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Original article

Lead optimization of 4-imidazolylflavans: New promising aromatase inhibitors

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ABSTRACT

Our previous studies have shown that several 7-substituted-4-imidazolylflavans are potent inhibitors of aromatase. These compounds were designed considering the anti-aromatase effect of some natural flavonoids and the importance of an azole ring for synthetic inhibitors such as letrozole or anastrozole towards binding to the heme iron of aromatase. In this study, we report the optimization of these lead compounds by the modulation of flavan A ring. The resulting 7,8-benzo-4-imidazolylflavans were tested in order to assess their ability to inhibit aromatase. Biological data concerning enantiomers obtained from the chiral separation of the racemate compound 4-imidazolyl-7-methoxyflavan are also presented.

1. Introduction

Breast cancer is the most common cancer diagnosed in women and represents the second leading cause of cancer-related deaths in women [1]. Nearly one-third of all breast cancers and two-thirds of postmenopausal breast cancers are hormone-dependent, estrogens playing a critical role in cancer cell proliferation. Therefore, several strategies were developed to remove the influence of these hormones on tumour growth; one of the most promising strategies is hormonotherapy based on aromatase inhibitors such as letrozole and anastrozole [2,3]. Aromatase, which is a cytochrome P450 enzyme, is responsible for the final step of the estrogen biosynthesis (i.e. the conversion of androgens to estrogens) and therefore is considered as a particularly attractive target for inhibition in the treatment of hormone-dependent breast cancer [4].

In addition to the large number of aromatase inhibitors that have arisen from medicinal chemistry efforts, some natural products such as flavonoids display an anti-aromatase activity [5,6]. Therefore, some research groups are pursuing drug discovery efforts exploiting flavonoids, including isoflavonoids [7,8], as core templates for novel aromatase inhibitors; with this aim, we have undertaken the modulation of A, B and C rings (see Scheme 1) of the flavan skeleton. Thus, some structure–activity relationships were evidenced through our different studies. First, a hydroxyl or a methoxy group on A ring at position 7 was found to be essential for activity [9,10]; then, we demonstrated that hydroxyl groups are also important on B ring at positions 3′ and/or 4′ [11]. Our investigations for the C ring modulation of flavan skeleton were directed towards the replacement of the flavanone 4-keto function by an azole ring (imidazolyl or triazolyl moiety) [12–14]. The introduction of an imidazolyl moiety resulted in 4-imidazolylflavans which are the most active molecules among the whole products we synthesized; some of them demonstrated a high potential against aromatase, exhibiting IC_{50} (40 nM) not far from that of letrozole (IC_{50} = 18 nM).

In order to optimize these lead compounds and to increase their interesting potency, we report in the course of the present paper, the synthesis of a new class of 4-imidazolylflavans substituted by a benzo ring at positions C-7 and C-8 (Scheme 1). This A ring modulation is based on the results of several studies which have shown that the presence of a benzo group at C-7 and C-8 on flavone skeleton is crucial for enhanced inhibitory activity [15,16]. Our previous work dealing with 7,8-benzoflavanones [17], confirmed this structure–activity relationship, the latter compounds being with the 8-prenylnaringenin [18,19], the most potent of the whole flavanones reported in the literature. In the present work, the synthesis of the non-substituted 7,8-benzo-4-imidazolylflavan was performed and cyano and hydroxyl groups were also introduced at the 4′ position of the B ring. First, the cyano group is common in several aromatase inhibitors such as anastrozole, letrozole or pyrazole based heterocycles [20]; in the azole family of aromatase inhibitors (letrozole, indole derivatives [21]), the presence of a *para*-cyano phenyl group is known to be important for activity. Secondly, we have previously demonstrated, that a 4′-hydroxy

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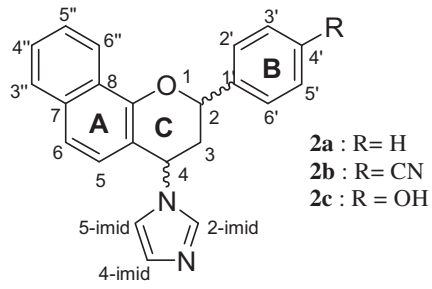


Scheme 1. Structure of the 7,8-benzo-4-imidazolylflavans **2a**, **2b** and **2c**.

substitution was responsible for an increase in aromatase inhibition in the case of a 4-imidazolyl-7-methoxyflavan [13].

These original 7,8-benzo-4-imidazolylflavans were obtained using the strategy described for the synthesis of previous 4-imidazolylflavans, involving chalcones, flavanones and flavan-4-ols as synthetic intermediates [12]. The synthesis and the characterization of the target compounds and of the 7,8-benzoflavan-4-ol intermediates are reported as well as the results of the biological evaluation against aromatase.

