H-3′ and H-5′), 7.14 (1H, d, $J = 8.4$ Hz, H-5), 7.26 (2H, d, $J = 8.5$ Hz, H-2′ and H-6′); ^{13}C NMR (100 MHz; CD_3OD): δ 40.1 (C-3), 64.4 (C-4), 74.2 (C-2), 103.8 (C-8), 109.7 (C-6), 116.2 (C-3′ and C-5′), 116.5 (C-4a), 128.8 (C-2′ and C-6′), 132.6 (C-5), 133.6 (C-1′), 157.4 (C-4′), 158.4 (C-8a), and 159.7 (C-7); ESP-MS (−40 V): *m*/*z* 257 $[M-H]^-$.

2,4-trans-4′,7-dihydroxy-4-imidazolylflavan (**3e**) was obtained in 16% yield. ^1H NMR (400 MHz; CD_3OD): δ 2.32 (1H, dt, $J = 2.3$ and 14.5 Hz, H-3_{eq}), 2.47 (1H, ddd, $J = 4.5$, 11.6, and 14.5 Hz, H-3_{ax}), 4.82 (1H, dd, $J = 1.5$ and 11.6 Hz, H-2), 5.46 (1H, br t, $J = 3.3$ Hz, H-4), 6.40 (1H, d, $J = 2.4$ Hz, H-8), 6.46 (1H, dd, $J = 2.4$ and 8.4 Hz, H-6), 6.77 (2H, d, $J = 8.6$ Hz, H-3′ and H-5′), 6.97 (1H, d, $J = 8.4$ Hz, H-5), 7.03 (1H, br s, H-4″), 7.09 (1H, br s, H-5″), 7.17 (2H, d, $J = 8.6$ Hz, H-2′ and H-6′), 7.59 (1H, br s, H-2″); ^{13}C NMR (100 MHz; CD_3OD): δ 39.2 (C-3), 52.8 (C-4), 74.3 (C-2), 104.3 (C-8), 110.3 (C-4a), 110.6 (C-6), 116.3 (C-3′/5′), 120.2 (C-4″), 128.7 (C-2′/6′), 129.2 (C-5″), 132.3 (C-1′), 132.4 (C-5), 138.1 (C-2″), 158.1 (C-4′), 158.7 (C-8a), and 160.7 (C-7); ESP-MS (+40 V): *m*/*z* 309 $[M + H]^+$.

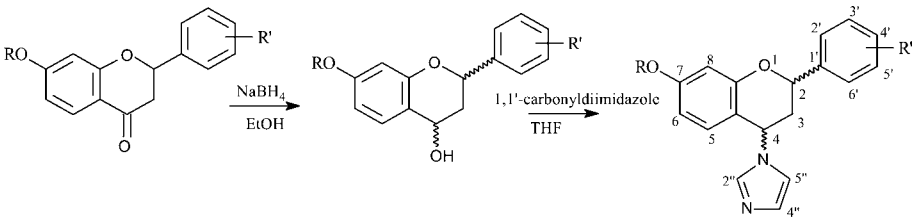
2.2. Biological tests

The inhibitory activities of the compounds towards aromatase were determined in vitro using human placental microsomes and [1,2,6,7-^{3}H]-androstenedione as previously described [4]. The IC_{50} values were determined graphically by testing compounds in six appropriate concentrations (0.02, 0.05, 0.07, 0.10, 0.50, and 1.00 μM) with each experiment performed in duplicate. The deviations were within ±5%.

3. Results and discussion

The route for the synthesis of the target compounds is outlined in Scheme 2. Flavanones **1a–1d** were prepared by cyclization of corresponding 2′-hydroxychalcones previously obtained by Claisen–Schmidt condensation between 2-hydroxy-4-methoxyacetophenone and appropriately substituted benzaldehydes [5]. For flavanone **1d**, condensation was carried out with 4-hydroxybenzaldehyde protected as tetrahydropyranyl ether. Flavanone **1e** was synthesized by cyclization of the 2′,4,4′-trihydroxychalcone, obtained by the condensation of 4-hydroxybenzaldehyde and 2,4-dihydroxyacetophenone. Both compounds were protected as their tetrahydropyranyl ethers.

Reduction of the 4′-substituted flavanones was carried out at a temperature of 6 °C because at room temperature, the alkaline medium resulting from the presence of $NaBH_4$ was responsible for the isomerization into the corresponding 2′-hydroxychalcones. Reduction by $NaBH_4$ of the flavanones **1a–1c** gave the 2,4-*cis*-flavan-4-ols



	R	R'
a	CH_3	3'-OH
b	CH_3	4'-Cl
c	CH_3	4'-CN
d	CH_3	4'-OH
e	H	4'-OH

Scheme 2. Synthesis of 4-imidazolylflavans **3a–3e**.

