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Synthesis and evaluation of 4-triazolylflavans as new aromatase inhibitors

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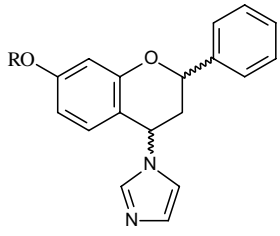
Abstract—Aromatase is a target of pharmacological interest for the treatment of estrogen-dependent cancers. Azole derivatives such as letrozole or anastrozole have been developed for aromatase inhibition and are used for the treatment of breast tumors. In this paper, four 4-triazolylflavans were synthesized and were found to exhibit moderate to high inhibitory activity against aromatase.

1. Introduction

Breast cancer, being the first cause of death in women between 40 and 50 years old, is an important public health problem. About 50% of breast cancers are considered to be estrogen dependent.[1] The final and the rate step in the production of estrogens is the conversion of androgens, androstenedione, and testosterone to estrone and estradiol, respectively, by aromatase, a cytochrome P450 enzyme.[2]

In postmenopausal women, aromatase inhibitors such as letrozole and anastrozole, have been shown to be useful in the second-line therapy of estrogen-dependent breast cancer and have recently been approved as first-line therapy in several countries.[3] These compounds are nonsteroidal inhibitors with an aza-hetero ring containing a sp^2 nitrogen that binds to the heme iron atom of aromatase. Previous studies revealed that inhibitory potency and selectivity for aromatase depend on the number and position of sp^2 nitrogen atoms in the heterocycle.[4–6]

In our search of new aromatase inhibitors, we synthesized the 4-imidazolyl-7-methoxyflavan **A** and 7-hydroxy-4-imidazolylflavan **B** (Fig. 1), which were found to present a significant anti-aromatase activity since they were shown to be more potent than aminoglutethimide, the first nonsteroidal inhibitor clinically used in breast cancer therapy.[7] Compounds **A** and **B** have to be considered as a result of the modulation of flavonoids, which are natural compounds widely distributed in the plant kingdom and with about the same inhibitory effect as aminoglutethimide against aromatase.[8,9] Some flavonoids are also known to be inhibitors of other steroidogenic enzymes such as the 17β-HSD type 1 (17β-hydroxysteroid dehydrogenase), which is involved in the regulation of the reversible interconversion of estrone to the potent estradiol.[10,11]



A : R = Me
B : R = H

Figure 1. Structure of 4-imidazolylflavans **A** and **B**.

Keywords: 4-Triazolylflavans; Aromatase inhibition; Breast cancer.
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The structure of the two molecules **A** and **B** consists of two parts, one being the imidazole part for interaction with the heme of aromatase and the second, the flavan skeleton, which mimics the steroid ring of the substrate.

With the aim to prepare new potent aromatase inhibitors, we undertook the replacement of the imidazole moiety by a triazole heterocycle, with the image of letrozole and anastrozole containing such a group. In this paper, we report the synthesis of the 4-triazolylflavans **1a**, **1b**, **2a**, and **2b** and their inhibitory potency against aromatase.

2. Chemistry

The synthetic pathway for compounds **1a**, **1b**, **2a**, and **2b** is described in Figure 2. The reduction of the commercially available 7-methoxyflavanone and 7-hydroxyflavanone was carried out with sodium borohydride in ethanol as previously described.[12] 2,4-*cis*-7-Methoxyflavan-4-ol and 2,4-*cis*-7-hydroxyflavan-4-ol were respectively, obtained and triazole moiety was introduced by direct reaction of these two flavan-4-ols with 1,1′-sulfinylditriazole in dry acetonitrile. 1,1′-Sulfinylditriazole was obtained through reaction between thionyl chloride and 1*H*-1,2,4-triazole.

For these triazole derivatives, two isomers could be isolated: the 1*H*-triazole and the 4*H*-triazole isomers, both with a 2,4-*trans*-configuration as previously established for the 4-imidazolylflavans.[7] For the 7-methoxy-substituted compounds, the 1*H*-triazole isomer **1a** was obtained in a 33% yield along with the 4*H*-triazole isomer **1b** in 34% yield, while for the 7-hydroxylated counterparts, we obtained 24% yield for the compound **2a** and 12% for the compound **2b**. However, we noticed that the 4*H*-triazole isomer **2b** undergoes a rearrangement to give the corresponding 1*H*-triazole derivative **2a**, as described by Bentley et al. for 1,2,4-triazol-4-yl-ethanols.[13] The mechanism proposed for such a rearrangement involves the electron releasing effect of the 7-hydroxy group, which by mesomerism, leads to the formation of the triazole anion (Fig. 3). Then, the nucleophilic attack of this anion, through the N-1 atom, provides the formation of the 1*H*-triazole derivative **2a**.