2. Results and discussion

2.1. Chemistry

2.1.1. General procedure of synthesis

In this work, three 7,8-benzo-4-imidazolylflavans were synthesized: the 7,8-benzo-4-imidazolylflavan (**2a**), the 4′-cyano derivative (**2b**) and the 4′-hydroxyl derivative (**2c**). The synthesis of these compounds is a multi-step process requiring three classes of intermediate products: 3′,4′-benzo-2′-hydroxychalcones, 7,8-benzoflavanones and 7,8-benzoflavan-4-ols. The synthesis pathway is depicted in Scheme 2. Benzochalcones and 7,8-benzoflavanones were described elsewhere [17].

2.1.2. 7,8-Benzoflavan-4-ols

Reduction of the 7,8-benzoflavanones was carried out at low temperature (6 °C) using $NaBH_4$; indeed, at room temperature, the alkaline medium resulting from the presence of $NaBH_4$ was responsible for the isomerization into the corresponding 2′-hydroxychalcones, as evidenced by NMR analysis. This reaction, except for the 4′-hydroxy derivative, was stereoselective since it led only to 2,4-*cis*-7,8-benzoflavan-4-ols. The hydride attack on the opposite side from the 2-phenyl group is responsible for this stereochemistry. The action of $NaBH_4$ on 7,8-benzo-4′-hydroxyflavanone led to a mixture of 2,4-*cis* and 2,4-*trans* isomers in a 1:1 ratio, as previously described for the reduction of 4′-hydroxy-7-methoxyflavanone and 4′,7-dihydroxyflavanone [13]. However, these two isomers were not separated.

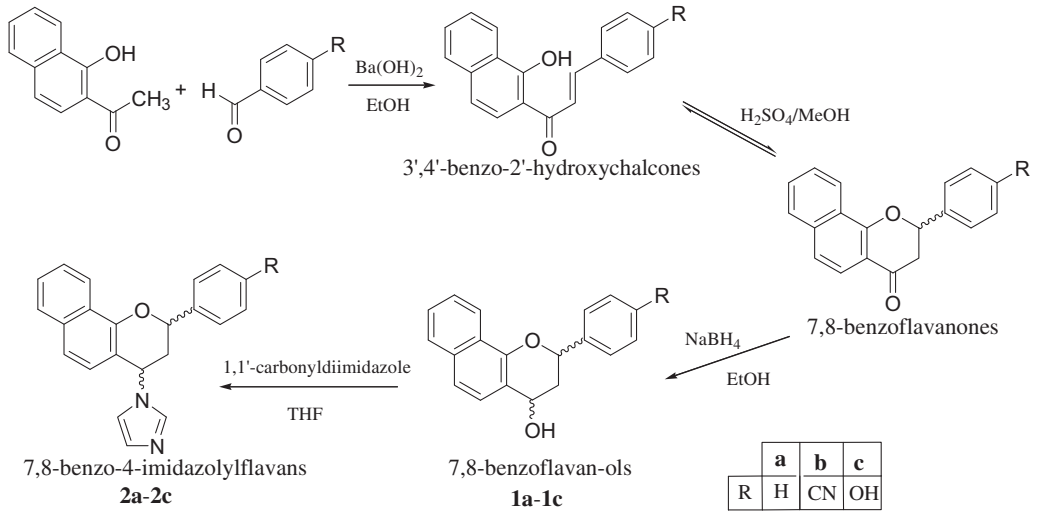
Excellent yields of these products were obtained (around 80%); nevertheless, flavan-4-ols deteriorate if not carefully handled and should be stored at low temperature. The instability may result from their possible intramolecular dehydration into flav-3-enes, as previously observed [22].

2.1.3. 7,8-Benzo-4-imidazolylflavans

In our previous studies, the substitution reaction on flavan-4-ols, using 1,1′-carbonyldiimidazole, led only to the 2,4-*trans*-4-imidazolylflavans [12,13]; however, in this work, the action of the same reagent on the 2,4-*cis*-7,8-benzoflavan-4-ols led also, for the first time, to the corresponding 2,4-*cis*-4-imidazolylflavans for compounds **2b** (4′-cyano) and **2c** (4′-hydroxy). Separation of the two stereoisomers of these 7,8-benzo-4-imidazolylflavans was undertaken but compound **2b** was the only one from which 2,4-*cis* and 2,4-*trans* isomers were obtained as pure molecules (1:4 ratio between the 2,4-*cis* and the 2,4-*trans* isomer).

