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New 7,8-benzoflavanones as potent aromatase inhibitors: Synthesis and biological evaluation

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Abstract—Some natural compounds such as flavonoids are known to possess a moderate inhibitory activity against aromatase, this enzyme being an interesting target for hormone-dependent breast cancer treatment. It has been demonstrated that the modulation of flavonoid skeleton could increase anti-aromatase effect. Therefore, new 7,8-benzoflavanones were synthesized and tested for their activity toward aromatase inhibition. It was observed that the introduction of a benzo ring at position C-7 and C-8 on flavanone skeleton led to new potent aromatase inhibitors, the resulting 7,8-benzoflavanones being until nine times more potent than aminogluthetimide (the first aromatase inhibitor used clinically).

1. Introduction

Flavonoids are a group of polyphenolic phytochemical compounds that occur ubiquitously in foods of plant origin. Over 4000 different naturally occurring flavonoids have been described.[1] Several studies have addressed the ability of flavonoids to interfere with the catalytic activity or expression of aromatase which is a cytochrome P-450 enzyme,[2] this enzyme being responsible for the final step of the estrogen biosynthesis (i.e. the conversion of androgens to estrogens). Estrogens are known to be important in the development of breast cancer in both pre- and postmenauposal women.

With reference to the mechanisms by which estrogens may increase the risk of breast cancer,[3–5] two general strategies have been developed to reduce the action of these hormones. The first strategy is to block estrogen receptors by anti-estrogens such as tamoxifen; the second pharmacological approach is to block estrogen synthesis through aromatase inhibition. The action level of aromatase inhibitors can explain their superiority relative to anti-estrogen drugs, this superiority being demonstrated by several studies.[4,5]

Besides the large number of synthetic aromatase inhibitors, a significant number of natural products such as flavonoids have been identified to possess also anti-aromatase effect with about the same activity as aminoglutethimide (AG), the first non-steroidal inhibitor used clinically. Among flavonoids, flavones and flavanones generally have a higher inhibitory effect than isoflavones and isoflavanones.[6]

Flavanones which possess a chiral center at C-2 are usually tested as racemic mixture; however, Kao et al. demonstrated that only the *S*-configuration isomer can bind to the active site of aromatase, which could explain the lower activity of flavanones relative to flavones.[7]

Several works have tried to establish the nature of flavonoid-aromatase interactions; we noticed that flavones bind to the active site of aromatase with an orientation in which A and C rings mimic rings D and C of androgen substrate, respectively.[7] Moreover, the presence of the C4-keto group on flavanone and flavone structures seems to be crucial for aromatase inhibition since its reduction to hydroxyl group causes complete loss of biological activity.[8] It appears that the keto group makes an interaction with the iron heme of aromatase.[7]

Keywords: 7,8-Benzoflavanones; Flavonoids; Aromatase inhibitors; Breast cancer.
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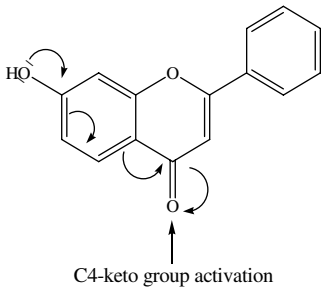
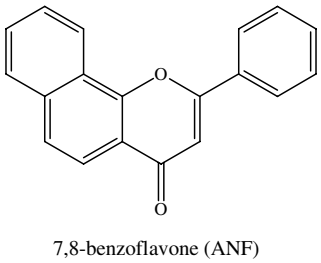


Scheme 1.



Scheme 3. Structure of 7,8-benzoflavone (ANF).

Other studies showed that the modulation of A, B, and C rings of flavan skeleton can increase significantly the inhibitory activity and many elements came out from these investigations. First, the A ring modulation of flavanone and flavone skeleton by hydroxylation at position 7 enhances the biological activity[8–10]; this effect could be explained by the activation of C4-keto group by the n–π–π–π conjugation caused by the hydroxyl group (Scheme 1). This activation seems to increase the connection between the C4 group and the heme iron of aromatase. Thus, 7-hydroxyflavone is 20 times more potent than flavone,[8] while the 7-hydroxyflavanone is six times more active than flavanone.[10] It appears also that hydroxylation at position 5 decreases the inhibitory activity; thus, 5-hydroxyflavone and 5,7-dihydroxyflavone (chrysin) are less active than flavone[8] and 7-hydroxyflavone,[7,9] respectively. The possible intra-molecular hydrogen bond between 5-OH group and C-4 keto may decrease the connecting potential of C4-keto with the heme iron of aromatase (Scheme 2).

