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# Design, Synthesis and Evaluation of 4-Imidazolylflavans as New Leads for Aromatase Inhibition

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**Abstract**—Two 4-imidazolylflavans were synthesized and their relative stereochemistry was established by $^{1}H$ and $^{13}C$ NMR data. These compounds were tested for their activity to inhibit aromatase. It was observed that the introduction of an imidazolyl group at carbon 4 on flavan nucleus led to potent molecules.


## Introduction

Non steroidal aromatase inhibitors are known to prevent the conversion of androgens to estrogens and thus play a significant role in the treatment of advanced breast cancer in postmenopausal women.[1] Today, a broad range of inhibitors has already been studied. Aminoglutethimide was the first non steroidal inhibitor clinically available. Then, the second generation of aromatase inhibitors, like the non steroidal fadrozole and the steroidal substrate analogue 4-hydroxyandrostenedione, was launched. Recently, a third generation of azole compounds with greater selectivity and potency has been developed including letrozole and anastrozole.[1] These two compounds have become the established second-line treatment for metastatic breast cancer after tamoxifen and have recently been approved as first-line therapy in several countries.[2] The structure of these non steroidal aromatase inhibitors can be regarded as consisting of two parts, one being the azole part with a nitrogen atom which binds to the heme iron atom of cytochrome P450 of aromatase and the other, the bulky aryl part which mimics the steroid ring of the substrate.

Besides synthetic aromatase inhibitors, some flavonoids which are natural compounds widely distributed in the plant kingdom, were found to inhibit aromatase with about the same activity as aminoglutethimide.[3–6] Thus, we demonstrated that 7-methoxyflavanone **1** and 7-hydroxyflavanone **2** were able to block aromatase activity ($IC_{50} = 8.0$ and 3.8 μM, respectively). Our own interest in both flavonoids and anti-breast cancer agents led us to design new aromatase inhibitors by taking the flavan nucleus as molecular skeleton on which to insert an imidazole ring supposed to be critical for the binding to the heme iron atom of cytochrome P450 of aromatase.

In the present paper, we report the synthesis and structural analysis of the 4-imidazolylflavans **5** and **6** and their biological evaluation against aromatase.

## Chemistry

The synthesis of the target compounds **5** and **6** is described in Scheme 1 and consisted in introduction of an imidazolyl group at carbon 4 on flavan nucleus. Stereoselective reduction by $NaBH_4$ of 7-methoxyflavanone **1** and 7-hydroxyflavanone **2** gave respectively 2,4-*cis*-7-methoxyflavan-4-ol **3** and 2,4-*cis*-7-hydroxyflavan-4-ol **4** as previously described.[7] Treatment of these two compounds with 1,1′-carbonyldiimidazole in THF led to the azole derivatives **5** (38%) and **6** (37%).

## Structural Analysis

The key signals and the associated coupling constants in the $^{1}H$ NMR spectra of the compounds **5** and **6** are

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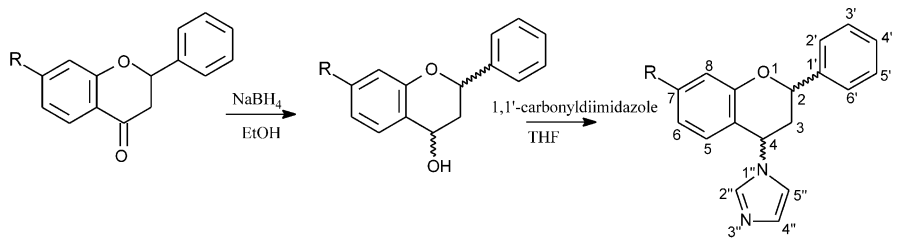


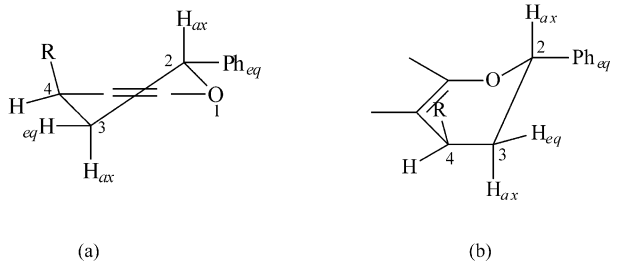

**Scheme 1.**

given in Table 1 except for the coupling constant between H-3eq and H-4 which could not be measured due to the broadening of the signals for these two protons. The assignment of stereochemistry to these 4-imidazolylflavans was readily made on the basis of the $^1$H NMR vicinal coupling constants. As previously described for 4-substituted flavans, the coupling constants for these two compounds were consistent with either the half-chair (a) or sofa (b) conformation of the heterocyclic ring in which the 2-aryl group was equatorial and H-2 was axial (Scheme 2).[7,8]