2.2. Aromatase inhibitory activity

The pharmacological data - IC_{50} values and the potencies (RP) relative to aminoglutethimide which is the first aromatase inhibitor used clinically - are summarized in Table 1. For compound **2a**, the 2,4-*trans* isomer was the only obtained; for derivative **2b**, the two isomers 2,4-*cis* and 2,4-*trans* were separated and evaluated as pure molecules. A 50/50 mixture of these two isomers was also made for measuring its inhibitory effect. Then, for compound **2c**, anti-aromatase activity was evaluated for a 50/50 mixture of the two isomers. Previous results concerning 7,8-benzoflavanones and 2,4-*trans*-4-imidazolyl-7-methoxyflavans are also reported (Table 1).



Scheme 2. Synthesis pathway for 7,8-benzo-4-imidazolylflavans (**2a**–**2c**).

Table 1
Aromatase inhibitory activity of 7,8-benzo-4-imidazolylflavans (**2a**–**2c**) *The relative potency (RP) was calculated from the ratio of IC_{50} values for the tested compounds to that measured for aminoglutethimide (AG) (IC_{50} = 5.8 μM).

B ring substitution	7,8-benzo flavanones	4-imidazolylflavans				
		7,8-benzo				2,4-*trans*–7-methoxy
	IC_{50}(μM)	Compound	stereochemistry	IC_{50}(μM)	RP/AG*	RP/AG*
not substituted	4.3	**2a**	2,4-*trans*	0.036	161	64
4′–CN	1.54	**2b**	2,4-*cis*	0.534	11	
			2,4-*trans*	0.034	170	39
			2,4-*trans*+2,4-*cis* (1:1)	0.078	74	
4′–OH	0.63	**2c**	2,4-*trans*+2,4-*cis* (1:1)	0.032	181	130

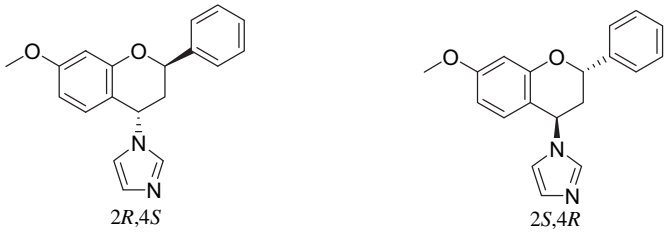
First, the derivatives show high inhibitory activity against aromatase with IC_{50} values from 0.03 to 0.53 μM and are more potent than aminoglutethimide (IC_{50} = 5.8 μM) exhibiting relative potencies (RP) from 11 to 180. Comparing the target compounds with their corresponding flavanones, it became apparent that replacement of the carbonyl function by an imidazolyl group lead to a strong increase in enzyme inhibition. Thus, the 2,4-*trans*-7,8-benzo-4-imidazolylflavan **2a** is 120 times more potent than the 7,8-benzoflavanone. This result is in agreement with those previously described for 7-hydroxy-, and 7-methoxyflavanones [12].

Besides, comparing the activities of 7,8-benzo-4-imidazolylflavans with those of their 7-methoxy counterparts, it is evident that a benzo ring at C-7 and C-8 positions of 4-imidazolylflavan skeleton is more attractive than a 7-methoxy group for an enhanced aromatase inhibition [13]. Thus, the 7,8-benzo-4-imidazolylflavans were found to be more potent than 7-methoxy analogs. These findings reinforce the results of many studies dealing with flavonoid inhibitory effect, like 7,8-benzoflavanones [17], or 7,8-benzoflavone [5,15,16], and demonstrating that an additional 7,8-benzo ring is of great interest to obtain potent aromatase inhibitors.

Then, for the first time, a 2,4-*cis*-4-imidazolylflavan was obtained and evaluated for anti-aromatase effect. A marked difference of activity was observed between the two isomers (2,4-*trans* and 2,4-*cis*) of compound **2b**; 2,4-*cis* isomer was found 15 times less potent than the 2,4-*trans* derivative. A 50/50 mixture of the two isomers was made and it was found to be twice less potent than the 2,4-*trans* isomer.