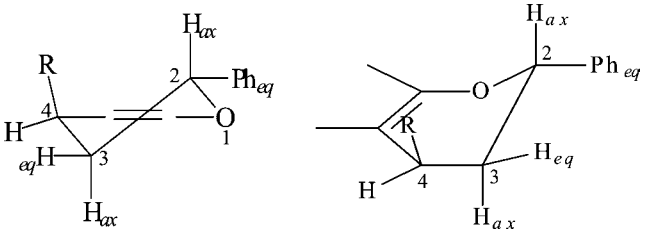
(**2a–2c**). The $NaBH_4$ reduction of the 4′-hydroxy-7-methoxyflavanone **1d** afforded the *cis* and *trans* isomers (**2d** and **2d′**, respectively) of 4′-hydroxy-7-methoxyflavan-4-ol, in a 2:1 ratio, which were separated by preparative HPLC using a diol column Merck LichroCART, Lichrospher 10μ (250 × 10 mm) with a mixture of hexane containing 5% isopropanol/methanol (97/3) as eluent. Reduction of the 4′,7-dihydroxyflavanone **1e** also gave the *cis* and *trans* isomers (**2e** and **2e′**, respectively) of 4′,7-dihydroxyflavan-4-ol, in a 1:3 ratio, which could be separated by HPLC on a reverse phase C18 column Waters Nova-Pak 4μ (100 × 8 mm) with H_2O/methanol (85/15). However, preparative chromatography was not performed and the condensation with 1,1′-carbonyldiimidazole was carried out on the mixture of the two isomers.

Treatment of the compounds **2a–2c** with 1,1′-carbonyldiimidazole in THF [10] led to the 2,4-*trans*-4-imidazolylflavans **3a–3c** while both the two isomers of 4′-hydroxy-7-methoxyflavan-4-ol, after condensation with 1,1′-carbonyldiimidazole, gave the 2,4-*trans*-4-imidazolylflavan **3d**. In the same way, the mixture of the two isomers of the 4′,7-dihydroxyflavan-4-ol led to the only 2,4-*trans*-4-imidazolylflavan **3e**.

The 2,4-*trans* configuration for these compounds was determined from the ^1H NMR vicinal coupling constants as previously described [7]. These constants are consistent with either the half chair (a) or sofa (b) conformation of the heterocyclic ring in which the H-2 is axial (Scheme 3). The coupling constant for the H-3_{ax} and H-2 is approximately 11 Hz, which corresponds to a *trans*-diaxial relationship between the protons. The coupling constant for the H-4 and H-3_{ax} in compounds **3a–3e** is 4.4 Hz, which corresponds to a quasi-equatorial position for the proton H-4 and allowed us to determine a 2,4-*trans* configuration.

It was observed that steric hindrance due to the 2-phenyl group influenced the type of substitution and was responsible for the 2,4-*trans*-stereochemistry of the 4-imidazolylflavans. From the 2,4-*cis*-flavan-4-ols, the inversion of configuration at the carbon C-4 was consistent with the occurrence of a S_N2 pathway while for the 2,4-*trans* isomers **2d′** and **2e′**, the reaction likely involved the formation of a carbocation followed by a nucleophilic attack by the azole on the only opposite side from the 2-phenyl group.

The IC_{50} values and inhibitory potencies of the compounds **3a–3e** relative to aminoglutethimide are reported in Table 1. The derivatives show high inhibitory activity against aromatase with IC_{50} values from 0.04 to 0.20 μM and are more potent than



Scheme 3. Conformation of the heterocyclic ring of 2,4-*trans*-4-imidazolylflavans.

Table 1
Aromatase inhibitory activity of compounds **3a–3e**

Compound	**3a**	**3b**	**3c**	**3d**	**3e**	Letrozole
IC_{50} (μM)	0.130	0.200	0.133	0.040	0.077	0.018
RP[a]/aminoglutethimide	40	26	39	130	68	289

[a] The relative potency (RP) was calculated from the ratio of IC_{50} values for compounds **3a–3e** to that measured for aminoglutethimide.

aminoglutethimide (IC_{50} = 5.2 μM), exhibiting relative potencies (RP) from 26 to 130.