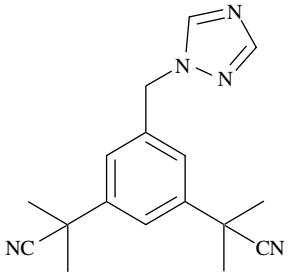
It was demonstrated that a 7-methoxy group is also favorable for activity; however, the 7-methoxyflavanone remains less active than the 7-hydroxyflavanone.[9,10]

Concerning the B ring modulation of 7-methoxyflavanone skeleton, we demonstrated that additional hydroxyl groups led to an increase in aromatase inhibition, the 3′,4′-dihydroxy-7-methoxyflavanone being the most active molecule. Thus, this latter compound was found to be twice more active than AG.[11]

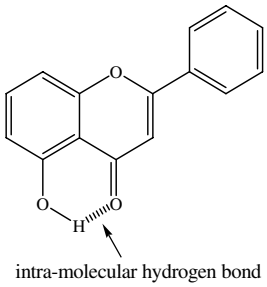
In the search of a new substitution pattern which could reinforce the interaction of flavanone skeleton with aromatase enzyme, we report in the course of the present study, the modulation of A ring of flavanone by the introduction of a benzo cycle at C7 and C8 leading to new 7,8-benzoflavanones. Indeed, the presence of such a substitution moiety on flavone skeleton was found to be essential for inhibitory effectiveness.

Thus, the 7,8-benzoflavone or α-naphthoflavone (ANF) (Scheme 3) has been extensively studied with regard to its inhibition of chemical carcinogenesis and of certain cytochrome P-450 mixed-function oxidase.[12] ANF, which is a synthetic flavonoid, has a similar affinity with aromatase to that of the natural substrates (androstenedione and testosterone); the study of its inhibition mechanism demonstrated that ANF is a competitive aromatase inhibitor.[13] Several comparative studies about flavonoid inhibitory effect against aromatase showed that ANF is the most potent inhibitor among the whole compounds tested.[13–15] We can notice that ANF is 15 and 136 times more potent than aminoglutethimide and flavone, respectively[14]; the 7-hydroxyflavone is also five times less potent than ANF.[15]

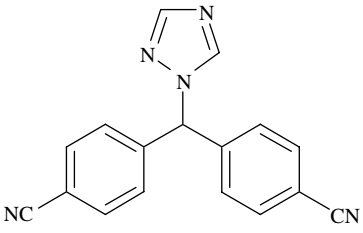
In the following, the preparation and the biological evaluation of new 7,8-benzoflavanones are described.



Scheme 4. Structure of anastrozole.



Scheme 2.

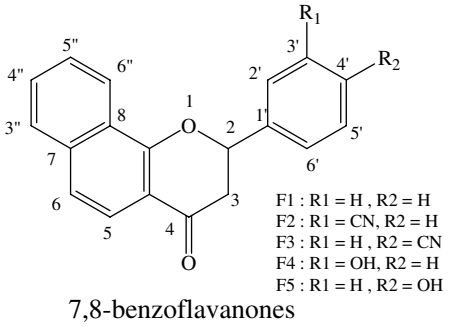


Scheme 5. Structure of letrozole.

2. Chemistry

2.1. General procedure of synthesis

Third generation aromatase inhibitors such as anastrozole (Scheme 4) and letrozole (Scheme 5) were 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.[16] Letrozole and anastrozole structure contains a cyano group, which is probably important for the biological activity.



Scheme 6. Structure of 7,8-benzoflavanones.

In order to determin the impact of this group on flavanone skeleton, we decided to synthesize two cyanobenzoflavanone derivatives. Additionally, two hydroxyl derivatives were also prepared with regard to the importance of hydroxyl groups for the flavonoid-enzyme interactions. Totally, five benzoflavanone derivatives were synthesized (Scheme 6): the non-substituted benzoflavanone (**F1**), two cyanobenzoflavanones (**F2** and **F3**), and two hydroxybenzoflavanones (**F4** and **F5**).

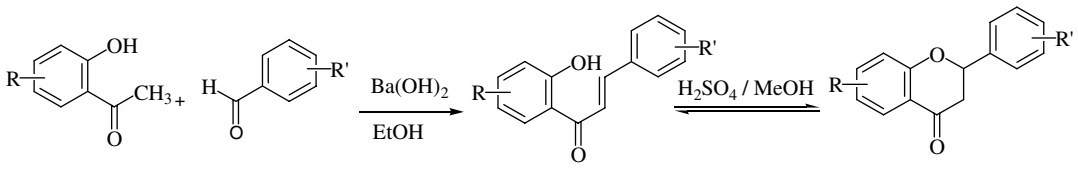
The 7,8-benzoflavanones (**F1**–**F5**) were obtained through the same synthesis pathway (Scheme 7) as described by Pouget et al.[11] and which is based on the cyclization of corresponding 2′-hydroxychalcones.