Finally, the influence of the 4′-substituent (B ring) on the aromatase inhibitory activity was investigated. By comparing activity of 2,4-*trans* isomers for compounds **2a** and **2b**, it appears that a cyano group at position 4′ do not increase the aromatase inhibition as previously noticed for the 4-imidazolyl-7-methoxyflavan derivatives [13]. On the contrary, an additional 4′-cyano group on the 7,8-benzoflavanone skeleton was previously found to enhance the anti-aromatase effect [17]. These findings may suggest a different mode of binding to the active site of the aromatase between flavanones and 4-imidazolylflavans. Then, it was important to study the influence of a 4′-hydroxyl group since the 4′-hydroxy substitution on 7,8-benzoflavanone [17], and on 4-imidazolyl-7-methoxyflavan [13], was shown to increase aromatase inhibition. Comparing the activities of the 50/50 mixture for compounds **2b** and **2c**, it appears that an additional 4′-hydroxyl group is more favorable to the anti-aromatase effect; thus, the 4′-hydroxy derivative is 2.5 times more potent than the 4′-cyano analog. Certainly, the separation between the two isomers (2,4-*trans* and 2,4-*cis*) of compound **2c** was not performed but considering that a 2,4-*trans* isomer is twice more potent than a 50/50 mixture of the two isomers, a great IC_{50} value for the 2,4-*trans* isomer of compound **2c** could be expected. Thus, this 2,4-*trans* compound could be, *in vitro*, as active as letrozole (IC_{50} = 0.018 μM), which is used as the first-line therapy for metastatic breast cancer. The introduction of this 4′-hydroxyl group on B ring of 7,8-benzo-4-imidazolylflavan scaffold seems very important to reinforce the interaction between the inhibitor and the aromatase active site. So, our prior interest is now to carry out further work to separate the two isomers of compound **2c** in order to obtain the 2,4-*trans* isomer as a pure molecule and to confirm the great influence of stereochemistry on biological activity.

It is also of importance to consider that our 4-imidazolylflavans were always tested as racemate compounds; to further investigate the influence of stereochemistry on biological activity, the first active 2,4-*trans*-4-imidazolyl-7-methoxyflavan that we synthesized, was separated into its enantiomers (2*S*,4*R* and 2*R*,4*S*) (Scheme 3) using chiral HPLC (Chiralpak®AD) with hexane/ethanol 80/20 as an eluent. Thus, pure enantiomers of a 2,4-*trans*-4-imidazolylflavan were tested, for the first time, against aromatase (Table 2). The (+) enantiomer was found to be a highly potent inhibitor, being 1.5 and 10 times more active than its racemate and the (−) enantiomer, respectively. This result confirms the importance of the chiral center configuration towards aromatase inhibition, as previously observed for vorozole [23] and for indole derivatives [21].



Scheme 3. Structure of the 2,4-*trans*-4-imidazolyl-7-methoxyflavan enantiomers.

3. Conclusion

With the aim to design new leads for aromatase inhibition, we are pursuing the modulation of flavan skeleton and we synthesized novel 7,8-benzo-4-imidazolylflavans which were tested against aromatase, exhibiting a higher inhibitory activity than 4-imidazolyl-7-methoxyflavans. Thus, the 2,4-*trans* isomer of 7,8-benzo-4′-cyano-4-imidazolylflavan **2b** is only twice less active than letrozole.

Table 2
Aromatase inhibitory activity of 2,4-*trans*-4-imidazolyl-7-methoxyflavan.

Compound	racemate	(+) enantiomer	(−) enantiomer
IC_{50} (μM)	0.091	0.060	0.600
RP/AG	64	97	10

Besides, a 2,4-*cis* isomer was obtained for the first time and was found to be less potent than the corresponding 2,4-*trans* isomer, which demonstrates the great influence of stereochemistry on biological effect. So, it is of interest to work further to the separation of the 2,4-*cis* and 2,4-*trans* isomers of compound **2c** since the 50/50 mixture was evaluated to be only twice less active than letrozole. We have also demonstrated for the first time the difference of activity between the two enantiomers (2*S*,4*R* and 2*R*,4*S*) of a 2,4-*trans*-4-imidazolylflavan. Therefore, a next step in this work is the chiral separation of all these racemic compounds, in order to enhance anti-aromatase effect by testing enantiomerically pure compounds. Further experiments directed towards X-ray crystallographic and molecular modeling studies, are currently undergoing, considering that 7,8-benzo-4-imidazolylflavans could probably result in novel drug candidates for the treatment of hormone-dependent breast cancer.

4. Experimental

4.1. General experimental procedures

Compounds were purified by preparative thin layer chromatography on Macherey–Nagel silica gel. NMR spectra were recorded on a Bruker 400 MHz spectrometer with Me_4Si as an internal standard. Mass spectrometric (ESI-TOF) analysis was carried out on a Waters Alliance system equipped with electrospray interface.

4.2. Synthesis

To a solution of 7,8-benzoflavanone in ethanol was added sodium borohydride (5 eq). The mixture was kept under nitrogen atmosphere at a temperature of 6 °C for 24–72 h, then evaporated under reduced pressure. Water was added, the pH was adjusted to 6 using 1M HCl and the resulting solution was extracted with chloroform. The organic layers were washed with water, dried over Na_2SO_4 and evaporated. Preparative TLC on silica gel afforded pure flavan-4-ols.