For the compound **5**, the occurrence of four protons at δ 2.38 (br dt, $J=2.6$ and 14.5 Hz), 2.48 (ddd, $J=4.4$, 11.2 and 14.5 Hz), 4.97 (dd, $J=2.2$ and 11.2 Hz) and 5.34 (br t, $J=3.7$ Hz) was characteristic for the heterocyclic ring of a 4-substituted flavan. The two highest field signals were assigned to H-3eq and H-3ax for which the geminal coupling constant was 14.5 Hz. Among the other constants for these two protons, the value 11.2 Hz was the only one which could arise from a *trans*-diaxial coupling and therefore corresponded to the constant between H-2 and H-3ax. This allowed H-2 to be assigned as the signal at 4.97 ppm and H-3ax as the signal at 2.48 ppm. Consequently, H-4 resonated at δ 5.34 ppm. In the same way, for the compound **6**, the signal at 5.02 ppm was assigned to H-2 and the signal at 5.42 ppm to H-4. Therefore, in the $^1$H NMR spectra of both compounds **5** and **6**, the lowest field signal was assigned to H-4 which was in accordance with previous results about 4-substituted flavans.[9] Then, the coupling constant between H-4 and H-3ax, which was equal to 4.4 Hz for both compounds **5** and **6**, was consistent with a quasi-equatorial position for the proton H-4 and allowed to determine a 2,4-*trans* configuration for these two compounds. This configuration was confirmed by the occurrence of the H-4 signal as a triplet; indeed, in the 2,4-*trans*-4-substituted flavans, $J_{3ax,4}$ and $J_{3eq,4}$ are sufficiently close for the H-4 signal to appear as a triplet.[8]

**Table 1.** Selected $^1$H NMR data for compounds **5** and **6**

| | δ H-3$_{eq}$ | δ H-3$_{ax}$ | δ H-2 | δ H-4 | $J_{3ax\text{-}3eq}$ | $J_{2-3ax}$ | $J_{2-3eq}$ | $J_{4-3ax}$ |
|---|---|---|---|---|---|---|---|---|
| **5** | 2.38 | 2.48 | 4.97 | 5.34 | 14.5 | 11.2 | 2.2 | 4.4 |
| **6** | 2.39 | 2.48 | 5.02 | 5.42 | 14.4 | 10.8 | 2.8 | 4.4 |




**Scheme 2.**

All $^{13}$C NMR signals could be assigned by the C–H correlation and long-range C–H COSY techniques. The C-4 resonance for compounds **5** and **6** at about 50 ppm was characteristic for a C–N type bond.

**2,4-*trans*-4-Imidazolyl-7-methoxyflavan 5.** Yield 38%; $^1$H NMR (400 MHz; $CDCl_3$): δ 2.38 (1H, br dt, $J=2.6$ and 14.5 Hz, H-3$_{eq}$), 2.48 (1H, ddd, $J=4.4$, 11.2 and 14.5 Hz, H-3$_{ax}$), 3.81 (3H, s, $OCH_3$), 4.97 (1H, dd, $J=2.2$ and 11.2 Hz, H-2), 5.34 (1H, br t, $J=3.7$ Hz, H-4), 6.57 (1H, d, $J=1.9$ Hz, H-8), 6.59 (1H, dd, $J=2.6$ and 9.0 Hz, H-6), 6.94 (1H, br s, H-4″), 7.04 (1H, d, $J=9.2$ Hz, H-5), 7.12 (1H, br s, H-5″), 7.34–7.40 (5H, m, Ph), 7.48 (1H, br s, H-2″); $^{13}$C NMR (100 MHz; $CDCl_3$): δ 38.3 (C-3), 51.0 (C-4), 55.4 ($OCH_3$), 73.1 (C-2), 101.6 (C-8), 109.2 (C-6), 109.6 (C-4a), 118.3 (C-4″), 126.1 (C-2′/6′), 128.4 (C-4′), 128.7 (C-3′/5′), 129.7 (C-5″), 131.2 (C-5), 136.8 (C-2″), 139.6 (C-1′), 156.4 (C-8a), 161.4 (C-7); *m*/*z* (EI), $M^+$ 306, (Found: $M^+$, 306.1354. $C_{19}H_{18}N_2O_2$ requires M, 306.1368).