2.1. Experimental procedure

To an ice-cooled suspension of 1,2,4-triazole (4 equiv) in dry acetonitrile, was added a solution of thionyl chloride

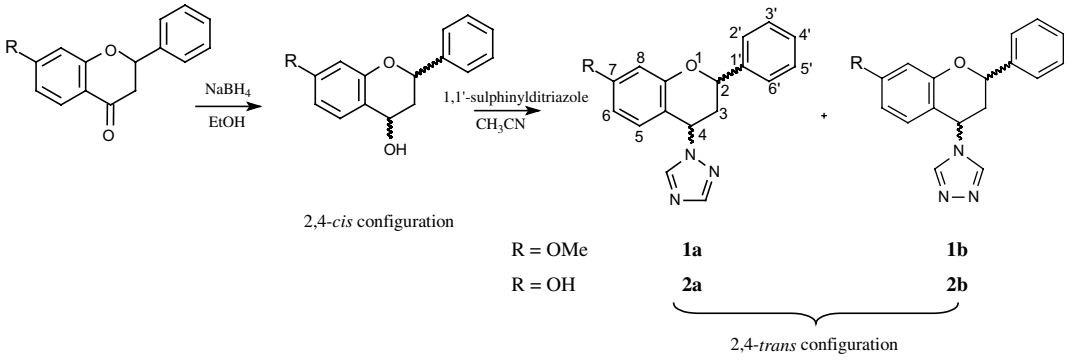

Figure 2. Synthesis of 4-triazolylflavans.

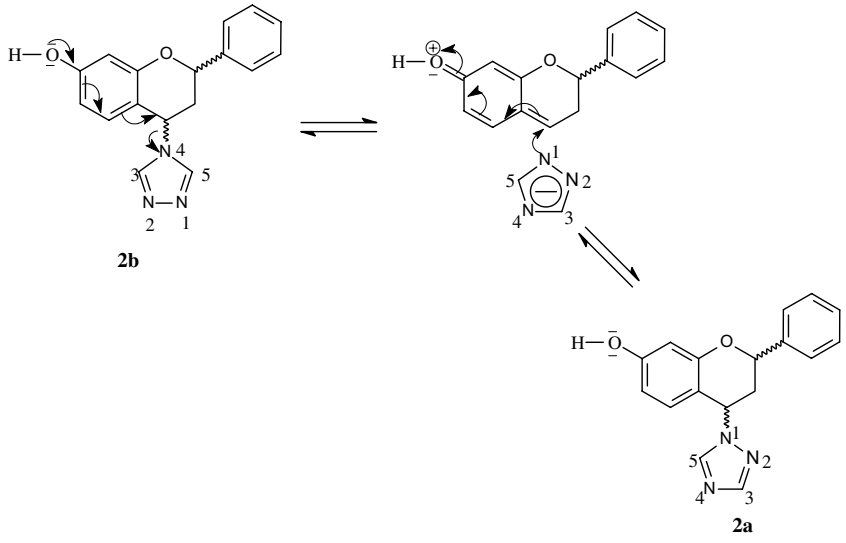

Figure 3. Rearrangement of the 4*H*-triazole isomer **2b** into the 1*H*-triazole derivative **2a**.

(1 equiv) in dry acetonitrile. The reaction was stirred under nitrogen at room temperature for 1 h. This mixture was added dropwise to a solution of flavan-4-ol (0.025 equiv) in dry acetonitrile and the reaction mixture was kept at room temperature under nitrogen atmosphere for 2 h. Acetonitrile was evaporated under reduced pressure and water was added to the residue. Extraction was performed with chloroform and compounds were purified via preparative TLC.

3. Structural analysis

3.1. 2,4-*trans*-7-Methoxy-4-1*H*-1,2,4-triazol-1-ylflavan (1a)

