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New Aromatase Inhibitors. Synthesis and Inhibitory Activity of Pyridinyl-Substituted Flavanone Derivatives

Christelle Pouget, Catherine Fagnere, Jean-Philippe Basly, Gerard Habrioux and Albert-José Chulia*

UPRES EA 1085 « Biomolécules et cibles cellulaires tumorales », Faculté de Pharmacie, 2 rue du Docteur Marcland, 87025 Limoges cedex, France

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Abstract—Two (*E*)-pyridinyl-substituted flavanone derivatives were synthesized and UV irradiation of these compounds afforded a *Z*-enriched mixture. These products were tested for their ability to inhibit the cytochrome P450 aromatase. It was observed that the introduction of a pyridinylmethylene group at carbon 3 on flavanone nucleus led to a significant increase of aromatase inhibitory effect. Moreover, configuration had a substantial influence on the aromatase inhibitory activity since (*E*)-isomers were found to be more active than (*Z*)-isomers.

Introduction

Besides antiestrogens and progestins, aromatase inhibitors are used today for the treatment of advanced breast cancer of postmenopausal women.[1] Aromatase is a cytochrome P450 enzyme which catalyzes the conversion of androgens to estrogens. For two decades, aminoglutethimide was the unique aromatase inhibitor used clinically but its lack of selectivity and potency brought many groups to develop new leads for inhibition of the cytochrome P450 aromatase. Among all the synthesized compounds, 2-(4-pyridinylmethylene)-1-tetralones **A**[2] and derivative **B**[3] appeared to be potent aromatase inhibitors in vitro. It has been shown, on the basis of the characteristics of the UV difference spectrum (type II), an interaction between the pyridin nitrogen atom of these compounds and the heme iron atom of the cytochrome.

Our previous studies[4,5] have been mainly focused on the flavonoids which are natural compounds inhibiting human aromatase activity.[6–8] Thus, we demonstrated that flavanone **1** and 7-methoxyflavanone **2** were potent aromatase inhibitory agents with an IC_{50} of 28.5 and 8.0 μM respectively while the IC_{50} of aminoglutethimide was measured at 5.2 μM.

Some structure–activity relationship studies allowed to determine the binding characteristics and the structural requirements for flavonoids to inhibit aromatase.[9] Thus, these compounds were found to 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 while the B ring is oriented in a similar position to that of the substrate's ring A and points towards the extrahydrophobic pocket, described by Laughton et al.,[10] within the active site.

In the present paper, structural modifications on the C ring of flavanones **1** and **2**, leading to compounds **3** and **4**, respectively, were undertaken. In the following, the synthesis of these 3-(4-pyridinylmethylene)flavanones and their inhibitory activity towards aromatase are described.

Chemistry

The synthesis of compounds **3** and **4** called upon the following sequence (Scheme 1) in which a flavanone was submitted to the action of pyridine-4-carboxaldehyde in the presence of piperidine as catalyst. Compounds **3** and **4** were obtained in modest yields and usually as a mixture of geometrical isomers (in a 20:1 ratio) which were separable by TLC on silica gel ($CHCl_3$ 3% MeOH). By ^1H NMR spectroscopy, it was shown that the major compounds were the *E*-isomers; due to the diamagnetic

*Corresponding author. Tel.: +33-555-435-834, Fax: +33-555-435-910; e-mail: jose.chulia@unilim.fr

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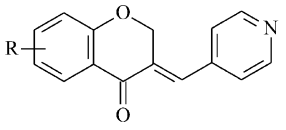
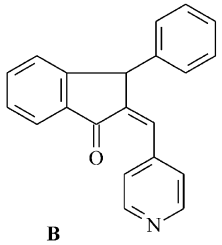


A



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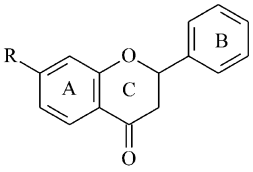

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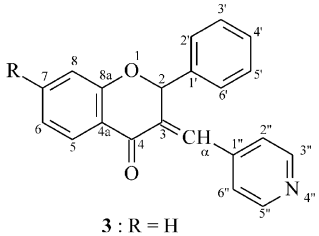
2 : R = OMe