To a solution of flavan-4-ol in anhydrous THF was added 1,1′-carbonyldiimidazole (4eq). The mixture was kept under nitrogen atmosphere at room temperature for 20–48 h. Subsequently, it was evaporated under reduced pressure. The residue was dissolved in chloroform and the organic layer was washed with water, dried over Na_2SO_4 and evaporated. The resulting 4-imidazolylflavans were purified *via* preparative TLC on silica gel.

4.3. Characterization of compounds

4.3.1. Non-substituted derivatives

2,4-cis-7,8-Benzoflavan-4-ol (**1a**) was obtained in an 81% yield, as a white amorphous solid; mp 168 °C. UV in MeOH λ_{max}: 218, 239, 296, 324 nm.^{1}H NMR (400 MHz; $CDCl_3$): δ 2.25 (1H, ddd, J = 9.9; 11.3 and 13.2 Hz, H-3ax), δ 2.66 (1H, ddd, J = 2.1; 6.4 and 13.3 Hz, H-3eq), δ 5.20 (1H, br t, J = 7.7 Hz, H-4), δ 5.35 (1H, dd, J = 1.7 and 11.3 Hz, H-2), δ 7.36 (1H, dt, J = 2.4 and 7.2 Hz, H-4′), δ 7.41–7.53 (5H, m, H-6; H-4″; H-5″; H3′ and H5′), δ 7.52 (2H, d, J = 7.2 Hz, H-2′ and H-6′), δ 7.58 (1H, d, J = 8.5 Hz, H-5), δ 7.77 (1H, br d, J = 7.9 Hz, H-3″), δ 8.23 (1H, br d, J = 8.6 Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 40.0 (C-3), δ 65.8 (C-4), δ 76.9 (C-2), δ 119.1 (C-4a), δ 120.4 (C-6), δ 122.2 (C-6″), δ 124.3 (C-5), δ 124.8 (C-8), δ 125.5 (C-5″), δ 125,9 (C-2′ and C-6′), δ 126.5 (C-4″), 127.4 (C-3″), δ 128.1 (C-4′), δ 128.6 (C-3′ and C-5′), δ 134.1 (C-7), δ 140.7 (C-1′), δ 149.6 (C-8a). HRMS (ESI+) calculated for $C_{19}H_{16}O_2Na$ $(M + Na)^+$ 299.1048, found 299.1056.

2,4-trans-7,8-Benzo-4-imidazolylflavan (**2a**) was obtained in a 21% yield, as a white amorphous solid; mp 170 °C.UV in MeOH λ_{max}: 216, 236, 291, 323 nm ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.50 (1H, dt, J = 2.6 and 14.4 Hz, H-3eq), δ 2.59 (1H, ddd, J = 4.6; 11.0 and 14.4 Hz, H-3ax), δ 5.18 (1H, dd, J = 2.4 and 11.0 Hz, H-2), δ 5.49 (1H, br t, J = 3.6 Hz, H-4), δ 6.70 (1H, br s, H-4imid), δ 7.12 (1H, br s, H-5imid), δ 7,14 (1H, d, J = 8.5 Hz, H-5), δ 7.35 to δ 7.59 (9H, m, H-6; H-2′; H-3′; H-4′; H-5′; H-6′; H-4″; H-5″ and H-2imid), δ 7.82 (1H, br d, J = 7.5 Hz, H-3″), δ 8.20 (1H, br d, J = 8.0 Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 38.5 (C-3), δ 51.6 (C-4), δ 73.5 (C-2), δ 110.9 (C-4a), δ 118.7 (C-4imid), δ 121.2 (C-6), δ 122.3 (C-6″), δ 125.2 (C-8), δ 126.0 (C-2′ and C-6′), δ 126.1 (C-5″), δ 126.5 (C-5), δ 127.5 (C-4″), δ 127.6 (C-3″), δ 128.4 (C-4′), δ 128.8 (C-3′ and C-5′), δ 129.7 (C-5imid), δ 134.7 (C-7), δ 137.0 (C-2imid), δ 139.8 (C-1′), δ 151.3 (C-8a). HRMS (ESI+) calculated for $C_{22}H_{19}N_2O$ $(M + H)^+$ 327.1497, found 327.1506.