2.2. Synthesis and characterization

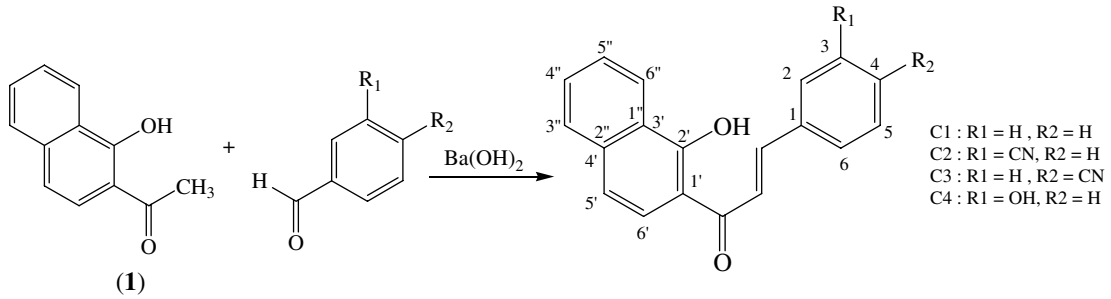
2.2.1. 3′,4′-Benzo-2′-hydroxychalcones

2.2.1.1. Synthesis. 3′,4′-Benzo-2′-hydroxychalcones were obtained by the Claisen–Schmidt condensation between 1-hydroxy-5,6-benzo-1-acetophenone (**1**) and appropriatly substituted benzaldehydes (Scheme 8). The preparation of 3′,4′-benzo-2′,4-dihydroxychalcone (**C5**) necessitated a protection step of 4-hydroxybenzaldehyde by 3,4-dihydro-α-pyrane (3,4-DHP), then the deprotection was carried out using APTS (Scheme 9).

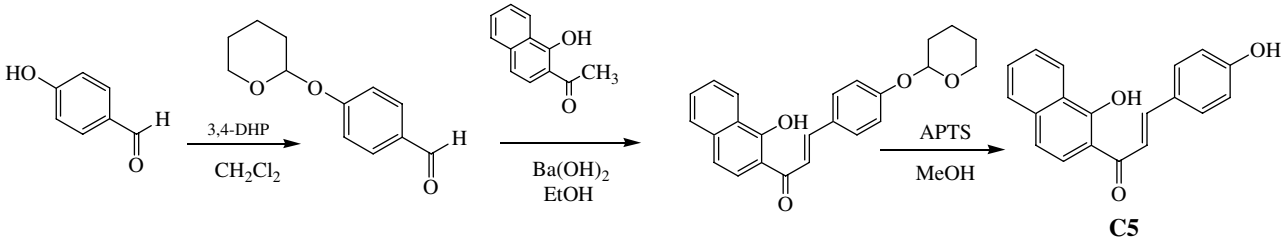
Benzochalcones were obtained with variable yields ranging from 35% to 85%. The synthesis of the non-substi-



Scheme 7. General synthesis of chalcones and flavanones.



Scheme 8. Synthesis of benzochalcones **C1**–**C4**.



Scheme 9. Synthesis of 3′,4′-benzo-2′,4-dihydroxychalcone **C5**.

Table 1. Characteristics of benzochalcone synthesis

Compound	Ratio (acetophenone/ benzaldehyde)	Reaction's time (h)	Yield (%)
C1	1/1.2	1.5	85
C2	1/1.5	4	35
C3	1/1.5	20	56
C4	1/2	29	45
C5	1/2	48	72

tuted benzochalcone was carried out with 1.2 equivalent of benzaldehyde, this ratio being used for the synthesis of previous chalcones.[11] However, the quantity of benzaldehyde was increased for the other benzochalcones (Table 1).

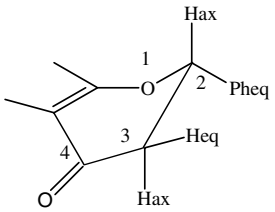
2.2.1.2. Structural analysis. Some characteristic ^{1}H NMR data are common between the five benzochalcone compounds. First, all proton signals are between 6.8 and 8.5 ppm, corresponding to aromatic and ethylenic protons. Then, all benzochalcones have two protons with a coupling constant of 15.5 Hz corresponding to a *trans*-ethylenic coupling.

2.2.2. 7,8-Benzoflavanones

2.2.2.1. Synthesis. The synthesis method for the 7,8-benzoflavanones is characterized by a chemical balance with corresponding chalcones, catalyzed by sulfuric acid (Scheme 10). 7,8-Benzoflavanones were obtained with satisfying yields (more than 59%) except for **F3** (yield = 25%). The presence of a cyano group at *para* position of B ring seems to be responsible for the lower yield. Indeed, the cyano group exerts a –M electronic effect which can decrease the Cβ affinity, to the detriment of the nucleophilic attack provoked by the oxygen of hydroxyl group at 2′ position.

2.2.2.2. Structural analysis. These compounds are characterized by three proton signals in the aliphatic zone corresponding to the heterocyclic ring protons, H-2, H-3eq, and H-3ax.