Yield 33%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.43 (1H, ddd, J = 4.4, 12.0, and 14.5 Hz, H-3ax), 2.73 (1H, br dt, J = 2.2 and 14.6 Hz, H-3eq), 3.82 (3H, s, OCH_3), 4.88 (1H, dd, J = 1.9 and 12.0 Hz, H-2), 5.53 (1H, dd, J = 2.2 and 4.2 Hz, H-4), 6.59 (1H, d, J = 2.4 Hz, H-8), 6.61 (1H, dd, J = 2.5 and 8.4 Hz, H-6), 7.11 (1H, d, J = 8.4 Hz, H-5), 7.32–7.41 (5H, m, Ph), 7.90 (1H, s, H-triazole), 8.04 (1H, s, H-triazole); ^{13}C NMR (100 MHz; $CDCl_3$): δ 36.0 (C-3), 54.3 (C-4), 55.4 (OCH_3), 73.1 (C-2), 101.9 (C-8), 108.2 (C-4a), 109.4 (C-6), 126.1 (C-2′/6′), 128.4 (C-4′), 128.7 (C-3′/5′), 131.3 (C-5), 139.5 (C-1′), 143.2 (CH-triazole), 152.6 (CH-triazole), 156.8 (C-8a), 161.8 (C-7); ESP-MS m/z, found: $[M+H]^+$ 308.1400. $C_{18}H_{18}N_3O_2$ requires $[M+H]^+$, 308.1399.

3.2. 2,4-*trans*-7-Methoxy-4-4*H*-1,2,4-triazol-4-ylflavan (1b)

Yield 34%; ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.36 (1H, br dt, J = 2.5 and 14.6 Hz, H-3eq), 2.55 (1H, ddd, J = 4.4, 11.6, and 14.6 Hz, H-3ax), 3.82 (3H, s, OCH_3), 4.90 (1H, dd, J = 2.0 and 11.6 Hz, H-2), 5.45 (1H, dd, J = 3.0 and 3.9 Hz, H-4), 6.59 (1H, d, J = 2.4 Hz, H-8), 6.61 (1H, dd, J = 2.5 and 8.4 Hz, H-6), 7.03 (1H, d, J = 8.4 Hz, H-5), 7.33–7.42 (5H, m, Ph), 8.16 (2H, s, H-triazole); ^{13}C NMR (100 MHz; $CDCl_3$): δ 38.0 (C-3), 50.2 (C-4), 55.5 (OCH_3), 72.8 (C-2), 102.0 (C-8), 108.0 (C-4a), 109.7 (C-6), 126.1 (C-2′/6′), 128.7 (C-4′), 128.8 (C3′/5′), 130.9 (C-5), 139.0 (C-1′), 142.3 (2 × CH-triazole), 156.7 (C-8a), 161.9 (C-7); ESP-MS m/z, found $[M+Na]^+$: 330.1220. $C_{18}H_{17}N_3O_2Na$ requires $[M+Na]^+$, 330.1218.

3.3. 2,4-*trans*-7-Hydroxy-4-1*H*-1,2,4-triazol-1-ylflavan (2a)

Yield 24%; ^{1}H NMR (400 MHz; CD_3 OD): δ 2.45 (1H, ddd, J = 4.8, 11.7, and 14.6 Hz, H-3ax), 2.59 (1H, br dt, J = 2.3 and 14.6 Hz, H-3eq), 5.08 (1H, dd, J = 2.2 and 11.7 Hz, H-2), 5.61 (1H, dd, J = 2.3 and 4.6 Hz, H-4), 6.43 (1H, d, J = 2.4 Hz, H-8), 6.47 (1H, dd, J = 2.4 and 8.4 Hz, H-6), 7.03 (1H, d, J = 8.4 Hz, H-5), 7.30–7.38 (5H, m, Ph), 8.06 (1H, s, H-triazole), 8.28 (1H, s, H-triazole); ^{13}C NMR (100 MHz; DMSO-d_6): δ 35.3 (C-3), 52.1 (C-4), 72.9 (C-2), 102.8 (C-8), 109.0 (C-4a), 109.2 (C-6), 121.1 (C-2′/6′), 128.0 (C-4′), 128.4 (C-3′/5′), 130.8 (C-5), 140.1 (C-1′), 143.5 (CH-triazole), 151.6 (CH-triazole), 155.7 (C-8a), 158.8 (C-7); ESP-MS m/z, found $[M+H]^+$: 294.1247. $C_{17}H_{16}N_3O_2$ requires $[M+H]^+$, 294.1243.

3.4. 2,4-*trans*-7-Hydroxy-4-4*H*-1,2,4-triazol-4-ylflavan (2b)

Yield 12%; ^{1}H NMR (400 MHz; pyridine-d_5): δ 2.52–2.56 (2H, m, H-3ax and H-3eq), 5.23 (1H, dd, J = 4.3 and 9.4 Hz, H-2), 5.72 (1H, br t, J = 3.5 Hz, H-4), 6.87 (1H, dd, J = 2.4 and 8.4 Hz, H-6), 6.97 (1H, d, J = 2.4 Hz, H-8), 7.11 (1H, d, J = 8.4 Hz, H-5), 7.32–7.39 (5H, m, Ph), 8.89 (2H, s, H-triazole); ^{13}C NMR (100 MHz; pyridine-d_5): δ 38.2 (C-3), 50.5 (C-4), 73.4 (C-2), 104.5 (C-8), 108.8 (C-4a), 111.2 (C-6), 126.9 (C-2′/6′), 128.8 (C-4′), 129.2 (C-3′/5′), 131.9 (C-5), 140.6 (C-1′), 143.5 (2 × CH-triazole), 157.2 (C-8a), 161.3 (C-7).