4.3.2. 4′-cyano derivatives

2,4-cis-7,8-Benzo-4′-cyano-flavan-4-ol (**1b**) was obtained in an 81% yield, as a white amorphous solid; mp 122 °C. UV in MeOH λ_{max}: 221, 237, 294, 323 nm ^{1}H NMR (400 MHz; CD_3OD) : δ 2.09 (1H, ddd, J = 10.3; 12.0 and 12.9 Hz, H-3ax), δ 2.58 (1H, ddd, J = 1.9; 6.4 and 13.0 Hz, H-3eq), δ 5.22 (1H, dd, J = 6.4 and 10.3 Hz, H-4), δ 5.46 (1H, dd, J = 1.7 and 11.9 Hz, H-2), δ 7.42 (1H, m, H-5"), δ 7.45 (1H, m, H-4"), δ 7.46 (1H, d, J = 8.8 Hz, H-6), δ 7.60 (1H, d, J = 8.6 Hz, H-5), δ 7.77 (2H, d, J = 8.3 Hz, H-2′ and H-6′), δ 7.78 (1H, m, H-3″), δ 7.81 (2H, d, J = 8.5 Hz, H-3′ and H-5′), δ 8.14 (1H, br d, J = 8.2 Hz, H-6").^{13}C NMR (100 MHz; CD_3OD) : δ 40.9 (C-3), δ 66.0 (C-4), δ 77.8 (C-2), δ 112.7 (C-4′), δ 119.7 (CN), δ 121.2 (C-4a), δ 121.5 (C-6), δ 122.8 (C-6"), δ 125.7 (C-5), δ 126.1 (C-8), δ 126.5 (C-5"), δ 127.4 (C-4"), δ 128.0 (C-2′ and C-6′), δ 128.6 (C-3"), δ 133.7 (C-3′ and C-5′), δ 135.5 (C-7), δ 148.2 (C-1′), δ 150.3 (C-8a). HRMS (ESI+) calculated for $C_{20}H_{15}NO_2Na$ $(M + Na)^+$ 324.1000, found 324.1005.

*4.3.2.1. 7,8-Benzo-4′-cyano-4-imidazolylflavan (**2b**).* 2,4-*cis* isomer : was obtained in a 7% yield, as a white amorphous solid; mp 220 °C.UV in MeOH λ_{max}: 221, 242, 282, 323 nm ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.54 (1H, dt, J = 11.6 and 13.2 Hz, H-3ax), δ 2.77 (1H, ddd, J = 1.8; 6.3 and 13.5 Hz, H-3eq), δ 5.53 (1H, br d, J = 10.5 Hz; H-2), δ 5.88 (1H, dd, J = 6.3 and 11.2 Hz, H-4), δ 6.82 (1H, d, J = 8.6 Hz, H-5), δ 6.85 (1H, br s, H-4*imid*), δ 7.12 (1H, br s, H-5*imid*), δ 7.40 (1H, d, J = 8.6 Hz, H-6), δ 7.53 (1H, m, H-5″), δ 7.55 (1H, m, H-4″), δ 7.69 (1H, br s, H-2*imid*), δ 7.71 (2H, d, J = 8.3 Hz, H-2′and H-6′), δ 7.77 (2H, d, J = 8.4 Hz, H-3′ and H-5′), δ 7.80 (1H, m, H-3″), δ 8.25 (1H, br d, J = 8.0 Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$) : δ 39.1 (C-3), δ 53.3 (C-4), δ 76.6 (C-2), δ 112.4 (C-4′), δ 114.3 (C-4a), δ 117.5 (C-4*imid*), δ 118.5 (CN), δ 121.7 (C-6), δ 121.9 (C-6″), δ 123.7 (C-5), δ 124.8 (C-8), δ 126.3 (C-5″), δ 126.6 (C-2′ and C-6′), δ 127.3 (C-4″), δ 127.7 (C-3″), δ 130.2 (C-5*imid*), δ 132.7 (C-3′ and C-5′), δ 134.3 (C-7), δ 137.2 (C-2*imid*), δ 144.9 (C-1′), δ 150.0 (C-8a). HRMS (ESI+) calculated for $C_{23}H_{18}N_3O$ $(M + H)^+$ 352.1450, found 352.1460.

2,4-*trans* isomer : was obtained in a 30% yield, as a white amorphous solid; mp 216 °C.UV in MeOH λ_{max}: 222, 241, 281, 323 nm ^{1}H NMR (400 MHz; $CDCl_3$) : δ 2.48 to δ 2.57 (2H, m, H-3eq and H-3ax), δ 5.22 (1H, dd, J = 4.7 and 9.2 Hz, H-2), δ 5.51 (1H, br t, J = 3,6 Hz, H-4), δ 6.97 (1H, br s, H-4*imid*), δ 7.15 (1H, br s, H-5*imid*), δ 7,17 (1H, d, J = 8.5 Hz, H-5), δ 7.49 (1H, d, J = 8.5 Hz, H-6), δ 7.51 (1H, br s, H-2*imid*), δ 7.57 (1H, m, H-5″), δ 7.59 (2H, d, J = 8.2 Hz, H-2′ and H-6′), δ 7.60 (1H, m, H-4″), δ 7.73 (2H, d, J = 8.4 Hz, H-3′ and H-5′), δ 7.84 (1H, br d, J = 8.4 Hz, H-3″), δ 8.31 (1H, br d, J = 8.2 Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 38.7 (C-3), δ 51.1 (C-4), δ 72.7 (C-2), δ 110.8 (C-4a), δ 112.3 (C-4′), δ 118.5 (C-4*imid*), δ 118.5 (CN), δ 121.7 (C-6), δ 122.0 (C-6″), δ 124.9 (C-8), δ 126.3 (C-5″), δ 126.5 (C-5), δ 126.7 (C-2′ and H-6′), δ 127.7 (C-4″), δ 127.8 (C-3″), δ 130.0 (C-5*imid*), δ 132.7 (C-3′ and C-5′), δ 134.7 (C-7), δ 136.9 (C-2*imid*), δ 145.1 (C-1′), δ 150.6 (C-8a). HRMS (ESI+) calculated for $C_{23}H_{18}N_3O$ $(M + H)^+$ 352.1450, found352.1449.