The H-3ax and H-3eq are coupled approximately with a constant of 17 Hz corresponding to a geminal coupling. Then, the value of the $J_{2,3ax}$ is so large (13 Hz) that it can only arise from a *trans*-diaxial coupling, thus H-2 is axial and the 2-phenyl group is equatorial. Finally, the coupling constant between H-2 and H-3eq which is close to 3.5 Hz confirms also the conformation of the heterocycle (Scheme 11).



Scheme 11. Conformation of the heterocyclic ring of flavanones.

Table 2. Aromatase inhibitory activity of tested compounds

Compound	IC_{50} (μM)	RP/AG
Flavanone	28	0.2
7-Methoxyflavanone	8.0	0.7
7-Hydroxyflavanone	3.8	1.4
7,8-Benzoflavanone	4.3	1.3
7,8-Benzoflavone	2.0	2.9

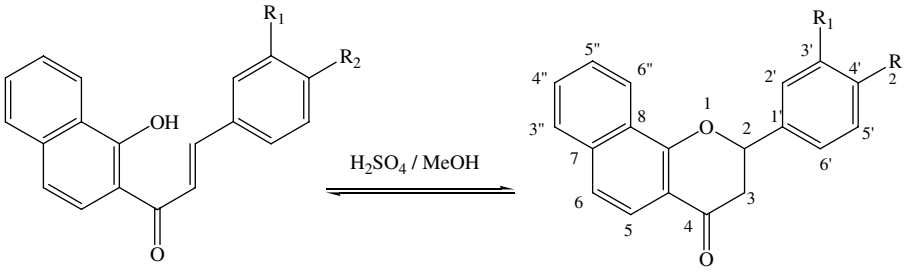
3. Biology

Inhibitory activity of compounds on aromatase was evaluated in vitro using human placental microsomes and [1,2,6,7-^{3}H] androstenedione as substrate. Aromatase inhibitory activity of compounds at five appropriate concentrations was expressed as percent inhibition of the aromatization of androstenedione which had an initial concentration of 40 nM. All experiments were performed in duplicate in order to determine the IC_{50} value. The pharmacological data for compounds **F1**–**F5** are summarized in Tables 2 and 3.

4. Results and discussion

First, our results (Table 2) confirm the relation between flavanones and flavones previously cited in this paper. Indeed, 7,8-benzoflavanone appears to be twice less potent than ANF; however, it possesses a similar activity to that of aminogluthetimide (5.8 μM). Then, we observed that the extension of A ring of flavanone by a benzo ring at positions 7 and 8 increases significantly the inhibitory activity. Thus, 7,8-benzoflavanone is seven times more potent than flavanone. Moreover, 7,8-benzoflavanone is twice more active than 7-methoxyflavanone; nevertheless, it possesses the same activity as 7-hydroxyflavanone.

The inhibitory activities of the whole 7,8-benzoflavanones and their 7-methoxy and 7-hydroxy analogs are listed



Scheme 10. Cyclization of 3′,4′-benzochalcones into corresponding 7,8-benzoflavanones.

Table 3. Anti-aromatase activity of 7,8-benzoflavanones compared to 7-hydroxy and 7-methoxyflavanones

Substitutents	7-Methoxyflavanones		7-Hydroxyflavanones		7,8-Benzoflavanones	
	IC_{50}	RP/AG	IC_{50}	RP/AG	IC_{50}	RP/AG
Non-substituted	8.0	0.7	3.8	1.4	4.3	1.3
3′-CN	/	/	/	/	1.37	4.2
4′-CN	/	/	/	/	1.54	3.7
3′-OH	3.5	1.5	3.4	1.5	0.61	9.3
4′-OH	3.7	1.4	2.1	2.5	0.63	9.0

in Table 3. The modulation of the B ring of the 7,8-benzoflavanone has highly increased the biological effect since 7,8-benzoflavanones are until nine times more potent than AG.

Introduction of an electron-withdrawing substituent such as the cyano group or electron-donating group such as the hydroxyl group on the B ring of 7,8-benzoflavanone increases the aromatase inhibitory effect. First, the presence of an hydroxyl group at 3′ or 4′ position is essential for high potency, the hydroxyl derivatives being seven times more potent than 7,8-benzoflavanone. We observed that hydroxyl groups on B ring exert a better effect on activity when they are associated to a 7,8-benzo ring rather than to a 7-methoxy or a 7-hydroxy group; indeed, the hydroxyl derivatives of both 7-methoxyflavanone and 7-hydroxyflavanone are at the most two times more potent than the corresponding flavanone.

Additionally, we observed that the cyano substituent plays also an important role for activity. The introduction of this latter group on B ring at position 3′ or 4′ of 7,8-benzoflavanone increases the inhibitory effect since cyano derivatives (**F2** and **F3**) are three times more potent than 7,8-benzoflavanone. However, we observed that hydroxyl compounds are two times more potent than their cyano analogs; so, the substituent nature influences the structure–activity relationship of 7,8-benzoflavanones.