4. Biological assay

Aromatase inhibitory activity of the compounds was determined in vitro using human placental microsomes and [1,2,6,7-^{3}H] androstenedione as the substrate.[8] The IC_{50} values and the potencies (RP) relative to aminoglutethimide of the triazolyl compounds and their corresponding imidazolyl derivatives **A** and **B** are given in Table 1. Compounds were tested in five appropriate concentrations (5, 1, 0.5, 0.2, and 0.1 μM for compounds **1a** and **2a**; 50, 25, 10, 5, and 1 μM for compound **1b**) with each experiment performed in duplicate in order to determine their IC_{50}. The concentration of the substrate [1,2,6,7-^{3}H] androstenedione was 40 nM.

5. Results and discussion

The target compounds were tested for aromatase inhibitory activity except for compound **2b**, which could not be evaluated due to its rearrangement into the corresponding 1*H*-triazole isomer. These derivatives demonstrated moderate to high inhibitory activity against aromatase since using our assay conditions, the IC_{50} ranged from 0.43 to 31.6 μM while the IC_{50} of aminoglutethimide was 5.2 μM.

First, a marked difference of activity was observed between the two triazole isomers **1a** and **1b**; 4*H*-triazole isomer was found 20 times less potent than the corresponding 1*H*-triazole derivative. Similar results have been already described by Marchand et al.[14] for 3-(azolylmethyl)-1*H*-indoles while Vinh et al.[15] observed that, in a benzofuran series, a 4*H*-triazolyl isomer displayed greater activity than the 1*H*-isomer. Further

Table 1. Aromatase inhibitory activity of 4-triazolylflavans

	1a	1b	2a	A	B
IC_{50} (μM)	1.4	31.6	0.43	0.091	0.041
RP (/aminoglutethimide)[a]	3.7	0.16	12.1	57.1	126.8

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

investigations must be followed to determine the influence of the sp^2 nitrogen position on the coordination potential with the heme iron of aromatase. Then, it appeared that the triazoles exhibited weaker inhibitory activity than the corresponding imidazole analogues. For example, compound **1a** appeared to be 15 times less potent than the corresponding 4-imidazolylflavan **A** while inhibitory potency of compound **2a** was 10 times lower than that of derivative **B**. Such findings were previously demonstrated by Marchand et al.,[14] Vinh et al.[16] as well as by Lang et al.[17] who compared, in a series of cyanophenyl derivatives, the activity of letrozole with that of the imidazolyl analogue.

For imidazolyl compounds, it has been demonstrated that the imidazole moiety interacts through a N: $\rightarrow$ Fe^{3+}-heme coordinate link via the N-3 atom. Introduction of an additional nitrogen in the heterocyclic ring decreases the coordination potential, probably as a result of the electron withdrawing effect of this additional electronegative nitrogen.

However, it should be noted that compounds like letrozole or anastrozole containing this triazole moiety are extremely potent aromatase inhibitors, becoming essential therapeutic agents against hormone-dependent breast cancer. In fact, Lang et al. demonstrated that the replacement of the imidazole moiety by a triazole one led on the one hand to lower in vitro inhibition and on the other hand to higher in vivo activity.[17] The increased in vivo activity of triazoles compared with imidazoles has been shown to result from increased metabolic stability of the triazole derivatives.[18] Additionally, the triazole derived aromatase inhibitors generally exhibit greater selectivity with respect to other steroidogenic and nonsteroidogenic P450 enzymes.[17] Therefore, further experiments on the in vivo inhibitory potency and on the selectivity toward other steroidogenic P450 enzymes will be performed regarding the potency of our compound **2a**.

Moreover, we demonstrated that the inhibitory effect depends on the substitution pattern since exchanging the 7-methoxy group for a 7-hydroxy one increased significantly the anti-aromatase activity. This result indicates that it is not only the aza-heterocycle, which determines activity but the overall ability of the entire structure to fit in the P450 aromatase active site. Therefore, we are currently investigating the influence of flavan skeleton on anti-aromatase effect, particularly the substitution of the phenyl group at C-2.

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