4.3.3. 4′-hydroxy derivatives

7,8-Benzo-4′-hydroxy-flavan-4-ol (**1c**) was obtained in an 81% yield, as a white amorphous solid; mp 190 °C. UV in MeOH λ_{max} : 221, 238, 284, 322 nm. HRMS (ESI+) calculated for $C_{19}H_{16}O_3Na$ $(M + Na)^+$ 315.0997, found 315.1008.

2,4-cis isomer : ^{1}H NMR (400 MHz; CD_3OD) : δ 2.17 (1H, ddd, J = 10.7; 12.6 and 13.0 Hz, H-3ax), δ 2.49 (1H, ddd, J = 1.7; 6.4 and 13.0 Hz, H-3 eq), δ 5.19 (1H, dd, J = 6.5 and 10.5 Hz, H-4), δ 5.23 (1H, dd, J = 1.2 and 12.6 Hz, H-2), δ 6.85 (2H, d, J = 8.5 Hz, H-3′ and H-5′), δ 7.37 (1H, m, H-5″), δ 7.38 (2H, d, J = 8.4 Hz, H-2′ and C-6′), δ 7.38−7.47 (2H, m, H-6 and H-4″), δ 7.59 (1H, d, J = 8.6 Hz, H-5), δ 7.74 (1H, br d, J = 7.3 Hz, H-3″), δ 8.09 (1H, br d, J = 8.0 Hz, H-6″). ^{13}C NMR (100 MHz; CD_3OD) : δ 41.0 (C-3), δ 66.6 (C-4), δ 78.7 (C-2), δ 116.3 (C-3′ and C-5′), δ 118.5 (C-4a), δ 120.9 (C-6), δ 123.1 (C-6″), δ 125.7 (C-5), δ 126.2 (C-5″), δ 126.6 (C-8), δ 127.2 (C-4″), δ 128.4 (C-3″), δ 128.8 (C-2′ and C-6′), δ 133.6 (C-1′), δ 135.5 (C-7), δ 151.1 (C-8a), δ 158.5(C-4′).

2,4-trans isomer : ^{1}H NMR (400 MHz; CD_3OD) : δ 2,19 (1H, ddd, J = 3.5; 11.4 and 15.0 Hz, H-3ax), δ 2.26 (1H, dt, J = 2.6 and 14.2 Hz, H-3 eq), δ 4.85 (1H, m, H-4), δ 5.27 (1H, dd, J = 2.8 and 11.5 Hz, H-2), δ 6.86 (2H, d, J = 8.5 Hz, H-3′ and H-5′), δ 7.38 (2H, d, J = 8.4 Hz, H-2′ and H-6′), δ 7.38−7.43 (3H, m, H-5; H-6 and H-5″), δ 7.45 (1H, m, H-4″), δ 7.76 (1H, br d, J = 7.6 Hz, H-3″), δ 8.17 (1H, br d, J = 7.7 Hz, H-6″). ^{13}C NMR (100 MHz; CD_3OD) : δ 40.1 (C-3), δ 64.7 (C-4), δ 74.7 (C-2), δ 116.3 (C-3′ and C-5′), δ 121.0 (C-6), δ 121.1 (C-4a), δ 123.2 (C-6″), δ 126.3 (C-5″), δ 126.6 (C-8), δ 127.6 (C-4″), δ 128.5 (C-3″), δ 128.7 (C-2′ and C-6′), δ 128.9 (C-5), δ 133.4 (C-1′), δ 135,6 (C-7), δ 151.6 (C-8a), δ 158.4(C-4′).

7,8-Benzo-4′-hydroxy-4-imidazolylflavan (**2c**) was obtained in a 46% yield, as a white amorphous solid; mp 180 °C.UV in MeOH λ_{max} : 221, 241, 283, 323 nm. HRMS (ESI+) calculated for $C_{22}H_{19}N_2O_2$ $(M + H)^+$ 343.1447, found 343.1444.