On the contrary, there is no significant difference of activity between 3′-CN and 4′-CN analogs or between 3′-OH and 4′-OH derivatives; therefore, the position of these substituents does not influence the inhibitory activity on 7,8-benzoflavanone skeleton.

5. Conclusion

In conclusion, the introduction of a benzo ring at C7 and C8 positions of flavanone skeleton led to a new class of synthetic flavanones (i.e. 7,8-benzoflavanones) which are potent aromatase inhibitors since they are up to nine times more active than AG. Moreover, the B ring modulation of the new 7,8-benzoflavanone skeleton through the introduction of hydroxyl or cyano group at position 3′ or 4′ increases significantly the biological effect; therefore, further investigations will concern other substituent groups on different positions of the B ring.

Our recent studies showed that the C ring modulation of 7-OMe and 7-OH flavanones, through the replacement of the C4-keto group by an imidazolyl moiety, led to highly potent aromatase inhibitors.[17] The resulting compounds (4-imidazolylflavans) demonstrated a high potential against aromatase; they proved to be more potent than AG, exhibiting relative potencies ranging from 40 to 130.[17] Further modulation of the C ring of the present 7,8-benzoflavanones could increase the inhibitory effect and lead to compounds as active as the third-generation aromatase inhibitors.

As a conclusion, the modulation of flavonoids provides new leads for aromatase inhibition such as 7,8-benzoflavanones. Our results should contribute substantially to establish a structure–activity relationship between aromatase and their inhibitors which is important in the drug design strategy.

6. Characterization

The identification of benzochalcones and benzoflavanones was carried out by NMR spectroscopy and electrospray mass spectrometric (ESI-TOF) analysis. NMR spectra were recorded on Bruker DPX Avance spectrometer using tetramethylsilane as internal standard. Mass spectrometry was carried out on a Waters Alliance system equipped with electrospray interface.

6.1. Characterization of 3′,4′-benzo-2′-hydroxychalcones

3′,4′-Benzo-2′-hydroxychalcone (**C1**) was obtained in 85% yield as a red solid, mp: 112 °C. R_f 0.83 (toluene 100%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.30 (1H, d, J = 8.2 Hz, H-5′), δ 7.44 (3H, m, H-3, H-4 and H-5), δ 7.53 (1H, m, H-5″), δ 7.63 (1H, m, H-4″), δ 7.69 (2H, m, H-2 and H-6), δ 7.74 (1H, d, J = 15.4 Hz, H-α), δ 7.77 (1H, br d, J = 7.8 Hz, H-3″), δ 7.84 (1H, d, J = 8,9 Hz, H-6′), δ 7.98 (1H, d, J = 15.5 Hz, H-β), δ 8.50 (1H, br d, J = 8.2 Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 113.6 (C-1′), δ 118.3 (C-5′), δ 120.7 (C-α), δ 124.0 (C-6′), δ 124.6 (C-6″), δ 125.6 (C-3′), δ 126.0 (C-5″), δ 127.4 (C-3″), δ 128.7 (C-2/C-6), δ 129.1 (C-3 and C-5), δ 130.2 (C-4″), δ 130.8 (C-4), δ 134.9 (C-1), δ 137.5 (C-4′), δ 145.1 (C-β), δ 164.5 (C-2′), δ 193.3 (C=O). HR-MS (ESI) measured for $C_{19}H_{15}O_2$ $(M+H)^+$: 275.1075.

3′,4′-Benzo-3-cyano-2′-hydroxychalcone (**C2**) was obtained in 35% yield as a red solid, mp: 200 °C. R_f 0.44 (toluene 100%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.32 (1H, d, J = 8.9 Hz, H-5′), δ 7.55 (1H, m, H-5″), δ 7.57 (1H, t, J = 7.8 Hz, H-5), δ 7.66 (1H, m, H-4″), δ 7.70

(1H, dt, $J = 1.2$ and 7.8 Hz, H-4), δ 7.76 (1H, d, $J = 15.5$ Hz, H-α), δ 7.78 (1H, br d, $J = 7.8$ Hz, H-3″), δ 7.81 (1H, d, $J = 9.0$ Hz, H-6′), δ 7.88 (1H, br dt, $J = 7.8$ Hz, H-6), δ 7.90 (1H, d, $J = 15.4$ Hz, H-β), δ 7.96 (1H, br t, H-2), δ 8.50 (1H, br d, $J = 8.3$ Hz, H-6″). ^{3}C NMR (100 MHz; $CDCl_3$): δ 113.3 (C-1′), δ 113.5 (C-3), δ 118.1 (CN), δ 118.5 (C-5′), δ 123.0 (C-α), δ 123.7 (C-6′), δ 124.6 (C-6″), δ 125.4 (C-3′), δ 126.1 (C-5″), δ 127.5 (C-3″), δ 129.9 (C-5), δ 130.6 (C-4″), δ 131.5 (C-2), δ 133.5 (C-4), δ 132.6 (C-6), δ 137.5 (C-4′), δ 136.1 (C-1), δ 141.9 (C-β), δ 164.7 (C-2′), δ 192.5 (C═O). HR-MS (ESI) measured for $C_{20}H_{14}O_2N$ $(M+H)^+$: 300.1037.