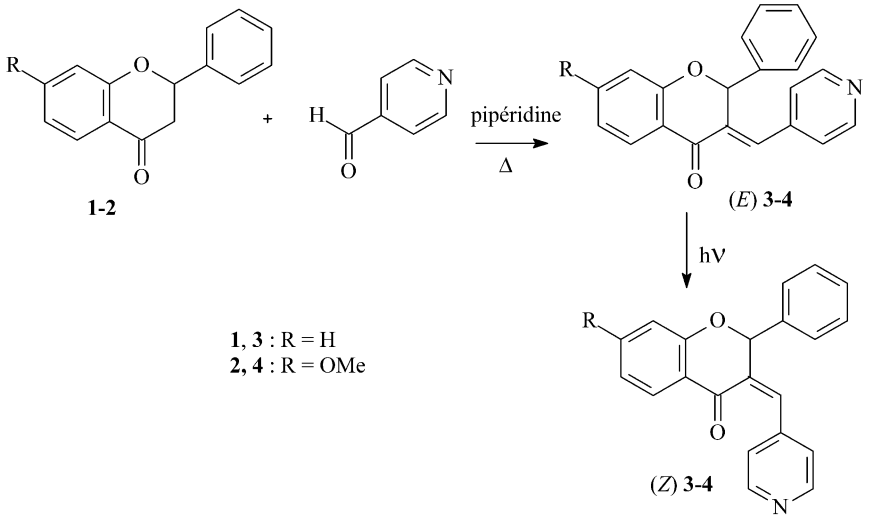
3 : R = H
4 : R = OMe

anisotropy of the carbonyl group, the vinylic proton H-α of these compounds gives a signal at a lower field than expected for an ordinary vinylic proton which indicates a *trans* configuration. The observed broadening of the signals for the vinylic proton H-α and the one at C-2 is attributed to a small long-range allylic coupling between these two protons. UV irradiation of the *E*-isomers **3** and **4** afforded a *Z*-enriched mixture (*Z*/*E*, 10:1, determined by ^{1}H NMR) (Scheme 1).

Results and Discussion

The inhibitory activities of the compounds **3** and **4** 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 and the potencies of the *E*-derivatives **3** and **4** and of the *Z*-enriched mixtures, relative to aminoglutethimide ($IC_{50} = 5.2$ μM) and to the corresponding flavanones, are given in Table 1. To determine IC_{50} values, compounds were tested in five appropriate concentrations with each experiment performed in duplicate.

The present work showed that an enhancement of the aromatase inhibitory effect of flavanones **1** and **2** could be achieved by the introduction of a 4-pyridinylmethylene group into the C-3 of their heterocyclic ring. Moreover, all products proved to be more potent aromatase inhibitors than aminoglutethimide used as reference compound. An investigation of the relationship between the configuration and aromatase inhibitory activity of compounds **3** and **4** revealed that the pure *E*-isomer was the most active. These findings may suggest that this restrained configuration could bring the pyridin nitrogen closer to the heme iron. Finally, as previously described for flavanones, the presence of a 7-methoxy group increased the inhibitory effect on aromatase activity. We hypothesized that this methoxy group could act as hydrogen bond acceptor and interact with aminoacid residues of the aromatase.



Scheme 1. Synthesis of compounds **3**–**4**.

Table 1. Aromatase inhibitory activity of compounds **3** and **4**

Compd	**3**		**4**	
	E-derivative	*Z*-enriched mixture	*E*-derivative	*Z*-enriched mixture
IC_{50} (μM)	0.80	3.3	0.62	1.6
RP[a]/aminoglutethimide	6.5	1.6	8.4	3.3
RP[a]/flavanone	35.6	8.6	12.9	5.0

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

These 3-(4-pyridinylmethylene)flavanones can be considered as new leads in the aromatase inhibitors area; therefore, their pharmacomodulation is currently ongoing. In particular, we are carrying out the substitution of the phenyl group at C-2 to occupy, in an optimal way, the extrahydrophobic pocket within the active site of the enzyme and to reinforce the interaction between the nitrogen atom of the inhibitors and the heme iron atom of the aromatase. The full details of the structure–activity relationships, chemical synthesis, molecular modeling and pharmacological studies of this novel series of compounds will be the subject of future publications.

Synthesis

Preparation of 3 and 4

Equivalent amounts of flavanone and pyridine-4-carboxaldehyde were allowed to react at 120 °C in the presence of few drops of piperidine for 15 min. Then, chloroform was added to the reaction mixture and the organic layer was washed twice with water, dried over sodium sulfate and evaporated to dryness. The residue was purified by preparative thin layer chromatography on silica gel with chloroform 3% methanol as eluent.

Preparation of the *Z*-enriched mixtures. The corresponding *E*-isomer was dissolved in EtOH and the solution was irradiated ($\lambda = 365$ nm) for 15 h.

***E*-3-(4-pyridinylmethylene)flavanone 3.** Yield 23%; λ_{max} (EtOH)/nm 282, 349; ν_{max} (CH_2Cl_2) cm^{-1}: 3058, 3035, 1675, 1603; δ_H (400 MHz; $CDCl_3$) 6.54 (1H, br s, H-2), 6.95 (1H, br d, $J = 8.9$ Hz, H-8), 6.99 (1H, br t, $J = 7.9$ Hz, H-6), 7.15 (2H, br s, H-2″ and H-6″), 7.28–7.46 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′ and H-7), 7.92 (1H, dd, $J = 7.8$ and 1.7 Hz, H-5), 7.97 (1H, br s, H-α), 8.64 (2H, br s, H-3″ and H-5″); δ_C (100 MHz; $CDCl_3$) 77.3 (CH, C-2), 118.8 (CH, C-8), 121.8 (Cq, C-4a), 122.1 (CH, C-6), 123.5 (2×CH, C-2″/6″), 127.4 (2×CH, C-2′/6′), 127.7 (CH, C-7), 128.9 (CH, C-4′), 129.0 (2×CH, C-3′/5′), 135.8 (CH, C-α), 135.9 (Cq, C-1′), 136.6 (CH, C-5), 137.5 (Cq, C-3), 141.5 (Cq, C-1″), 150.4 (2×CH, C-3″/5″), 159.0 (Cq, C-8a), 182.0 (Cq, C-4); ESP-MS (+40 V) m/z 314 $[M + H]^+$.