2,4-*cis* isomer : ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.59 to δ 2.76 (2H, m, H-3ax and H-3eq), δ 5.46 (1H, br d, J = 10.3 Hz, H-2), δ 6.05 (1H, dd, J = 6.2 and 11.2 Hz, H-4), δ 6.64 (1H, d, J = 8.6 Hz, H-5), δ 6.86 (2H, d, J = 8.5 Hz, H-3′ and H-5′), δ 6.95 (1H, br s, H-4*imid*), δ 7.23 (1H, br s, H-5*imid*), δ 7.39 (1H, d, J = 8.6 Hz, H-6), δ 7.46 (2H, d, J = 8.5 Hz, H-2′ and H-6′), δ 7.49 to δ 7.60 (2H, m, H-4″ and H-5″), δ 7.88 (1H, br d, J = 7.6 Hz, H-3″), δ 7.91 (1H, br s, H-2*imid*), δ 8.16 (1H, br d, J = 7.9 Hz, H-6″), δ 9.49 (1H, br s, 4−OH). ^{13}C NMR (100 MHz, DMSO-d6): δ 36.7 (C-3), δ 52.5 (C-4), δ 77.1 (C-2), δ 115.3 (C-3′ and C-5′), δ 116.2 (C-4a), δ 118.3 (C-4*imid*), δ 120.3 (C-6), δ 121.6 (C-6″), δ 123 0.7 (C-5), δ 124.5 (C-8), δ 125.9 (C-5″), δ 126.8 (C-4″), δ 127.5 (C-3″), δ 127,8 (C-2′ and C-6′), δ 128.9 (C-5*imid*), δ 130.4 (C-1′), δ 133.5 (C-7), δ 137.1 (C-2*imid*), δ 149.7 (C-8a), δ 157.3 (C-4′).

2,4-*trans* isomer : ^{1}H NMR (400 MHz; DMSO-d_6) : δ 2.45 (1H, dt, J = 2.6 and 14.6 Hz, H-3eq), δ 2.59 to δ 2.76 (1H, m, H-3ax), δ 5.19 (1H, dd, J = 1.9 and 11.1 Hz, H-2), δ 5.66 (1H, br t, J = 3.8 Hz, H-4), δ 6.81 (2H, d, J = 8.5 Hz, H-3′ and H-5′), δ 6.97 (1H, br s, H-4*imid*), δ 7.14 (1H, d, J = 8.5 Hz, H-5), δ 7.23 (1H, br s, H-5*imid*), δ 7.32 (2H, d, J = 8.5 Hz, H-2′ and H-6′), δ 7.49 to δ 7.60 (3H, m, H-4″, H-5″and H-6), δ 7,71 (1H, br s, H-2*imid*), δ 7,82 (1H, br d, J = 8.0 Hz, H-3″), δ 8.09 (1H, br d, J = 8.3 Hz, H-6″), δ 9.47 (1H, br s, 4−OH). ^{13}C NMR (100 MHz; DMSO-d_6) : δ 36.5 (C-3), δ 50.1 (C-4), δ 73.2 (C-2), δ 112.7 (C-4a), 115.2 (C-3′ and C-5′), δ 119.0 (C-4*imid*), δ 120.0 (C-6), δ 121.7 (C-6″), δ 124.2 (C-8), δ 125.8 (C-5″), δ 126.7 (C-5), δ 127.1 (C-4″), δ 127.6 (C-3″), δ 127.7 (C-2′ and C-6′), δ 128.5 (C-5*imid*), δ 130.1(C-1′), δ 133.9 (C-7), δ 137.7 (C-2*imid*), δ 150.3 (C-8a), δ 157.4 (C-4′).

4.4. Aromatase assay

Inhibitory activity of the tested compounds on aromatase was evaluated *in vitro* following Le Bail *et al.* procedure [9], using human placental microsomes and [1,2,6,7-^{3}H] androstenedione as substrate. All enzymatic experiments were performed at 37 °C in 0.1 M phosphate buffer. The final incubation mixture (1 mL) contained 3 mM glucose-6-phosphate, 0.5 mM NADP and 0.1 unit glucose-6-phosphate dehydrogenase in the presence or absence of inhibitor. The steroids were extracted from the incubation mixture, identified and quantified using HPLC and Packard Flow Scintillation Analyzer 150 TR. Inhibitory activity of these compounds at five appropriate concentrations was expressed as percent inhibition of the aromatization of androstenedione which had an initial concentration of 40 nM. In order to determine the IC_{50} value, all experiments were performed in duplicate. The IC_{50} value of the reference molecules, aminoglutethimide and letrozole are 5.8 μM and 0.018 μM respectively.

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