3′,4′-Benzo-4-cyano-2′-hydroxychalcone (**C3**) was obtained in 56% yield as a red solid, mp: 218 °C. R_f 0.57 (toluene 100%). ^{1}H NMR (400 MHz; DMSO-d_6): δ 7.47 (1H, d, $J = 8.9$ Hz, H-5′), δ 7.61 (1H, m, H-5″), δ 7.74 (1H, m, H-4″), δ 7.94 (1H, br d, $J = 8.0$ Hz, H-3″), δ 7.95 (2H, d, $J = 8.3$ Hz, H-3 and H-5), δ 7.96 (1H, d, $J = 15.3$ Hz, H-β), δ 8.17 (2H, d, $J = 8.2$ Hz, H-2 and H-6), δ 8.31 (1H, d, $J = 9.1$ Hz, H-6′), δ 8.32 (1H, d, $J = 15.4$ Hz, H-α), δ 8.36 (1H, br d, $J = 8.3$ Hz, H-6″). ^{3}C NMR (100 MHz; DMSO-d_6): δ 112.6 (C-4), δ 113.4 (C-1′), δ 118.4 (C-5′), δ 118.6 (CN), δ 123.7 (C-6″), δ 124.3 (C-3′), δ 124.4 (C-α), δ 125.1 (C-6′), δ 126.3 (C-5″), δ 127.7 (C-3″), δ 129.8 (C-2 and C-6), δ 130.7 (C-4″), δ 132.7 (C-3 and C-5), δ 137.2 (C-4′), δ 138.9 (C-1), δ 142.9 (C-β), δ 163.4 (C-2′), δ 193.3 (C═O). HR-MS (ESI) measured for $C_{20}H_{14}O_2N$ $(M+H)^+$: 300.1025.

3′,4′-Benzo-2′,3-dihydroxychalcone (**C4**) was obtained in 45% yield as a red solid, mp: 163 °C. R_f 0.59 CH_2Cl_2–EtOAc 9.75:0.25. ^{1}H NMR (400 MHz; CD_3OD): δ 6.89 (1H, dt, $J = 2.6$ and 6.4 Hz, H-4), δ 7.18 (1H, m, H-2), δ 7.27 (1H, m, H-6), δ 7.28 (1H, m, H-5), δ 7.37 (1H, d, $J = 8.9$ Hz, H-5′), δ 7.53 (1H, m, H-5″), δ 7.65 (1H, m, H-4″), δ 7.81 (1H, br d, $J = 8.2$ Hz, H-3″), δ 7.88 (2H, br s, H-β and H-α), δ 8.02 (1H, br d, $J = 9.0$ Hz, H-6′), δ 8.43 (1H, br d, $J = 8.2$ Hz, H-6″). ^{3}C NMR (100 MHz; CD_3OD): δ 114.8 (C-1′), δ 116.1 (C-2), δ 119.2 (C-4), δ 119.6 (C-5′), δ 121.5 (C-6), δ 121.7 (C-α), δ 125.1 (C-6″), δ 125.5 (C-6′), δ 126.5 (C-3′), δ 127.0 (C-5″), δ 128.6 (C-3″), δ 131.2 (C-4″), δ 131.3 (C-5), δ 137.6 (C-1), δ 139.0 (C-4′), δ 146.6 (C-β), δ 159.2 (C-3), δ 164.9 (C-2′), δ 195.2 (C═O). HR-MS (ESI) measured for $C_{19}H_{15}O_3$ $(M+H)^+$: 291.1021.

3′,4′-Benzo-2′,4-dihydroxychalcone (**C5**) was obtained in 72% yield as a red solid, mp: 183 °C. R_f 0.21 (toluene–EtOAc 9.5:0.5). ^{1}H NMR (400 MHz; CD_3OD): δ 6.87 (2H, d, $J = 8.6$ Hz, H-3 and H-5), δ 7.35 (1H, d, $J = 8.8$ Hz, H-5′), δ 7.52 (1H, m, H-5″), δ 7.63 (1H, m, H-4″), δ 7.67 (2H, d, $J = 8.6$ Hz, H-2 and H-6), δ 7.77 (1H, d, $J = 15.4$ Hz, H-α), δ 7.80 (1H, br d, $J = 8.2$ Hz, H-3″), δ 7.92 (1H, d, $J = 15.3$ Hz, H-β), δ 8.02 (1H, d, $J = 9.0$ Hz, H-6′), δ 8.41 (1H, br d, $J = 8.4$ Hz, H-6″). ^{3}C NMR (100 MHz; CD_3OD): δ 114.8 (C-1′), δ 117.0 (C-3 and C-5), δ 118.3 (C-α), δ 119.4 (C-5′), δ 125.1 (C-6″), δ 125.5 (C-6′), δ 126.6 (C-3′), δ 126.8 (C-5″), δ 127.8 (C-1), δ 128.6 (C-3″), δ 131.1 (C-4″), δ 132.2 (C-2 and C-6), δ 138.8 (C-4′), δ 147.0 (C-β), δ 162.0 (C-4), δ 164.6 (C-2′), δ 195.1 (C═O). HR-MS (ESI) measured for $C_{19}H_{15}O_3$ $(M+H)^+$: 291.1020.