***Z*-3-(4-pyridinylmethylene)flavanone 3.** δ_H (400 MHz; $CDCl_3$) 6.13 (1H, br s, H-α), 6.56 (1H, br s, H-2), 7.04 (1H, br t, $J = 7.9$ Hz, H-6), 7.08 (1H, br d, $J = 8.4$ Hz, H-8), 7.38 (2H, br s, H-2″ and H-6″), 7.30–7.54 (6H, m, H-2′, H-3′, H-4′, H-5′, H-6′ and H-7), 7.89 (1H, dd, $J = 7.9$ and 1.7 Hz, H-5), 8.60 (2H, br s, H-3″ and H-5″); δ_C (100 MHz; $CDCl_3$) 84.2 (CH, C-2), 118.1 (CH, C-8), 122.0 (CH, C-6), 122.3 (Cq, C-4a), 123.6 (2×CH, C-2″/6″), 127.2 (2×CH, C-2′/6′), 127.8 (CH, C-7), 128.9 (CH, C-4′), 129.0 (2×CH, C-3′/5′), 136.5 (Cq, C-1′), 136.5 (CH, C-5), 137.2 (Cq, C-3), 137.6 (CH, C-α), 142.4 (Cq, C-1″), 149.7 (2×CH, C-3″/5″), 160.2 (Cq, C-8a), 183.1 (Cq, C-4).

***E*-7-methoxy-3-(4-pyridinylmethylene)flavanone 4.** Yield 25%; λ_{max} (EtOH)/nm 293, 339; ν_{max} (CH_2Cl_2) cm^{-1}: 3061, 3031, 2941, 2841, 1672, 1604; δ_H (400 MHz; $CDCl_3$) 3.81 (3H, s, OCH_3), 6.38 (1H, d, $J = 2.4$ Hz, H-8), 6.50 (1H, br s, H-2), 6.54 (1H, dd, $J = 8.8$ and 2.4 Hz, H-6), 7.13 (2H, br s, H-2″ and H-6″), 7.31–7.45 (5H, m, H-2′, H-3′, H-4′, H-5′ and H-6′), 7.87 (1H, d, $J = 8.8$ Hz, H-5), 7.94 (1H, br s, H-α), 8.62 (2H, br s, H-3″ and H-5″); δ_C (100 MHz; $CDCl_3$) 55.7 (OCH_3), 77.6 (CH, C-2), 101.8 (CH, C-8), 110.5 (CH, C-6), 115.6 (Cq, C-4a), 123.4 (2×CH, C-2″/6″), 127.4 (2×CH, C-2′/6′), 128.9 (CH, C-4′), 129.0 (2×CH, C-3′/5′), 129.6 (CH, C-5), 135.2 (CH, C-α), 135.9 (Cq, C-1′), 137.7 (Cq, C-3), 141.7 (Cq, C-1″), 150.3 (2×CH, C-3″/5″), 161.1 (Cq, C-8a), 166.7 (Cq, C-7), 180.5 (Cq, C-4); ESP-MS (+40 V) m/z 344 $[M + H]^+$.

***Z*-7-methoxy-3-(4-pyridinylmethylene)flavanone 4.** δ_H (400 MHz; $CDCl_3$) 3.85 (3H, s, OCH_3), 6.10 (1H, br s, H-α), 6.50 (1H, br s, H-2), 6.52 (1H, d, $J = 2.4$ Hz, H-8), 6.60 (1H, dd, $J = 8.9$ and 2.4 Hz, H-6), 7.38 (2H, br s, H-2″ and H-6″), 7.30–7.47 (5H, m, H-2′, H-3′, H-4′, H-5′ and H-6′), 7.83 (1H, d, $J = 8.9$ Hz, H-5), 8.59 (2H, br s, H-3″ and H-5″); δ_C (100 MHz; $CDCl_3$) 55.7 (OCH_3), 84.6 (CH, C-2), 101.0 (CH, C-8), 110.6 (CH, C-6), 116.2 (Cq, C-4a), 123.7 (2×CH, C-2″/6″), 127.2 (2×CH, C-2′/6′), 128.9 (CH, C-4′), 129.0 (2×CH, C-3′/5′), 129.6 (CH, C-5), 136.7 (Cq, C-1′), 137.1 (CH, C-α), 137.3 (Cq, C-3), 142.7 (Cq, C-1″), 149.6 (2×CH, C-3″/5″), 162.2 (Cq, C-8a), 166.5 (CH, C-7), 181.7 (Cq, C-4).

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