**2,4-*trans*-7-Hydroxy-4-imidazolylflavan 6.** Yield 37%; $^1$H NMR (400 MHz; DMSO-$d_6$): δ 2.39 (1H, br dt, $J=3.2$ and 14.4 Hz, H-3$_{eq}$), 2.48 (1H, ddd, $J=4.4$, 10.4 and 14.4 Hz, H-3$_{ax}$), 5.02 (1H, dd, $J=2.8$ and 10.8 Hz, H-2), 5.42 (1H, t, $J=4.0$ Hz, H-4), 6.38 (1H, d, $J=2.0$ Hz, H-8), 6.42 (1H, dd, $J=2.0$ and 8.4 Hz, H-6), 6.91 (1H, d, $J=8.4$ Hz, H-5), 6.93 (1H, br s, H-4″), 7.13 (1H, br s, H-5″), 7.34–7.40 (5H, m, Ph), 7.57 (1H, br s, H-2″), 9.43 (1H, br s, OH); $^{13}$C NMR (100 MHz; DMSO-$d_6$): δ 37.6 (C-3), 50.2 (C-4), 73.3 (C-2), 103.5 (C-8), 109.9 (C-6), 110.3 (C-4a), 119.0 (C-4″), 126.6 (C-2′/6′), 128.4 (C-4′), 128.9 (C-3′/5′), 129.1 (C-5″), 131.3 (C-5), 137.1 (C-2″), 140.6 (C-1′), 156.1 (C-8a), 159.3 (C-7); *m*/*z* (EI), $M^+$ 292 (found: $M^+$, 292.1200. $C_{18}H_{16}N_2O_2$ requires M, 292.1211).

## Biological Assay

The inhibitory activities of the compounds **5** and **6** towards aromatase were determined in vitro using human placental microsomes and [1,2,6,7-$^3$H]-androstenedione

**Table 2.** Aromatase inhibitory activity of compounds **5** and **6**

| Compd | **5** | **6** |
|---|---|---|
| $IC_{50}$ (μM) | 0.091 | 0.041 |
| RP[a]/aminoglutethimide | 57 | 127 |
| RP[a]/flavanone | 88 | 93 |

[a]RP, relative potency calculated from the $IC_{50}$ values.

as previously described.[5] The $IC_{50}$ values and the potencies of the compounds **5** and **6**, relative to aminoglutethimide ($IC_{50} = 5.2$ μM) and to the corresponding flavanones, are given in Table 2. To determine $IC_{50}$ values, compounds **5** and **6** were tested in five appropriate concentrations with each experiment performed in duplicate.

## Results and Discussion

The target compounds **5** and **6** were tested for in vitro inhibitory activity against aromatase; these two derivatives demonstrated high potential against aromatase since under our assay conditions, the $IC_{50}$ were 0.091 and 0.041 μM, respectively. They proved to be more potent than aminoglutethimide, exhibiting relative potencies RP = 57 and 127, respectively. The $IC_{50}$ value for compound **6** was not significantly different from that obtained for the well-known non steroidal inhibitor fadrozole ($IC_{50} = 0.019$ μM).

Comparing the azole derivatives with their corresponding flavanones, it became apparent that replacement of the carbonyl function by an imidazolyl group led to a strong enhancement in enzyme inhibition. Further, the inhibitory potency depended on the substitution pattern; thus, exchanging the 7-methoxy group for a hydroxy substituent resulted in a marked increase of inhibitory potency. This result is in agreement with that described for flavanones.[5]

In conclusion, we have described the synthesis of new 4-imidazolylflavans and biochemical studies have shown that compounds **5** and **6** were highly potent as aromatase inhibitors. Therefore, they can be considered as new leads and further investigation is currently undergoing to explore the flavanone scaffold as skeleton of our molecules for inhibiting aromatase. In particular, we will carry out the substitution of the phenyl group at C-2 to reinforce the interaction between the nitrogen atom of the inhibitors and the heme iron atom of cytochrome P450 of aromatase. The full details of the structure–activity relationships, chemical synthesis, molecular modeling and biochemical studies of this novel series of compounds will be the subject of future publications from these laboratories.

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