6.2. Characterization of 7,8-benzoflavanones

7,8-Benzoflavanone (**F1**) was obtained in 59% yield as a colorless oil. R_f 0.28 (toluene 100%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 2.99 (1H, dd, $J = 3.2$ and 16.8 Hz, H-3eq), δ 3.18 (1H, dd, $J = 13.5$ and 16.8 Hz, H-3ax), δ 5.69 (1H, dd, $J = 3.1$ and 13.5 Hz, H-2), δ 7.40–7.48 (4H, m, H-6, H-3′, H-4′ and H-5′), δ 7.52 (1H, m, H-5″), δ 7.58 (2H, br d, $J = 8.0$ Hz, H-2′ and H-6′), δ 7.62 (1H, m, H-4″), δ 7.81 (1H, br d, $J = 8.2$ Hz, H-3″), δ 7.91 (1H, d, $J = 8.7$ Hz, H-5), δ 8.34 (1H, br d, $J = 8.4$ Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 44.1 (C-3), δ 80.2 (C-2), δ 115.5 (C-4a), δ 121.3 (C-6), δ 121.7 (C-5), δ 123.7 (C-6″), δ 124.9 (C-8), δ 126.1 (C-2 and C-6), δ 126.3 (C-5″), δ 127.9 (C-3″), δ 128.8 (C-4′), δ 128.9 (C-3′ and C-5′), δ 129.7 (C-4″), δ 137.6 (C-7), δ 138.8 (C-1′), δ 159.8 (C-8a), δ 191.6 (C-4). HR-MS (ESI) measured for $C_{19}H_{15}O_2$ $(M+H)^+$: 275.1072.

3′-Cyano-7,8-benzoflavanone (**F2**) was obtained in 59% yield as a colorless oil. R_f 0.22 (toluene 100%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.01 (1H, dd, $J = 3.4$ and 16.8 Hz, H-3eq), δ 3.12 (1H, dd, $J = 13.1$ and 16.8 Hz, H-3ax), δ 5.72 (1H, dd, $J = 3.4$ and 13.1 Hz, H-2), δ 7.48 (1H, d, $J = 8.6$ Hz, H-6), δ 7.56 (1H, m, H-5″), δ 7.61 (1H, t, $J = 7.8$ Hz, H-5′), δ 7.66 (1H, m, H-4″), δ 7.72 (1H, dt, $J = 1.3$ and 7.8 Hz, H-4′), δ 7.79 (1H, br dt, $J = 7.8$ Hz, H-6′), δ 7.83 (1H, br d, $J = 8.1$ Hz, H-3″), δ 7.90 (1H, d, $J = 8.7$ Hz, H-5), δ 7.93 (1H, br t, $J = 1.6$ Hz, H-2′), δ 8.33 (1H, br d, $J = 8.3$ Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 43.9 (C-3), δ 79.1 (C-2), δ 113.3 (C-3′), δ 115.6 (C-4a), δ 118.4 (CN), δ 121.6 (C-5), δ 121.8 (C-6), δ 123.4 (C-6″), δ 124.6 (C-8), δ 126.6 (C-5″), δ 128.0 (C-3″), δ 129.7 (C-2′), δ 129.8 (C-5′), δ 130.0 (C-4″), δ 130.3 (C-6′), δ 132.4 (C-4′), δ 137.7 (C-7), δ 140.5 (C-1′), δ 159.2 (C-8a), δ 190.4 (C═O). HR-MS (ESI) measured for $C_{20}H_{14}O_2N$ $(M+H)^+$: 300.1035.