The influence of the B ring substitution pattern on the aromatase inhibitory effect was investigated by comparing activities of compounds **3a–3d** with that of the 2,4-*trans*-4-imidazolyl-7-methoxyflavan **A_2** (IC_{50} = 0.091 μM). It appears that a cyano group or a chlorine atom at position 4′ did not enhance the aromatase inhibition as previously described for the 7-methoxyflavanone skeleton [5]. These results suggest that the 4-imidazolylflavans and known azole compounds bind to the active site of aromatase in a different way. For the azole compounds, para-substitution of a phenyl group by halogen atoms or a cyano group led to a significant enhancement in inhibitory activity [8,9]. The presence of a 3′-hydroxy group did not increase the anti-aromatase effect of these imidazolylflavans whereas this group was found to be essential for enhanced aromatase inhibitory activity of flavanones [5]. In contrast, the 4′-hydroxy substitution was shown to be responsible for an increase in aromatase inhibition. Thus, the compound **3d** is only 2.2-fold less active than letrozole (IC_{50} = 0.018 μM), which is used as the first-line therapy for metastatic breast cancer.

The 7-hydroxy-4-imidazolylflavan **A_1** (IC_{50} = 0.041 μM) was more potent than the 7-methoxy counterpart **A_2**. For this reason, we synthesized the 4′,7-dihydroxy-4-imidazolylflavan **3e**. Unfortunately, this compound was found to be less active than expected (IC_{50} = 0.077 μM) even though inhibitory potency relative to aminoglutethimide was equal to 68. In contrast to what was observed for the 7-methoxyflavan skeleton, the presence of an additional 4′-hydroxyl group led to a decrease in aromatase inhibition. This result is in agreement with those previously established for the 7-hydroxyflavanone scaffold since we demonstrated that 4′,7-dihydroxyflavanone was less potent than 7-hydroxyflavanone (unpublished results). These findings may suggest a different mode of binding to the active site of aromatase between 4-imidazolyl-7-methoxyflavans and the 7-hydroxy analogues.

4. Conclusion

The synthesis of five new 4-imidazolylflavans, which were found to be highly potent as aromatase inhibitors, has been described. This work allowed us to obtain a preliminary biological profile of the series indicating which compounds might be suitable for further investigation and in which direction optimization efforts should be pursued. The selectivity towards other P450 enzymes and the in vivo activity must be investigated to answer the question whether these compounds can be considered

as novel drug candidates for the treatment of hormone-dependent breast cancer. The next step in this work is to separate the most active racemic compounds by chiral HPLC, so as to enhance aromatase inhibitory activity of these 4-imidazolylflavans by testing enantiomerically pure compounds.

Acknowledgments

The authors are grateful to the Region Limousin for the grants awarded to C. Pouget and S. Yahiaoui and to Y. Champavier for acquiring the NMR spectra.

References

[1] M. Clemons, S. Danson, A. Howell, Cancer Treat. Rev. 28 (2002) 165–180.
[2] A.M.H. Brodie, V.C.O. Njar, Steroids 65 (2000) 171–179.
[3] P.E. Goss, K. Strasser, J. Clin. Oncol. 19 (2001) 881–894.
[4] J.C. Le Bail, T. Laroche, F. Marre-Fournier, G. Habrioux, Cancer Lett. 133 (1998) 101–106.
[5] C. Pouget, C. Fagnere, J.P. Basly, A.E. Besson, Y. Champavier, G. Habrioux, A.J. Chulia, Pharm. Res. 19 (2002) 286–291.
[6] Y.C. Kao, C. Zhou, M. Sherman, C.A. Laughton, S. Chen, Environ. Health Persp. 106 (1998) 85–92.
[7] C. Pouget, C. Fagnere, J.P. Basly, G. Habrioux, A.J. Chulia, Bioorg. Med. Chem. Lett. 12 (2002) 2859–2861.
[8] M. Lang, C. Batzl, P. Furet, R. Bowman, A. Häusler, S. Bhatnagar, J. Steroid Biochem. Mol. Biol. 44 (1993) 421–428.
[9] M. Okada, T. Yoden, E. Kawaminami, Y. Shimada, M. Kudoh, Y. Isomura, H. Shikama, T. Fujikura, Chem. Pharm. Bull. 44 (1996) 1871–1879.
[10] M. Le Borgne, P. Marchand, B. Delevoye-Seiller, J.M. Robert, G. Le Baut, R.W. Hartmann, M. Palzer, Bioorg. Med. Chem. Lett. 9 (1999) 333–336.