7,8-Benzo-4′-cyanoflavanone (**F3**) was obtained in 25% yield as a colorless oil. R_f 0.18 (toluene–EtOAc 9.75:0.25). ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.02 (1H, dd, $J = 3.6$ and 16.8 Hz, H-3eq), δ 3.12 (1H, dd, $J = 12.9$ and 16.8 Hz, H-3ax), δ 5.76 (1H, dd, $J = 3.6$ and 12.9 Hz, H-2), δ 7.48 (1H, d, $J = 8.7$ Hz, H-6), δ 7.56 (1H, m, H-5″), δ 7.66 (1H, m, H-4″), δ 7.71 (2H, d, $J = 8.2$ Hz, H-2′ and H-6′), δ 7.79 (2H, d, $J = 8.4$ Hz, H-3′ and H-5′), δ 7.83 (1H, br d, $J = 8.1$ Hz, H-3″), δ 7.90 (1H, d, $J = 8.7$ Hz, H-5), δ 8.33 (1H, br d, $J = 8.4$ Hz, H-6″). ^{13}C NMR (100 MHz; $CDCl_3$): δ 43.9 (C-3), δ 79.3 (C-2), δ 112.7 (C-4′), δ 115.6 (C-4a), δ 118.4 (CN), δ 121.6 (C-5), δ 121.8 (C-6), δ 123.4 (C-6″), δ 124.6 (C-8), δ 126.6 (C-5″), δ 126.7 (C-2′ and C-6′), δ 128.0 (C-3″), δ 129.9 (C-4″), δ 132.8 (C-3′ and C-5′), δ 137.7 (C-7), δ 140.5 (C-1′), δ 159.2 (C-8a), δ 190.4 (C═O). HR-MS (ESI) measured for $C_{20}H_{14}O_2N$ $(M+H)^+$: 300.1029.

7,8-Benzo-3′-hydroxyflavanone (**F4**) was obtained in 86% yield as a colorless oil. R_f 0.37 (CH_2Cl_2–EtOAc 9.75:0.25). ^{1}H NMR (400 MHz; DMSO-d_6): δ 2.97 (1H, dd, J = 3.3 and 16.8 Hz, H-3eq), δ 3.26 (1H, dd, J = 12.6 and 16.7 Hz, H-3ax), δ 5.84 (1H, dd, J = 3.3 and 12.5 Hz, H-2), δ 6.80 (1H, dt, J = 1.6 and 7.9 Hz, H-4′), δ 7.03 (1H, br d, J = 7.4 Hz, H-6′), δ 7.04 (1H, br s, H-2′), δ 7.26 (1H, t, J = 7.9 Hz, H-5′), δ 7.55 (1H, d, J = 8.6 Hz, H-6), δ 7.62 (1H, m, H-5″), δ 7.72 (1H, m, H-4″), δ 7.78 (1H, d, J = 8,6 Hz, H-5), δ 7.95 (1H, br d, J = 8.1 Hz, H-3″), δ 8.27 (1H, br d, J = 8.2 Hz, H-6″), δ 9.51 (1H, s, 3′-OH). ^{13}C NMR (100 MHz; DMSO-d_6): δ 42.7 (C-3), δ 79.3 (C-2), δ 113.1 (C-2′), δ 115.1 (C-4a), δ 115.3 (C-4′), δ 116.7 (C-6′), δ 120.7 (C-6), δ 121.1 (C-5), δ 122.9 (C-6″), δ 124.2 (C-8), δ 126.5 (C-5″), δ 127.9 (C-3″), δ 129.5 (C-5′), δ 129.7 (C-4″), δ 136.8 (C-7), δ 140.2 (C-1′), δ 157.5 (C-3′), δ 158.8 (C-8a), δ 190.9 (C=O). HR-MS (ESI) measured for $C_{19}H_{15}O_3$ $(M+H)^+$: 291.1018.

4′-Hydroxy-7,8-benzoflavanone (**F5**) was obtained in 66% yield as a colorless oil. R_f 0.20 (CH_2Cl_2–EtOAc 9.8:0.2). ^{1}H NMR (400 MHz; acetone-d_6): δ 2.90 (1H, dd, J = 3.0 and 16.7 Hz, H-3eq), δ 3.27 (1H, dd, J = 13.3 and 16.7 Hz, H-3ax), δ 5.76 (1H, dd, J = 3.0 and 13.2 Hz, H-2), δ 6.96 (2H, d, J = 8.6 Hz, H-3′ and H-5′), δ 7.51 (1H, d, J = 8.7 Hz, H-6), δ 7.54 (2H, d, J = 8.6 Hz, H-2′ and H-6′), δ 7.57 (1H, m, H-5″), δ 7.68 (1H, m, H-4″), δ 7.83 (1H, d, J = 8.7 Hz, H-5), δ 7.91 (1H, br d, J = 8.2 Hz, H-3″), δ 8.28 (1H, br d, J = 8.4 Hz, H-6″). ^{13}C NMR (100 MHz; acetone-d_6): δ 44.1 (C-3), δ 81.2 (C-2), δ 116.2 (C-3′ and C-5′), δ 116.4 (C-4a), δ 121.6 (C-6), δ 122.4 (C-5), δ 124.2 (C-6″), δ 125.9 (C-8), δ 126.1 (C-2′ C-6′), δ 127.2 (C-5″), δ 128.8 (C-3″), δ 130.4 (C-4″), δ 130.9 (C-1′), δ 138.4 (C-7), δ 158.7 (C-4′), δ 160.4 (C-8a), δ 191.8 (C=O). HR-MS (ESI) measured for $C_{19}H_{15}O_3$ $(M+H)^+$: 291.1019.

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